P461

P461 -Characterisation of Basal Myogenic Gene and Protein Expression in Skeletal Muscle of Advanced Chronic Kidney Disease Patients

Mr Tom O'Sullivan[1], Dr Luke Baker[1], Professor Alice Smith[1], Dr Emma Watson[1]
[1]*University Of Leicester, Leicester, United Kingdom*

Introduction:
Chronic kidney disease (CKD) is associated with skeletal muscle weakness and wasting which increases mortality risk and adversely impacts quality of life, physical function and activity. Skeletal muscle contains myogenic precursor cells, also known as satellite cells (SCs), which are capable of re-entering the cell cycle in response to stimuli such as tissue damage and in some cases exercise. Following proliferation, myoblasts undergo terminal differentiation and eventually fuse to existing fibres, thereby providing new myonuclei. This process is known as myogenesis and is regulated by a series of transcription factors collectively referred to as Myogenic Regulatory Factors (MRFs). We recently found an absence of up-regulation in MRF expression in non-dialysis CKD patients following acute resistance exercise. However, comparison of the basal gene and protein expression of these MRFs between CKD patients and healthy controls (HCs) is lacking from the literature. Thus, the current investigation aimed to characterise MRF's in pre-dialysis CKD patients in comparison to matched healthy controls (HC).
Methods:
Skeletal muscle biopsies were taken at rest from CKD patients (n=21) and age matched HC (n=16). Tissue samples were homogenised and RNA extracted, reversed transcribed, with the resultant cDNA used for RT-PCR with specific primers for Pax7, MyoD, Myf5 and Myogenin. Threshold cycle (Ct) was normalised to an internal control (18S) and analysed according to 2-ΔCT. Tissue lysates were prepared for protein analysis via Western Blotting using stain free gels and monoclonal antibodies raised against Pax7 and MyoD, relative to loading control. Statistical significance was determined through the use of parametric analysis (mean ± SD, $p \leq 0.05$).
Results:
Group demographics reported no significant difference across age and gender (CKD; 62.00 ± 12.48 yr, 57% female vs. HC; 58.94 ± 13.45 yr, 64% female), whereas eGFR was significantly lower in CKD patients (26.29 ± 7.58 ml.min.1.732 vs 75 ± 6.83 ml.min.1.732, $p < 0.001$). Gene expression of Pax7 (4.7-fold, $p = 0.02$), MyoD (5.5-fold, $p = 0.01$), Myf5 (4.6-fold, $p = 0.03$) and Myogenin (4.7-fold, $p = 0.03$) was significantly higher in biopsy samples from HC, compared to CKD. However, no significant difference was noted in the protein expression of Pax7 and MyoD between the two groups ($p > 0.05$).
Conclusions:
This data indicates considerable down-regulation of gene expression of MRFs responsible for myogenesis in CKD skeletal muscle at rest. This includes Pax7, the canonical SC marker in human skeletal muscle, and genes required for myoblast proliferation (Myf5), myogenic determination (MyoD) and differentiation (Myogenin). In contrast, protein expression of Pax7 and MyoD in CKD patients did not differ from that of HC. Impaired expression of these genes may underlie the lack of myogenic response to exercise previously reported in this population, which could contribute to muscle wasting and weakness. Future work should seek to compare the myogenic response to exercise in CKD patients with HC, to elucidate potential targets for therapeutic intervention to counteract muscle losses in this patient population.