Abstract T3 Table 1 The effects of ECVE exposure on MMP-9 and CXCL8 release from blood neutrophils after 6 hr, and CXCL8 release from alveolar macrophages after 24 hr. Data presented as mean (sd)

Neutrophils				Macrophages	
MMP-9		CXCL8		CXCL8	
0 ECVE	0.003 ECVE	0 ECVE	0.003 ECVE	0 ECVE	0.003 ECVE
		175	348	1647	6000
44782 (25809)	184585 (104617)	(248)	(364)	(2457)	(7602)

We have investigated the effects of e-cigs on human innate immune cells *in vitro*.

Methods Blood neutrophils from six healthy non-smokers were exposed to e-cig vapour extract (ECVE) for 6 hr. MMP-9 and CXCL8 release were measured by ELISA and MMP-9 activity was measured by zymography. p38 MAPK activation was also measured, along with neutrophil shape change and CD11b and CD66b expression by flow cytometry. Finally, we measured CXCL8 release from alveolar macrophages isolated from resected lung tissue from three ex-tobacco smokers exposed to ECVE for 24 hr.

Results Exposure of neutrophils to ECVE increased MMP-9 and CXCL8 release with the maximal effect observed at an optical density (OD) of 0.003 (Table 1). This was observed along with an increase in MMP-9 gelatinase activity and increased p38 MAPK activation.

Furthermore, neutrophil shape change, and dual CD11b and CD66b expression increased in response to ECVE treatment compared to untreated cells.

Following a similar trend, 0.003 (OD) ECVE caused an increase in CXCL8 release from alveolar macrophages.

Discussion We have shown that e-cig exposure causes an inflammatory response from neutrophils and macrophages. The effects discussed here are similar to those caused by tobacco cigarettes. Based on these findings, the use of e-cigs may pose a risk to public health.

T4 PNEUMOCOCCAL CONJUGATE VACCINE REDUCES RATE, DENSITY AND DURATION OF EXPERIMENTAL HUMAN PNEUMOCOCCAL COLONISATION: FIRST HUMAN CHALLENGE TESTING OF A PNEUMOCOCCAL VACCINE

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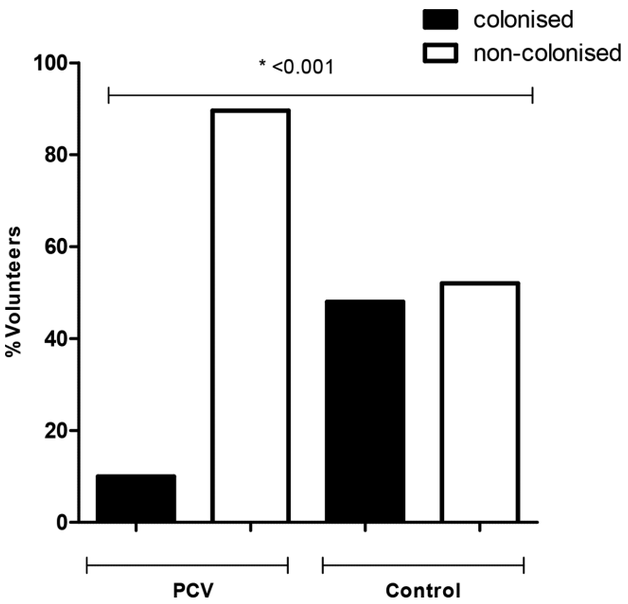
Objective To determine the effect of the Pneumococcal Conjugate Vaccine (PCV-13) on experimental pneumococcal colonisation compared to Hepatitis A vaccination (control) in healthy participants.

Design Double blind randomised controlled trial.

Setting Clinical Research Unit in the Royal Liverpool University Hospital.

Participants 99 healthy participants aged 18–50 years were randomly assigned to receive PCV-13 (n = 49) or Hepatitis A (n = 50) vaccination according to a randomisation plan in blocks of ten.

Interventions Participants previously vaccinated with PCV or control were inoculated after 4 weeks with 80,000C FU/100 µl pneumococcal bacteria (6B) 100 µl per naris. Participants were followed up for 21 days to determine pneumococcal colonisation by culture of nasal wash samples.



Abstract T4 Figure 1 Percentage of participants' colonised and non-colonised by 6B pneumococcus assessed using classical microbiology according to vaccination group at any time point

Main outcome measures The primary outcome measure was the culture of type 6B pneumococcus at either day 2, 7, 14 or 21 following inoculation. Secondary outcome measures included the density and duration of pneumococcal colonisation post inoculation of 6B and the presence of any other naturally acquired pneumococcal strains.

Results The PCV group showed a significantly reduced experimental colonisation rate 5/48 compared to the control group 23/48 (<0.001) [Figure 1]. Both the density and duration of colonisation were reduced in the PCV group compared to the control group following inoculation. The area under the density-time curve (total exposure) was significantly reduced in the PCV compared to control group (mean 8902 vs 267580 p = 0.0179).

Conclusion PCV reduces pneumococcal colonisation rate, density and duration in healthy adults. The Experimental Human Pneumococcal Colonisation (EHPC) model is a safe, effective and efficient method of analysing the efficacy of vaccination on pneumococcal colonisation. We suggest that this novel EHPC model can now be used as a platform for future pneumococcal vaccine testing, using small sample sizes and shorter time scales than community studies in order to reduce time and cost to market. We recommend that carriage rate, density and duration are all measured in these studies.

Trial registration.

EudraCT: 2012-005141-20.

ISRCTN: 45340436.

T5 OPG REGULATES PULMONARY ARTERIAL SMOOTH MUSCLE CELL PROLIFERATION AND THE EXPRESSION OF PAH-ASSOCIATED GENES VIA FAS

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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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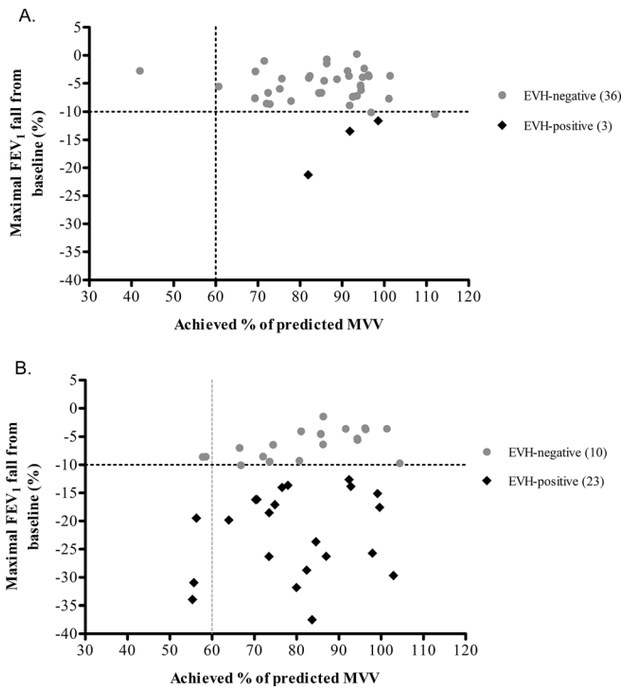
Objectives Exercising in a provocative environment (e.g. indoor swimming pool) at sustained high minute ventilation rates may increase the prevalence of airway dysfunction in athletic populations. The purpose of the study was to evaluate the impact of environmental differences in the prevalence of airway 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