**Introduction** Muscle wasting, that is present in a subgroup of patients with COPD, is an independent predictor of health related quality of life and survival. The two-dimensional fluorescence difference in gel electrophoresis (2D-DIGE) technology is now recognised as an accurate method to determine and quantify proteins.

**Methods and results** With the aim of identifying proteins potentially involved in the process of muscle wasting, we performed 2D-DIGE protein expression profiling in the *vastus lateralis* of 10 patients with COPD and low fat free mass index (FFMI) ($COPD_L$) ($FEV_1$ 33 ± 4.3%pred, FFMI 15 ± 0.2 $Kg.m^{-2}$) in comparison with both 8 patients with preserved FFMI ($COPD_N$) ($FEV_1$ 47 ± 7.3%pred, FFMI 19 ± 0.6 $Kg.m^{-2}$) and 9 age and gender-matched healthy sedentary subjects (C) ($FEV_1$ 96 ± 4.0% pred, FFMI 20 ± 0.9 $Kg.m^{-2}$). Data analysis was performed using DeCyder software and for protein identification MALDI_-TOF mass spectrometry (MS).

Ten proteins, whose expression was significantly changed in $COPD_L$, were identified; serum albumin (ALBU), heat shock protein beta-1 (HSPB1), peroxiredoxin-6 (PRDX6), Alpha-crystallin B chain (CRYAB) and Alpha-1-antitrypsin (A1AT) were increased while Histone-lysine N-methyltransferase (DOT1L), Troponin T (TNNT1), Myozenin-1 (MYOZ1), Myosin light chain 1 (MYL1) and mitochondrial ATP synthase subunit alpha (ATPA) were decreased.

**Conclusion** Our results showed a down-regulation of structural muscle proteins, proteins involved in myofibrillogenesis, cell cycle arrest and energy production and up-regulation of proteins reacting to cell stress and proteins involved in oxidative stress protection.

Supported by Chief Scientist Office (CSO) Scotl*06/S1103/5* and FIS *PI08/0320*.

## S143 PREMATURE AGEING AND SKELETAL MUSCLE DYSFUNCTION IN COPD PATIENTS: DEVELOPMENT OF A CELL CULTURE MODEL

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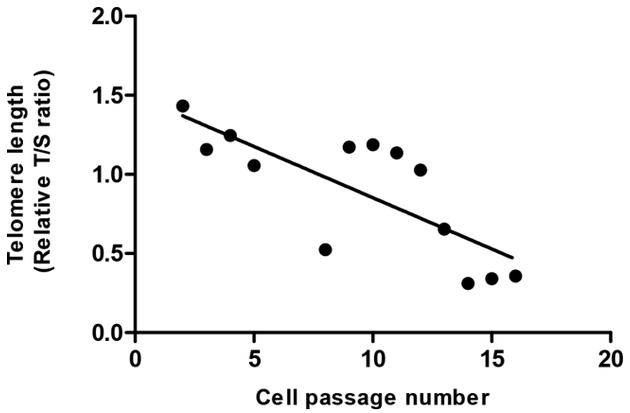
10.1136/thoraxjnl-2014-206260.149

**Introduction** COPD is a disease of accelerated ageing, as increased cellular-ageing (senescence) occurs in the lungs of these patients. We aim at developing a primary skeletal muscle cell culture (Human skeletal muscle satellite cells [HSKMC]) model of muscle ageing to study the cascade of events that occur in muscle senescence *in vitro* and to explore the effect of inflammation (TNF alpha) and oxidative stress ($H_2O_2$), two of the putative mechanisms related to muscle dysfunction and/wasting, on muscle differentiation and on protein loss in differentiated cells.

**Methods and results** HSKMC were cultured to senescence when the cells stopped replicating. DNA was isolated from cells in serial passages of culture. Telomere length, a marker of biological ageing, was measured by qPCR and expressed as the ratio of telomere repeat copy number to single gene copy number in the experimental sample relative to a control sample (relative T/S ratio) (n = 3). Preliminary results show a progressive shortening of telomere length with cellular ageing when comparing early (passage 2, 1.433 ± 0.05 relative T/S ratio) with a later passage (passage 15, 0.340 ± 0.2 relative T/S ratio) (Fig 1).




**Abstract S143 Figure 1** Telomere Length analysis in HSKMC

**Conclusion** We have developed a novel *in vitro* model of ageing skeletal muscle cells, which will help us to assess the role of accelerated ageing in muscle dysfunction and/wasting in COPD patients.

Dr Lakhdar was funded by an LTERS fellowship grant.

## S144 QUALITY OF LIFE IN IDIOPATHIC PULMONARY ARTERIAL HYPERTENSION IS ASSOCIATED WITH QUADRICEPS FUNCTION AND SIZE

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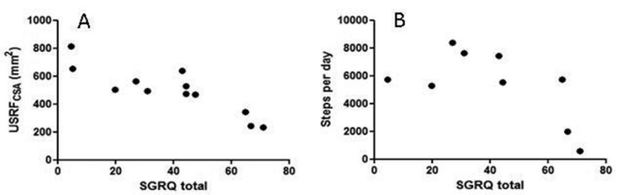
10.1136/thoraxjnl-2014-206260.150

**Introduction** Despite recent improvements in therapy patients with idiopathic pulmonary arterial hypertension (IPAH) still suffer with significantly reduced quality of life (QOL). Muscle dysfunction and low physical activity are emerging as important complications of the disease. Separately rehabilitation programs have been shown to cause an improvement in both QOL and muscle strength but the direct relationship between these factors has not as yet been documented in this condition.

**Aims** We aimed to define the relationship between QOL and muscle function, size and physical activity in patients with IPAH.

**Methods** In 12 patients with IPAH we measured quadriceps maximal volitional capacity (QMVC), ultrasound cross sectional area of the rectus femoris ($US_{RF}CSA$), fat free mass index by bioelectrical impedance (FFMI), physical activity using the Sensewear armband (steps per day, total energy expenditure (TEE), and active energy expenditure (AEE)). They were also asked to complete the St. George's respiratory questionnaire (SGRQ). Correlations were performed with Pearson's or Spearman's test.




**Abstract S144 Figure 1** St. George's respiratory questionnaire total score plotted against A: Ultrasound rectus femoris cross sectional area ($USRF_{CSA}$)(n = 12, Spearman's r = -0.88, p = 0.0002); B: Steps per day (n = 9, Spearman's r = -0.61, p = 0.08); in patients with IPAH

**Results** In the 12 patients with data so far available in this study QOL measured by the total SGRQ score correlated significantly with QMVC/BMI (r = -0.75, p = 0.005), FFMI (r = -0.71, p = 0.009) and $USRF_{CSA}$ (r = -0.88, p = 0.0002) (Figure 1A). There was no significant correlation between total SGRQ score and Sensewear measured steps per day (r = -0.62, p = 0.08) (Figure 1B) TEE (r = -0.62, p = 0.08) or AEE (rho = -0.41, p > 0.05) in the 9 patients with data available. Furthermore there was no significant correlation between BNP or resting echocardiographic parameters and total SGRQ QOL

**Discussion** We have shown that muscle size and function are directly related to QOL in patients with IPAH. This work suggests that muscle function may be an important determinant of QOL in these patients, making it a potential target for therapeutic intervention. Further data is needed to define the association between physical activity and QOL in patients with IPAH.

# Cough – mechanisms and therapies

## P1 A NOVEL CAPSAICIN COUGH CHALLENGE IN HEALTHY ADULTS; BEYOND THE C5

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10.1136/thoraxjnl-2014-206260.151

**Introduction** We have developed a novel cough challenge methodology and previously showed that evoked maximal cough responses, defined as $E_{max}$, better discriminate health from disease than traditional endpoints.[1] It is unclear how other factors influence $E_{max}$ or how it relates to the low cough rates observed in health. We therefore aimed to investigate the variability, repeatability and influences on $E_{max}$ in a larger group of healthy volunteers.

**Objective** To assess maximum cough responses to capsaicin in a group of healthy adults representing a wide range of ages.

**Method** Doubling doses of capsaicin 0.49 to 1000[micro]M) were inhaled sequentially up to the maximum tolerated dose. Four inhalations of each dose were administered 30 seconds apart and the number of coughs evoked within 15 seconds was recorded. The maximum number of coughs evoked by any dose of capsaicin ($E_{max}$) and the dose that elicited half of the $E_{max}$, defined as $ED_{50}$, were calculated. General linear models were used to assess the influence of subject demographics on these endpoints.

**Results** Forty seven healthy volunteers performed the capsaicin challenge; median age 38 years (range 20–74), 17 males, median $FEV_1$ 103% predicted (97–115), median BMI 25.0 (22.2–28.6), and median total cough rate 0.2 c/h (0.0–0.1). The median $E_{max}$ was 11 coughs (IQR, 8–19) with an $ED_{50}$ of 15.6[micro]M (7.8–109.4). The intraclass correlation coefficients for $E_{max}$ and $ED_{50}$ were 0.89 and 0.96 respectively which were highly significant (p < 0.001). Age, gender, $FEV_1$ and BMI had no significant influence on $E_{max}$. In contrast, gender (p < 0.001) and BMI (p = 0.029) both significantly influenced $ED_{50}$ explaining 41.7% of the variation. Those subjects with a higher BMI and females tended to have a lower $ED_{50}$. Objective 24 h cough frequency did not correlate with either $E_{max}$ or $ED_{50}$.

**Conclusion** Data collected to date has demonstrated that in healthy volunteers, $E_{max}$ and $ED_{50}$ are stable measures over time however $E_{max}$ has the advantage of being independent of patient factors. Intere stingly, objective cough frequency in healthy volunteers seems to be unrelated to capsaicin evoked coughing.

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## P2 CHARACTERISATION OF Aδ- AND C-FIBRES INNERVATING GUINEA-PIG AIRWAYS

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10.1136/thoraxjnl-2014-206260.152

**Introduction and objectives** Activation of afferent fibres from the lungs are involved in the cough reflex. *In vitro* studies in guinea-pig have demonstrated that there are various sub-types of Aδ-fibres (RAR's, nociceptive and cough)[1] and C-fibres (either nodose or jugular ganglia derived),[1,2] however, little is known about their equivalent characteristics *in vivo*. This study aims to characterise the responsiveness of airway sensory nerves, *in vivo*, ultimately providing a better insight into understanding the role/contribution of the various types/subtypes of Aδ and C-fibres in airway reflexes such as cough.

**Methods** Male guinea-pigs were anaesthetized with urethane (i.p. 1.5 g $kg^{-1}$), paralysed and artificially ventilated via a tracheal cannula. A vagus nerve was isolated: single fibres were identified as originating from Aδ- and C-fibres using several criteria. Action potentials were recorded[3] and agents were administered to the airways by aerosol.

**Results** Fibre-types were classified according to their conduction velocities (Table 1). All C-fibres examined were activated by capsaicin, whereas in the Aδ-fibres studies there were both capsaicin responsive and non-responsive fibres, irrespective of their CV range. All fibres exposed to CA responded strongly. There were marked differences in the responsiveness to the TRPV4 agonist, GSK1016790A: Aδ-fibres from all subgroups responded vigorously, but the C-fibres examined were not activated. Interestingly, administration of hypotonic solutions activated all of the Aδ-fibres, but had no effect on C-fibres. In contrast, all C-fibres responded to the TRPA1 agonist, acrolein, with no effect on Aδ-fibres.

**Conclusion** Several vagalafferent nerve subtypes have been identified in guinea-pig airways *in vivo*, although the classification does not appear as obvious to that observed *in vitro*. It is clear that there is a marked variation in their sensitivity to TRP channel agonists, TRPV1, TRPA1 and TRPV4, which have all been shown to evoke cough in a preclinical model in conscious guinea-pigs. It seems probable, therefore, that the different afferent pathways all regulate cough to a greater or lesser degree depending on the nature of the stimulus and underlying cause of the cough.

### REFERENCES

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2 Weigand 2012: *J Physiol*-590, (16):4109–4120
3 Adcock 2003: *Brit J Pharmacol*-138:407–416

**Abstract P2 Table 1** Characteristics of vagal afferent neuronal subtypes innervating the airways and lungs of guinea pigs *in vivo*

| C-fibres CV = < 1 m $s^{-1}$ | Aδ-fibres CV = 2.3–7.14 m $s^{-1}$ | | Aδ-fibres CV = >8 m $s^{-1}$ (Classical RARs) | |
|---|---|---|---|---|
| Capsaicin- responsive | Capsaicin-responsive (nociceptor) | Capsaicin non-responsive (mechano-receptor?) | Capsaicin-responsive | Capsaicin non-responsive |