Divergent biological effects of vitamin D metabolites on skeletal muscle cells

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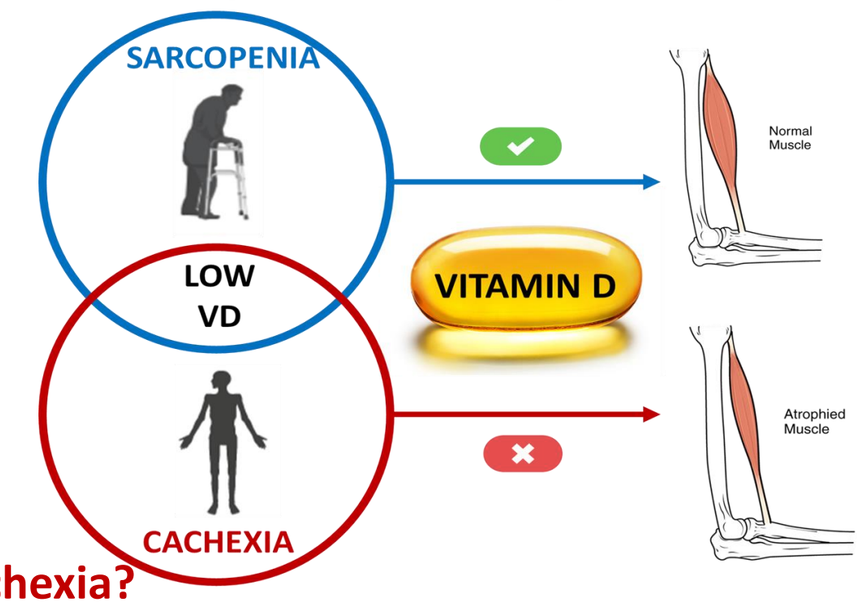
The Scientific Question

Skeletal muscle wasting represents the main overlapping features between sarcopenia and cachexia.

A deficit in vitamin D (VD) commonly co-occurs with both these muscle wasting-inducing conditions.

Due to the well-recognized relationship between VD/VDR and skeletal muscle mass and functionality, VD supplementation has been proposed as a therapeutic strategy to treat muscle wasting in both sarcopenia and cachexia. However, while several studies substantiated the efficacy of vitamin D intake in maintaining or improving muscle strength and function in elderly subjects, vitamin D supplementation is ineffective to counteract muscle wasting in cancer cachexia associated with low levels of plasmatic vitamin D, both in human and in rodent experimental models (Fig. 1).

Fig. 1 Scientific background.



Why is VD supplementation ineffective in restoring skeletal muscle in cancer cachexia?

Preliminary results

Studying the direct effects of vitamin D on C2C12-derived myotubes treated with pro-atrophic cytokines, as a model of skeletal muscle wasting, we demonstrated that:

1) not only 1,25VD is biological active, but also VD3, 25VD, and 24,25VD;
2) the effects of VD3 and 25VD are direct, do not depend on the intracellular conversion to 1,25VD;
3) VD3 and 25VD have protective effects, while 1,25VD has an atrophic activity per se. Also, 24,25VD itself has divergent effects, either pro-atrophic or anti-atrophic, depending on its concentration (Fig. 2).

	VD3	25VD	1,25VD	24,25VD
Anti-atrophic effect	✓	✓	✗	✓ (low doses)
Atrophy induction	✗	✗	✓	✓ (high doses)

Fig. 2 Direct effects of VD metabolites on C2C12 myotubes.

The divergent effect of VD on C2C12 trophism highlighted the urgency to elucidate the different mechanisms by which VD system impact on skeletal muscle

VD hydroxylases: cachexia vs sarcopenia

The *in vivo* metabolism of VD is extremely complex due to the presence in various tissues of VD hydroxylases and VDR, whose expression can be further modulated by VD metabolites themselves.

We hypothesize that the positive outcome of VD supplementation *in vivo* can results from the balance between pro- and anti-atrophic VD metabolites

To test this hypothesis, we analyzed the expression of the main hydroxylases and VDR in aged and cachectic mice vs. young healthy controls (Fig. 3).

To induce cachexia $1x10^6$ Lewis lung carcinoma cells were subcutaneously injected in the posterior lumbar region of C57BL/6 mice, and after 4 weeks mice were suppressed and tissues harvested.

Fig. 5 Used animal models, N=8 for each group.

VD metabolites impact differently on mitochondria

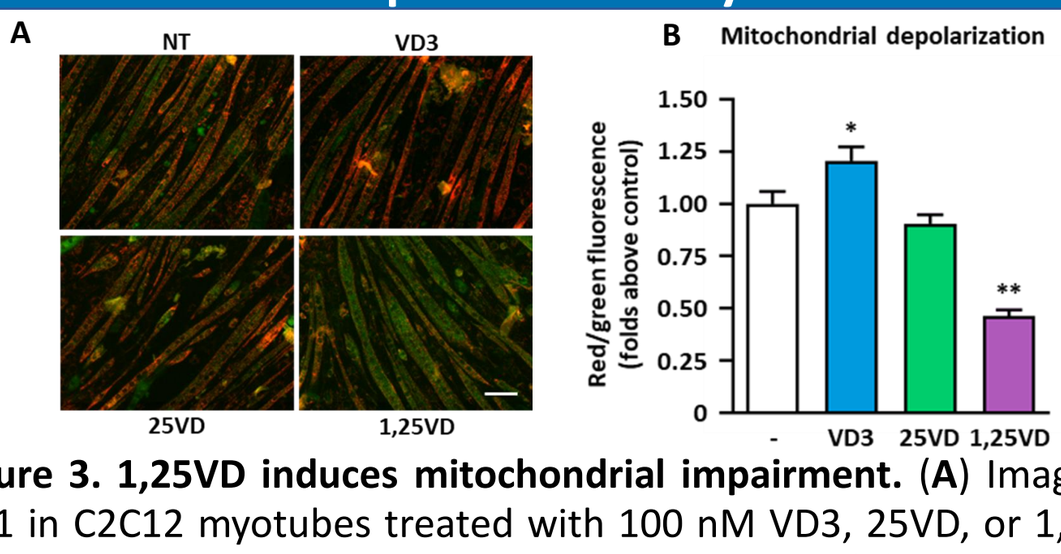


Figure 3. 1,25VD induces mitochondrial impairment. (**A**) Images of JC-1 in C2C12 myotubes treated with 100 nM VD3, 25VD, or 1,25VD for 24 h in serum-free medium, scale bar 100 µm; (**B**) Quantification of the ratio of red/green fluorescence in treated cells compared to vehicle-treated controls. *P<0.05; **P<0.01 compared to vehicle-treated cells

VD metabolites and ROS production

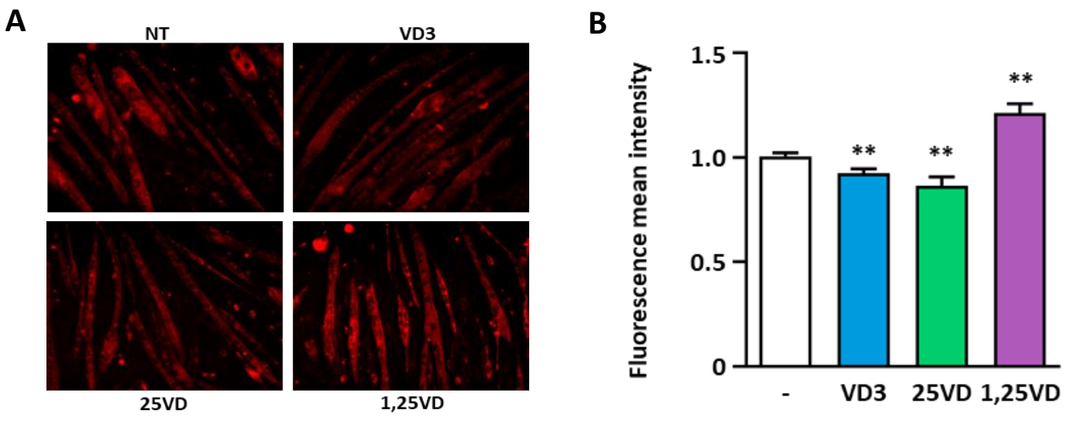


Figure 4. 1,25VD induces ROS production. After treatment with 100 nM VD3, 25VD, and 1,25VD for 24 h in serum-free medium, (**A**) the total reactive oxygen species species (ROS) generation was evaluated by CellROX Deep Red reagent. (**B**) The resulting fluorescence was acquired using a fluorescence microscope and quantified as fluorescence mean intensity; scale bar 100 µm; **P<0.01 compared to vehicle-treated control cells.

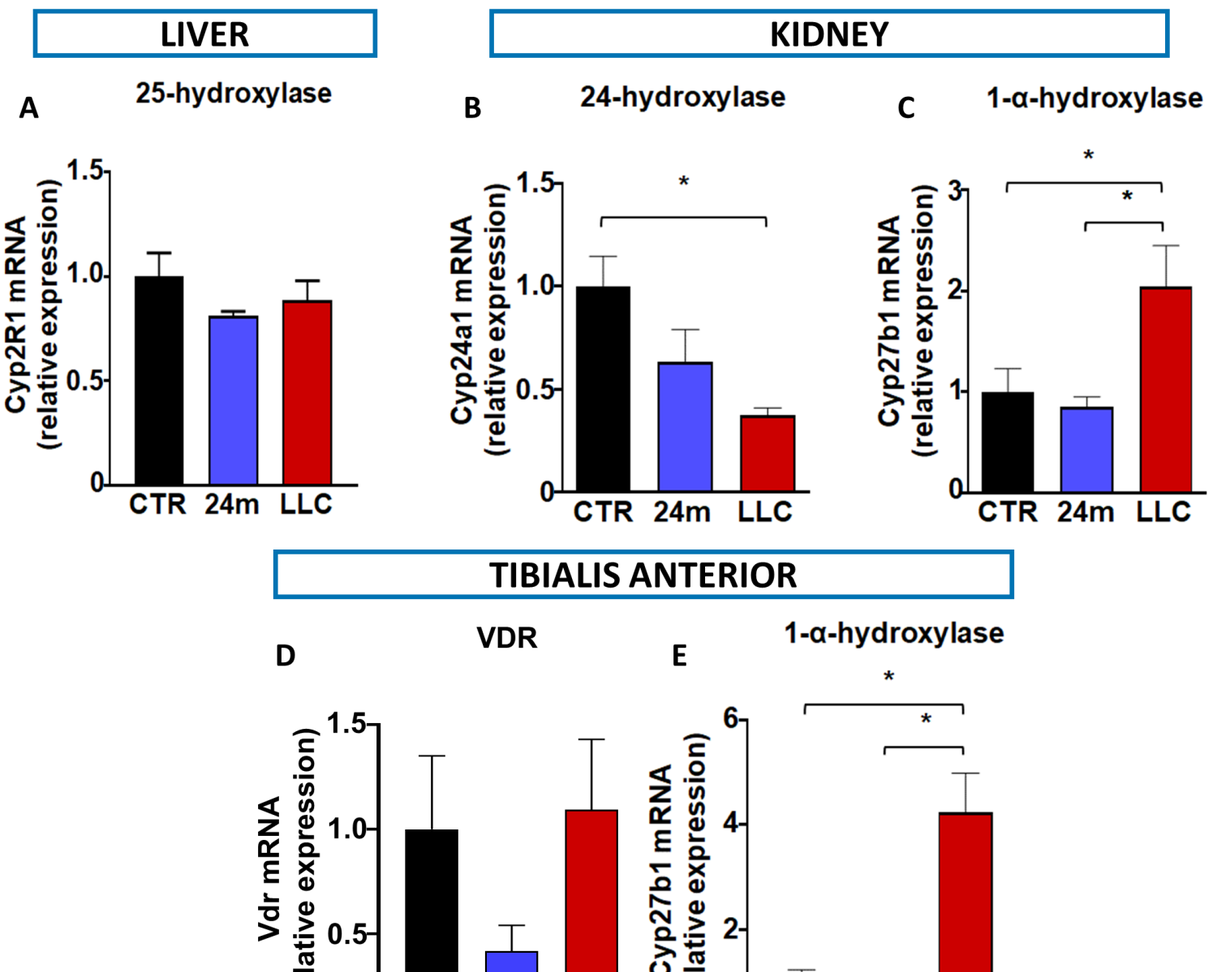


Figure 6. Differential expression of VD hydroxylases and VDR in aged vs. cachectic mice. VD hydroxylase and vitamin D receptor (VDR) expression was evaluated in three exprerimental group: control mice (CTR), 24-month-old mice (24m) and tumor bearing mice injected with LLC (LLC). The expression of 1-α-hydroxylase (*Cyp27b1*), 25-hydroxylase (*Cyp2R1*), 24-hydroxylase (*Cyp24a1*), and vitamin D receptor (*Vdr*) was assessed by real-time PCR in liver, kidney and *tibialis anterior* muscle.

Conclusion

In cachectic mice 1-a expression was significantly upregulated in both kidney and TA muscle (Fig. 6 C and E), suggesting a sustained systemic and local increase in the production of the pro-atrophic 1,25VD. Also, the reduced 24-hydroxylases expression in kidney of cachectic mice (Fig. 6 B) may imply an impairment in 1,25VD further processing and excretion.

We postulate that the efficacy of vitamin D supplementation in vivo depends on the balance between atrophic (1,25VD) and protective (VD3 and 25VD) metabolites, and that the altered expression of specific vitamin D hydroxylases in aging vs. other pathologies affecting skeletal muscle homeostasis might swing the balance.

UPO AGING PROJECT
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