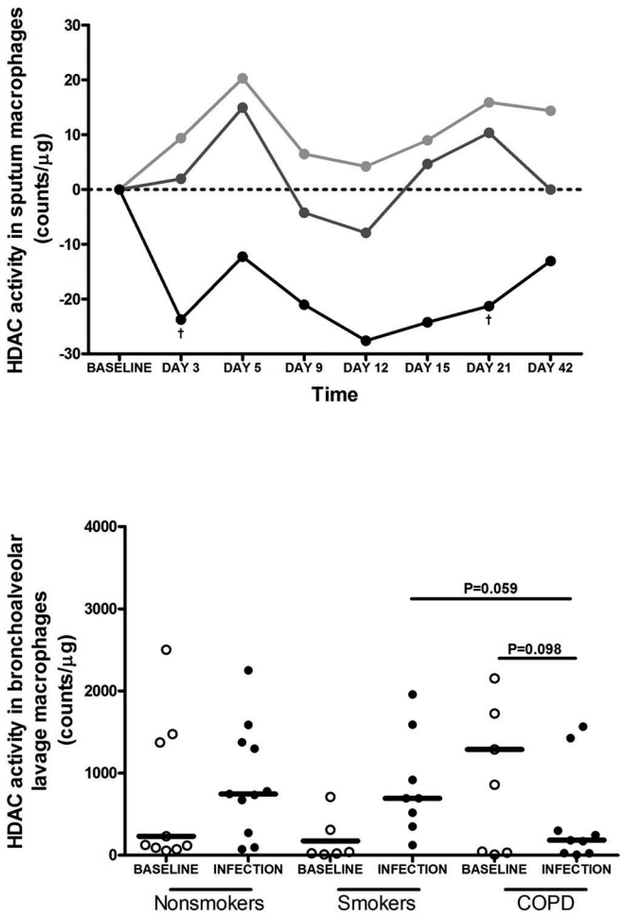

Abstract S112 Figure 1.

was significantly lower in the COPD subjects compared to non-smokers on days 5 and 42 (P < 0.05), and there was a trend towards lower levels of HDAC in BAL macrophages at infection compared to the non-smokers (P = 0.095) and smokers (P = 0.059) (Figure 1).

Lower sputum macrophage HDAC2 activity at baseline was associated with greater sputum virus load (r = -0.82, P = 0.022) and higher sputum levels of neutrophil elastase (r = -0.81, P = 0.022) and TNF- (r = -0.79, P = 0.028).HDAC2 activity in BAL macrophages at infection correlated inversely with peak NL virus load (r = -0.8, P = 0.0096), peak sputum GM-CSF (r = -0.67, P = 0.0499), TNF- (r = -0.72, P = 0.03), neutrophil elastase (r = -0.67, P = 0.0499) and sputum nitrite levels (r = -0.78, P = 0.0125).

Conclusions Following rhinovirus infection HDAC2 activity in airway macrophages is reduced and relates to airway inflammatory markers. Restoring HDAC activity is a potential therapeutic option for COPD exacerbations.

S113 HAEMOPHILUS INFLUENZAE STIMULATION OF ALVEOLAR MACROPHAGES FROM COPD PATIENTS; EFFECTS OF CORTICOSTEROIDS

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Background The lower airways of COPD patients are often chronically colonised by bacteria such as Non-typeable *Haemophilus influenza* (NTHI). Bacteria are a common cause of COPD exacerbations. Corticosteroids are often used to prevent and treat COPD exacerbations.

The aim of this study was to investigate the effect of corticosteroids on the *in vitro* inflammatory response of COPD alveolar macrophages (AM) to NTHI infection. We also investigated the cell signalling pathways activated by NTHI infection.

Methods AM from 12 COPD patients and 9 smoking controls were infected with live NTHI at multiplicity of infection (MOI) of 100:1 (bacteria: AM) for 24 hours. AM were pre-treated with dexamethasone (up to 1 M) for 1 hour. Supernatants were analysed for TNF- , IL-6, IL-8 and IL-10 by ELISA. AM protein was extracted for Western blot analysis of nuclear factor B (NF B), p38 and extracellular regulated mitogen activated protein kinases (p38 and ERK) activation.

Results NTHI stimulated release of TNF- , IL-6, IL-8 and IL-10 (p < 0.05) from both COPD patients and controls.

TNF- , IL-6 and IL-10 production was significantly inhibited by dexamethasone at 1 and 0.1 M (p < 0.05). Inhibition of TNF- and IL-6 release was significantly higher in AM from smokers compared to COPD patients. Dexamethasone had no effect on IL-8 production (see table 1).

NTHI infection activated NF B, p38 and ERK MAPK signalling pathways in AM.

Conclusion NTHI infection stimulated COPD AM to release inflammatory cytokines which are only partially responsive to corticosteroids; importantly, there was no suppression of the neutrophil chemoattractant IL-8. The production of this corticosteroid resistant chemokine is associated with NF- B and MAPK activation; these signalling pathways drive bacteria induced inflammation in COPD airways.

Abstract S113 Table 1. Dexamethasone inhibition of NTHI induced mediator production in alveolar macrophages.

	Percentage inhibition by 1 M dexamethasone	
Cytokine	**COPD**	**Smokers**
TNF-	42.5% ***#	67.3% ***#
IL-6	26% **#	43.2% **#
IL-8	-29%	16.2%
IL-10	44% ***	38.7% **

,* = significant bellow dimethyl sulfoxide (DMSO) control (p< 0.01, 0.001)
= significant difference between COPD and Smokers (p<0.05)

S114 SIMVASTATIN IMPROVES NEUTROPHIL MIGRATORY TARGETING IN COPD: *IN VITRO* STUDIES SUPPORTING STATIN USE AS A POTENTIAL ADJUVANT THERAPY

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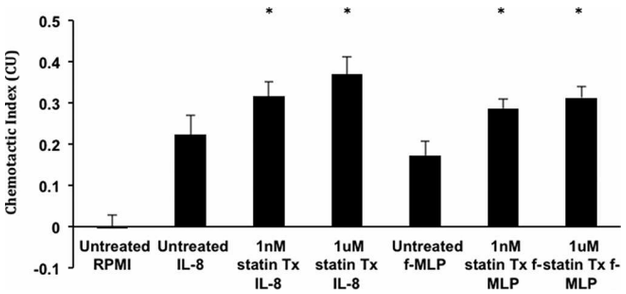
Introduction Statin use in COPD is associated with a reduction in all cause mortality, with greatest reductions seen in patients with the highest inflammatory burden. However, the mechanism for these effects is poorly understood, as statin treatment has not been found to lower systemic inflammation and *in vitro* studies of cellular effects use concentrations that exceed the therapeutic range. Neutrophils are key effector cells in COPD, and correlate with disease severity and inflammation. Recent *in vitro* studies



Abstract S114 Figure1 Simvastatin improves neutrophil migration in COPD. Legend. Isolated neutrophils from COPD patients (n = 13) migrated towards IL8 (100nM) or fMLP (100nM) following incubation with carrier control or Simvastatin (1nM or 1uM). Measurements were taken from 10 randomly selected cells from each individual. The average results for each subject were calculated, and an overall average was used for comparisons across groups using analysis of variance. Bars represent the mean migratory parameter with standard deviation shown as the error line. * = significant difference in migratory parameter from carrier control data across groups ($p < 0.05$).

have shown neutrophil migratory accuracy to be reduced in COPD. This is thought to contribute to the destruction of lung parenchyma and the poor responses seen in infective exacerbations. We aimed to characterise neutrophil migration in COPD and assess whether physiologically relevant concentrations of simvastatin altered neutrophil migration.

Methods Neutrophils were isolated from COPD patients and healthy smoking age-matched controls (age > 60yrs, n = 13 per group) and incubated with 1nM - 1μM Simvastatin or with a carrier control before migratory dynamics were assessed towards IL8 and fMLP using time-lapse photography. Data is expressed as means with standard deviation in parentheses.

Results COPD neutrophils displayed reduced chemotaxis (directional speed of migration) and reduced chemotactic accuracy (Chemotactic Index - a vector analysis of migratory tracks) compared to cells from healthy age-matched controls (HC) in the presence of IL-8 and f-MLP, replicating previous work. For example, Chemotactic Index: IL8; HC, 0.42CU (0.03), COPD 0.22CU (0.05), p = 0.002: fMLP; HC, 0.34CU (0.05), COPD, 0.18CU (0.03) p = 0.014).

Treatment with Simvastatin significantly improved the chemotactic ability of COPD neutrophils in a dose response with greatest improvement seen at the highest concentration (e.g. Chemotaxis to IL8, Carrier control 0.8um/min (0.2), 1nM Simvastatin 1.3um/min (0.2), p = 0.04; 1uM Simvastatin 1.4um/min (0.2), p = 0.004). A similar improvement was seen in Chemotactic Accuracy (e.g. Chemotactic Index to fMLP, Carrier control 0.17CU (0.03), 1nM Simvastatin 0.26CU (0.02), p = 0.018; 1uM Simvastatin 0.31CU (0.03), p = 0.002).

Conclusions Migratory accuracy of circulating neutrophils is reduced in COPD patients compared with healthy, smoking, age-matched controls but can be restored by treatment with therapeutic concentrations of Simvastatin *in vitro*. Our data suggest statin therapy might be an adjuvant intervention in COPD, modulating neutrophil responses.

S115 THE EFFECTS OF HYPOXIA ON NEUTROPHIL-MEDIATED TISSUE DAMAGE IN THE LUNG

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Sites of infection and inflammation are profoundly hypoxic, requiring neutrophils to function under low oxygen tensions. Although neutrophils are well adapted and can rely on glycolytic metabolism, hypoxia still impairs the neutrophil oxidative burst, reduces bacterial killing and delays apoptosis.[1] As neutrophil proteases have been implicated in lung diseases such as COPD, we hypothesised that hypoxia might also promote neutrophil degranulation, with an enhanced potential for neutrophil-mediated tissue injury.

Neutrophils isolated from healthy volunteers were subjected to normoxia (20 kPa) or hypoxia (3 kPa) and subsequently activated with GM-CSF (10 ng/ml) and the bacterial tri-peptide fMLP (100 nM). A549 cells and ciliated human primary bronchial epithelial (NHBE) cells were exposed to neutrophil supernatants, the extent of cellular damage was determined by MTT assay (A549 cells), EM ultrastructure and cleaved caspase 3 staining. Ciliary function was also investigated using video microscopy in the ciliated NHBE cells.

Hypoxic incubation for 4 hours resulted in a 3–5 fold increase in neutrophil degranulation; this was evident for active elastase, MPO, MMP-9 and lactoferrin and hence occurred from all granule sub-types. Supernatants from hypoxic neutrophils induced 50% more cell death in A549 cells compared to supernatants from normoxic neutrophils. NHBE cells exposed to supernatants from hypoxic versus normoxic neutrophils suffered more cellular damage (EM; images were scored for cytoplasmic blebbing, vacuole formation and dead cells), an increase in LDH activity (from 35.7 ± 6 to 50.2 ± 0.7 nmol/min/ml, was indicative of cell death), increased cleaved caspase 3 staining was shown to be an indicator of apoptosis and there was a substantial increase in the proportion of dyskinetic and immotile cilia.

In conclusion; hypoxia induced a destructive neutrophil phenotype with delayed apoptosis, impaired bacterial killing and increased release of histo-cytotoxic proteases. This phenotype may be important for understanding the mechanisms of chronic inflammatory diseases like COPD.

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Physiological measurement of breathlessness and breathing

S116 BREATHLESSNESS IN COPD IS ASSOCIATED WITH ALTERED COGNITIVE PROCESSING IN THE MEDIAL PREFRONTAL CORTEX

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Introduction Breathlessness is the main cause of suffering in COPD. Its brain mechanisms remain poorly understood, yet may represent a novel therapeutic avenue. Until now, functional magnetic resonance imaging (FMRI) studies of breathlessness have been limited to experiments in healthy volunteers. FMRI demonstrates that imagination of painful events engages the same brain networks responsible for perception of physical pain. We