inhaled flow of 28 l/min. Radioactive counts were measured using a gamma camera. FP drug concentration was determined using HPLC.
Results Percentage FP deposition and radioactive counts (R) were well matched on all ACI stages (particle size ranges given) for the three monodisperse aerosol distributions (table 1, N = 3 experiments each). The ratios of FP%/R% were constant on each ACI stage (particle size range) for each monodisperse aerosol size.
Conclusions Our in vitro experiments show monodisperse FP drug aerosols can be successfully radiolabelled and that the radiolabel does not alter the particle size distribution of the drug formulation. This validation allows for future precise in vivo scintigraphic investigations, using monodisperse aerosols, of the perceived clinical importance of targeting inhaled corticosteroid formulations to different lung regions in different disease states and severities.

P133 IMPLICATIONS OF NOT WELL-CONTROLLED ASTHMA: UK RESULTS FROM A EUROPEAN SURVEY

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Introduction and Objectives A European patient survey in 2006 showed that the majority of treated asthma patients (55%) are poorly controlled.[1] A 2008 survey[2] aimed to reassess the level of asthma control in Europe. We investigated if asthma control in the UK had improved and the implications for patients and clinicians of rescue use of ⩾2 puffs/week.
Methods A detailed population-based cross-sectional survey carried out in 2008 in France, Germany, Italy, Spain and UK was administered to a sample of individuals drawn from an internet panel. The Asthma Control Test (ACT)[3] was used to assess level of control: at least well-controlled (ALWC) asthma (score 20–25) and not well-controlled (NWC) asthma (score ⩽19). The SF-12 questionnaire was used to assess health-related quality of life.
Results Of 15 000 UK respondents, 1523 were diagnosed asthma patients (mean age 45.3 years). 1362 (89.4%) of asthma respondents were receiving treatment for asthma. Of these, 697 (51.2%) were NWC in 2008 compared with 45% in 2006.[1] 590 (84.7%) of the NWC patients used rescue medication ⩾2 puffs/week. Table 1 compares NWC and ALWC patients.
Conclusions The level of asthma control in the UK has not improved since 2006.[1] NWC patients experienced significantly more night-time awakenings and visited their physician more frequently than those with ALWC asthma. Furthermore, these patients were less able to perform physical (eg, housework, sports) and social activities. This survey suggests that rescue medication use of ⩾2 puffs/week is a useful way of identifying NWC patients for further assessment (eg, using validated tools such as ACT) to ensure that these patients are optimally managed and treated according to BTS/SIGN guidelines, with the goal of improving quality of life and asthma control in the UK.

Asthma Control Test is a trademark of QualityMetric Inc. GlaxoSmithKline purchased the survey data from Consumer Health Sciences International.

1. **Desfougeres JL**, *et al*. Abstract 1589, ERS, 2007.
2. **Walters R**, *et al*. Abstract 167, EAACI, 2009.
3. **Nathan RA**, *et al*. *J Allergy Clin Immunol* 2004;**113**:59–65.
4. **GINA Report**. 2008.

Abstract P133 Table 1

	NWC patients	ALWC patients	p Value
% with night-time awakenings (⩾1/week)	58.2%	1.8%	<0.001
Mean visits to physician about asthma in past 12 months	2.53	1.10	<0.001
SF-12 Physical Functioning score	39.84	47.19	<0.001
SF-12 Social Functioning score	39.25	46.52	<0.001

ALWC, at least well-controlled; NWC, not well-controlled.

COPD: α_1-antitrypsin deficiency and other manifestations

P134 POLYMICROBIAL AIRWAY COLONISATION AND SYSTEMIC INFLAMMATION IN STABLE COPD

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Introduction The presence of lower airway bacteria in stable state chronic obstructive pulmonary disease (COPD) is associated with a heightened inflammatory response.[1] In most cases, only a single potentially pathogenic microbe (PPM) is isolated but about 20% of patients will have co-colonisation with two PPMs. We hypothesised that the magnitude of inflammation would differ according to the number of PPMs present.
Methods Patients with COPD who had been followed in our cohort for >1 year were sampled at 6-monthly baseline visits. Patients were symptomatically stable at these visits and had not had an exacerbation or antibiotic use in the preceding 6 weeks. Serum was taken for the acute phase response mediators interleukin 6 (IL6) and C-reactive protein (CRP) and spontaneously expectorated sputum was collected for standard microbiological culture. To ensure that the samples reflected the stable state, they were subsequently excluded from analysis if an exacerbation occurred within 2 weeks of the visit. Salivary sputum (>25 epithelial cells/low power field) was also excluded.
Results There were 196 patients with 479 sputum samples (47% 0 PPM, 42% 1 PPM, 11% 2 PPM). Their mean age was 68.0 years (SD 8.1); 61% were male; forced expiratory volume in 1 s 47.1 (18.3)% predicted and 49.8 (36.7) pack-years smoking history.
Conclusions CRP was significantly higher in dual versus single PPM airway colonisation in the stable state and this may be related to bacterial load (table 1). Dual pathogen colonisation may have a contributory role in the pathogenesis of systemic complications and declining lung function, both of which have been linked to elevated systemic inflammatory markers.

1. **Wilkinson TMA**, Patel I, Wilks M, *et al*. Airway bacterial load and FEV_1 decline in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003;**167**:1090.

Abstract P134 Table 1

Inflammatory markers	0 PPM (median (IQR))	n	1 PPM	n	2 PPM	n	p Value*
CRP (g/dl)	4.0 (2.0–7.8)	69	4.9 (2.0–8.0)	53	13.0 (6.0–22.0)	9	0.01
Serum IL6 (pg/ml)	4.6 (2.9–8.3)	77	5.3 (3.4–8.4)	58	6.0 (5.2–10.1)	15	0.26

CRP, C-reactive protein; IL, interleukin; PPM, potentially pathogenic microbe.
*Comparisons were made between sputum samples containing one or two PPMs using the Mann-Whitney U test.

P135 ASSESSMENT OF OSTEOPOROSIS RISK IN PATIENTS WITH COPD: A COMMUNITY-BASED STUDY

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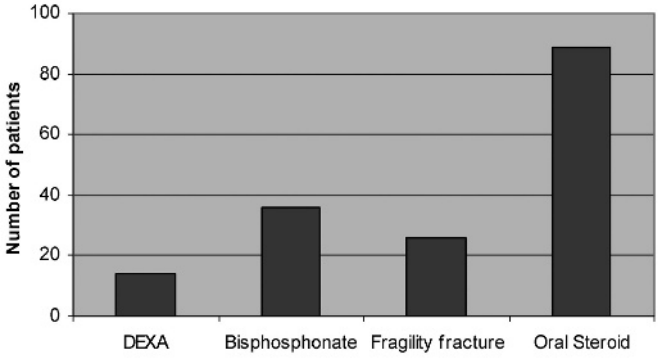
The Royal College of Physicians (RCP) guidelines on steroid-induced osteoporosis highlight that patients taking high-dose steroid inhalers are at risk of glucocorticoid-induced osteoporosis. In addition "FRAX" (the WHO fracture risk assessment tool) indicates that COPD is an important risk factor for osteoporosis and is included in the 10-year fracture probability assessment.

This is one of the first community-based studies which aimed to assess whether patients with COPD on high-dose steroid inhalers were being investigated and treated for osteoporosis. We looked at whether patients had been considered for a dual energy *x* ray absorptiometry (DEXA) scan of the lumbar spine and hip to assess bone density and bone protection agents. This prospective study used a questionnaire which was filled in by the respiratory nurse specialists. It asked the patient the following questions: type of residence, history of previous fragility fracture, whether a DEXA scan had been done, number of courses of oral steroids in the past year and if they took a bisphosphonate.

100 patients with COPD on high-dose steroid inhalers (64 women, 36 men) were included in the study. Their ages ranged from 46 to 91 years. 89% had taken at least one course of oral steroids to control their airways disease in the past year. In total, only 14% had a DEXA and 36% were taking a bisphosphonate (fig 1). Just over a quarter of patients had a previous fragility fracture, most of whom had taken at least one course of oral steroids. Of the patients with a fragility fracture, only 54% were taking a bisphosphonate and 15% had a DEXA scan. These figures indicate that patients were being managed suboptimally with regard to preventing fragility fractures.



Abstract P135 Figure 1 Assessment of osteoporosis risk in 100 patients with chronic obstructive pulmonary disease.

In conclusion, patients with COPD are at increased risk of fragility fractures, however only a small percentage are investigated for osteoporosis. More patients should be started on bisphosphonates based on risk profile alone. An easily accessible referral service needs to be implemented and awareness increased. In the future larger studies need to be instigated in order to better assess this issue.

P136 DOES SEVERITY OF COPD DETERMINE THE RISK OF DEPRESSION?

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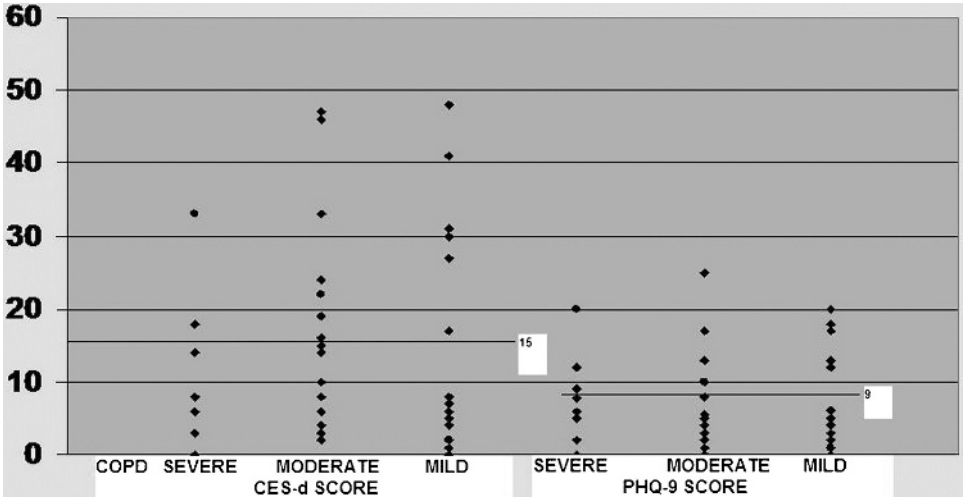
Introduction When assessing patients with chronic obstructive pulmonary disease (COPD), NICE guidelines recommend screening for depression.[1] We wanted to know if the severity of COPD correlated with the degree of depression in order to determine how best to screen the patients.

Method A prospective cohort study was performed in 50 patients with COPD. For each patient we checked forced expiratory volume in 1 s (FEV_1) and FEV_1 predicted (FEV_1p). We asked each patient to complete a questionnaire for Center for Epidemiological Studies depression score (CES-d) and Patient Health Questionnaire-9 (PHQ-9). We also noted any history of treatment for depression and asked about co-morbidities. CES-d score >15 or a PHQ-9 score >9 are consistent with depression.

Results 47 patients completed spirometry and questionnaires. 7/47 (15%) patients had severe COPD (FEV_1/FEV_1p <30), 23/47 (49%) patients had moderate COPD (FEV_1/FEV_1p 30–50%) and 17/47 (36%) patients had mild COPD (FEV_1/FEV_1p 50–80%) (fig 1). We found a strong positive correlation between CES-d and PHQ-9 scores. Mean (SD) CES-d result for severe COPD was 13.42 (9.99), for moderate COPD was 13.83 (13.01) and for mild COPD was 13.53 (15.65). Mean (SD) PHQ-9 result for severe COPD was 7.71 (6.75), for moderate COPD was 5.57 (6.09) and for mild COPD was 5.76 (7.09). We also found that 16/47 patients in all severity groups had CES-d score >15 and 13/47 had PHQ-9 score >9. Only 8/47 patients were being treated for depression at the time of the study

Comment We feel that the strong correlation between CES-d and PHQ-9 scores validates their use as screening tools for depression in COPD patients. We realise that depression in COPD is multi-factorial, but we expected patients with more severe disease would



Abstract P136 Figure 1

have been depressed; this does not appear to be the case. We also suggest that we were not good at detecting depression in patients with COPD, given that 38–50% in our study with high depression scores had no treatment.

1. NICE. Guideline CG12. Chronic obstructive pulmonary disease.

P137 REDUCED EXPRESSION AND ACTIVITY OF MYOCARDIN-RELATED TRANSCRIPTION FACTORS CONTRIBUTES TO FIBRE TYPE CHANGES IN THE QUADRICEPS OF PATIENTS WITH COPD

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Introduction and Objectives Skeletal muscle atrophy is a systemic consequence of chronic obstructive pulmonary disease (COPD) associated with increased morbidity and mortality. Muscle atrophy is often associated with a fibre type switch from slow fatigue resistant type I fibres to fast type II fibres. The underlying molecular mechanisms that regulate muscle mass are not understood. Serum response factor (SRF) is a transcription factor that regulates muscle-specific gene expression. In mice, reduced SRF expression has been shown to induce premature muscle ageing and atrophy, associated with an increase in expression of IIa MHC. In muscle, the myocardin-related transcription factors MRTFA and MRTFB function as potent co-activators of SRF activity. The activity of the MRTFs on SRF is inhibited by FHL2 in cardiac muscle, and we have previously shown that another FHL protein, FHL1, is inversely correlated with physical activity in patients with COPD. We hypothesise that the reduced expression or activity of MRTFs inhibits SRF activity in patients with COPD, contributing to the fibre type switch and skeletal muscle atrophy.

Methods 41 COPD patients and 19 healthy age-matched controls underwent percutaneous quadriceps biopsy. Levels of MRTFA, MRTFB, type I and type IIa MHC mRNA were quantified by qPCR. FHL1/MRTF protein interactions were analysed by co-immunoprecipitation. The effect of FHL1 on MRTF activity was determined by co-transfection and luciferase assay.

Results MRTFA but not MRTFB mRNA levels were reduced in patients (2.4-fold, $p = 0.014$) compared with controls. Type I MHC transcripts were reduced in patients (3-fold $p<0.001$) whereas IIa MHC transcripts were increased (1.7-fold $p<0.001$). MRTFA expression was strongly correlated with type I MHC in controls but not in patients ($p<0.01$). FHL1 bound to MRTFA in immunoprecipitation experiments but did not affect the activity of MRTFA in cultured cells. However, FHL1 did inhibit the activity of MRTFB in the same system.

Conclusions Reduced MRTFA levels and MRTFB activity in patients with COPD are likely to inhibit the activity of SRF. As SRF inhibition increases type IIa gene expression, it is likely that these changes contribute to the fibre type switch seen in patients with COPD and to the loss of type IIx fibres.

P138 MULTIPLE-FREQUENCY BIOELECTRICAL IMPEDANCE ANALYSIS AND QUADRICEPS STRENGTH IN PATIENTS WITH COPD

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Introduction Quadriceps weakness is well recognised in chronic obstructive pulmonary disease (COPD) and predicts mortality in severe disease. Single frequency (usually 50 kHz) bioelectrical impedance values depend on cell membranes acting as incomplete capacitors and on conduction through body water. Regression equations which include height, weight and gender can then be used to calculate fat-free mass (FFM). At low frequencies (5 kHz) current does not penetrate cell membranes, however at high frequencies (200 kHz) both intracellular and extracellular spaces are penetrated. Therefore, the ratio of the impedance at these frequencies is thought to give an index of extracellular and total body water. This ratio may be influenced by acute or chronic illness.

Aims Our aim was to evaluate the association between multiple-frequency bioelectrical impedance analysis and quadriceps strength in stable COPD patients.

Methods We measured FFM and whole body bioelectrical impedance at four frequencies (5, 50, 100 and 200 kHz) using a BodyStat QuadScan 4000 in stable COPD patients and healthy controls. The ratio of impedance at 200 kHz and at 5 kHz (Z_{200}/Z_5) was recorded. Quadriceps strength was measured by maximal voluntary contraction (QMVC). FFM was determined using impedance at 50 kHz and a disease-specific regression equation.

Results We studied 35 patients with stable COPD (mean (SD) forced expiratory volume in 1 s 43.5 (24.7)% predicted) and 23 age-matched controls. Patients were significantly weaker than controls (mean (SD) QMVC 26.8 (9.7) kg vs 38.3 (11.7) kg, $p<0.0001$), but there was no significant difference in Z_{200}/Z_5 (mean 0.813 vs 0.798, $p = 0.14$). There was a correlation between QMVC and Z_{200}/Z_5 in all subjects ($n = 58$; $r^2 = 0.41$, $p<0.0001$); in the COPD group ($n = 35$; $r^2 = 0.52$, $p<0.0001$) and in controls ($n = 23$; $r^2 = 0.29$, $p = 0.008$). QMVC also correlated with FFM in the COPD group ($r^2 = 0.497$, $p<0.0001$) and in controls ($r^2 = 0.404$, $p = 0.001$). A model containing both FFM and Z_{200}/Z_5 explained 76% of the variance in QMVC in COPD patients, 57% in healthy controls and 72% in the whole population.

Conclusion Z_{200}/Z_5 is associated with quadriceps weakness both in patients and healthy subjects independent of FFM. Further work is needed to confirm the physiological basis of this relationship and establish the value of this non-invasive technique in the clinical setting.

Acknowledgements Quadscan equipment provided by Bodystat Ltd.

P139 RELATIONSHIPS BETWEEN CO-MORBIDITIES, EXACERBATION FREQUENCY AND FEV_1 IN COPD

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Introduction Co-morbidity is known to be common in chronic obstructive pulmonary disease (COPD), however the impact on exacerbation frequency is not well understood. This study aimed to ascertain the nature and prevalence of co-morbid conditions in COPD patients in the UK in relation to exacerbation frequency and forced expiratory volume in 1 s (FEV_1).

Methods We retrospectively analysed the recruitment records of 355 well characterised COPD patients and 44 controls (current and ex-smokers with normal lung function) enrolled into the London COPD cohort. Self-reported co-morbid diagnoses, medication, age, gender, FEV_1%predicted and validated exacerbation frequency (for those who had completed ⩾1 year symptom diary card data) were analysed using χ^2 and Mann-Whitney U tests as well as Spearman rank correlation where appropriate.

Results COPD patients had significantly more co-morbid diagnoses than controls: median (IQR) 3 (2–5) vs 2 (1–2), respectively ($p = 0.007$). Co-morbidities were more common in patients with milder COPD (higher FEV_1%predicted) (rho = 0.249, $p = 0.01$).

Abstract P139 Table 1

	COPD (n = 355)	Controls (n = 44)	p Value
Age (mean ± SD)	68.7 ± 8.9	68.8 ± 6.0	0.951
FEV_1% predicted (mean ± SD)	49.1 ± 19.0	92.6 ± 16.7	<0.001
Median (IQR) number of co-morbid diagnoses	3 (2–5)	2 (1–3)	0.007
Hypertension	37.4%	22.7%	0.059
Previous vascular event	25.4%	11.3%	0.040
Osteoarthritis	19.1%	25.0%	0.359
Ischaemic heart disease	17.8%	6.8%	0.066
Atrial fibrillation or flutter	8.7%	2.3%	0.040
Gastro-oesophageal reflux/peptic ulcer	8.1%	6.7%	0.352
Diabetes	7.3%	9.1%	0.773
Hypercholesterolaemia	6.8%	9.1%	0.568

There was no significant correlation between exacerbation frequency and number of co-morbidities (rho = −0.09, p = 0.182). There was a significantly higher prevalence of vascular events (MI, CVA, AAA repair, PVD) in COPD patients than controls (25.4% vs 11.3%, p = 0.04). Atrial fibrillation/flutter (AF) was also significantly more common in COPD than controls (8.7% vs 0%, p = 0.04). Higher prevalence of hypertension (37.4% vs 22.7%) and ischaemic heart disease (17.8% vs 6.8%) were observed in COPD but did not reach statistical significance (p = 0.059 and p = 0.066, respectively). Although osteoarthritis, diabetes, gastro-oesophageal reflux and hypercholesterolaemia were common, they were not significantly different between the two groups (table 1). Diagnoses not expected to be more common in COPD such as hysterectomy and appendicectomy were equivalent in the two groups, providing evidence for comparable patient recollection and clinician recording with COPD and control subjects.

Conclusions Co-morbidities are more prevalent in COPD than in controls, particularly cardiovascular conditions such as AF and previous vascular events. Self-reported co-morbidity does not correlate with exacerbation frequency in COPD. We have shown, for the first time, less co-morbidity with more severe COPD. This could be explained by a survival effect secondary to higher mortality rates among more severe COPD patients with multiple co-morbidities.

P140 INCREASED ARTERIAL STIFFNESS IN PATIENTS WITH α_1-ANTITRYPSIN DEFICIENCY

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Introduction and Objectives Aortic pulse wave velocity (aPWV), the gold standard measure of arterial stiffness, is an independent predictor of future cardiovascular disease and events.[1] Previously, we have demonstrated increased aPWV in patients with COPD compared with matched controls,[2] and we hypothesised that patients with α_1-antitrypsin deficiency (AATD) would present similarly.

Methods Adult patients with PiZZ AATD (n = 19) and age-, sex- and smoking-matched controls (n = 20) all free of known cardiovascular disease were studied at clinical stability. All underwent spirometry, large artery haemodynamics and blood sampling (interleukin 6, fasting lipids and glucose).

Results Heart rate, mean arterial pressure (MAP), fasting lipids and glucose were similar in patients and controls (table 1). Aortic PWV was greater in AATD patients (p = 0.03). Mean (SD) circulating interleukin 6 was greater in patients (3.2 (1.6) pg/ml) than controls (2.2 (1.6) pg/ml; p = 0.015). In all subjects aPWV was positively related to $\log_{10}$IL6 (r = 0.50, p = 0.001). Significant independent variables of aPWV in all subjects were age, FEV_1%, heart rate and MAP (all p<0.001).

Abstract P140 Table 1

	Controls (n = 20)	Patients (n = 19)
Age (years)	61.1 (9.1)	59.2 (12.1)
Men, n (%)	13 (65%)	12 (63%)
Smoking (pack-years)†	5.5 (0–70)	10.0 (0–60)
FEV_1 (% predicted)	100.8 (12.5)	42.7 (23.3)**
Heart rate (bpm)	68.2 (12.4)	75.7 (12.5)
Peripheral MAP (mm Hg)	100.4 (10.2)	101.5 (9.2)
Aortic PWV (m/s)	8.5 (1.6)	9.9 (2.1)*
Augmentation index (%)	23.7 (8.8)	26.1 (6.5)
Total cholesterol (mmol/l)	5.8 (1.0)	5.7 (1.4)

FEV_1, forced expiratory volume in 1 s; MAP, mean arterial pressure; PWV, pulse wave velocity.
Values are mean (SD) unless otherwise stated.
*p<0.05; **p<0.001.
†Median (range).

Conclusions Patients with AATD have increased aPWV compared with age- and sex-matched healthy controls despite similar low smoking exposure, in the setting of similar MAP, lipids and glucose. This indicates increased cardiovascular risk as in non-AATD COPD patients.

1. **Laurent S**. *Eur Heart J* 2006;**27**:2588–605.
2. **Sabit R**. *Am J Respir Crit Care Med* 2007;**175**:1259–65.

P141 BONE MINERAL DENSITY AND FAT-FREE MASS IN PATIENTS WITH α_1-ANTITRYPSIN DEFICIENCY

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Introduction and Objectives Studying α_1-antitrypsin deficiency (AATD) patients allows exploration of bone mineral density (BMD) and fat-free mass (FFM) in chronic obstructive pulmonary disease (COPD) while minimising the confounding effect of smoking. Previously we have demonstrated a low BMD and FFM in patients with COPD compared with matched controls,[1] and we hypothesised that AATD patients would present similarly.

Methods Adult patients with PiZZ AATD (n = 19) and age-, sex- and smoking-matched controls (n = 20) were studied at clinical stability. BMD at hip and lumbar spine together with body composition (DXA, Hologic Discovery), spirometry, 6 Minute Walk Distance (6MWD), physical activity scores and circulating markers of bone formation and resorption were measured.

Results BMD and FFMI were lower in patients than controls (table 1). Osteoporosis was present in 8/19 patients (2/20 controls) and osteopenia in 8/19 patients (4/20 controls). Significant independent variables in all subjects of hip BMD were FEV_1% pred (p = 0.001) and FFMI (p = 0.027) with an r^2 of 0.52, and of lumbar spine BMD was FFMI alone (p<0.001) with r^2 of 0.37. Circulating markers of bone formation (P1NP and osteocalcin) and bone resorption (CTX) were similar between patients and controls. There was a strong linear association between markers of bone formation and resorption

Abstract P141 Table 1

	Controls (n = 20)	Patients (n = 19)
Age (years)	61.1 (9.1)	59.2 (12.1)
Men, n (%)	13 (65%)	12 (63%)
Smoking (pack-years)†	5.5 (0–70)	10.0 (0–60)
FEV_1 (% predicted)	100.8 (12.5)	42.7 (23.3)**
6MWD (m)	496.5 (80.1)	312.1 (133.4)**
FFMI (kg/m^2)	18.6 (2.6)	15.7 (1.7)**
BMD hip (g/cm^2)	1.03 (0.20)	0.79 (0.12)**
BMD lumbar spine (g/cm^2)	1.12 (0.20)	0.88 (0.13)**

BMD, bone mineral density; FEV_1, forced expiratory volume in 1 s; FFMI, fat-free mass; 6MWD, six minute walk distance.
Values are mean (SD) unless stated otherwise.
*p<0.05, **p<0.001.
†Range.

(p<0.001). In patients, P1NP (r = −0.56), osteocalcin (r = −0.67) and CTX (r = −0.48) were inversely related with BMD total hip (all p<0.05) but were unrelated to BMD lumbar spine.

Conclusions Patients with AATD have lower BMD and FFMI than age- and sex-matched controls and a greater proportion have new diagnoses of osteoporosis mirroring previous studies in non-AATD COPD.[1] The mechanism of BMD loss in these patients is likely to be multifactorial and may reflect a degree of deconditioning. The inverse association of bone formation and resorption markers with hip BMD suggests potential increased bone turnover.

1. **Bolton CE**. *Am J Respir Crit Care Med* 2004;**170**:1286–93.

P142 POLYMERS OF Z α_1-ANTITRYPSIN ARE INDUCED BY PULMONARY INFLAMMATION

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Alpha-1 antitrypsin (AT) is an important inhibitor of neutrophil elastase (HLE). Z antitrypsin (Glu342Lys) (Z-AT) polymerises within the hepatocyte and the subsequent severe plasma deficiency exposes the lungs to uncontrolled elastolysis and premature emphysema. We have shown that polymers of Z-AT (pZ-AT) are found in emphysematous alveolar walls and are co-localised with neutrophils. pZ-AT are ineffective inhibitors of HLE and are also chemotactic to neutrophils, suggesting a novel role for pZ-AT in Z-AT related emphysema. The factors that promote polymerisation in the lung are unknown and their recognition could identify new treatment strategies. We investigated whether pulmonary inflammation would promote production of pZ-AT.

Lung inflammation was induced by intranasal lipopolysaccharide (LPS) in 8-week-old female C57BL/6 mice and heterozygous littermates overexpressing human Z-AT. Mice were killed 1, 2 and 5 days (9 per group) after receiving 20 mg LPS or PBS. Concentrations of pZ-AT were assessed in bronchoalveolar lavage (BAL) fluid and lung homogenates (LH) by ELISA (monoclonal antibody to pZ-AT (ATZ11)). pZ-AT was increased in BAL fluid and LH following LPS instillation; d1 BAL fluid median (IQR): LPS-Z 806 (1178–693) ng/ml vs 433 (749–297) for PBS-Z (p = 0.013); d1 LH LPS-Z 437 (522–406) ng/mg lung vs 298 (346–223) for PBS-Z (p = 0.003); d2 LPS-Z mice 903 (1078–872) vs 573 (663–483) for PBS-Z (p<0.001). Western blot/non-denaturing PAGE confirmed the classical ladder formation of polymeric AT in Z-AT mice. BAL fluid and LH of LPS Z mice had significantly higher neutrophil numbers and HLE activity, respectively, compared with LPS-C57BL/6 mice: d1 HLE LPS-Z 5.36 (5.99–4.87) ng/mg lung vs LPS-C57BL/6, 3.51 (4.17–2.82) (p<0.001); d2 LPS-Z 5.4 (6.0–4.9) vs LPS-C57BL/6, 2.95 (3.15–2.8) (p = 0.003). Total protein in BAL fluid was significantly higher in LPS-Z d1, 2 compared with LPS-C57BL/6 (p = 0.026 and p = 0.008, respectively).

This is the first demonstration that pulmonary inflammation can promote polymerisation of Z-AT. Polymers are associated with neutrophilic influx and lung injury. These findings suggest that pulmonary infections result in an exaggeration of the anti-elastase:elastase imbalance in Z-AT individuals and that the frequency of pulmonary infections may explain some of the heterogeneity in Z-AT individuals. Aggressive treatment of both pathogens and the inflammatory response may reduce the development of polymers and lung damage in Z-AT individuals.

P143 ROLE OF MUSCARINIC RECEPTORS IN REGULATION OF NEUTROPHILIC CHEMOKINE RELEASE FROM HUMAN LUNG MACROPHAGES

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Background Macrophages are the principal pulmonary inflammatory cell and contribute to lung inflammation in numerous diseases such as chronic obstructive pulmonary disease (COPD). However, glucocorticosteroids are largely ineffective at inhibiting inflammatory mediator release from these cells in COPD. Macrophages express muscarinic receptors but their role in the inflammatory response is unclear. They may, however, lead to release of neutrophil chemoattractants, thereby driving inflammation.

Hypothesis Stimulating muscarinic receptors on human lung macrophages leads to release of neutrophil chemoattractants; anti-muscarinic agents may therefore have an anti-inflammatory component.

Methods Human lung macrophages were isolated from lung resection tissue and exposed to increasing concentrations of carbachol, a stable analogue of acetylcholine. Release of neutrophil chemoattractants was assessed by chemotaxis assays using neutrophils from both non-smoking controls and patients with COPD. CXCL8 levels were measured by ELISA.

Results Neutrophils from both healthy and COPD subjects migrated towards macrophage supernatants. When macrophages were treated with carbachol for 24 h, neutrophils from healthy subjects showed a significant concentration-dependent increase in migration (baseline: 62 ± 8 cells/field vs carbachol treatment: 168 ± 25 cells/field, n = 9, p<0.01), but neutrophils from COPD patients migrated less than cells from control subjects. Supernatants from macrophages pretreated with AFDX-116, a selective M2 receptor antagonist, but not 4-DAMP, a selective M3 receptor antagonist, resulted in a concentration-dependent inhibition of neutrophil migration (carbachol: 143 ± 8 cells/field vs AFDX-116 1 μM: 91 ± 9 cells/field, n = 4, p<0.05). In order to determine the nature of the chemoattractant, neutrophils were exposed to a selective CXCR2 antagonist (SB225002) prior to assay. Neutrophil migration to supernatants from treated macrophages was significantly inhibited in the presence of SB225002 (SB225002: 82 ± 11% fall in migration vs carbachol, n = 3, p<0.05). However, the CXCR2 chemokine CXCL8 was not increased in carbachol-treated macrophage supernatants.

Conclusions Stimulation of M2 muscarinic receptors on human lung macrophages causes release of neutrophil chemoattractant(s) that act via CXCR2. This is not CXCL8. Furthermore, anti-muscarinic medications may exert anti-inflammatory effects by reducing chemoattractant release from macrophages, and hence may be of additional benefit in COPD where glucocorticosteroids are ineffective.