mechanisms linking cough and reflux are not well understood. The aim of this study was to investigate the relationships between measures of micro-aspiration, reflux and cough in these patients.
Methods: Simultaneous 24 h oesophageal impedance/pH and cough monitoring were carried out on 78 chronic cough patients presenting to a specialist clinic. Pepsin quantification was performed in sputum and bronchoalveolar lavage (BAL) samples using a plate ELISA, based on a mono-specific antibody to porcine pepsin. 12 BAL samples from healthy controls were used for comparison.
Results: The mean number of reflux events in 24 h was within the normal range (64 (±23); 29 patients (37%) had reflux disease (>73 episodes/24 h)). Median 24-h cough rate was 10.4 coughs/h (range 0.3–70.2). There was no significant difference in median BAL pepsin concentrations in cough patients and controls (18.2 ng/ml (range 0–56.4) and 9.3 ng/ml (range 0–46.9), respectively, $p = 0.27$) or in cough patients with and without reflux ($p = 0.34$). The median sputum pepsin concentration in cough patients was 17.3 ng/ml (range 0–55.4), with a trend towards higher concentrations in those with reflux ($p = 0.07$). Using multiple linear regression and correcting for the effects of age and gender, cough rate was significantly related to the number of reflux episodes ($p<0.001$). Using a similar model cough rate was also predicted by sputum pepsin ($p = 0.002$) but BAL pepsin in this model was not significant ($p = 0.20$). The slope of this relationship was negative, indicating that patients with higher cough rates had less pepsin in their sputum.
Conclusions: Despite physiological levels of reflux in the majority of patients, cough rates were significantly predicted by the number of reflux events. Pepsin concentrations did not support a role for micro-aspiration in chronic cough, rather subjects with higher cough rates had less sputum pepsin, suggesting that cough may protect from micro-aspiration or clear pepsin from the proximal airways. Reflux appears to be a trigger for cough, but micro-aspiration does not appear to occur. An abnormal oesophageal sensitivity to physiological levels of reflux may be present, explaining the low sputum pepsin levels.

S47 CHRONIC COUGH AND ESOMEPRAZOLE: A DOUBLE-BLIND, RANDOMISED PLACEBO-CONTROLLED PARALLEL STUDY

H Fathi, S Faruqi, R Thompson, C Wright, AH Morice. *Department of Academic Medicine, University of Hull, Castle Hill Hospital, Cottingham, UK*

Background: Gastro-oesophageal reflux has been implicated in the pathogenesis of a significant proportion of patients with chronic non-specific cough. Guidelines on the management of chronic cough suggest a therapeutic trial of anti-reflux medications. Esomeprazole at a dose of 20 mg twice daily is a proton pump inhibitor (PPI) licensed for the long-term treatment of reflux in adults. We wanted to evaluate the effects of treatment with esomeprazole on chronic cough.
Method: This was a prospective, single-centre, randomised, double-blind, placebo-controlled, parallel design trial conducted over 8 weeks. 50 adult (>18 years) patients who were non-smokers and had chronic non-specific cough (>3 months duration) were randomly assigned into this trial. All patients had a normal spirometry. Patients completed a Leicester cough questionnaire (LCQ), a validated cough-related quality of life questionnaire, at the beginning and at the end of the study. The LCQ scores varied from 3–21 with higher scores representing better quality of life. Subjective self-assessment cough score from 0–9 (cough score) and a similar score for symptoms of gastro-oesophageal reflux (reflux score) were obtained at baseline and at the end of the study. Citric acid cough challenge and a laryngoscopic examination (obtaining a reflux finding score (RFS)) were performed at baseline and the end of the study. The primary outcome of the study was improvement in cough.
Results: 15 men and 23 women with a mean age of 56.8 years completed the study. Their mean duration of cough was 5 years. The treatment and placebo groups were comparable. There was a significant improvement in the reflux score seen in patients on the treatment arm ($p = 0.03$). There was no significant improvement either in the cough score, LCQ or in the RFS in the treatment arm as compared with placebo.
Conclusion: PPI are suggested as first line treatment in patients with chronic cough which is possibly caused by extra oesophageal reflux. Our results show that treatment with a PPI ameliorates symptoms of ''heart burn'', the characteristic feature of gastro-oesophageal reflux. No differences in symptoms of cough were observed on treatment with a PPI.

Systemic aspects of chronic obstructive pulmonary disease

S48 OSTEOPOROSIS AND LOSS OF BONE MINERAL DENSITY IN CORTICOSTEROID-NAIVE MALE PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

[1]JM Duckers, [1]CE Bolton, [2]BA Evans, [3]W Evans, [4]MD Stone, [1]DJ Shale. *[1]Department of Respiratory Medicine, Cardiff University, Cardiff, UK; [2]Department of Child Health, Cardiff University, Cardiff, UK; [3]Department of Medical Physics, University Hospital of Wales, Cardiff, UK; [4]Bone Research Unit, Cardiff University, Cardiff, UK*

Background: Previously, we reported an increased prevalence of loss of bone mineral density (BMD) in patients with chronic obstructive pulmonary disease (COPD) with a third having undetected osteoporosis.[1] Here, we hypothesised that loss of BMD occurs early in COPD progression and is independent of corticosteroid therapy.
Methods: Thirty corticosteroid-naive (inhaled or oral) male patients with COPD and 15 age-matched male controls were studied. Spirometry, incremental shuttle walking test (ISWT), BMD at hip and lumbar spine together with body composition (DXA, Hologic, Discovery) were performed.
Results: Previously undetected osteoporosis[2] was present in 17% of patients (3% control) and osteopenia in 57% of patients (33% control). Results are presented in the table. In patients FFMI was related to BMD hip ($r = 0.506$, $p = 0.004$) but not BMD lumbar spine ($r = 0.310$, $p>0.05$). There was a trend that ISWT in patients was related to BMD hip ($p = 0.06$).
Conclusions: Loss of BMD was evident in 74% of corticosteroid-naive male patients. The loss of BMD was more prominent at the hip compared with the lumbar spine. This difference may reflect loss of physical activity and subsequent deconditioning as suggested by the loss of fat-free mass and shorter ISWT distance in these patients. Bone thinning in COPD appears to occur early in the genesis of lung disease, to be independent of corticosteroid therapy and is associated with physical deconditioning, which should be targeted for intervention at initial diagnosis.

Abstract S48 Table Characteristics of patients and control subjects

	Patients	Controls
Smoking pack years median (range)	47.5 (10–150)	30 (10–80)*
FEV_1 % predicted	63.7(17.9)	92.9 (10.6)**
FFMI (kg/m^2)	18.3 (2.4)	20.4 (1.7)*
ISWT (m)	424 (171)	563 (221)*
BMD hip (g/cm^2)	0.93 (0.14)	1.05 (0.17)*
BMD lumbar spine (g/cm^2)	1.03 (0.20)	1.13 (0.22)

*$p<0.05$; **$p<0.001$. BMD, bone mineral density; FFMI, fat-free mass index; ISWT, incremental shuttle walking test.

Abstract A49 Table

	Controls n = 15	Patients n = 30
Smoking pack years median (range)	30 (10–80)	47.5 (10–150)*
BMD hip (g/cm^2)	1.05 (0.17)	0.93 (0.14)*
BMD lumbar spine (g/cm^2)	1.13 (0.22)	1.03 (0.20)
IL-6 (ng/l)†	3.1 (2.2)	4.8 (2.8)
OPG (pmol/l)†	6.5 (1.9)	8.5(1.4)
Osteocalcin (μg/l)†	23.0 (1.4)	22.7 (1.5)
PINP (μg/l)†	40.9 (1.4)	45.6 (1.9)
CTX (μg/l)†	0.41 (1.5)	0.38 (1.9)
25 Hydroxyvitamin D (μg/l)†	16.1 (1.4)	11.4 (1.9)*

Mean (SD) unless otherwise documented. *p<0.05 compared with control. †Geometric mean (SD). BMD, bone mineral density; CTX, bone resorption; OPG, osteoprotegerin; P1NP, procollagen type 1 intact N-terminal propeptide.

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1. **Bolton CE,** *et al*. *Am J Respir Crit Care* 2004.
2. **World Health Organisation, 1994**.

S49 METABOLIC BONE PROFILING IN MALE PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

[1]JM Duckers, [1]DJ Shale, [2]BA Evans, [2]C Elford, [3]WD Fraser, [4]MD Stone, [1]CE Bolton. *[1]Department of Respiratory Medicine, Cardiff University, Cardiff, UK; [2]Department of CHild Health, Cardiff University, Cardiff, UK; [3]Unit of Clinical Chemistry, School of Clinical Sciences, Liverpool University, Liverpool, UK; [4]Bone Research Unit, Cardiff University, Cardiff, UK*

Background: Low bone mineral density (BMD) and osteoporosis have been reported in patients with chronic obstructive pulmonary disease (COPD). However, potential confounding factors have always been implicated—female gender, smoking and corticosteroid use. We hypothesised that other factors associated with COPD contributed to a low BMD and explored markers of bone formation and resorption together with bone status and other metabolic profiling in a group of male patients with COPD.
Methods: Thirty male patients with confirmed COPD, naive to corticosteroids (oral or inhaled), and 15 "smoker" controls matched for age were recruited. GOLD distribution (n) was I: 5; II: 20; III: 5. BMD (Hologic, Discovery), spirometry and circulating bone and inflammatory markers were measured.
Results: Osteoporosis was present in 17% of patients (3% of controls); osteopenia in 57% patients (33% controls). BMD hip was lower in patients (at all three sites and total) compared with controls, see table. BMD lumbar spine was similar between groups. Circulating markers of bone formation (osteocalcin and P1NP), bone resorption (CTX) and osteoprotegerin (OPG; decoy receptor of RANKL and product of osteoblast function) were similar between patients and controls, see table. Osteoprotegerin was higher in the patients with osteoporosis, when corrected for age compared with other patients. Other markers did not differentiate. The receiver operator characteristic curve for osteoprotegerin to predict osteoporosis was 0.832. There was an inverse association of osteocalcin ($r = -0.51$), P1NP ($r = -0.67$), CTX ($r = -0.57$), osteoprotegerin ($r = -0.41$) with BMD hip, all $p<0.05$ and similar correlations with t score hip but not with lumbar spine in patients. Total testosterone concentrations were not different nor were the proportion with low testosterone (both 15%). Free T4 and TSH were similar. 25 Hydroxyvitamin D was significantly lower in patients (see table). 24 patients and nine controls had vitamin D levels less than 20 μg/l.
Conclusions: In this well described steroid-free cohort of male patients with mild–moderate COPD, we report lower BMD and a greater prevalence of osteoporosis compared with controls. The strong associations of the bone markers to BMD at the hip are an important first step in unravelling the underlying potential mechanisms of COPD-related osteoporosis and warrant further investigation.

S50 FOUR AND A HALF LIM PROTEIN 1 EXPRESSION AND AKT PHOSPHORYLATION IN QUADRICEPS MUSCLE IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE PATIENTS AND ITS RELATION TO MUSCLE STRENGTH AND PHYSICAL ACTIVITY

J Riddoch Contreras, SA Sathyapala, GS Marsh, MI Polkey, PR Kemp. *Imperial College, London, UK*

Introduction: Muscle atrophy is a common systemic feature of advanced chronic obstructive pulmonary disease (COPD). Muscle disuse, caused by reduced physical activity imposed by breathlessness, is a possible aetiological factor. Four and a half LIM proteins (FHL), which are abundantly expressed in skeletal muscle, are associated with hypertrophic signalling in young animals. One well-characterised hypertrophy signalling pathway involves insulin-like growth factor 1 (IGF-1) and phosphorylated Akt. Recently, FHL1 mutations have been implicated in distinct hereditary myopathies such as scapuloperoneal myopathy, X-linked postural muscle atrophy and human reducing body myopathy. These data suggest that FHL1 is a regulator of muscle mass but FHL1 expression has not been investigated in COPD.
Aim: To determine FHL1 expression in quadriceps muscle of COPD patients compared with age-matched controls.
Methods: Lung function, quadriceps strength (isometric maximal voluntary contraction, MVC in kg), physical activity over 12 h/day for 2 days using the Dynaport ADL accelerometer to give averaged locomotion time (Lo_t; min/12 h), were determined in 42 COPD patients and 21 healthy controls before percutaneous needle biopsy of the vastus lateralis. Biopsies were analysed for FHL1 messenger RNA using real-time PCR. FHL1 and phospho-Akt protein levels were quantified by Luminex assay. Statistical analysis of group differences was performed using the Mann–Whitney U test and correlations with calculation of Spearman's rank correlation coefficient.
Results: FEV_1 in the patients was 35 ± 16% predicted and 108 ± 13% predicted in controls. Quadriceps strength (MVC corrected for weight) was not significantly lower in patients (0.43 ± 0.1) than controls (0.45 ± 0.1, $p>0.11$). Although there was no significant difference in FHL1 mRNA expression in muscle from COPD patients (6.8 ± 0.49 AU) and controls (7.4 ± 0.82 AU), FHL1 expression was inversely correlated with locomotion time and MVC/wt in patients ($p>0.005$ and 0.014, respectively) but not in controls. Phospho-Akt protein levels were inversely proportional to MVC/wt ($p>0.001$) in patients but not in controls.
Conclusions: The data suggest that quadriceps muscle FHL1 expression is associated with reduced activity and muscle strength in COPD patients, conversely to animal models. Furthermore, the data suggest that hypertrophy signalling in the patients does not produce a restoration of muscle strength.

S51 THE EFFECTS OF HYPOXIC CHALLENGE ON COAGULATION AND INFLAMMATION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

[1]R Sabit, [1]P Thomas, [1]DJ Shale, [2]P Collins, [1]SJ Linnane. *[1]Department of Respiratory Medicine, Llandough Hospital, Penarth, UK; [2]Department of Haematology, University Hospital of Wales, Cardiff, UK*

Introduction: Hypobaric hypoxia is one possible explanation for increased venous thromboembolism (VTE) risk during air travel. Patients with chronic obstructive pulmonary disease (COPD) are also at increased risk of VTE, especially during exacerbations. The reasons for this increased risk are unknown, but may involve a

hypercoagulable state secondary to hypoxia and/or heightened systemic inflammation. We investigated the effects of hypoxia on markers of coagulation and systemic inflammation in patients with COPD.

Methods: Twenty clinically stable patients with COPD, who were not receiving an inhaled corticosteroid, were recruited. Patients were randomly assigned to receive either medical air or 100% nitrogen through a 40% venturi mask at a flow rate of 10 l/minute for 2 h. All patients had spirometry prior to testing. Oxygen saturations, blood pressure and heart rate were measured throughout the study. Blood was sampled for thrombin anti-thrombin complex (TAT), prothrombin activation fragments 1+2 (F_{1+2}), von Willebrand factor (vWF), D-dimer and IL-6 at baseline and at 2 h. Non-parametric data were $\log_{10}$ transformed. Measurements at baseline and after 2 h testing were compared using the paired student t test.

Results: Patients (14 male), had a mean (SD) age of 68.8 years (8.3) and a mean (SD) FEV_1 of 1.75 litres (0.53). Patients in the hypoxia and control groups were similar in terms of age, gender, pack years smoked and severity of airflow obstruction. Baseline TAT, F_{1+2}, vWF, D-dimer and IL-6 levels and oxygen saturations were also similar between the groups. In the control group, there was no change in markers of coagulation or systemic inflammation over the 2-h period. In patients who underwent hypoxic challenge, there was an increase in $\log_{10}$ TAT ($p<0.001$), $\log_{10} F_{1+2}$ ($p<0.01$) and $\log_{10}$ IL-6 ($p<0.01$), whereas D-dimer and vWF levels were similar. Changes in serum IL-6 were related to changes in F_{1+2} ($r = 0.65$, $p<0.05$) but not TAT ($r = 0.39$, $p = 0.09$).

Conclusions: This single blind placebo-controlled pilot study demonstrates that a 2-h hypoxic challenge results in an increase in markers of coagulation and systemic inflammation, which are linked and which may explain the increased risk of VTE in COPD patients who are experiencing an acute exacerbation.

Abstract S51 Table

	Baseline	After 2 h	p Value
Oxygen saturations	94.4 ± 2.3	92.0 ± 3.5	0.096
TAT	10.2 ± 3.16	130.4 ± 3.6	<0.001
F_{1+2}	334.3 ± 2.47	1878.0 ± 3.93	0.01
D-dimer	173.1 ± 90.0	204.4 ± 89.6	0.10
vWF	124.2 ± 20.5	1167 ± 18.3	0.39
IL-6	3.17 ± 1.36	4.67 ± 1.40	0.002

F_{1+2}, prothrombin activation fragments 1+2; TAT, thrombin anti-thrombin complex; vWF, von Willebrand factor.

S52 ENDOTHELIAL DYSFUNCTION IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE: IMPAIRED ENDOGENOUS FIBRINOLYSIS

[1]JD Maclay, [1]DA McAllister, [2]NL Mills, [2]DE Newby, [1]W MacNee. *[1]ELEGI Labs, University of Edinburgh, Edinburgh, UK; [2]Cardiovascular Research, University of Edinburgh, Edinburgh, UK*

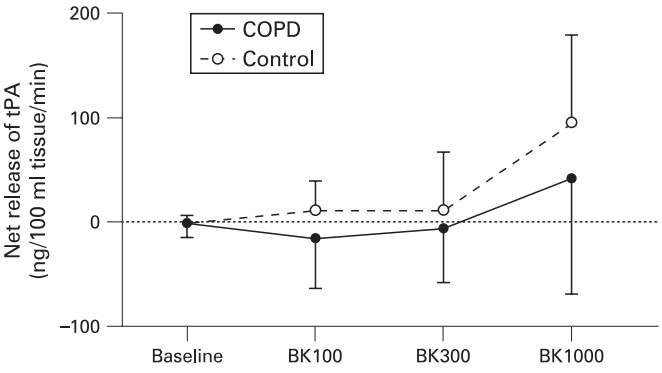
Introduction: Chronic obstructive pulmonary disease (COPD) is now established as a condition that not only affects the lungs, but also has systemic features. Cardiovascular disease is common in COPD, and contributes significantly to both morbidity and mortality. Dysfunction of the systemic vascular endothelium contributes to the formation and progression of atherosclerotic plaques. Stable and unstable coronary artery disease, smoking, air pollution and systemic inflammation have all been associated with impairment of endothelial function.

Hypothesis: Patients with COPD have impaired endothelial fibrinolytic and vasomotor function in comparison with age, sex and smoking-matched controls.

Methods: 17 male ex-smokers with and without COPD, with no history of cardiovascular disease were recruited. Forearm blood flow (FBF) in response to endothelial-dependent vasodilators (bradykinin and acetylcholine) and endothelial-independent vasodilators (sodium nitroprusside and verapamil) were measured using venous occlusion plethysmography. Bradykinin was used to stimulate endothelial tissue plasminogen activator (tPA) release. Venous blood was collected at baseline and following each infusion of bradykinin for tPA antigen.

Results: COPD patients were well-matched with controls regarding age and smoking history. Baseline blood flow was the same in COPD patients as in controls and remained so prior to each intra-arterial infusion. There were no differences in FBF responses to endothelial-dependent vasodilators between the two groups (COPD vs control: bradykinin, peak response, 16.0 vs 15.6 ml/100 ml of tissue/minute; $p = 0.55$; acetylcholine, peak response, 5.7 vs 6.5 ml/100 ml of tissue/minute; $p = 0.52$). There was no difference in FBF responses to endothelial-independent vasodilators between the two groups (sodium nitroprusside, peak response, 10.1 vs 9.6 ml/100 ml of tissue/minute; $p = 0.37$; verapamil, peak response, 11.5 vs 12.1 ml/100 ml of tissue/minute; $p = 0.67$). Bradykinin caused a dose-dependent increase in tPA antigen in both groups. Baseline net tPA antigen release was similar in each group, but bradykinin-stimulated release of tPA was reduced in the COPD group ($p = 0.035$) (see fig).

Conclusion: COPD is associated with impaired endothelial fibrinolytic function, which may contribute to the increased cardiovascular risk associated with this condition. There was no impairment of endothelial-dependent or independent vasomotor function.



Abstract S52 Figure Net tPA release.

Novel mechanisms in interstitial lung disease

S53 GALECTIN-3 REGULATES EPITHELIAL TO MESENCHYMAL TRANSITION IN LUNG EPITHELIAL CELLS

AC MacKinnon, SL Farnworth, M Gibbons, SJ Forbes, T Sethi. *University of Edinburgh, Edinburgh, UK*

Galectin-3 is a beta-galactoside binding animal lectin of approximately 30 kDa, which is highly expressed in fibrotic tissue of diverse aetiologies. Mice deficient in galectin-3 develop reduced fibrosis in several models of organ fibrosis in vivo. Galectin-3 is secreted by macrophages and is a potent mitogen for fibroblasts in vitro. In the chronic inflammatory milieu macrophages interact with other cell types including cells of mesenchymal origin (fibroblasts), which transdifferentiate into matrix-secreting myofibroblasts, with resultant scar formation and disruption of tissue architecture. Our previous work has demonstrated that fibroblasts deficient in galectin-3 fail to differentiate into myofibroblasts in