patients using immunofluorescence for the markers CX3CR1 and CD68.

Methods Formalin fixed paraffin embedded tissue blocks were obtained from an area of the lung as far distal to the tumour as possible from COPD patients, smokers (S) and healthy non-smokers (HNS) undergoing lung resection for lung carcinoma. Sections were labelled with an anti-CX3CR1 antibody and detected using an Alexafluor conjugated secondary antibody. Immunohistochemical detection of CD68 (enzymatic non-biotin amplification technique) confirmed the macrophage phenotype of CX3CR1+ cells.

Results All CX3CR1+ cells expressed CD68. The diameters of COPD macrophages were greater than controls (Table 1). Intravascular CX3CR1+CD68^{+} macrophages were observed in COPD and S (Table 1).

Abstract P105 Table 1

	COPD (n = 9)	S (n = 9)	HNS (n = 6)
25th percentile (μm)	11.6	10.3	10
Median (μm)	13.9	12.2*	12.1*
75th percentile (μm)	16.5	14.4	13.9
Vessels with intra-vascular macrophages (%)	15.6	22.2	0

The Kruskal-Wallis test with application of Dunn's post-test was used to determine the statistical significance of differences observed in the alveolar macrophage diameter between the three groups. *p < 0.0001 against COPD.

Conclusion Increased macrophage size in COPD may be linked to altered function. Pulmonary intravascular macrophages have been observed in other mammalian species and may promote pulmonary inflammation through direct release of cytokines into the pulmonary circulation.

P106 TISSUE FACTOR PATHWAY INHIBITOR (TFPI) IS CLEAVED BY MULTIPLE PROTEASES IN COPD LUNGS TO AFFECT CIRCULATING TFPI LEVELS

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Background Tissue factor pathway inhibitor (TFPI) attenuates intravascular coagulation, a function limited by its proteolysis. Airway inflammation in COPD is associated with protease activity and intravascular thrombotic events, yet the link between proteolysis of TFPI in the airways and intravascular thrombosis in COPD is unexplored.

Aims To explore the presence and processing of TFPI in COPD airways and its relationship to plasma TFPI levels.

Methods COPD sputum and blood were collected at exacerbation and when stable. *In vitro* cleavage of TFPI was explored by incubation with proteases and Western blotting. TFPI presence and cleavage in sputum was detected by Western blotting. To determine the main protease/s involved in TFPI cleavage, sputum was spiked with recombinant TFPI in the presence of protease inhibitors, followed by Western blotting.

Results TFPI was cleaved *in vitro* by Matrix Metalloproteinase (MMP)-12, Neutrophil Elastase (NE) and urokinase-type plasminogen activator (uPA) to <20.

Conclusion TFPI is cleaved by NE in COPD airways, leading to lower circulating levels. Further studies are needed to determine if lower circulating TFPI levels lead to increased intravascular thrombotic events in COPD.

P107 FUNCTIONAL SIGNIFICANCE OF THE NITRIC OXIDE-ASYMMETRIC DIMETHYLARGININE-DIMETHYLARGININE DIMETHYLAMINOHYDROLASE (NO-ADMA-DDAH) AXIS IN TGF-β MEDIATED EPITHELIAL-MESENCHYMAL TRANSITION

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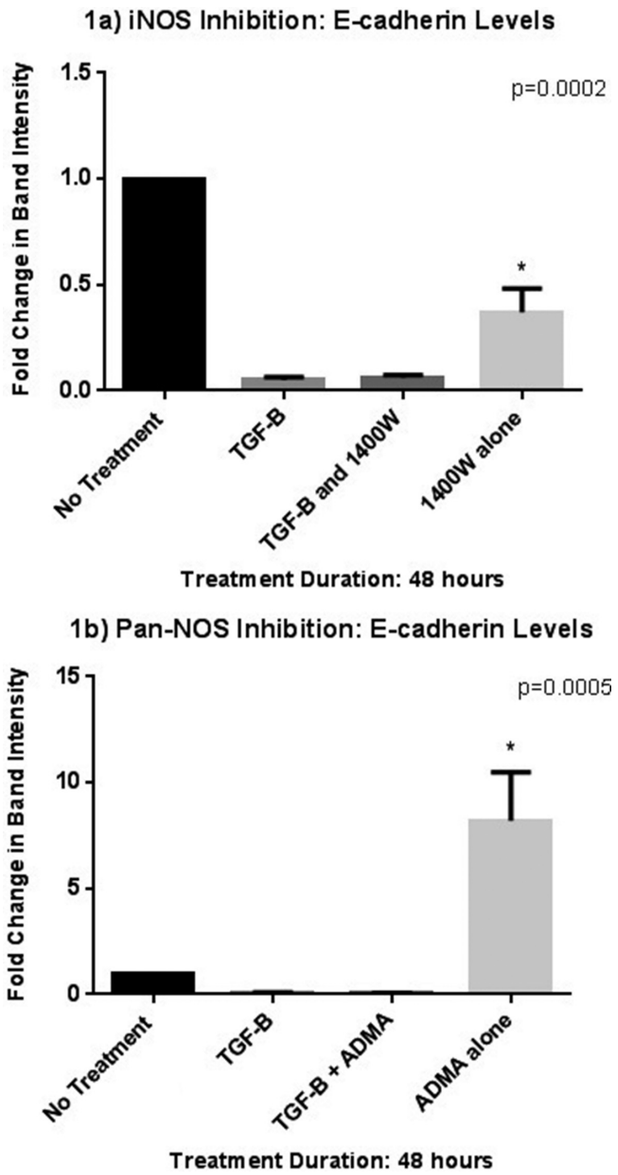
Background Transforming growth factor (TGF)-β is a key mediator of epithelial-mesenchymal transition (EMT), a pathogenetic



Abstract P107 Figure 1 Fold change in band intensity of E-cadherin levels corrected for tubulin on Western blotting: 1a) Treatment with 140W, 1b) Treatment with ADWA (p values = 0.0002 and 0.0005 respectively on one-way ANOVA)

mechanism in idiopathic pulmonary fibrosis (IPF). Nitric oxide (NO) may potentiate TGF-β/Smad-signalling and increased levels of NO and one isoform of its generating enzyme, inducible nitric oxide synthetase (iNOS), are observed in experimental models of IPF. Asymmetric dimethylarginine (ADMA) competitively inhibits iNOS, and is hydrolysed by dimethylarginine dimethylaminohydrolase (DDAH) 1 and 2. Our prior data suggests that regulation of NO production via inhibitory methylarginines may play a role in IPF. The role of NOS inhibition on the NO-ADMA-DDAH axis in TGF-β mediated EMT is unknown.

Methods Human type II alveolar epithelial cells (A549) were serum starved for 24 hrs before stimulation with 5 ng/ml TGF-β (control); co-treatment with TGF-β 5 ng/ml and 100 uM/ml 1400 W (highly selective iNOS inhibitor); and 1400 W treatment alone. A separate experiment was performed with 100 uM/ml exogenous ADMA (pan-NOS inhibitor). A profile indicating transformation from an epithelial to mesenchymal phenotype (E-cadherin, a-SMA), and expression and protein levels of DDAH isoforms and NOS enzymes was assessed by qRT-PCR and Western blotting.

Results TGF-β mediated EMT was confirmed by significant changes in protein levels in both 1400 W and ADMA experiments respectively: decreased E-cadherin ($p = 0.0002$, $p = 0.0005$) and increased a-SMA ($p = 0.009$, $p = 0.003$). Protein levels of DDAH2 ($p = 0.01$, $p = 0.0024$) and iNOS ($p = 0.01$, $p = 0.0083$) were increased. In the presence of either TGF-β and 1400 W or TGF-β and ADMA co-treatments; the mesenchymal pattern of changes in E-cadherin and α-SMA, and the elevation in DDAH2 and iNOS levels, were not attenuated. Treatment with either 1400 W or ADMA alone resulted in significantly elevated E-cadherin levels compared to TGF-β control or co-treatments (Figure 1). Pan-NOS inhibition with ADMA alone resulted in a several fold increase in E-cadherin levels compared to no treatment ($p = 0.0005$) (Figure 1b).

Conclusion TGF-β mediated transition towards a mesenchymal phenotype is not attenuated by NOS inhibition. However, our results suggest that regulation of NO production by ADMA promotes E-cadherin expression via an iNOS independent mechanism and may play a role in the maintenance of an epithelial phenotype in IPF.

Sleep services: current delivery and future directions

P108 QUALITY OF LIFE, DIET AND EXERCISE MEASUREMENTS IN OBESE INDIVIDUALS WITH AND WITHOUT VENTILATORY FAILURE

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Introduction Obesity is associated with reduced quality of life (QOL), particularly physical health. In addition obesity has been linked to reduced exercise and high calorie diet. We aimed to describe these factors in obese individuals with and without ventilatory failure, and investigate the hypothesis that ventilatory failure would have a negative impact on QOL.

Methods QOL, diet and exercise was assessed as part of an open cross-sectional study of ventilatory failure in obese subjects referred either for assessment of sleep disordered breathing or bariatric surgery.

The SF-12 was completed; a validated questionnaire to assess QOL giving summary scores for physical health (PCS) and mental health (MCS), and compared to data from a large non-obese UK cohort.[1]

Participants underwent actigraphy (*SenseWear BodyMedia)* and from this the daily energy expenditure was estimated. A sedentary lifestyle was defined as <5000 steps/day.

Participants completed a validated food frequency questionnaire, which calculates daily dietary calorie intake from patient reported three month food habits. This was compared to UK guideline recommended daily maximum intake.

Arterial base excess was measured as a marker of ventilatory failure and the correlations between quality of life indices and arterial base excess were calculated.

Results 72 individuals with a mean age of 52.0 years (SD 8.9) and median BMI of 46.7 kg/m^2 (IQR 39.5, 52.6) participated in the study. Median duration of actigraphy was 23.2 days (IQR 21.2, 23.4).

Arterial base excess was significantly but weakly correlated to MCS ($r = 0.33$, $p = 0.01$) but not to PCS ($r = 0.05$, $p = 0.74$).

Abstract P108 Table 1 Results of SF-12, actigraphy, food frequency questionnaire and arterial blood gasses

	N	Study mean or median	SD or IQR	95% confidence interval of difference from comparison mean	P value
PCS	58	38.0	11.3	-16.4, -11.3	<0.0001
MCS	58	41.2	10.6	-10.2, -5.0	<0.0001
Energy expenditure (kCal)	58	2977	566		
Daily steps	59	3169	2141, 5242	*71.2% were 'sedentary' (<5000 steps/day)*	
Dietary energy (kCal) *Men*	26	2434	1760, 3348	*46.1% were above recommended daily allowance for men (>2500kCal/d)*	
Dietary energy (kCal) *Women*	26	2812	2171, 3494	*76.9% were above recommended daily allowance for women (>2000kCal/d)*	
Arterial base excess (mmol/l)	72	2.08	2.41	*48.6% had a raised arterial base excess (>2 mmol/l)*	
Arterial $PaCO_2$ (kPa)	72	5.57	0.80	*22.2% had a raised $PaCO_2$ (>6 kPa)*	

Conclusions Obesity had a large negative impact on both physical and mental QOL not reproducibly reported elsewhere. Ventilatory failure was only a weak predictor of mental, but not physical QOL scores. The majority of participants were sedentary and dietary calorie intake was higher than the recommended daily allowance for most women and a significant number of men. Actigraphy energy expenditure estimates exceeded patient reported dietary intake, which is probably due to patient underreporting. This highlights the clinical importance of considering mental health, physical activity and diet together when obese individuals are seen in a tertiary centre.

REFERENCE

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