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Vitamin D_3 protects against glucocorticoid-induced muscle weakness and bone loss through a mechanism that requires VDR signaling only in skeletal muscle

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ABSTRACT

Vitamin D_3 has beneficial effects in skeletal muscle and can prevent falls leading to reduced bone fracture risk. Earlier findings showed that administration of 1,25-dihydroxyvitamin D_3 (1,25D) prevents muscle atrophy induced by glucocorticoids (GC) in mice. However, it remains unknown whether these effects are due to direct actions of the hormone in muscle. To answer this question, we generated a mouse model of inducible, skeletal muscle-specific deletion of the Vitamin D_3 receptor (VDR) in mature mice. VDRf/f;human skeletal muscle α-actin (HSA)-Cre+/- and littermate control VDRf/f;HSA-Cre-/- mice (C) were treated with tamoxifen (2mg/d 1x/d for 5d) at 3mo of age. At 4mo, mice were implanted with slow-release pellets with 2.1mg/kg/d prednisolone or placebo and were treated with 50ng/kg/d 1,25D or vehicle 5x/wk for 4wks, N=13-21. Mice were fed a regular Vitamin D_3-containing diet and maintained in a 12h light/dark cycle. The excised VDR form was detected only in skeletal muscle (plantaris and tibialis anterior-TA), but not in kidney, intestine, or bone (other target tissues), nor in any tissues from C mice. Adult-onset deletion of the VDR in muscle did not change body weight, lean body mass, skeletal muscle weight (TA, soleus), or *in vivo* muscle strength (plantarflexion torque testing), as no differences were detected between VDRf/f;HSA-Cre+/- vs C mice receiving placebo/vehicle or placebo/1,25D. GC did not alter body weight or lean body mass, but it decreased TA weight and *in vivo* muscle strength to a similar extent in VDRf/f;HSA-Cre+/- and C mice. These indexes of muscle atrophy induced by GC were prevented by 1,25D only in C but not in VDRf/f;HSA-Cre+/- mice. These findings demonstrate that VDR signaling is dispensable for physiological muscle function in vitamin D replete animals, but it is required for the prevention of GC-induced muscle atrophy by 1,25D. Because of the postulated crosstalk between muscle and bone and our previous findings showing that 1,25D also prevents GC-induced bone loss, we examined next whether the response to Vitamin D_3 in bone was impacted by the loss of VDR in skeletal muscle. Remarkably, whereas 1,25D prevented the decrease in total BMD induced by GC in C mice, 1,25D did not prevent the bone loss in VDRf/f;HSA-Cre+/- mice. Taking together, these findings suggest that VDR signaling in skeletal muscle confers protective actions against GC-induced atrophy not only in skeletal muscle, but also in bone.

INTRODUCTION

Excess of glucocorticoids (GC), either endogenous as in aging or due to administration as immunosuppressants, leads to loss of bone and skeletal muscle mass. Muscle weakness, in turn, reduces body balance and increases falls, thus increasing the risk of bone fractures.

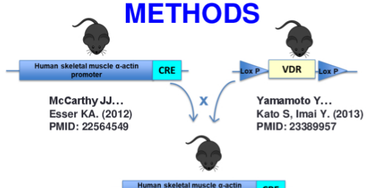
Vitamin D_3 is known to have beneficial effects on skeletal muscle and may prevent falls.[1,2] However, the role of Vitamin D_3 receptor (VDR) signaling in muscle remains controversial as some, but not all, investigations support direct hormonal actions on muscle cells.[3,4]

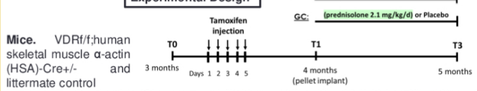
Our earlier work showed that $1,25(OH)_2$ Vitamin D_3 (1,25D) prevents GC-induced atrogene expression in skeletal muscle *in vivo*, in muscle organ cultures *ex vivo*, and prevents reductions in C2C12 myotube diameter *in vitro*[5-6]. Taken together, these findings suggest that the beneficial actions of Vitamin D_3 are mediated by direct hormonal effects on skeletal muscle cells.

OBJECTIVE

To investigate whether the musculoskeletal protective effects of 1,25D against GC-induced atrophy and weakness are due to direct actions in muscle

METHODS





Mice. VDRf/f;human skeletal muscle α-actin (HSA)-Cre+/- and littermate control VDRf/f;HSA-Cre-/- mice (C) were treated with tamoxifen (2mg/d 1x/d for 5d) at 3mo of age. At 4mo, mice were implanted with 2.1mg/kg/d prednisolone or placebo, as published (7). 1,25D (calcitriol) was administered 5x/wk at 50ng/kg/d. Four weeks after pellet implantation mice were sacrificed.

Genotyping. Genomic DNA was extracted from tissues indicated in the figures, followed by a PCR reaction using the following primers: VDR excised amplicon, P1 (AAA GAC ACT GGC TGC CAA CC), and P2 (TGA CAG TGC CCT GTT CTT CC); HSA-CRE forward (CCC GCA GAA CCT GAA GAT G) and reverse (GAC CCG GCA AAA CAG GTA G); Cx43, forward (TCA TGC CCG GCA CAA GTG AGA C) and reverse (TCA CCC CAA GCT GAC TCA ACC G). PCR products were analyzed.

Body weight, Lean Body Mass, and BMD. Lean body mass and BMD of the total were measured by DXA, as previously published (7).

Muscle function testing. *In vivo* muscle function was quantified using the 1205A Whole Mouse/Rat Test System by plantarflexion torque testing, as published (7).

Statistical analysis. Data are expressed as mean ± standard deviations with appropriate analysis indicated in the figure legends.

Study approval. All animal procedures were approved by the Division of Laboratory Animal Medicine of the University of Arkansas for Medical Sciences.

ACKNOWLEDGMENTS

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Fig. 1. Tamoxifen induces VDR deletion only in skeletal muscle of VDRf/f; HSA-CRE+/- mice and not in other tissues.

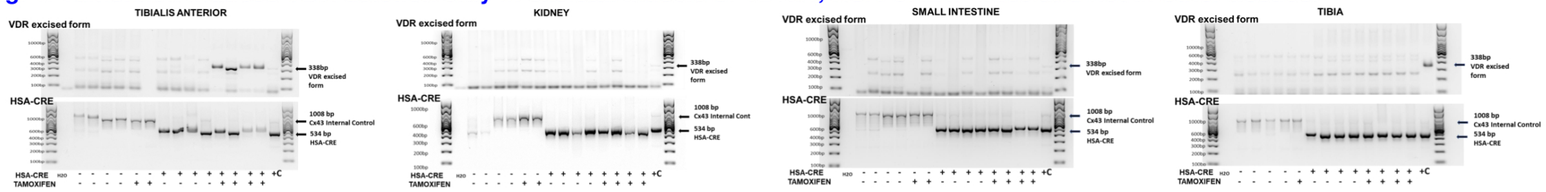


Fig. 2. Adult-onset deletion of the VDR in skeletal muscle did not alter body weight, lean body mass, or muscle weight.

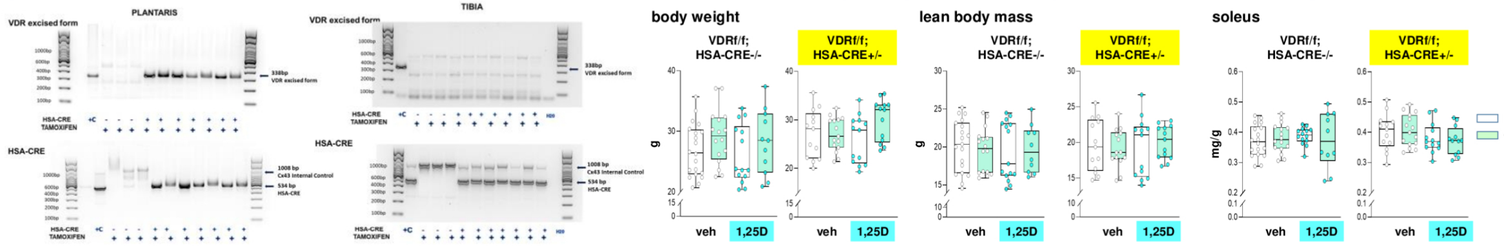
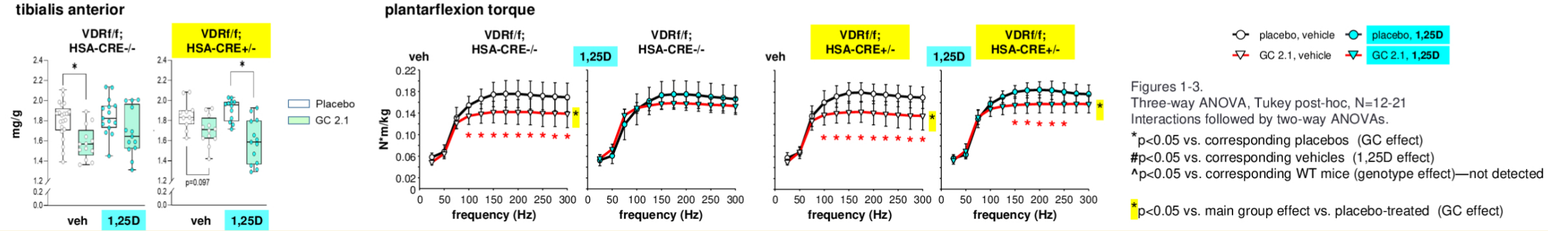


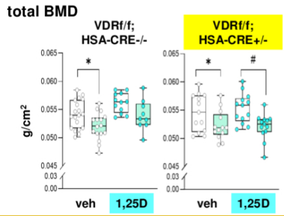
Fig. 3. Protection from GC-induced muscle loss and weakness by 1,25D is dependent on VDR expression in skeletal muscle.



Figures 1-3.
Three-way ANOVA, Tukey post-hoc, N=12-21
Interactions followed by two-way ANOVAs.
*p<0.05 vs. corresponding placebos (GC effect)
#p<0.05 vs. corresponding vehicles (1,25D effect)
^p<0.05 vs. corresponding WT mice (genotype effect)—not detected

*p<0.05 vs. main group effect vs. placebo-treated (GC effect)

Fig. 4. 1,25D failed to prevent GC-induced bone loss in mice lacking muscle VDR



Three-way ANOVA, Tukey post-hoc, N=12-21, Interactions followed by two-way ANOVAs.

*p<0.05 vs. corresponding placebos
#p<0.05 vs. corresponding vehicles

SUMMARY OF RESULTS

1. The excised form of VDR was only detected in skeletal muscle of CRE positive mice (VDRf/f; HSA-CRE+/-), but not in other target tissues (kidney, intestine, or bone), or in tissues from control littermate mice (VDRf/f; HSA-CRE-/-).
2. Tamoxifen-induced deletion of the VDR in skeletal muscle did not alter body weight, lean body mass, or skeletal muscle weight.
3. 1,25D prevented the decrease in skeletal muscle weight and strength induced by GC only in control mice but not in mice lacking the VDR in skeletal muscle.
4. 1,25D failed to prevent bone loss induced by GC in mice lacking the VDR in skeletal muscle.

CONCLUSION

1,25D prevents GC-induced muscle atrophy and weakness by direct actions on skeletal muscle, which might also affect bone.