VITAMIN D PROMOTES MYOGENIC DIFFERENTIATION AND INDUCES AN ANTIFIBROTIC PHENOTYPE IN PRIMARY CULTURES OF SKELETAL MUSCLE DERIVED SATELLITE CELLS AND FIBROBLASTS

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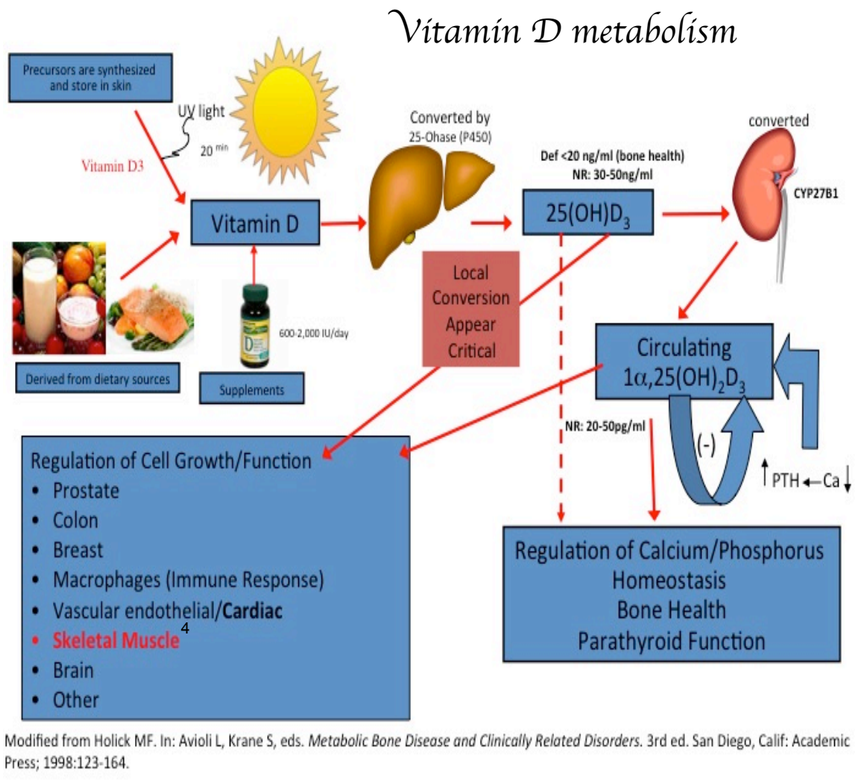
Background I

- Skeletal muscle wasting is a serious public health problem associated with aging, Chronic Kidney Disease (CKD), and AIDS[1].
- Vitamin D (VD) is most widely recognized for its regulation of calcium and phosphate homeostasis in relation to bone development and maintenance, and for its synergistic effects on target organs such as PTH glands.
- Recently, it has been shown to improve muscle performance and reduce falls in VD deficient older adults[2,3]. However, little is known of the underlying molecular mechanism or the role it plays in association with myogenic differentiation and on muscle fibrosis.

Background II



Modified from Holick MF. In: Avioli L, Krane S, eds. *Metabolic Bone Disease and Clinically Related Disorders.* 3rd ed. San Diego, Calif: Academic Press; 1998:123-164.

Objectives

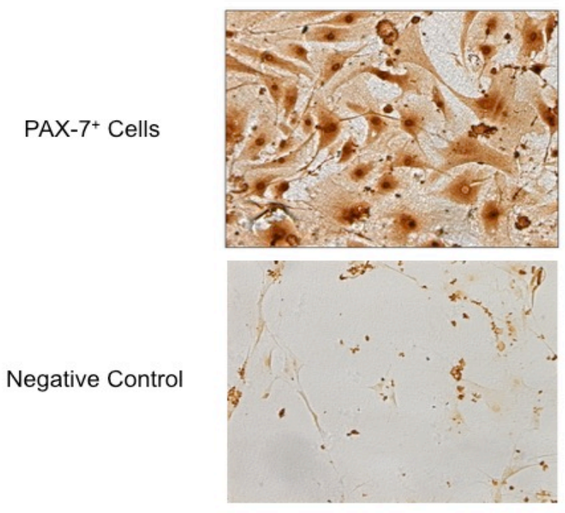
- To examine the effects of 1,25-D_3, the active form of Vitamin D, also known as 'calcitriol' on:
  - Myogenic cell differentiation of muscle stem cells (satellite cells), and
  - The generation of an anti-fibrotic phenotype in skeletal muscle derived fibroblast cells.

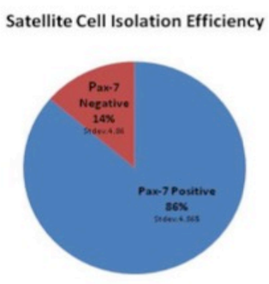
Materials & Methods

- Primary cultures of skeletal muscle derived satellite cells and fibroblasts were isolated from the tibialis anterior, soleus and gastrocnemius muscles of 2-month-old C57/BL6 male mice and then treated with or without 1,25-D_3 in a time course manner.
- Expression of Vitamin D receptor (VDR), collagen I, III, pro and anti-fibrotic factors, muscle lineage and angiogenic markers were assessed by Immunocytochemistry (ICC), PCR arrays and confirmed by Real time qPCR and western blots.

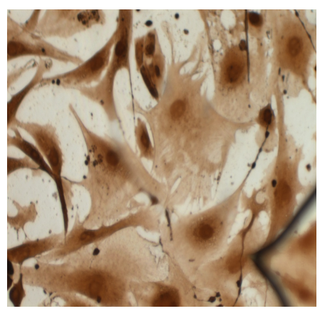
Results I

Isolation of Satellite Cells from Skeletal Muscle Yields an 86% Efficiency Demonstrated by PAX-7 Expression by ICC

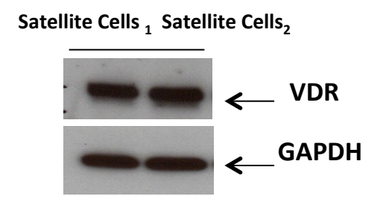




Satellite Cells Express the VDR



ICC



Western Blot

Results II

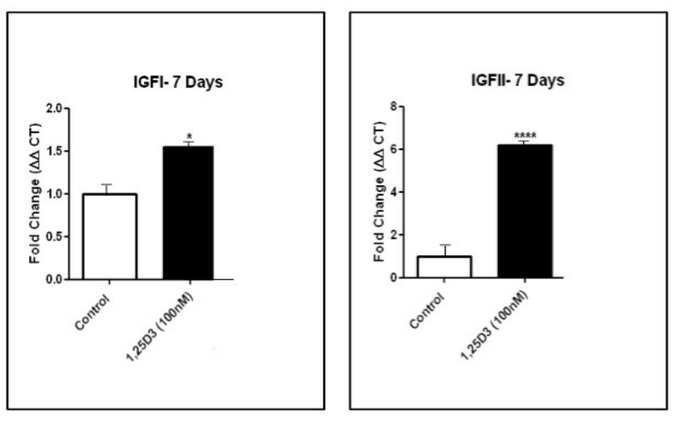
Myogenesis Array Results after 7 days of continuous Incubation of Satellite cells with 1,25-D

Ref. Seq	Symbol	Description	Fold Δ	
NM_007554	Bmp4	Bone morphogenetic protein 4	+2.7	*
NM_008006	Fgf2	Fibroblast growth factor 2	+2.52	*
NM_010512	Igf1	Insulin-like growth factor 1	+1.62	←
NM_010514	Igf2	Insulin-like growth factor 2	+6.01	←
NM_010834	Mstn	Myostatin	-2.6	←
NM_013599	Mmp9	Matrix metallopeptidase 9	+3.2	← Anti-fibrotic
NM_030679	Myh1	Myosin, heavy polypeptide 1, skeletal muscle, adult	+3.99	Markers of Skeletal Muscle Cell Differentiation
NM_009405	Tnni2	Troponin I, skeletal, fast 2	+1.93	
NM_011620	Tnnt3	Troponin T3, skeletal, fast	+2.2	

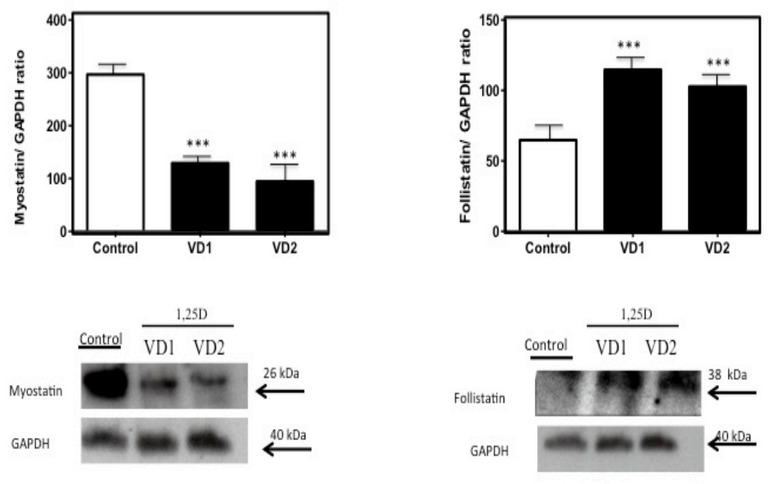
*Umemoto T, Furutani Y, Murakami M, Matsui T, Funaba M. Endogenous Bmp4 in myoblasts is required for Myotube formation in C2C12 cells. Biochim Biophys Acta. 2011 Dec;1810(12):1127-35.

*Florini, J.R., D.Z. Ewton, S.A. Coolican. 1996. Growth hormone and the insulin-like growth factor system in myogenesis. *Endocr. Rev.* 17:481–517.

Expression of IGF-1 and IGF-2 by Real Time PCR



Expression of Mstn and Fst by Real Time PCR and Western Blots

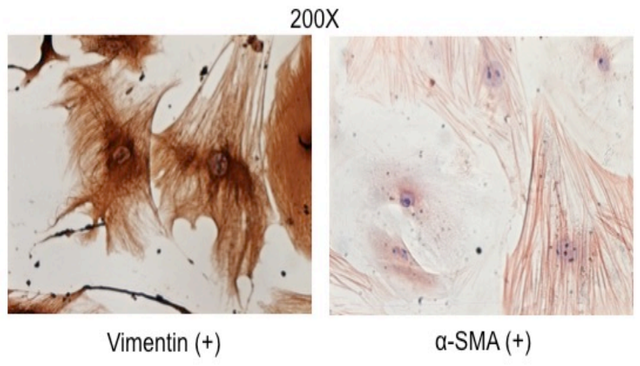


Characterization of Primary Cultures of Fibroblasts from Skeletal Muscle

✓ **Isolation of fibroblasts:**

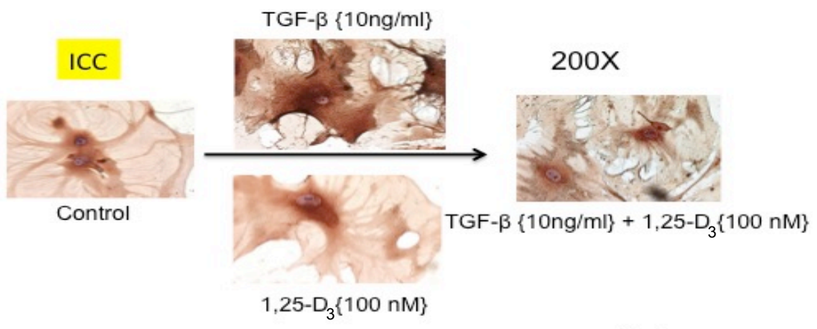
- Cells were allowed to adhere for 2h to remove large debris, macrophages and fibroblasts that adhere to the plastic, the non-adherent cells (satellite cells) were transfer to another flask.
- Fibroblast were detached with a cell scraper and transfer to 6-well plates for characterization.

200X

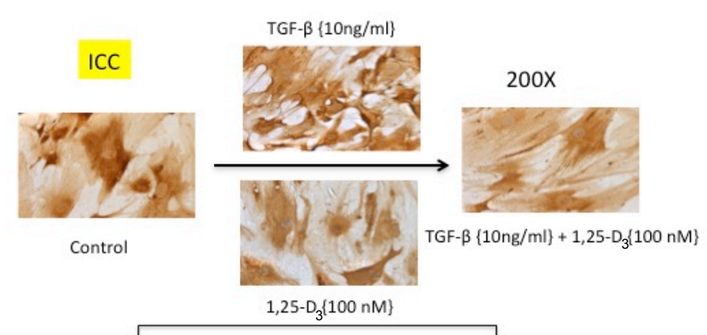


Vimentin (+) α-SMA (+)

Vitamin D decreases the Expression of Collagen I in primary cultures of Fibroblast treated with TGF-β



Vitamin D decreases the Expression of Collagen III in primary cultures of Fibroblast treated with TGF-β



Conclusions

- The efficiency of satellite cells isolation determined by PAX-7+ cells was 86%.
- It was confirmed that satellite cells expressed VDR.
- Addition of 1,25-D_3 (100nM) to satellite cells induces:
  a) Increase expression of Troponin-I and II,
  b) Increase expression of Bmp4
  c) Increase expression of IGF-I and IGF-II,
  d) Increase expression of Follistatin (Myostatin inhibitor) and
  e) A decrease expression of Mstn (Myostatin- a key negative regulator of muscle mass).
- Fibroblast isolated with a 90% efficiency were characterized by Vimentin+ and α-SMA+ cells showed a decreased expression of collagen I and III after being challenged with TGF-β alone or in combination with 1,25-D_3.

Summary

- Vitamin D possesses a clear myogenic effect on satellite cells (adult muscle stem cells) in charge of reconstituting the muscle after muscle injury or muscle waste.
- Vitamin D also possesses an anti-fibrotic effect on fibroblasts of muscle origin.
- This study provides a mechanistic justification for Vitamin D replenishment in:
  - Muscle waste conditions such as: AIDS, cancer, congestive heart failure and renal failure, characterized by lost of muscle mass and excessive collagen deposition (fibrotic process) and also in
  - Vitamin D deficient older adults who are known to have age-related loss of muscle mass and an increased rate of falls.

References

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2. Glerup H, Mikkelsen K, Poulsen L, Hass E, Overbeck S, Andersen H, Charles P, Eriksen EF. 2000. Hypovitaminosis D myopathy without biochemical signs of osteomalacic bone involvement. Calcif Tissue Int 66:419–424.
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