# Cellular studies in obstructive lung disease

## S49 INCREASED SKELETAL MUSCLE-SPECIFIC MICRORNA-1 IN THE BLOOD OF COPD PATIENTS

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**Introduction** Quadriceps muscle dysfunction is an important prognostic comorbidity in COPD. MicroRNAs (miRs) are small non-coding RNAs that regulate gene expression. Skeletal muscle expresses a number of tissue-specific microRNA including miR-1, which modulates muscle phenotype. MicroRNAs can be secreted from cells and maintained in blood within exosomes. Elevated levels of circulating miR-1 have been demonstrated in a number of human and animal models of muscle disease. We hypothesised that plasma levels of miR-1 would be elevated in COPD patients and would correlate with important physiological parameters.

**Methods** 103 COPD patients and 25 controls were studied. MiR-1 was quantified in stored plasma samples using q-RT PCR.[1] MiR-16 and miR-122 were quantified as negative controls. Results were normalised to an exogenous spiked-in control.

**Results** Characteristics as mean (SD); COPD patients: M: 67, F: 36, age=66.47 (8.4), $FEV_1$ % pred= 43.5 (18.6), 6-minute walk (6MW) = 394 (120). Controls: M: 14, F: 11, age=67 (8.1), $FEV_1$ % pred=111.2 (13.1), 6MW=613 (83). Plasma miR-1 was significantly elevated in COPD patients, p=0.002. There was no difference in miR-16 and miR-122. MiR-1 was negatively associated with $FEV_1$ % predicted (r =−0.3, p<0.001) and with Tlco (r=−0.3, p<0.001), but it was not possible to distinguish between GOLD stages using ANOVA. However, if patients were sub-divided into early GOLD stage COPD (1 and 2) or late GOLD stage COPD (3 and 4), miR-1 was significantly higher in the latter group (p=0.02). The plasma level of miR-1 was inversely correlated with daily activity measured as locomotion time (r= −0.25, p<0.01) but miR-1 levels were not associated with any muscle phenotype or with muscle-specific gene expression.

**Conclusion** Our results show that stable COPD patients have elevated plasma levels of muscle-specific miR-1. The increase in miR-1 may be due to increased muscle degradation or turnover in the COPD patients studied. Our work raises the possibility of using other muscle-specific microRNAs in the future as potential biomarkers of muscle dysfunction in patients with COPD.

**REFERENCE**
1. **Kroh.** Methods **50** 2010:298−301.

## S50 NITRATIVE STRESS IS INCREASED IN COPD EXACERBATIONS FOLLOWING EXPERIMENTAL RHINOVIRUS INFECTION

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**Introduction and Objectives** The majority of acute exacerbations of COPD are associated with viral infection and rhinoviruses are the most frequently detected species. Exacerbations represent a major unmet health need and mechanisms are poorly understood. The association between nitrative stress and virus induced exacerbations of COPD are unclear. To investigate this we used an experimental rhinovirus challenge study.

**Methods** Experimental rhinovirus challenge was performed in COPD (GOLD stage II) subjects (COPD, n=9), and non-obstructed control smokers (Smk, n=10) and non-smokers (NS, n=11). Rhinovirus infection was confirmed with quantitative PCR performed on nasal lavage and sputum samples collected at baseline and days 3, 5, 9, 12, 15, 21 and 42 post virus inoculation. Nitrite concentration was measured in sputum supernatant using the Griess assay, as a marker of total nitrative stress.

**Results** At baseline the geometric mean (95% CI) nitrite levels were similar between the groups studied (NS 5.13 (3.27 to 8.05); Smk 2.82 (1.37 to 5.80) and COPD 4.17 (3.04 to 5.72); p=0.281). Nitrite levels were significantly higher in COPD subjects on day 15 when compared to both control groups (geometric mean (95% CI) NS 7.94 (7.59 to 8.31); Smk 7.59 (4.41 to 13.04) and COPD 20.98 (13.92 to 31.36); p=0.008). (Abstract S50 figure 1). At every time point sampled there was a significant increase in nitrite concentration from baseline in COPD subjects, but not controls. The area under the curve for nitrite concentration over the time course for NS, Smk and COPD subjects was 18, 43 and 76 respectively (p<0.05).

**Conclusions** Rhinovirus infection is associated with increased nitrative stress in COPD subjects compared to smoking and non-smoking controls. This may play a role in COPD exacerbations.
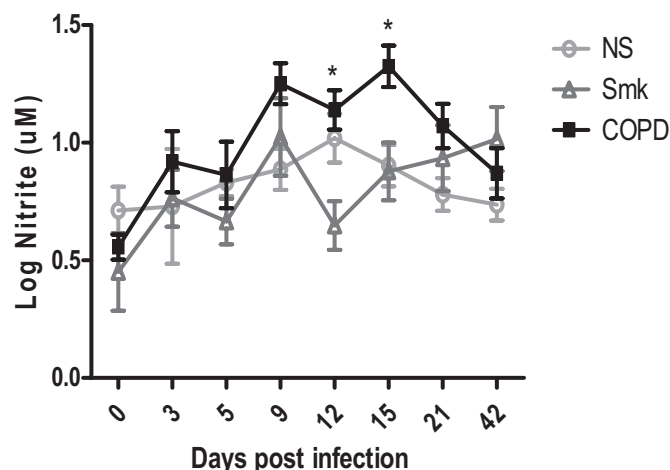


Abstract S50 Figure 1 Time course of sputum supernatant nitrite levels in COPD subjects and non-obstructed non-smoking (NS) and smoking (Smk) controls. Data presented mean +/-SEM. (* One-way ANOVA p<0.05).

## S51 REDUCING INTRACELLULAR AGGREGATION AND IMPROVING THE SECRETION OF Z ALPHA-1 ANTITRYPSIN

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The Z variant (Glu342Lys) of $\alpha_1$-antitrypsin (AT) is one of the serpinopathies; it polymerises and accumulates in the hepatocyte endoplasmic reticulum (ER) resulting in neonatal hepatitis and liver cirrhosis. The secretory defect leaves the lungs vulnerable to elastolysis and early-onset emphysema. Prevention of polymerisation of Z-AT can be achieved in vitro by targeting strand 4a of the AT molecule. Here we evaluate whether an inhibitor of polymerisation; Ac-TTAI-$NH_2$ (4M) would inhibit Z-AT polymerisation in a cell model. HEK293 cells were transfected with Z-AT (Z-AT cells) or control M-AT (M-AT cells). ELISA demonstrated significantly reduced Z-AT secretion, 242(SEM±63) ng/ml compared to M-AT, 2449±130 ng/ml (p≤0.001), due to retention of Z-AT polymers in inclusion bodies. This was confirmed by electron microscopy demonstrating distension of the rough ER, and ELISA and Immunoblot with a polymer specific antibody (ATZII). 4M significantly reduced the amount of polymers in inclusions in a dose-dependent