Vitamin D Supplementation induces myogenic differentiation in skeletal muscle cells isolated from Vitamin D deficient patients.

Introduction

Patients with advanced Chronic Kidney Disease (CKD) experience skeletal muscle wasting which negatively impacts upon quality of life, morbidity and mortality. However, the causes of this muscle wasting are poorly understood and as such, there are no adopted therapies. Vitamin D deficiency is common in CKD and is known to be associated with reduced muscle function in frail and elderly populations, but its contribution to reduced muscle mass in CKD has not been investigated. We examined the morphological changes in skeletal muscle cells isolated from CKD patients with confirmed vitamin D deficiency when incubated with different concentrations of Vitamin D (1α,25(OH)2D3) to determine if vitamin D supplementation can induce myogenic differentiation.

Methods

Four CKD patients (mean eGFR 26, range 15-36ml/min/1.73m2; mean age 54, range 27-78 years) with Vitamin D deficiency (mean 25[OH]D 12.5, range 9.2-19.1 ng/mL determined using the Endocrinology Society classification system) analysed by high-pressure liquid chromatography tandem mass spectrometry (LC-MS/MS), consented to give a skeletal muscle biopsy. Muscle tissue was homogenised and satellite cells isolated and grown to confluence in HamsF10 medium with 20% serum. Once confluent, cells were switched to MEM with 2% serum for five days and supplemented with either 0nmol, 10nmol, or 100nmol 1α,25(OH)2D3 that was changed every day. Cells were then fixed in 4% paraformaldehyde and stained with and an immunofluorescent antibody (Alexa Fluor 488) against Desmin. Images were captured using a FLoid imaging station and fusion index and myotube diameter determined using Image J.

Results

Both 10nm and 100nm 1α,25(OH)2D3 significantly increased myotube diameter compared to control (23.4 ± 6.7 µm, P<0.001; and 26.0 ± 9.4 µm, P<0.001 respectively vs 15.7 ± 5.8 µm). 100mol 1α,25(OH)2D3 also significantly increased myotube fusion index above that seen in 10nm and control conditions (100nm 51.3 ± 15% vs 10nm 34.0 ± 7.7 P=0.016) (100nm 51.3 ± 15% vs control 29.6 ± 7.8% P = 0.025). There was no improvement in myotube fusion index with 10nm 1α,25(OH)2D3 compared to control (P=0.25).

Key Conclusions

In myotubes derived from satellite cells isolated from Vitamin D deficient CKD patients, 100nm 1α,25(OH)2D3 significantly increased myotube diameter and fusion index. This suggests that vitamin D signalling seems to play a role in muscle regeneration and Vitamin D deficiency may be an important factor in skeletal muscle wasting and dysfunction in CKD.