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Vitamin D Receptor Expression in Human Muscle Tissue Decreases With Age

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ABSTRACT: Intracellular 1,25-dihydroxyvitamin D receptor (VDR) is expressed in human skeletal muscle tissue. However, it is unknown whether VDR expression in vivo is related to age or vitamin D status, or whether VDR expression differs between skeletal muscle groups.

Introduction: We investigated these factors and their relation to 1,25-dihydroxyvitamin D receptor (VDR) expression in freshly removed human muscle tissue.
Materials and Methods: We investigated biopsy specimens of the gluteus medius taken at surgery from 20 female patients undergoing total hip arthroplasty (mean age, 71.6 ± 14.5; 72% > 65 years) and biopsy specimens of the transversospinalis muscle taken at surgery from 12 female patients with spinal operations (mean age, 55.2 ± 19.6; 28% > 65 years). The specimens were obtained by immunohistological staining of the VDR using a monoclonal rat antibody to the VDR (Clone no. 9A7). Quantitative VDR expression (number of VDR positive nuclei) was assessed by counting 500 nuclei per specimen and person. Serum concentrations of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were assessed at day of admission to surgery.
Results: All muscle biopsy specimens stained positive for VDR. In the univariate analyses, increased age was associated with decreased VDR expression ($r = 0.5$: $p = 0.004$), whereas there were no significant correlations between VDR expression and 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D levels. VDR expression did not differ between patients with hip and spinal surgery. In the multivariate analysis, older age was a significant predictor of decreased VDR expression after controlling biopsy location (gluteus medius or the transversospinalis muscle), and 25-hydroxyvitamin D levels (linear regression analysis: β-estimate $= -2.56$; $p = 0.047$).
Conclusions: Intranuclear immunostaining of the VDR was present in muscle biopsy specimens of all orthopedic patients. Older age was significantly associated with decreased VDR expression, independent of biopsy location and serum 25-hydroxyvitamin D levels.
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Key words: 1,25-dihydroxyvitamin D_3 receptor, skeletal muscle tissue, vitamin D deficiency, elderly, aging

INTRODUCTION

THE CENTRAL ROLE of vitamin D in calcium homeostasis is well established. Specific receptors for 1,25-dihydroxyvitamin D (VDR) are present in bone and the gastrointestinal tract, where calcium flow is most active.[1,2]

In addition, vitamin D receptors are present in skeletal muscle,[3,4] but their precise physiological function and relevance to normal muscle physiology is not well understood. It has been suggested that the VDR in muscle tissue is a nuclear receptor that binds 1,25-dihydroxyvitamin D with high affinity and elicits its actions to regulate protein synthesis.[3,5]

Muscle weakness is a prominent feature of the clinical syndrome of vitamin D deficiency, which can be reversed by substitution.[6,7] In the elderly population, vitamin D deficiency has been associated with muscle weakness,[8,9] increased susceptibility to falls,[10,11] and fractures.[12,13] Although these potential effects of vitamin D deficiency have been observe in separate studies, a shared pathway is likely to be present. We speculate that falls and therefore nonvertebral fractures associated with falls may in part be explained by vitamin D deficiency causing clinically important muscle weakness. This is supported by recent randomized controlled trials indicating improved body sway in ambulatory elderly women,[14] and better muscle strength and reduced risk of falling in institutionalized vitamin D–deficient elderly women supplemented with vitamin D and calcium.[10] Furthermore, Glerup et al.[15] suggested that, in vitamin D–deficient subjects, severely impaired muscle function may be present even before biochemical signs of bone disease develop.

It is not known whether low serum vitamin D or some unknown factor associated with aging leads to a decreased stimulation of its specific receptor in muscle or whether age

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itself leads to a decreased expression of the receptor. One of these possibilities or a combination may in part explain sarcopenia occurring with age and the adverse outcomes among older persons such as muscle weakness, falls, and fractures.

In accordance with early findings of Simpson et al.,[3] indicating that young cultured skeletal myocytes expressed more VDR than old myocytes, Horst et al.[16] found that aging is associated with diminished expression of the VDR in rat intestine and bone tissue. It is unknown whether age or vitamin D levels are related to the expression of VDR in human muscle tissue in vivo. In addition, it is unclear whether VDR expression differs between skeletal muscle groups. Therefore, we investigated the influence of age, muscle group and vitamin D levels on the expression of the VDR in freshly removed human muscle tissue.

MATERIALS AND METHODS

Patients

Muscle biopsy specimens were taken during elective orthopedic surgery from 20 female patients undergoing total hip arthroplasty and 12 female patients undergoing spinal surgery between September 1999 and April 2000. The mean age in the 20 patients with hip surgery was 71.6 ± 14.5 years, and in patients with spinal surgery was 55.2 ± 19.6 years. In the hip surgery group, 72.2% were >65 years old, and in the spinal surgery group, 27.7% were >65 years old.

Patients who underwent total hip arthroplasty had as a primary reason for surgery a diagnosis of hip osteoarthritis (70%) or an osteoporotic hip fracture (30%). Reasons for spinal surgery were spinal fixation, decompression, or removal of prior fixation hardware. All operations were performed at the Department of Orthopedic Surgery, University of Basel, Switzerland. Written informed consent was obtained from all patients. The hospital ethics committee approved the study protocol.

Muscle biopsy

The biopsy specimens were of the gluteus medius or transversospinalis muscle and were 5 × 5 × 3 mm. Specimens were imbedded in tissue tek (Miles, Elkhart, IN, USA), snap frozen, and stored at −80°C. Sections of 6 μm thickness were cut on a cryostat and visualized by immunohistochemical and hematoxylin-eosin staining.

The sections were incubated with an anti-VDR rat monoclonal antibody (Biomol, Plymouth, MA, USA; immunogen: partially purified chicken intestinal cytoplasmic 1,25-dihydroxyvitamin D receptor protein; clone no. 9A7; Ab, at 1:250, in DAKODiluent; DAKO, Glostrup, Denmark). The antibody recognizes the VDR epitope C-terminal to the DNA binding zinc finger domain. It reacts with occupied and unoccupied receptors and cross-reacts with all avian and mammalian VDR. The antibody does not cross-react with glucocorticoid and estrogen receptors or serum and cytosolic vitamin D–binding proteins. It has been used successfully in the immunohistochemical investigation of the VDR in various tissues, such as human skin,[17] human duodenum,[18] human cervical tissue,[19] avian chondrocytes,[20] and rat reproductive tissues.[21]

Controls were incubated with PBS and with rat-IgG (gift from Dr M Knopf, Institute of Immunology, Basel; 1:1000, DAKO diluent). Sections were evaluated at 630× magnification. For each sample, immunohistochemical evidence of intranuclear expression of VDR was evaluated by counting 500 nuclei. The nuclei of all biopsy specimens were counted once by a physician investigator, blinded to all information about the patient, using a grid at random. In addition, a subgroup of 15 biopsy specimens chosen at random were analyzed a second time by the same physician investigator. The two measurements were highly correlated (correlation: $r = 0.86$ for VDR positive nuclei). Immunohistochemical investigations were described in detail previously.[4]

Vitamin D metabolites

The 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D serum concentrations were measured by radioimmunoassays (Nichols Institute Diagnostics, San Juan Capestrano, CA, USA). Vitamin D deficiency was defined as 25-hydroxyvitamin D serum concentrations below 30 ng/ml.[22]

Statistical analysis

The statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). Values are expressed as mean ± SD. Wilcoxon rank-sum test was used to compare VDR expression between hip and spinal surgery patients. Linear regression analysis included age, 25-hyroxyvitamin D, and location as possible predictors for VDR expression (number of positive nuclei). Results with p values <0.05 were considered statistically significant, and all tests were two-sided.

RESULTS

Intranuclear immunostaining of VDR was detected in muscle cells of all patients investigated. Mean values for patients' age, VDR positive nuclei, serum 25-hydroxyvitamin D, and 1,25-dihydroxyvitamin D are depicted for groups with hip and spinal surgery in Table 1.

Epidermal skin (Fig. 1A) was used as a positive control, with intensive immunhistochemical staining of the VDR in all epidermal cell layers, and was absent when treated with PBS instead of the primary antibodies or polyclonal rat-IgG (Fig. 1B). The monoclonal mouse antibody to VDR exhibited immunoreactivity in human skeletal muscle cells with a lower number of positive nuclei in an older patient (Fig. 1C) compared with a younger patient (Fig. 1D).

Univariate analysis revealed no significant difference between VDR expression (number of positive nuclei) of spinal surgery patients and patients with hip arthroplasty. Correlation studies between VDR expression and 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D were weak and not significant [VDR positive nuclei by 25(OH)D: $r = 0.002$; $p = 0.994$; VDR positive nuclei by 1,25(OH)2D: $r = -0.108$; $p = 0.650$). Only age was negatively correlated with the number of positive nuclei ($r = -0.5$; $p = 0.004$). Among subjects who had both vitamin D metabolites assessed, 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D were highly correlated ($r = 0.8$; $p < 0.0001$), and neither vitamin metabolites were correlated with age.

TABLE 1. MEAN AGE, VDR IMMUNOSTAINING, 25-HYDROXYVITAMIN D, AND 1,25-DIHYDROXYVITAMIN D SERUM CONCENTRATIONS IN SUBJECTS WITH HIP SURGERY AND PATIENTS WITH SPINAL SURGERY

Subgroups	*Number*	*Parameter*	*Mean*	*SD*	*Minimum*	*Maximum*
Hip surgery	20	Age (years)	71.6	14.5	31	91
	20	VDR-positive nuclei*	346.5	119.2	60	484
	18	25(OH)D (ng/ml)†	16.7	9.6	4.4	42.8
	12	$1,25(OH)D_2$ (pg/ml)‡	29.3	13.8	5.7	54
Spinal surgery	12	Age (years)	55.2	19.6	24	75
	12	VDR positive nuclei*	390.9	54.4	289	479
	11	25(OH)D (ng/ml)†	12.9	5.6	6.4	24.6
	3	$1,25(OH)D_2$ (pg/ml)‡	37.2	14.8	21.5	51

* Number of VDR-positive nuclei per 500 counted nuclei.
† To convert values for 25(OH)D to nanomoles per liter, multiply by 2.5.
‡ Normal values for $1,25(OH)_2D$ are uncertain.

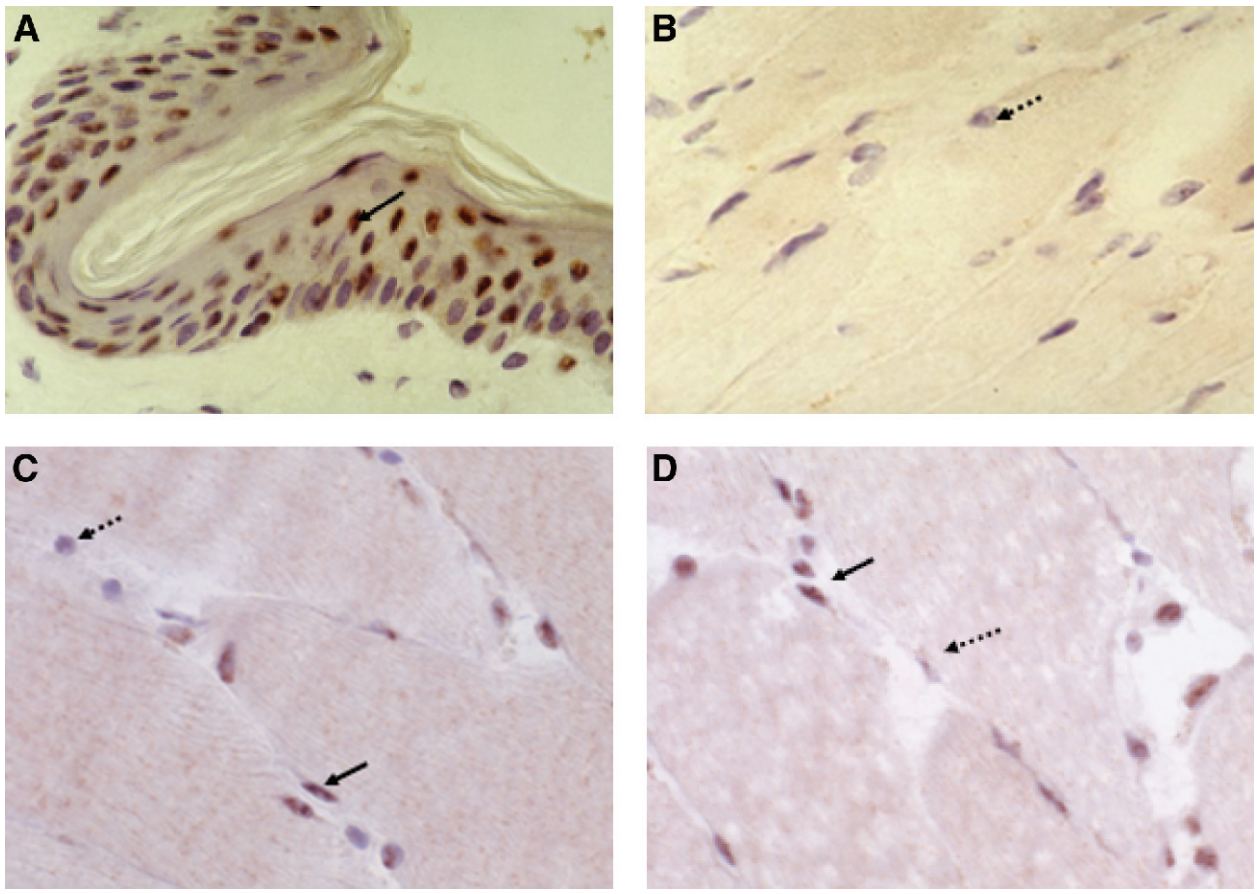

FIG. 1. Nuclei with immunohistochemical evidence of intranuclear expression of VDR show brownish staining. Examples for VDR negative nuclei are indicated by dashed arrows and examples for VDR positive nuclei are indicated by solid arrows. (A) Positive control with intensive immunohistochemical staining of the VDR in epidermal cell layers. In B, staining was absent when handled with PBS or polyclonal rat-IgG instead of the primary antibodies. (C) VDR expression in a muscle biopsy of a 91-year-old patient with hip arthroplasty. (D) VDR expression in a biopsy of a 51-year-old patient with hip arthroplasty.

In the multivariate model, only older age was a significant determinant of decreased VDR expression in human muscle tissue after adjustment for 25-hydroxyvitamin D and location (β estimate = -2.56; $p = 0.047$; Fig. 2).

DISCUSSION

In this study, we found that in situ VDR expression in skeletal muscle decreases significantly with age. Our findings confirm a previous study by Simpson et al.,[3] who

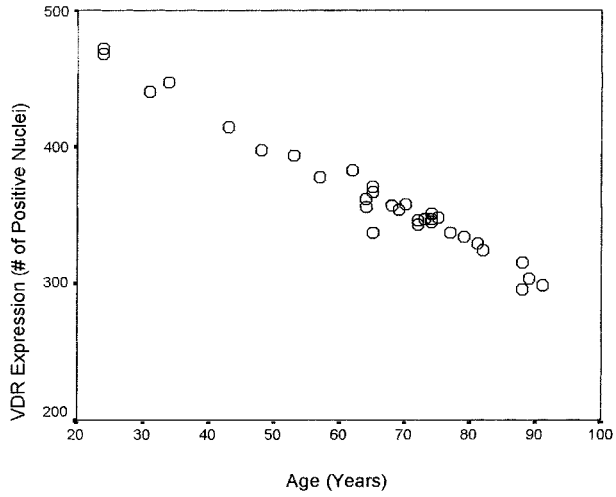

FIG. 2. Number of VDR positive nuclei by age. Scatter plot gives predicted number of VDR positive nuclei by age controlling for biopsy location (gluteus medius or transversospinalis muscle) and 25-hydroxyvitamin D serum levels based on the linear regression model (age: β estimate $= -2.56$; $p = 0.047$).

showed that young cultured skeletal myocytes expressed more VDR than old myocytes.

Aging is associated with a decrease in muscle strength.[23–25] Contributing factors are functional impairment on the motorneuron level[26] and a progressive reduction in the area of muscle tissue occupied by type II fibers,[27,28] the latter possibly being linked to the VDR in muscle tissue.[7] The underlying mechanism might be that decreased VDR expression reduces the functional response of the muscle cells to 1,25-dihydroxyvitamin D. Alternatively, decreased vitamin D levels, as frequently found in older persons,[29–31] may lead to decreased expression of the VDR because of a decrease in stimulation and thus down-regulation of the receptor. Over time, this may impair protein synthesis in the muscle cells,[5,7] resulting in a decrease in type II fibers,[9] and eventually sarcopenia.[7] Irrespective of the mechanism by which age is associated with a decrease in VDR, our findings are consistent with previous studies showing a decrease of VDR expression with age in other calcium-dependent organs, such as the intestine and bone in rats[16] and mucosal VDR in human intestine.[32]

In rodents, VDR expression in the duodenum[2] and kidney[33] has been found to be regulated by 1,25-dihydroxyvitamin D serum levels. Similarly, Sorensen et al.[9]showed that treatment with 1-α-hydroxyvitamin D (1 μg/day for 3 months) resulted in an increase in the cross-sectional area and relative number of type II muscle fibers in osteoporotic elderly women. This, together with evidence from clinical trials on improved body-sway,[14] muscle strength, and reduced risk of falling[10] in elderly women treated with vitamin D (800 IU/day), may indicate that the VDR in muscle could be stimulated in a clinically meaningful way even in the elderly.

In this study, however, we were not able to show a relationship between serum 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D and VDR expression in human muscle tissue. Our results agree with the cross-sectional results of Kinyamu et al.,[34] who did not find a relationship between 25-hydroxyvitamin D or 1,25-dihydroxyvitamin D levels and mucosal VDR levels in the intestine of young or elderly women. The lack of association in our study may be because of the relatively low 25-hydroxyvitamin D levels observed in most of our patients. In addition, we obtained 1,25-dihydroxyvitamin D levels only from a subsample of participants. Also, the antibody to the VDR used in our study binds to both occupied and unoccupied VDR, whereas the unoccupied VDR may be a more sensitive measure. Furthermore, the cross-sectional design of the study might not be ideal to investigate the relationship between VDR expression and serum vitamin D concentrations. Therefore, a positive association between VDR expression and serum 25-hydroxyvitamin D and serum 1,25-dihydroxyvitamin D cannot be excluded from our study. Future studies are required to prospectively assess the influence of vitamin D supplementation on the expression of the VDR in muscle tissue.

Another question of interest was whether VDR expression differs between muscle groups. In this study, the expression of VDR did not differ between patients who underwent hip surgery and those with spinal surgery after controlling for age and 25-hydroxyvitamin D levels. Both muscle groups were proximal striated muscles, which may explain their similar expression of the VDR

There are limitations to our study, including its cross-sectional design and the inability to study the effect of gender because all biopsy specimens were collected in women.

In conclusion, we found a significant decrease in VDR expression with age in freshly removed muscle tissue of female orthopedic patients undergoing hip or spine surgery. Older age was a significant determinant of decreased VDR expression independent of biopsy location and serum 25-hydroxyvitamin D. These results may suggest that the age-related decline in muscle strength observed in other studies could in part be explained by a decrease in VDR expression. However, future studies are needed to prospectively address the association between VDR expression in muscle and age, and whether VDR expression could be increased by vitamin D supplementation.

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