Introduction and objectives Pulmonary arterial hypertension (PAH) is a fatal lung disease characterised by progressive pulmonary vascular remodelling, a key component of which is the proliferation and migration of pulmonary arterial smooth muscle cells (PA-SMCs). Although current therapies are good at alleviating symptoms, they do not reverse the underlying pulmonary vascular remodelling. We have previously demonstrated that the secreted glycoprotein, osteoprotegerin (OPG, TNFRSF11B), is elevated within pulmonary vascular lesions and serum from idiopathic PAH (IPAH) patients and induces PA-SMC proliferation and migration *in vitro*. Furthermore, genetic deletion or antibody blockade of OPG can prevent and reverse disease in preclinical animal models. However, how OPG signals to mediate PA-SMC phenotype remains unclear. We therefore aimed to characterise the OPG signalling cascade in PA-SMCs, and identify the receptor through which this is mediated.
Methods PA-SMCs were stimulated with 0.2% FCS and OPG (50 ng/ml) for 10 and 60 min. Phosphorylation targets were identified by Kinex antibody microarray (Kinexus, Canada). An RNA expression microarray (Agilent) was performed on PA-SMCs following 6-hour OPG stimulation. OPG binding partners were identified following reverse transfection of HEK293 cells with 2054 human membrane proteins (Retrogenix, Sheffield, UK). Interactions were confirmed in PA-SMCs by co-immunoprecipitation. PA-SMCs were pre-treated with Fas neutralising antibody (1500 ng/ml), TRAIL antibody (1500 ng/ml) or both antibodies, 30 min before OPG stimulation. Proliferation was assessed after 72 h.
Results OPG induced significant activation of CDK4 and CDK5, HSP27 and ERK1/2, and significant decrease in phospho-mTOR. OPG significantly altered the expression of 57 PAH-associated genes, including TRAIL. Four novel OPG interactions with IL1RAcP, Fas, TMPRSS11D and GAP43 were identified and we confirmed OPG interaction with IL1RAcP and Fas in PA-SMC. Fas RNA expression was elevated in IPAH PA-SMCs and protein expression was elevated in the right ventricle and pulmonary artery from IPAH patients. Fas blockade reduced OPG-induced proliferation by ~40%, which was further reduced by simultaneous TRAIL blockade. Fas blockade also prevented OPG-induced PDGFRA and TNC RNA expression.
Conclusions These studies begin to reveal the intracellular signalling mechanisms and receptor through which OPG induces PA-SMC proliferation, further highlighting the therapeutic potential of targeting OPG in PAH.

T6 IMPACT OF ENVIRONMENTAL DIFFERENCES IN THE PREVALENCE OF AIRWAY DYSFUNCTION IN ELITE ATHLETES: GB BOXING VS. GB SWIMMING

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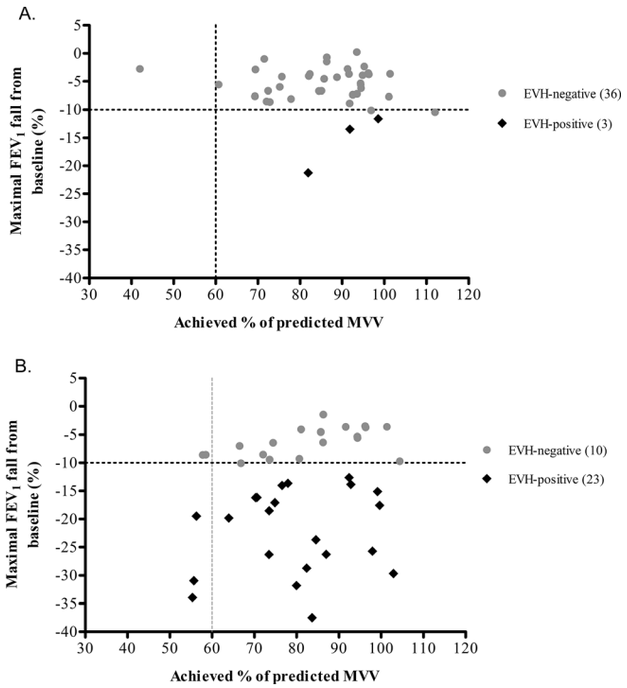
Objectives Exercising in a provocative environment (e.g. indoor swimming pool) at sustained high minute ventilation rates may increase the prevalence of airway dysfunction in athletic populations. The purpose of the study was to evaluate the impact of environmental differences in the prevalence of airway dysfunction in two cohorts of elite GB athletes.
Methods Airway dysfunction was evaluated in the GB boxing (n = 39, Mean (SD) age: 22.0 (3.2) yrs.) and swimming squads (n = 33, Mean (SD) age: 21.0 (3.0) yrs.). All participants completed a Eucapnic Voluntary Hyperpnoea (EVH) challenge test, an indirect bronchoprovocation test, to characterise airway dysfunction (defined as abnormal if >10% fall in FEV_1 post-challenge). Fraction of exhaled Nitric Oxide (FeNO) was measured and participants completed a symptom and medication questionnaire.
Results The prevalence of airway dysfunction was greater in elite swimmers (70%) than boxers (8%) ($p < 0.001$) (Figure 1). The EVH assessment process revealed missed and incorrect diagnosis of airway dysfunction; specifically 65% (17 of 26) of those with airway dysfunction had no prior diagnosis of asthma or exercise induced bronchoconstriction. Moreover, a prior diagnosis of asthma was not supported by testing in 9% (4 of 46) of the athletes. These athletes were prescribed one or a combination of short-acting β2-agonists, long-acting β2-agonists and inhaled corticosteroids. Neither symptoms nor baseline lung function were predictive of a positive EVH-challenge in swimmers. No correlation between change in lung function or airway dysfunction and FeNO value.
Conclusions The prevalence of airway dysfunction was nine fold greater in elite swimmers when compared with boxers. This finding emphasises the high proportion of EVH-positive elite swimmers and the importance of strategies needed to ensure their respiratory health is optimised. These results also suggest that airway dysfunction is not only related to intensity and frequency of exertional hyperpnoea but also environmental conditions.



Abstract T6 Figure 1 Maximal fall in FEV_1 post-EVH challenge showing tests that attained 60%MVV (vertical line and tests, that were above and below the 10% drop in FEV_1 cut-off value (horizontal line) for a positive test. Panel A, represents GB Boxing and Panel B, represents GB Swimming