S140 GDF-15 DOWN-REGULATION OF MUSCLE MICRORNA DRIVES INCREASED SENSITIVITY TO TGF-β SIGNALLING; A NOVEL MECHANISM IN INTENSIVE CARE UNIT ACQUIRED WEAKNESS

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Introduction Intensive care unit acquired weakness (ICUAW) is common and associated with significant morbidity. We previously identified GDF-15, a TGF-β super-family member, as a potential driver of acute muscle wasting in a novel human model of ICUAW (Crit Care Med 2013;2013;41:982). In the current study we investigated the potential mechanisms by which GDF-15 may contribute to the development of ICUAW.

Dysregulation of muscle microRNAs has been described in muscle disorders. MicroRNAs are essential for muscle homeostasis and their expression can be influenced by inflammatory cytokines. Furthermore muscle microRNAs may down-regulate TGF-β signalling. However, the function of microRNAs in ICUAW has not previously been described. We hypothesised that down-regulation of muscle microRNAs, driven by GDF-15, would lead to increased sensitivity to TGF-β signalling in muscle of patients with ICUAW.

Methods We conducted an observational study of 20 patients with ICUAW and 7 elective surgical controls. Subjects underwent rectus femoris muscle biopsy and blood sampling. Muscle specimens were examined for mRNA and microRNA expression of target genes by qPCR. Plasma samples were tested for GDF-15 concentration (ELISA). Histology samples were stained for pSMAD2/3 nuclear positivity. To examine the effects of GDF-15 on target genes, differentiated C2C12 myotubes were treated with GDF-15 for 4 days. The effect of over-expression of miR-181a in C2C12 myoblasts on TGF-β signalling was also examined.

Results Compared with controls, patients with ICUAW had greater GDF-15 mRNA expression in muscle (median 2-fold higher; p = 0.006) and concentration in plasma (median 7239 *vs.* 2454 pg/ml; p = 0.001). MicroRNAs involved in muscle homeostasis were significantly lower in muscle from patients with ICUAW. Both log[GDF-15 mRNA] and log[plasma GDF-15] were significantly negatively correlated with log[microRNA expression]. GDF-15 treatment of myotubes significantly elevated expression of muscle atrophy-related genes and down-regulated expression of muscle microRNAs. miR-181a suppressed TGF-β responses in myoblasts, suggesting increased sensitivity to TGF-β in ICUAW muscle. Consistent with this, nuclear phospho-SMAD2/3 and CYR61 mRNA expression were increased in ICUAW muscle.

Discussion By suppressing expression of muscle microRNAs GDF-15 may increase sensitivity to TGF-β signalling, thus promoting muscle wasting in ICUAW. This study identifies both GDF-15 and associated microRNA as potential therapeutic targets in ICUAW.

S141 TUMOUR NECROSIS FACTOR RECEPTOR 1 SHEDDING IS RELATED TO ACUTE SKELETAL MUSCLE WASTING IN CRITICAL ILLNESS

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Introduction Muscle wasting occurs early and rapidly in critically ill patients. It results from a decrease in muscle protein synthesis and an increase in its breakdown, with muscle necrosis a common associated finding. The drivers for such atrophy and necrosis remain poorly understood, as do the related regulatory pathways. We hypothesised that systemic and intracellular cytokines play a role in this process.

Methods The UK-MUSCLE study prospectively studied the wasting response (change in Rectus Femoris cross sectional area (RF_{CSA}) using serial ultrasound) in critically ill patients admitted to the ICU. Cytokine profiles (high sensitivity cytokine chip array, Randox, Ireland) were analysed in serum samples from 62 of these patients (days 1 and 7) and in contemporaneous Vastus Lateralis biopsies of 35 patients. Tumour Necrosis Factor (TNF)-α, TNF receptor (TNFR) 1 and 2, interleukin (IL)1a, il1b, IL-2, IL-4, IL-6, IL-8, IL-10, Vascular Endothelial growth Factor (VEGF), Interferon (IFN)-γ, Monocyte Chemoattractant Protein-1(MCP-1) and Epidermal Growth Factor were assayed. Muscle necrosis was determined by hematoxylin and eosin staining of the Vastus Lateralis biopsies.

Results Intramuscular TNFR1 concentrations increased over 7 days (0.83 ± 1.1 μg/L to 2.07 ± 2.65 μg/L; p = 0.042), as did intramuscular interleukin-10 (22.69 ± 26.5 ng/L to 59.8 ± 80.0 ng/L; p = 0.005). Increases in serum IL-1a (0.52 ± 0.26 ng/L to 0.57 ± 0.28 ng/L, p = 0.03), VEGF (166.86 ± 231.7 ng/L to 246.6 ± 236.7 ng/L, p < 0.001) and MCP-1 (886.8 ± 685.0 ng/L to 386.49 ± 469.7 ng/L, p < 0.001) were seen as well as a decrease in IL-6 (322.2 ± 422.7 ng/L to 78.55 ± 184.4 ng/L, p < 0.001), il-10 (22.73 ± 51.1 ng/L to 7.61 ± 15.3 ng/L, p = 0.03), IFN- γ (4.76 ± 11.0 ng/L to 1.28 ± 2.02 ng/L, p = 0.02) and MCP-1 (886.8 ± 685.0 ng/L to 386.49 ± 469.7 ng/L, p < 0.001). Neither myonecrosis nor change in RF_{CSA} was related to that in intramuscular cytokines by linear and logistical-regression analysis, using 10% loss as a cut off. Loss in RF_{CSA} over 10 days was very weakly correlated with serum TNFR1 concentration on days 1 (r^2=0.12; p < 0.01) and 7 (r^2=0.09; p = 0.02) and with serum IL-10 concentration (r^2=0.19; p < 0.01). Myofibre necrosis was unrelated to serum cytokine profile.

Discussion Soluble TNFR1 is associated with the degree of muscle wasting in critical illness. This relationship may be causal as TNF1R signalling leads to activation of nuclear factor kappa beta and apoptosis. Whilst no evidence was seen for intramuscular inflammation, increased intramuscular IL-10 may be protective, in its anti-inflammatory role.

S142 VASTUS LATERALIS PROTEOMIC ANALYSIS IN MUSCLE WASTED PATIENTS WITH COPD USING TWO-DIMENSIONAL FLUORESCENT ELECTROPHORESIS

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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.

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

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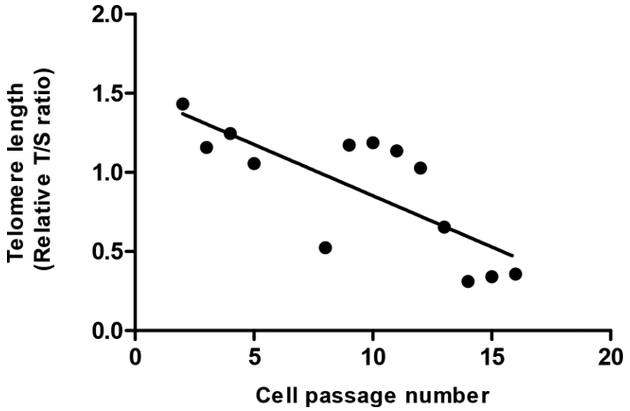
Introduction COPD is a disease of accelerated ageing, as increased cellular-ageing (senescence) occurs in the lungs of these patients. We aim at developing a primary skeletal muscle cell culture (Human skeletal muscle satellite cells [HSKMC]) model of muscle ageing to study the cascade of events that 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.

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

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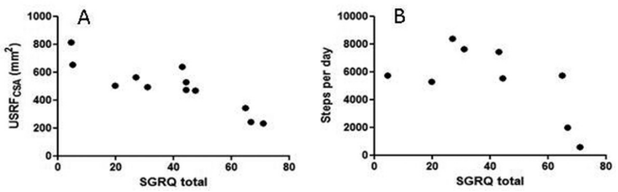
Introduction Despite recent improvements in therapy patients with idiopathic pulmonary arterial hypertension (IPAH) still suffer with significantly reduced quality of life (QOL). Muscle dysfunction and low physical activity are emerging as important complications of the disease. Separately rehabilitation 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

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