aqueous layer was recovered into a fresh tube and mixed with a half volume of 100% ethanol. Samples were applied to RNeasy Mini spin columns. RT-PCR, using Taqman low-density arrays, was used to determine gene expression.

Results The mean (SD) age and forced expiratory volume in 1 second of asthmatics was 36 (13.4) years and 101.16 (15.47)% predicted respectively and for healthy volunteers was 36 (7.2) years and 92.16 (17.43)% predicted. *MMP25* expression was significantly ($p = 0.04$) higher and *MMP15* expression was significantly ($p = 0.04$) lower in asthmatics compared to healthy volunteers. *ADAM28* was significantly ($p = 0.03$) higher and *ADAM17* and *ADAMTS15* expression were significantly lower in asthma ($p = 0.007$ & 0.008 respectively). *TIMP2* expression was significantly ($p = 0.007$) lower in the asthma. There were no significant changes in expression of any of the metalloproteinases or their inhibitors after montelukast therapy.

Conclusion We have studied a wide range of known MMPs, ADAMs, ADAMTSs and inhibitors with a refined technique and successfully increased the yield from induced sputum samples. Significant differences were found between healthy volunteers and asthmatic patients for gene expression of some metalloproteinases/TIMPs. This technique could be used in the future when evaluating gene expression in asthma.

P188 PREVALENCE OF SERUM EOSINOPHILIA AT TIME OF ADMISSION WITH AN EXACERBATION OF COPD

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COPD is generally viewed as a disease driven by neutrophilic inflammation but up to 40% of COPD patients have an inflammatory pattern that includes elevated eosinophils (Saha, 2006) and there has been recent interest in the role of eosinophils in the aetiology and pathophysiology of exacerbations of COPD. Increased eosinophilic airway inflammation has been reported during exacerbations of COPD and peripheral eosinophils levels have been used as a surrogate to predict response to corticosteroid therapy. Treatment strategies with oral and inhaled steroids to reduce sputum eosinophils in COPD reduce exacerbation rates compared to

Abstract P188 Figure 1.

a conventional care and there has been interest in using anti-eosinophil therapy to modify the clinical course of exacerbations.

Data were collected on 66 patients admitted with an acute exacerbation of COPD between Nov 2011 and Feb 2012 as part of an assessment of a discharge bundle. The mean age of the patients was 72 (range 49–91) and mean FEV1 33% predicted.

Serum eosinophils were measured routinely in full blood counts performed at the time of admission. 20 patients (30%) had no detectable eosinophils, and 6 (9%) had raised eosinophil counts (normal range $0.04\text{--}0.40 \times 10^9/\text{l}$). The median eosinophil count was 0.07. One patient had an eosinophil count of 15.24 on admission, having previously had intermittently mildly elevated counts (up to 1.57) since at least 2000. Excluding this patient, the mean (SE) eosinophil count was 0.37 (0.23). The distribution of serum eosinophil counts is shown in fig 1.

In this group of patients, serum eosinophilia (>0.4) was seen in only 9% of patients at the time of admission. This may have been affected by prior self management with oral steroids.

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P189 MITOCHONDRIAL DYSFUNCTION IN MUSCLE AND AIRWAY COMPARTMENTS IN COPD: PRELIMINARY FINDINGS

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Introduction & Rationale Oxidative stress may underlie both pulmonary and non-pulmonary manifestations of COPD and may result from mitochondrial dysfunction. We hypothesised that if oxidative stress arose from the lung and ‘spilled over’ to cause non-pulmonary disease (e.g. skeletal muscle weakness) then greater evidence of mitochondrial dysfunction should be evident in the lung.

Objectives We measured mitochondrial function in endobronchial and skeletal muscle biopsies from COPD patients and healthy smokers matched for smoking history, age and sex.

Methods and Measurements We have so far biopsied 4 control smokers with normal FEV₁ and FEV₁/FVC ratio, and 4 GOLD II COPD patients out of a planned total of 40. Bronchoscopy with endobronchial biopsies (EB) and percutaneous muscle biopsy of the vastus lateralis (VL) were obtained on the same day; additional phenotypic measurements included lung function, quadriceps strength and 6-minute walk (6MW) distance. Mitochondria were isolated and mitochondrial reactive oxygen species (ROS) and membrane potential (MP) were measured using MitoSOX Red and the carbocyanine dye JC-1 respectively and intracellular ROS determined by 2'-7'-dichlorofluorescein diacetate (DCF) staining.

Results

Mean ± SEM	COPD		Controls	
Age (years)	63 ± 1		61 ± 3	
FEV ₁ (% predicted)	72 ± 2		99 ± 6	
QMV (kg)	33 ± 8		49 ± 6	
Smoking (pack-years)	42 ± 10		34 ± 6	
6MW (m)	490 ± 47		635 ± 50	
	VL	EB	VL	EB
Mitochondrial ROS (RFU)	42196 ± 9819	244947 ± 78170	11983 ± 1408	93621 ± 17981
Mitochondrial MP (units)	4.6 ± 1.0	10.3 ± 2.5	4.9 ± 1.3	13.6 ± 1.5
Intracellular ROS (RFU)	22131 ± 7195	14820 ± 5014	727 ± 297	752 ± 165

GOLD stage II COPD patients had reduced lung function, quadriceps strength and 6MW test despite a similar smoking history. There was increased mitochondrial and intracellular ROS in both skeletal muscle and bronchial biopsies of COPD patients compared to controls. There was a trend for reduced MP in COPD EB mitochondria.

Conclusion The presence of excessive ROS in cells from a lung and a non-lung compartment support the existence of a generalised dysfunction of mitochondria in established COPD resulting in increased mitochondrial oxidative stress.

P190 CONTRASTING TECHNIQUES FOR THE STUDY OF COPD LUNG MICRO-STRUCTURE WITH X-RAY MICRO-COMPUTED TOMOGRAPHY

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Background Chronic obstructive pulmonary disease (COPD) is a severely debilitating lung condition characterised by airway obstruction within the distal respiratory tree. Micro-CT imaging is a novel radiographic method that can generate a three-dimensional reconstruction of human lung micro-structure at resolutions approaching 1 μ m. This has revealed an obliteration of terminal bronchioles that may begin before patients have symptoms. A significant challenge when imaging wax-embedded COPD lung tissue is improving the contrast-to-noise for reasonable scan durations. The low contrast within the tissue can result in image analysis taking weeks to perform so addressing this

Abstract P190 Figure 1. After segmentation. Small airways and accompanying vessels were almost entirely segmented after 0.1% PTA staining by quickly increasing the threshold applied to the sub-volume (see the higher threshold values displayed on the histogram). The blue marker shows where the airway cross section was taken (bottom left). Measuring internal circumference proved to be highly precise.

issue is critical if micro-structure is to be studied in a more robust and less time-consuming fashion.

Aims The study's aim was to compare and quantify the effects of different contrasting techniques on the ability of micro-CT to visualise small airways <2 mm in diameter and micro-vasculature in COPD human lung tissue.

Methods Samples were obtained from formalin-fixed sub-pleural lung tissue resected from a patient with moderate COPD and were incubated in 0.1% phosphotungstic acid (PTA), 25% Lugol's iodine, 1% silver nitrate or left unstained to act as a control. Post-incubation, samples were embedded in epoxy resin or paraffin wax and then imaged with a 225kV HMX CT scanner at Southampton University with an average voxel size of 7.6 μ m. The data was then analysed in Image J and VGI StudioMax.

Results Staining with 0.1% PTA and 25% Lugol's iodine significantly improved x-ray contrast ($p < 0.01$) with most intense staining occurring in the small airways and micro-vessels. Staining with 1% silver nitrate failed to improve contrast ($p = 0.110$). PTA staining enabled small airway and vascular occlusions to be three-dimensionally characterised, providing reliable quantification of the micro-structure. Fast and simple image segmentation taking 10 minutes was required to effectively map out most of the branching network of small airways and micro-vessels. Visualising micro-structure in uncontrasted control samples required complex image analysis which took four hours to complete.

Conclusion PTA staining is a simple and effective technique at increasing x-ray contrast and reducing noise in COPD lung tissue. This greatly improves the level of visualisation of micro-structure in COPD tissue, providing more efficient and reliable analysis.

P191 BETA-2 ADRENOCEPTOR GENOTYPE AND RESPONSE TO PROPRANOLOL IN PATIENTS WITH PERSISTENT ASTHMA

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Background The arginine-16-glycine (Arg16Gly) beta-2 adrenoceptor (ADR) polymorphism is associated with worse outcomes in patients exposed to regular beta-agonists. We therefore wished to know if Arg16Gly conferred a similar effect in response to beta-antagonists in asthma.

Methods and observations We have performed a retrospective composite analysis of two randomised controlled trials looking at effects of Arg16Gly on the chronic response to propranolol in $n = 25$ mild to moderate corticosteroid treated persistent asthmatics. We evaluated chronic dosing effects of propranolol given for at least 4 weeks (80mg dose at least 2 weeks) on pulmonary

Abstract P191 Table 1.

	ArgArg or ArgGly n=15		GlyGly n=10		Genotype Comparison
	Change from Baseline (SEM)	P-value	Change from Baseline (SEM)	P-value	P-value
FEV1 (%)	-3.9 (1.2)	0.006	-2.3 (3.4)	0.51	0.66
FEF25-75 (%)	-5.2 (2.1)	0.025	+2.0 (3.2)	0.55	0.06
R5 (%)	+20.7 (5.5)	0.003	+6.0 (10.3)	0.57	0.27
Recovery FEV1 (%)	-14.3 (3.0)	<0.001	-8.4 (2.8)	0.014	0.18