

Transcriptomics Identify a Unique Intermittent Hypoxia Mediated Profile in Obstructive Sleep Apnea

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Obstructive sleep apnea (OSA) is associated with systemic hypertension and cardiovascular comorbidities (1), but the underlying pathophysiology is not well understood. In our Supplemental Oxygen in OSA (SOX) randomised-control trial, recently published in this journal, supplemental oxygen virtually abolished the morning rise in blood pressure normally seen during continuous positive airway pressure (CPAP) withdrawal (2). This suggests that intermittent hypoxia (IH) is the dominant cause of morning blood pressure elevations during CPAP withdrawal, rather than recurrent arousals. However, following CPAP withdrawal we did not observe differences in secondary outcome measures of systemic inflammation (C-reactive protein) and sympathetic activation (urinary normetadrenaline). The role of IH-mediated oxidative stress and inflammation in the development of OSA-mediated cardiovascular disease is controversial (3, 4).

In this present study we used a hypothesis-free approach to identify potential unique transcriptomic profiles from whole blood leucocytes that were activated by IH, such as that occurring when CPAP is withdrawn, and to explore the effects of supplemental oxygen (which largely attenuated this IH) on these profiles.

We utilised samples from the SOX trial. Briefly patients with known moderate to severe OSA, previously established on CPAP, were withdrawn from CPAP onto supplemental oxygen or supplemental air (sham) for 14 nights, in a randomised cross-over design. Supplemental oxygen markedly attenuated IH; the median (1st, 3rd quartiles) oxygen

desaturation index was 32.5 /h (25.6, 47.0) during sham and 6.4 /h (4.0, 14.7) during supplemental oxygen.

Morning blood samples were collected before and after 14 nights in both arms. Whole blood leucocytes were extracted and stored for subsequent RNA extraction using LeukoLOCK™ kits. Following leucocyte RNA extraction, 3' RNA sequencing was performed (*Lexogen*) on the Hiseq 4000 platform (*Illumina*) with an average of 5 million reads/sample. Reads were aligned using Htseq v0.6.1 with DESEQ2 v1.10.1 for normalisation of data and differential expression (<http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html>). Gene set enrichment analysis was performed using the Hallmark Molecular Signatures Database (MsigDB) within the JavaGSEA version 2 platform (<http://software.broadinstitute.org/gsea/index.jsp>). These are 50 of the most well defined biological processes or states.

After false discovery rate (FDR) correction (Benjamin-Hochberg), 25 genes were significantly upregulated following CPAP withdrawal onto sham, compared to baseline (*Figure 1*). These included several genes (TRAFD1, PPP4C, ZFAND3, HPSA8, ITGAX, and HSPA1A) involved in nuclear factor kappa-beta (NFκβ) signalling, which is an IH-driven inflammatory pathway (5, 6). In contrast, there were no significantly differentially expressed genes following CPAP withdrawal onto supplemental oxygen.

Next, we looked at whether any Hallmark MsigDB gene pathways were enriched in our datasets. As shown in *Figure 2*, fifteen pathways were upregulated following CPAP withdrawal onto sham. Of these, seven pathways were only upregulated with CPAP withdrawal onto sham, and not supplemental oxygen. These included the “inflammatory response”, “interferon alpha response”, “interferon gamma response”, and “TGF beta signalling”. Interferon alpha and gamma pathways are upstream modulators of NFκβ. Eight pathways were significantly upregulated following CPAP withdrawal in both arms including pathways relating to oxidative stress (“reactive oxygen species” and “oxidative phosphorylation”) and inflammatory pathways (“TNFA signalling”, “IL6/JAK/STAT3 signalling”). There were five pathways that were only upregulated with CPAP withdrawal onto supplemental oxygen, and not sham. These included “MTORC1 signalling” and “DNA repair” pathways.

We report for the first time that, following CPAP withdrawal, the return of OSA results in upregulation of several genes in circulating leucocytes; and importantly that no single gene was upregulated following CPAP withdrawal in the presence of supplemental oxygen (that attenuates IH), suggesting that the IH is the driving factor. Further pathway analysis demonstrated enrichment of several pro-inflammatory pathways, such as “inflammatory response”, “interferon alpha”, and “interferon gamma” with sham, that was not seen in the supplemental oxygen arm. The upregulation of these genes and gene pathways with sham, but not with supplemental oxygen, suggests that IH may lead to cardiovascular disease through the activation of inflammatory processes, possibly through NFκβ signalling, as previously suggested (3). There were also inflammatory NFκβ-mediated pathways that were

enriched in both arms (“TNFA signalling” and “IL6/JAK/STAT3 signalling”) but, although not a direct comparison, the normalised enrichment scores were lower in the supplemental oxygen arm, again suggesting IH-driven, NFκβ-mediated inflammation in OSA.

In contrast, pathways relating to oxidative stress were similarly enriched with CPAP withdrawal in both arms. There are several possible explanations for this result. First, it could be that oxidative stress is caused by non-hypoxia mediated mechanisms, such as sleep deprivation (7), or sleep fragmentation, which occurred to an equal extent in both arms. Second, although supplemental oxygen attenuated the IH, it did not fully abolish it. Therefore, it could be that smaller oscillations in oxygen saturations still lead to cyclical deoxygenation and re-oxygenation sufficient to trigger oxidative stress (8). Third, there could be different mechanisms inducing oxidative stress, with intermittent hyperoxia in the supplemental oxygen arm and intermittent hypoxia in the sham arm, both causing oxidative stress.

Some pathways were only significantly upregulated following CPAP withdrawal onto supplemental oxygen, including the “DNA repair” pathway. This pathway has been implicated in hyperoxic damage (9), and supplemental oxygen may have potentially deleterious effects, as has been shown in the context of acute myocardial infarction (10).

In summary, our results have identified that there are discernible alterations in the transcriptome of circulating leucocytes following CPAP withdrawal. Some caveats must be

applied to our conclusions, principally, that transcriptomic changes were identified in leucocytes, and it would be unwise to extrapolate these findings to other tissues. Nevertheless, the changes observed in gene expression support a pro-inflammatory role of IH in OSA and suggest that supplemental oxygen may attenuate inflammation in OSA. The role of the transcriptional changes we observed in the large blood pressure effect seen in the main SOX study is uncertain (2). In any case, the IH-mediated inflammation demonstrated in this study could contribute to the development of atherosclerosis and related cardiovascular morbidities, known to be associated with uncontrolled OSA. Further confirmatory work is needed to fully understand the physiological effects, long-term efficacy, safety, and tolerability of supplemental oxygen, to establish if it is a viable treatment option when CPAP is not tolerated.

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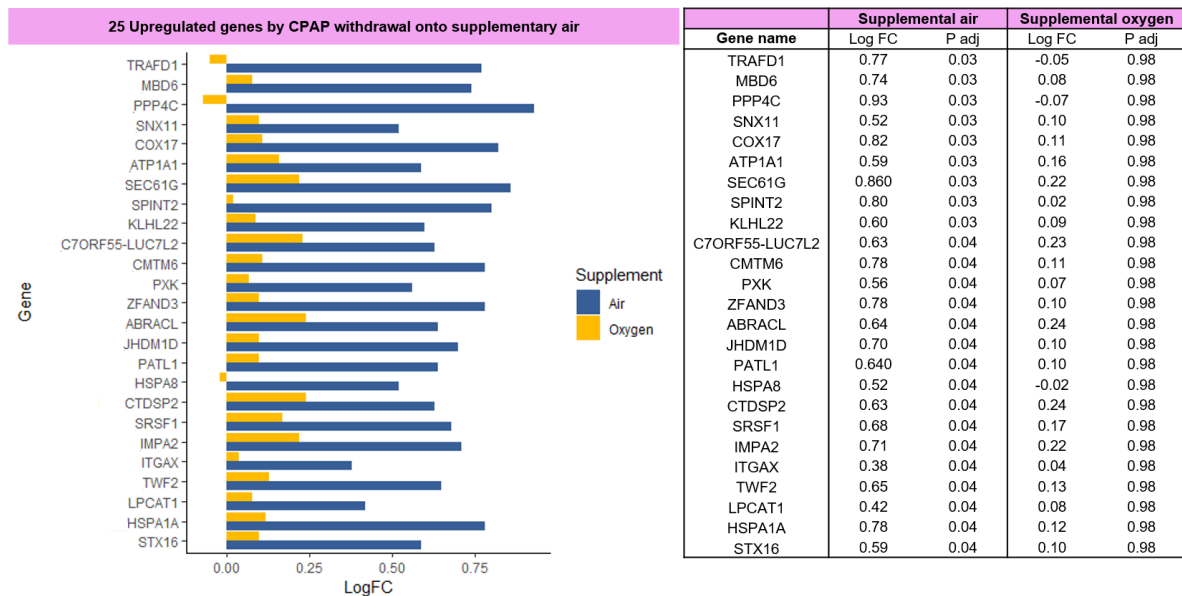


Figure 1: Bar chart showing the twenty-five genes that were significantly upregulated following CPAP withdrawal onto supplemental air (sham), compared to baseline. Blue bars represent the log fold change for CPAP withdrawal onto air and yellow bars represent the log fold change for the same genes with CPAP withdrawal onto supplemental oxygen. The log fold changes and adjusted *p* values are shown on the right of this figure. LogFC=log fold change to the base 2, *P* adj is the *p* value following adjustment for false discoveries.

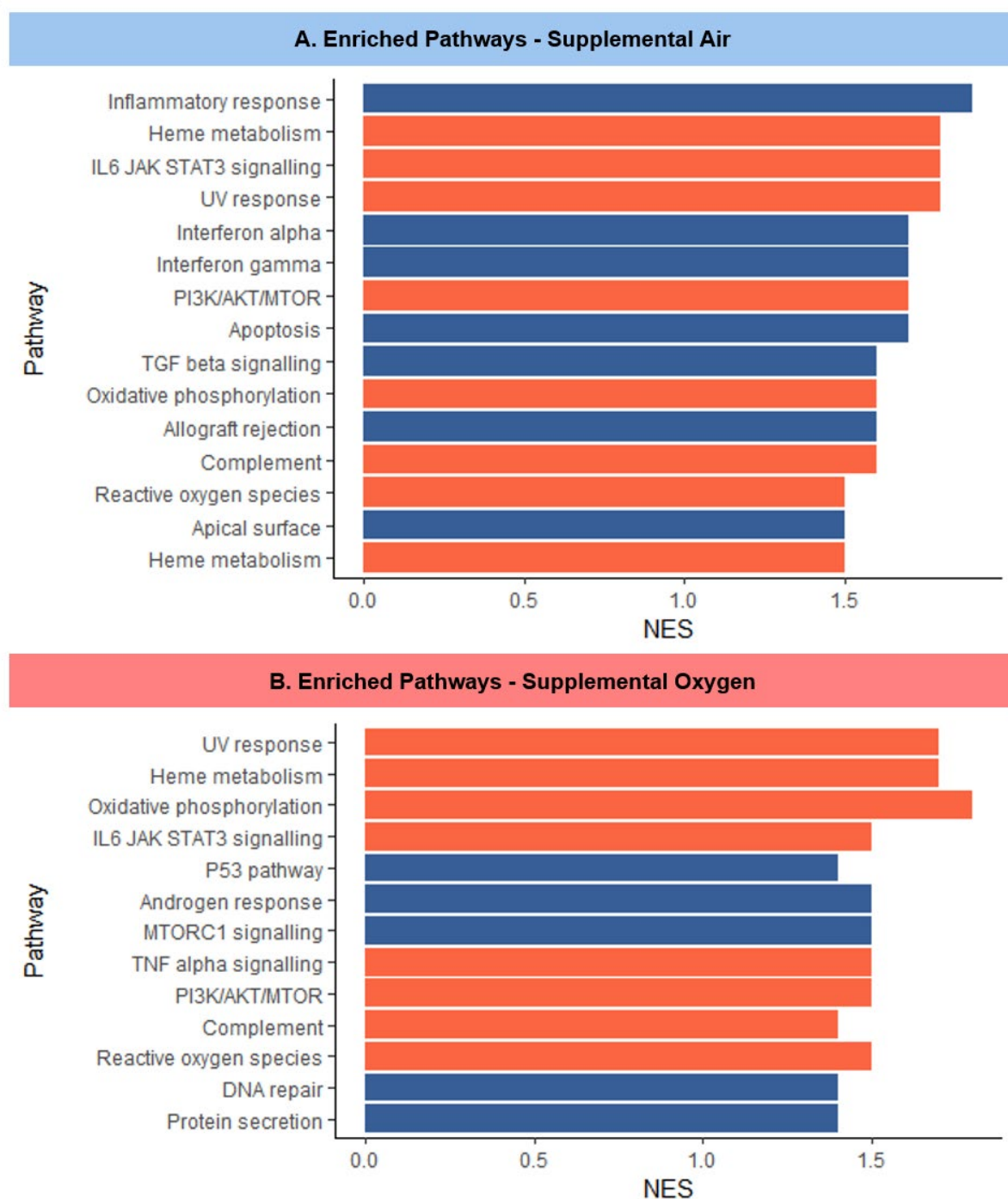


Figure 2: Bar charts showing A) Significantly enriched (upregulated) pathways of genes with CPAP withdrawal onto supplemental air and B) Significantly enriched (upregulated) pathways of genes with CPAP withdrawal supplemental oxygen. Red bars signify significantly enriched pathways common to both arms. NES=normalised enrichment score.

Due to the small number of samples a stringent FDR (false discovery rate corrected) q-value <0.05 was considered significant.