

## Spoken sessions

**Abstract S50 Table 1. Demographics of patients with PH and HP.**

	n = 19
Age at diagnosis	54 (42, 62) years
Female	14
Duration of disease prior to onset of PH	6 (2, 9) years
Alive	7
Time of onset of PH until time of death	8 (2.8, 20) months
BNP at diagnosis	35 (10.75, 152) pmol/L
RHC	
mPAP	36 (31.25, 44.25) mmHg
PVR	8 (5.3, 11.7) WU
CO	3 (3.1, 5.7) L/min
PCWP/LVEDP	8 (5.2, 13) mmHg
Echo	
RVSP	71 (53, 87) mmHg
TR vel	372 (350, 439) cm/s
PacT	72 (67, 101) ms
PFTs	
FEV1 % predicted	53 (42, 73) %
FVC % predicted	54 (43, 80) %
TLCoc % predicted	25 (18, 27) %
KCoc % predicted	47(31, 63) %
Treatment	
Sildenafil	7
Sildenafil and endothelin antagonist	3

**Results** Nineteen consecutive patients with PH and HP were identified. Fifteen had chronic HP as evidenced by fibrosis on HRCT, whilst 4 had sub-acute disease. Demographic and baseline findings are listed in table 1. Right ventricular (RV) dysfunction was found in 14 patients. Ten were treated with targeted therapy (sildenafil +/- endothelin receptor antagonist), of these 7 had evidence of RV dysfunction. Median BNP fell between pre and first post sildenafil measurement from 80 to 27 pmol/L but failed to reach statistical significance ( $p = 0.11$ ). RV function improved in 2 of 6 patients with available follow up echocardiogram at a median of 4 months. There was no consistent change in RVSP, TR velocity or pulmonary acceleration time with treatment. Twelve patients died, a median of 8 months (0.2 - 46) from diagnosis of PH. Although not reaching statistical significance, the median arterial  $\text{pO}_2$  was higher in survivors (7.25 versus 5.85 kPa;  $p = 0.11$ ).

**Conclusion** Chronic and sub-acute HP can be complicated by PH. Mortality in this group was extremely high despite targeted therapy.  $\text{PO}_2$  was generally lower in those who died. The diagnosis of PH in HP patients may well be a pre-terminal event. Further work is needed to understand which patients may respond to advanced therapies.

### REFERENCES

1. A Wells and N Hirani (2008) Interstitial Lung Disease Guidelines. *Thorax*. 63v1-v58
2. D S Koschel et al. (2012) *Lung*. 190(3):295–302

## Mechanisms of muscle wasting

### S51 KLOTHO IS ASSOCIATED WITH SKELETAL MUSCLE DYSFUNCTION AND OXIDATIVE STRESS IN COPD

<sup>1</sup>MS Patel, <sup>1</sup>AV Donaldson, <sup>1</sup>SA Natanek, <sup>2</sup>PLB Bruijnzeel, <sup>1</sup>NS Hopkinson, <sup>1</sup>W D-C Man, <sup>3</sup>PR Kemp, <sup>1</sup>MI Polkey, <sup>1</sup>NHRI Respiratory Biomedical Research Unit at the Royal Brompton and Harefield NHS Foundation Trust and Imperial College, London, United Kingdom; <sup>2</sup>INR, AstraZeneca, Mölndal, Sweden; <sup>3</sup>Imperial College London, London, United Kingdom

Kingdom; <sup>2</sup>INR, AstraZeneca, Mölndal, Sweden; <sup>3</sup>Imperial College London, London, United Kingdom

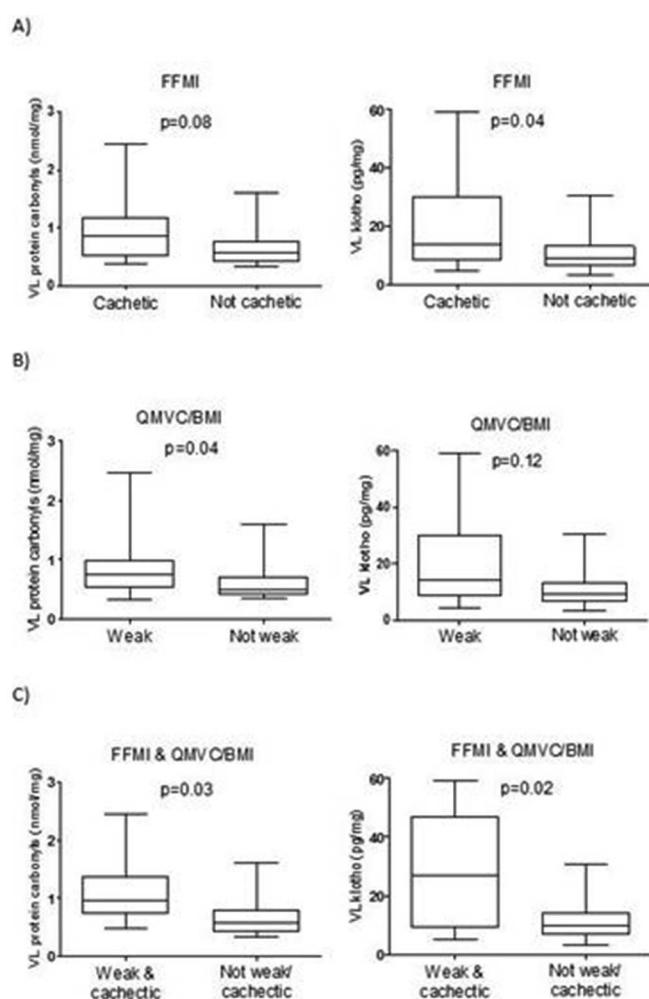
10.1136/thoraxjnl-2013-204457.58

**Introduction** Klotho is a transmembrane protein; klotho null mice develop sarcopenia and emphysema. We have previously found serum soluble klotho levels to be lower in COPD patients (ATS 2013). We hypothesised that klotho protects against muscle dysfunction by reducing oxidative stress which is a known feature of skeletal muscle dysfunction in COPD.

### Aims

1. To relate quadriceps klotho levels to protein carbonylation and muscle strength in COPD patients.
2. To determine the effect of smoking cessation on *vastus lateralis* protein carbonylation and klotho.

**Methods** In 47 COPD patients (mean  $\text{FEV}_1$  53 (23)%pred), we measured fat-free mass index (FFMI) and quadriceps strength (as QMVC/BMI). Klotho and protein carbonylation were assessed in *vastus lateralis* protein extracts. 7 current smokers (3 with COPD) had measurements before and after successful smoking cessation. Differences were assessed by Mann-Whitney and



**Abstract S51 Figure 1.** Vastus lateralis Protein carbonylation (left panel)and klotho levels(right panel) in COPD patients A) with ( $n = 12$ )or without ( $n = 35$ ) reduced FFMI, B) with ( $n = 24$ ) or without ( $n = 23$ ) reduced QMVC/BMI and C) with ( $n = 6$ ) or without ( $n = 41$ ) reduced FFMI and QMVC/BMI.

relationships by Spearman<sup>2</sup>. Response to smoking cessation was determined by paired Wilcoxon.

**Results** *Vastus lateralis* protein carbonyls were related to klotho levels ( $r = 0.34$ ,  $p = 0.02$ ). Patients with a low FFMI ( $n = 12$ ) had increased klotho levels compared to those who did not, 14.0 (8.5, 30.0) v 9.2 (6.5, 13.2) pg/mg;  $p = 0.04$ , however, protein carbonyls were not different ( $p = 0.08$ ). Conversely, patients with muscle weakness defined as a QMVC/BMI below 1.2 ( $n = 24$ ) had higher protein carbonylation, 0.76 (0.52, 0.99) v 0.51 (0.40, 0.71) nmol/mg;  $p = 0.04$ , but no difference in klotho (see figure 1). Patients with both a low FFMI and QMVC/BMI ( $n = 6$ ) had elevated levels of klotho and protein carbonyls ( $p = 0.02$  and  $p = 0.03$  respectively). In those completing smoking cessation, protein carbonyls, tended to fall, 0.75 (0.64, 1.24) to 0.61 (0.50, 0.71) nmol/mg;  $p = 0.08$ , however, klotho levels did not change ( $p = 0.38$ ).

**Conclusions** Klotho levels are related to oxidative stress within the quadriceps. Klotho levels are unexpectedly higher in those with reduced FFMI without increased oxidative stress. However, when there is loss of muscle mass and strength, increased klotho levels in muscle are accompanied by increased oxidative stress. Klotho may have a protective role against chronic oxidative stress in the skeletal muscle of patients with a reduced FFMI.

is consistent with COPD patients having a greater inflammatory response to exercise stimulus than controls.

## REFERENCES

- Lewis A, et al *Thorax* 2012;67:26–34
- Donaldson AVJ et al *Thorax* 2013: EPUB 28/06/2013

**Abstract S52 Table 1. Table of characteristics.**

Mean (SD) or Median (IQR)			significance of difference
	COPD	Control	*( $p < 0.05$ ) ** ( $p = <0.01$ ) ***( $p = <0.001$ )
Number	20	11	
Male (female)	13 (7)	6 (5)	
Age (years)	65.9 (9.1)	62.8 (7.2)	ns
FFMI (kg/m <sup>2</sup> )	18.2 (2.7)	17.9 (3.1)	ns
FEV1 (% pred)	56.5 (22.4)	103.6 (13.7)	
Best MVC (kg)	31.38 (9.5)	31.0 (6.5)	ns
average daily step count	4721 (6421,2453)	9140 (12635, 5682)	**
Workload, (watt, % pred)	69.9 (28.8)	146 (34.1)	***
V'Q2 L/min max	76.0 (21.6)	110.5 (16.9)	***
exercise (% pred)			

**S52 MIR-181 INCREASES IN THE QUADRICEPS MUSCLE OF COPD PATIENTS AFTER AN ACUTE BOUT OF EXERCISE**

<sup>1</sup>AVJ Donaldson, <sup>1</sup>RJ Tanner, <sup>1</sup>CA Davey, <sup>1</sup>N Hopkinson, <sup>1</sup>W D-C Man, <sup>2</sup>PR Kemp, <sup>1</sup>MI Polkey; <sup>1</sup>NIHR Respiratory Biomedical Research Unit of Royal Brompton and Harefield NHS Foundation Trust and Imperial College, London, UK; <sup>2</sup>Imperial College, London, UK

10.1136/thoraxjnl-2013-204457.59

**Introduction** MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression and have been implicated in the control of skeletal muscle phenotype. Our group has previously shown that muscle-specific miRs are altered, and correlate with physiological parameters in the quadriceps<sup>1</sup> and plasma<sup>2</sup> of COPD patients. MiRs may therefore have a mechanistic role in the development of skeletal muscle dysfunction in COPD.

We hypothesised that muscle-specific miRs are involved in skeletal muscle adaptation to exercise and that changes in quadriceps miR expression after acute exercise would differ between patients and controls.

**Methods** 20 COPD patients and 11 controls were studied. Fasted quadriceps biopsies were taken before and one hour after an incremental, symptom limited, cycle exercise test. Muscle-specific miR-1,-499,-133, and -206 as well as miR-181, a more widely expressed miR associated with inflammatory responses, were quantified using qPCR. Paired analyses were performed using paired T-test or paired Wilcoxon.

**Results** See table of characteristics (Table 1)

*Muscle miR results:*

MiR-1, -499, -133 did not change with exercise. MiR-181 increased x1.5 in the COPD patients only one hour after exercise ( $p = 0.017$ , controls  $p = 0.83$ ). There was also a trend for miR-206 to increase in the COPD group only ( $p = 0.07$ ).

**Conclusions** Muscle-specific miRs do not change one hour after an acute bout of exercise in COPD patients or controls. However, miR-181 increased in the quadriceps of COPD patients. MiR-181 is not restricted to, but functions in muscle, and in addition has been shown to be linked to inflammatory signalling. The difference in the response of miR-181 expression to exercise

**S53 STUDYING FIBRE SPECIFIC GENE EXPRESSION IN COPD USING LASER CAPTURE MICRO-DISSECTION IN HUMAN SKELETAL MUSCLE**

<sup>1</sup>D Mohan, <sup>2</sup>A Lewis, <sup>1</sup>MS Patel, <sup>1</sup>K Curtis, <sup>1</sup>R Tanner, <sup>2</sup>P Kemp, <sup>1</sup>MI Polkey; <sup>1</sup>NIHR Respiratory Biomedical Research Unit, Royal Brompton & Harefield NHS Foundation Trust and Imperial College, London, UK; <sup>2</sup>Section of Molecular Medicine, National Heart and Lung Institute, Imperial College London, London, UK

10.1136/thoraxjnl-2013-204457.60

**Background** Approximately 30% of patients with Chronic Obstructive Pulmonary Disease (COPD) exhibit peripheral skeletal muscle dysfunction and a shift towards type II glycolytic fibres in the quadriceps compared to healthy controls (Nanatek et al, 2013). Previous work to elucidate the molecular mechanisms underlying these changes has relied on whole biopsy samples and may have missed fibre-specific pathways; thus a method to evaluate fibre specific signalling pathways would be useful.

**Objective** To describe a novel laser capture micro-dissection (LCM) method to examine fibre-specific signalling in quadriceps biopsies.

**Methods** First larger Intercostal muscle biopsies were used to validate the methodology since they yielded more RNA. Fibres were classified as type-2 positive or type-2 negative based on immunoreactivity with a type-2 fibre specific anti-myosin Heavy Chain Alexa FLUOR 488 antibody. The type-2 negative fibre population was hence assumed to contain type-1 fibres, which was confirmed by the type-2 negative fibre population exhibiting a higher myhc7/2 mRNA ratio and expressing higher levels of genes associated with type-1 fibres, e.g. TNNT-1 and STARS, and lower levels of genes associated with type-2 fibres, e.g. TGF-B, myostatin, GAPDH and HDAC-4 ( $n = 2$ ).

We then examined OCT-embedded *vastus lateralis* muscle biopsy specimens. 10micron cryosections underwent fixation with 4% paraformaldehyde before immunostaining. LCM (PALM Microbeam, Zeiss, UK) was used to capture type I and type II fibre populations, before RNA extraction with RNeasy FFPE kit (Qiagen, USA) and rtPCR to obtain cDNA. Sybr-II