Protein degradation rates are comparable in cultured skeletal muscle cells from human patients with CKD and healthy controls

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Introduction

- Patients with advanced Chronic Kidney Disease (CKD) experience significant muscle wasting that negatively impacts upon quality of life, morbidity and mortality. However, the causes of this are poorly understood.
- Previous studies from animal studies have shown that this is largely due to an increase in skeletal muscle protein degradation (PD) rates with equivocal data on a suppression of protein synthesis.
- However, this effect has not been studied directly in the skeletal muscle of human CKD patients.

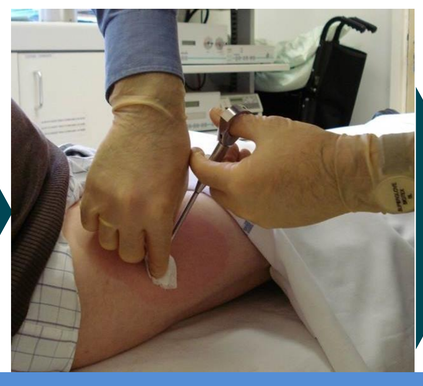
Aim

To investigate the basal PD rates in primary skeletal muscle cells from patients with CKD not yet requiring dialysis compared with those from age and sexed matched healthy controls (HC).

Methods

4 CKD patients
(Age 57, range 42-68 years; eGFR 23 range 14-29ml/min/1.73m^2)

3 Healthy Controls
(Age 65, range 36-68)



Vastus lateralis muscle biopsy

Satellite cells were dissociated from muscle tissue using 1mg/ml collagenase IV, filtered through a 70μm sterile filter and purified using the pre-plating technique

Cells were grown to 50-70% confluence in HamsF10 containing 1% Penicillin/streptomycin/fungizone solution and 20% FBS and maintained at 37^{o}C and 5% CO_2

For experimentation cells were switched to DMEM containing 2% horse serum and 1% penicillin/streptomycin for 1 week until mature myofibres had formed

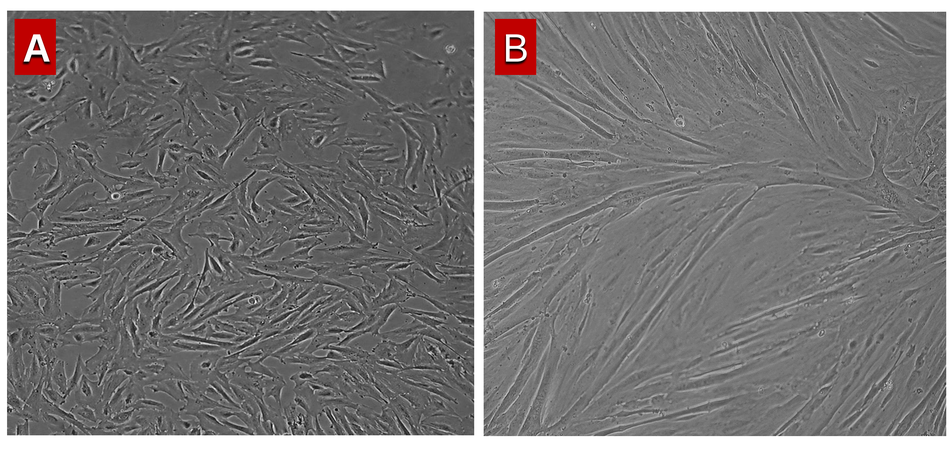
Experimentation

Myotubes were first prelabelled with 2µci/mL ^{3}H-L-Phe for 72h

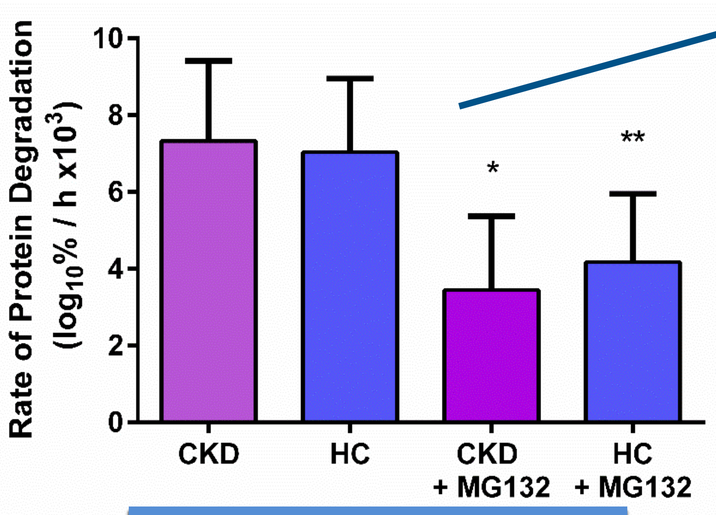
Then incubated with test media at pH7.4 which also contained unlabelled Phe

Rates of PD were measured from the release of ^{3}H into the medium at 9h,24h and 48h and are expressed as $\log_{10}$ of the % of initial cellular ^{3}H

Results



Representative images of of A) myoblasts and B) myotubes established from a biopsy of a CKD patient



There were no differences in the rates of PD in CKD patients (7.3±2.0 $\log_{10}$% / h x10^3) compared to controls (7.0±1.9 $\log_{10}$% / h x10^3; P=0.8)

The addition of MG132 at pH 7.4 significantly reduced PD rates in both patients and controls 59% and 47% respectively

* = P<0.001 vs CKD
** = P<0.05 vs HC value

Discussion

- **These preliminary results imply that in isolation and under basal conditions human primary muscle cells from CKD patients and matched controls show comparable rates of protein degradation.**
- **This appears to be in contradiction with previous studies from animal models that have shown that CKD promotes elevated rates of protein degradation that over time cause a loss of skeletal muscle mass.**
- **These results may also indicate 1) CKD does not bring out permanent changes in the protein turnover activity of myocytes and 2) previously observed increases in PD were elicited by the uraemic environment rather than a fundamental alteration in myocyte metabolism**

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