

A Randomized Study on the Effect of Vitamin D₃ Supplementation on Skeletal Muscle Morphology and Vitamin D Receptor Concentration in Older Women

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Context: Studies examining whether vitamin D supplementation increases muscle mass or muscle-specific vitamin D receptor (VDR) concentration are lacking.

Objective: Our objective was to determine whether vitamin D₃ 4000 IU/d alters muscle fiber cross-sectional area (FCSA) and intramyonuclear VDR concentration over 4 months.

Design and Setting: This was a randomized, double-blind, placebo-controlled study in a single center.

Participants: Participants were 21 mobility-limited women (aged ≥ 65 years) with serum 25-hydroxyvitamin D (25OHD) levels of 22.5 to 60 nmol/L.

Main Outcome Measures: Baseline and 4-month FCSA and intramyonuclear VDR were measured from vastus lateralis muscle cross-sections probed for muscle fiber type (I/IIa/IIx) and VDR using immunofluorescence.

Results: At baseline, mean (\pm SD) age was 78 ± 5 years; body mass index was 27 ± 5 kg/m², 25OHD was 46.3 ± 9.5 nmol/L, and a short physical performance battery score was 7.95 ± 1.57 out of 12. At 4 months, 25OHD level was 52.5 ± 17.1 (placebo) vs 80.0 ± 11.5 nmol/L (vitamin D [VD]; $P < .01$), and change in 25OHD level was strongly associated with percent change in intramyonuclear VDR concentration-independent of group ($r = 0.87$, $P < .001$). By treatment group, percent change in intramyonuclear VDR concentration was $7.8\% \pm 18.2\%$ (placebo) vs $29.7\% \pm 11.7\%$ (VD; $P = .03$) with a more pronounced group difference in type II vs I fibers. Percent change in total (type I/II) FCSA was $-7.4\% \pm 18.9\%$ (placebo) vs $10.6\% \pm 20.0\%$ (VD; $P = .048$).

Conclusion: Vitamin D₃ supplementation increased intramyonuclear VDR concentration by 30% and increased muscle fiber size by 10% in older, mobility-limited, vitamin D-insufficient women. Further work is needed to determine whether the observed effect of vitamin D on fiber size is mediated by the VDR and to identify which signaling pathways are involved. (*J Clin Endocrinol Metab* 98: E1927–E1935, 2013)

Low vitamin D status has been associated with reduced muscle mass, strength, and performance in older adults (1–5). Several intervention studies have reported that vitamin D supplementation increases appendicular muscle strength and improves physical function particularly in older women with low vitamin D status (6–9). Experiments *in vitro* have examined potential mechanisms by which vitamin D acts on skeletal muscle cells (10–13). Administration of vitamin D, as 1,25-dihydroxyvitamin D, stimulated key pathways of muscle growth and differentiation in C2C12 myoblasts (10, 12, 14) and acted directly on these cells via a nuclear vitamin D receptor (VDR) (10, 11). These studies have also demonstrated that concentration of the intramyonuclear VDR increases after both 1,25-dihydroxyvitamin D and 25-hydroxyvitamin D (25OHD) administration. Yet, clinical studies examining effects of parent vitamin D compounds on human muscle fibers and concentration of VDR in muscle, particularly in older adults, are lacking.

We conducted a pilot study to test the hypothesis that oral vitamin D₃ 4000 IU daily compared with placebo alters total and/or subtype muscle fiber cross-sectional area (FCSA) and intramyonuclear VDR concentration over a 4-month period in mobility-limited women aged 65 years and over with moderately low baseline vitamin D status. The study also aimed to examine the effects of vitamin D on the proportion of type I and/or II muscle fibers and urine nitrogen (UNi) excretion (a marker of muscle breakdown) and to confirm previously reported effects of vitamin D₃ on muscle strength and physical function.

Subjects and Methods

Study design and subjects

This was a randomized, double-blind, placebo-controlled study conducted at the Metabolic Research Unit at the Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University. Subjects were randomized to take an oral vitamin D₃ capsule 4000 IU daily or matching placebo for 4 months. The study obtained a fasting blood draw, a 24-hour urine collection, muscle performance measures, and a muscle biopsy of the vastus lateralis at baseline and 4 months. A 4-month duration was selected to evaluate changes in muscle tissue and simultaneously have a steady state in 25OHD level after the change in intake (15). The vitamin D₃ 4000 IU daily dose was chosen as a high yet safe dose of supplementation to minimize risk of underdosing (proof-of-principle study). According to the 2011 Institute of Medicine report, 4000 IU daily is the safe upper limit for supplementation (16).

Ambulatory, community-dwelling, postmenopausal women 65 years of age and over were recruited from direct mailings and advertisements. All subjects were required to maintain their usual level of physical activity and their habitual diet during the 4-month study to limit the impact of physical activity and dietary variation on skeletal muscle. Subjects with active parathyroid

disease, chronic kidney disease, nephrolithiasis, malignancy, liver disease, malabsorption, diabetes, unstable heart disease, severe osteoarthritis, and neurodegenerative disease were excluded. Additional exclusions consisted of a vitamin D intake >400 IU/d or a 25OHD level <22.5 or >60 nmol/L; a calcium intake >1000 mg/d (due to risk of hypercalcemia) or an abnormal serum calcium or 24-hour urinary calcium >275 mg (due to risk of hypercalciuria); medications such as hormone replacement therapy in the last 6 months, oral glucocorticoids in the last month, diuretics, antiseizure medications, drugs to treat osteoporosis in the last year, and prescribed antiplatelet and anticoagulant medications; travel to latitudes below 35°N; and use of tanning beds, wheelchair, walker, and nasal oxygen.

We selected women who were defined as at moderate risk for disability based on a short physical performance battery (SPPB) score of ≤9 (out of a possible 12 points) (17), to target a population who would stand to benefit from this intervention. The SPPB involves a balance assessment (open, semitandem, and tandem stance), a timed 4-m walk, and a chair rise test (timed 5 rises) and is predictive of future disability.

Ninety-four women were screened, and 70 were excluded (38 scored >9 on the SPPB, 10 had 25OHD levels above or below target range, 4 used exclusion medications, 9 had excluded medical conditions, 1 was enrolled in another study, 1 had poor venous access, and 7 were not agreeable to study procedures). Twenty-four eligible subjects were randomized to receive placebo (n = 13) or vitamin D₃ 4000 IU daily (n = 11). Only 21 of these 24 subjects were included in the analyses because they had muscle biopsies at 4 months (1 subject in each group declined biopsy and 1 subject in the vitamin D group had insufficient muscle on final biopsy for analysis). The Tufts Medical Center-Tufts University Health Sciences Campus Institutional Review Board approved the study, and written informed consent was obtained from each subject.

Study capsules

Vitamin D₃ and matching placebo capsules were purchased from Tishcon Corporation. The 4000-IU daily dose of vitamin D₃ was independently analyzed by Covance, Inc to confirm content. To keep the randomization concealed, the placebo was an identical opaque capsule packed with microcrystalline cellulose. Adherence to study capsules was assessed via a daily compliance calendar, routine pill counts by nursing staff, and a follow-up 25OHD level at 4 months.

All subjects took vitamin D₃ (or placebo) 4000 IU orally once daily in the morning immediately after breakfast. A safety spot urine was analyzed for calcium and creatinine on day 30 of the study. If the calcium to creatinine ratio exceeded 0.325 (corresponding to a 24-hour urine calcium [UCa] of 350 mg, the upper limit of the normal range) (18), the study pills were discontinued and a repeat spot urine and serum calcium level were drawn within the following 7 days. If the repeat UCa normalized and serum calcium was normal, pills could be resumed, but repeat testing on pills was performed during the following 2 and 4 weeks.

Biochemical measurements

Blood was drawn after a 12-hour overnight fast between 7:00 and 10:00 AM. All samples from individual subjects were batched for analyses, with the exception of the spot UCa and urine creatinine (UCr) measurement on day 30 (a safety measurement).

Serum 25OHD was measured with RIA kits from DiaSorin with coefficients of variation (CV) of 5.6% to 7.7%. The 24-hour UCr was measured on an automated clinical chemistry analyzer (Olympus AU400; Olympus America Inc). The CV for this assay ranged from 3.0% to 6.0%. The 24-hour UCa was measured by direct-current plasma emission spectroscopy (Beckman SpectraSpan VI Direct Current Plasma Emission Spectrophotometer; Beckman Instruments) with a CV of 3% to 5%. The 24-hour UNi was measured with a model FP-2000 nitrogen/protein analyzer (LECO), which employs a Dumas combustion method and detection using a thermal conductivity cell. It measures nitrogen with a precision of 15 ppm.

Muscle performance

Immediately after a standardized breakfast, muscle strength was quantitatively assessed by 1 repetition maximum (1RM) measures of knee extension in the nonbiopsied leg using Keiser pneumatic resistance training equipment. The 1RM is defined as the maximum load that could be moved only once throughout the full range of motion while maintaining proper form (19). After 1RM measurement and a 5-minute rest, assessment of individual knee extension average muscle power (in watts) was made. Performance of the average power measurement has been previously described (20). Briefly, subjects were instructed to complete 5 knee extension repetitions each separated by 30 seconds as quickly as possible through their full range of motion at both 40% and 70% of 1RM for knee extension. The average power at 40% and 70% of 1RM was recorded. Knee extension 1RM and average power measures were performed at baseline and at 4 months. The intraclass correlation coefficient for repeated 1RM testing of knee extension is 0.92 and for average power testing is 0.86 in previous reports from our group (20). The SPPB was performed at baseline and 4 months to capture change in physical function.

Muscle biopsy

Muscle biopsies were obtained from the nonexercised vastus lateralis muscle at the level of the midhigh under local anesthesia (xylocaine 1%) with a 5-mm Duchenne biopsy needle and suction approximately 1 hour after a standardized breakfast and 24 hours after the last dose of the study capsule. The specimens were mounted in a vinyl cryomold (Tissue-Tek) and secured using a viscous mounting medium (O.C.T.; Tissue-Tek) and then frozen in isopentane/liquid nitrogen slurry.

Immunohistochemistry

We employed a multistaining immunofluorescent technique to identify fiber type and intramyonuclear VDR as described in detail previously (21). Briefly, muscle tissue specimens were cut in 7- μ m serial sections with a cryostat microtome (Leica CM1850; Leica Microsystems). Slides were probed with a primary mouse/antihuman VDR/NR1H1 monoclonal antibody (clone H4537; Perseus Proteomics, Inc), a type IIa (N2.261 IgG₁) myosin heavy chain isoform-specific antibody, mouse antibodies against human type I (A4.951 IgG₁; A4.840 IgM) and IIx (212F IgG₁) myosin heavy chain, and a rabbit antihuman antibody (IgG) raised against laminin to facilitate identifying individual muscle fibers. Slides were mounted with 4',6-diamidino-2-phenylindole-containing mounting medium to stain myonuclei (Vectashield H-1500; Vector Laboratories, Inc). Control sections were processed independently as described above, without

primary, secondary or both antibodies (blank control). No staining signal higher than the natural self-fluorescence of the sections was observed (results not shown).

Fiber cross-sectional area

After the staining protocol, digital imaging was performed through $\times 100$ final magnification. Nikon NIS-AR (3.01) was employed for data acquisition. Image processing, including identification of fiber type in all fibers on the muscle cross-section, was performed using Adobe Photoshop CS3. The circumference of each fiber was outlined using NIH ImageJ software (version 1.37) to generate FCSA. Criteria used in the selection of muscle fibers to measure FCSA has been described previously (22). Image analyses were performed by 3 coauthors (L.C., S.N., and M.D.M.) who were blinded to treatment assignment. As an assessment of agreement between operators for average FCSA measurement, the mean absolute deviation was less than 0.03. The relative proportion of type I, IIa, IIx, and hybrid (ie, IIa-x) muscle fibers was recorded in each cross-section manually using Adobe Photoshop CS3. Criteria for muscle fibers to be included in total cross-sectional fiber number has been described previously (22). Because many individual muscle cross-sections had a substantial number of hybrid fiber subtypes (ie, IIa-x), we were not able to examine changes in type IIa and IIx fibers separately, but instead analyzed changes in the broader fiber classifications of type I or II for our analysis.

Intramyonuclear VDR concentration

A subset of 14 subjects (placebo, $n = 8$; vitamin D, $n = 6$) had sufficient muscle tissue available for VDR concentration analyses. Nonoverlapping high-magnification ($\times 400$) fields selected on the basis of lack of tissue artifacts were used to count myonuclei. On average, the combined field areas included 40% of the muscle cross-section at baseline and 4 months. Because vastus lateralis muscle has a mixed muscle fiber type distribution, the fields examined had similar muscle fiber type I to type II ratios (30%–70%) to those of the whole sections. The mean (\pm SD) total myonuclei per subject evaluated for VDR was 576 ± 223 at baseline and 602 ± 349 at 4 months. Using a multistaining protocol (21), we colocalized 4',6-diamidino-2-phenylindole myonuclei staining with VDR-positive staining. Bright-field overlaid images were used to aid assignment of VDR-positive myonuclei to a particular fiber. We measured the relative number of VDR-positive myonuclei within each muscle fiber subtype in all fields. We calculated the ratio of VDR-positive myonuclei to total myonuclei by muscle fiber subtype from fields evaluated. These analyses were performed by one of the coauthors (S.N.) who was blinded to treatment assignment.

Statistical analysis

Unblinding occurred after biochemical, muscle histology, and muscle performance analyses were completed. UCa and UNi were normalized to UCr excretion to control for incomplete 24-hour urine collections (23). Mean baseline values of clinical characteristics (age, body weight, body mass index [BMI], and daily calcium and protein intake), biochemical measurements (25OHD, UCa/Cr, and UNi/Cr), FCSA (total, type I, and type II), proportion of fiber type (type I, and type II), percent of VDR-positive myonuclei out of total myonuclei, and the mean changes in these values from baseline to the final 4-month visit were compared across groups with *t* tests for 2 independent samples.

Table 1. Baseline Characteristics in the Total Sample (n = 21) and the Subset With VDR Measurements (n = 14)

Characteristics	Total Sample		Subset	
	Placebo (Mean ± SD)	Vitamin D ₃ (Mean ± SD)	Placebo (Mean ± SD)	Vitamin D ₃ (Mean ± SD)
n	12	9	8	6
Caucasian, n	11	7	8	5
Age, y	80 ± 5	76 ± 4 ^a	79 ± 3	77 ± 4
Height, cm	154.3 ± 9.9	162.2 ± 7.3	153.6 ± 8.4	159.1 ± 6.5
Weight, kg	60 ± 9	77 ± 15 ^b	58 ± 10	77 ± 16 ^c
BMI, kg/m ²	25 ± 3	29 ± 7	24 ± 3	31 ± 7 ^d
Dietary protein intake, g/d ^e	82.7 ± 73.9	63.3 ± 30.1	50.2 ± 27.0	59.0 ± 21.5
Dietary calcium intake, mg/d ^e	1316 ± 1257	963 ± 663	752.8 ± 246.8	635.5 ± 486.9
SPPB score	8.17 ± 1.27	7.56 ± 1.88	8.38 ± 0.92	8.33 ± 0.82
40% of 1RM average power, W ^f	39.71 ± 14.45	43.52 ± 16.02	38.01 ± 14.53	45.50 ± 14.84
70% of 1RM average power, W ^f	51.44 ± 19.78	48.08 ± 23.12	48.50 ± 13.43	48.19 ± 22.00

^a $P = 0.03$, value compared with placebo.

^b $P = .005$, value compared with placebo.

^c $P = .02$, value compared with placebo.

^d $P = .046$, value compared with placebo.

^e One subject in the placebo group did not complete the food frequency questionnaire.

^f One subject in the placebo group was unable to perform the knee extensor measure due to knee pain.

Pearson correlation coefficients were used to describe linear associations. Two-sided P values $< .05$ were considered to indicate statistical significance. Statistical analyses were conducted using SAS Enterprise Guide version 4.2 (SAS Institute, Inc). Based on a previous study (24), our planned sample size of 12 participants per group provided over 80% power to detect a difference in FCSA of 740 μm^2 at the two-tailed 0.05 α -level.

Results

Subjects and intervention

Baseline characteristics of the 21 subjects are listed in Table 1. The vitamin D group was modestly younger and weighed more than the placebo group. Mean baseline serum 25OHD was 45.8 ± 10.8 nmol/L in the entire cohort and did not differ significantly by group (Table 2). As expected, there was a significant increase in 25OHD level in the vitamin D (36.4 ± 13.6 nmol/L) compared with placebo group (4.2 ± 11.9 nmol/L; $P < .001$).

Biochemical measurements and muscle performance

Baseline urine biochemistry values did not differ significantly in the 2 groups (Table 2). Percent change in UNi/Cr was $-10.9\% \pm 15.9\%$ in the vitamin D group compared with $2.2\% \pm 17.3\%$ in the placebo group ($P = .09$). Baseline (Table 1) and 4-month changes in 40% and 70% of 1RM average power in knee extension (data not shown) did not differ significantly in the 2 groups ($P > .28$). Likewise, changes in total SPPB score did not differ significantly by group (placebo, 0.67 ± 1.56 , vs vitamin D, 0.67 ± 1.32 , $P = 1.0$).

Muscle morphology

The 2 groups had similar mean baseline total, type I, and type II FCSA and relative proportion of type I and II fibers (Table 2). Percent change in total (combined type I and II) FCSA was $10.6\% \pm 20.0\%$ in the vitamin D group and $-7.4\% \pm 18.9\%$ in the placebo ($P = .048$; Figure 1). Percent changes in type I and type II-specific FCSA did not differ significantly in the 2 groups ($P > .30$; Figure 1), although there was a suggestion of type II preference. There was no association of age or BMI with baseline or 4-month change in FCSA. Percent changes in the relative proportion of type I and type II fibers in the muscle cross-sections did not differ significantly by group (data not shown).

Intramyonuclear VDR concentration

Clinical characteristics of the 14 subjects with VDR measurements are shown in Table 1. The ratio of VDR-positive myonuclei to total myonuclei was analyzed as a measure of intramyonuclear VDR concentration. At baseline, subjects in both groups had similar proportions of VDR-positive myonuclei on sampled cross-sections (Table 3). Percent change in VDR concentration was $29.7\% \pm 11.7\%$ in the vitamin D group and $7.8\% \pm 18.2\%$ in the placebo ($P = .025$; Figure 2). There was no association of age or BMI with baseline or 4-month change in VDR concentration. In the groups combined, the 4-month change in 25OHD level was strongly associated with percent change in intramyonuclear VDR concentration independent of group ($r = 0.87$, $P < .001$; Figure 3).

Fiber type-specific analyses revealed a significant increase in percent VDR-positive myonuclei in type II fi-

Table 2. Serum and Urine Biochemistry, FCSA, and Relative Proportion of Fiber Subtype Measurements at Baseline and 4 Months in the 2 Groups (n = 12 in Placebo; n = 9 in Vitamin D Group)

	Baseline Mean \pm SD	Final Mean \pm SD
Serum		
25OHD, nmol/L		
Placebo	48.3 \pm 8.8	52.5 \pm 17.1
Vitamin D ₃	43.6 \pm 10.3	80.0 \pm 11.5 ^a
24-h urine corrected for creatinine		
UNi/Cr, mmol/mmol		
Placebo	83.4 \pm 22.7	84.8 \pm 28.0
Vitamin D ₃	80.7 \pm 20.7	70.7 \pm 18.1
UCa/Cr, mmol/mol		
Placebo	326.1 \pm 120.2	324.4 \pm 207.2
Vitamin D ₃	393.1 \pm 208.5	440.3 \pm 247.1
FCSA		
Total (type I and II) muscle, μm^2		
Placebo	2985 \pm 455	2770 \pm 705
Vitamin D ₃	2836 \pm 618	3101 \pm 711
Type I muscle, μm^2		
Placebo	3860 \pm 635	3755 \pm 959
Vitamin D ₃	3624 \pm 915	3860 \pm 1223
Type II muscle FCSA, μm^2		
Placebo	2212 \pm 516	2171 \pm 555
Vitamin D ₃	2138 \pm 436	2434 \pm 520
Relative percentages of muscle fiber subtypes ^c		
Percent of type II muscle fibers, %		
Placebo	57.7 \pm 11.2	64.5 \pm 12.6
Vitamin D ₃	59.9 \pm 10.5	60.8 \pm 8.3

^a Differs from placebo group at $P < .001$.

^b Differs from placebo group at $P = .048$.

^c The percentage of type I fibers is not shown as it is the inverse of the type II.

bers after vitamin D₃ vs placebo ($P = .002$; Figure 2). There was also an increase in VDR concentration in type I fibers in the vitamin D group, but it was not statistically significant at the .05 level when compared with the placebo group.

Adverse events

One subject in the vitamin D group had a transient high spot UCa/UCr ratio, which resolved on follow-up testing

on study pills. No additional adverse events related to the study intervention were reported during the study.

Discussion

In our study of older mobility-limited women with moderately low vitamin D status, supplementation with vitamin D₃ resulted in a 30% increase in intramyonuclear

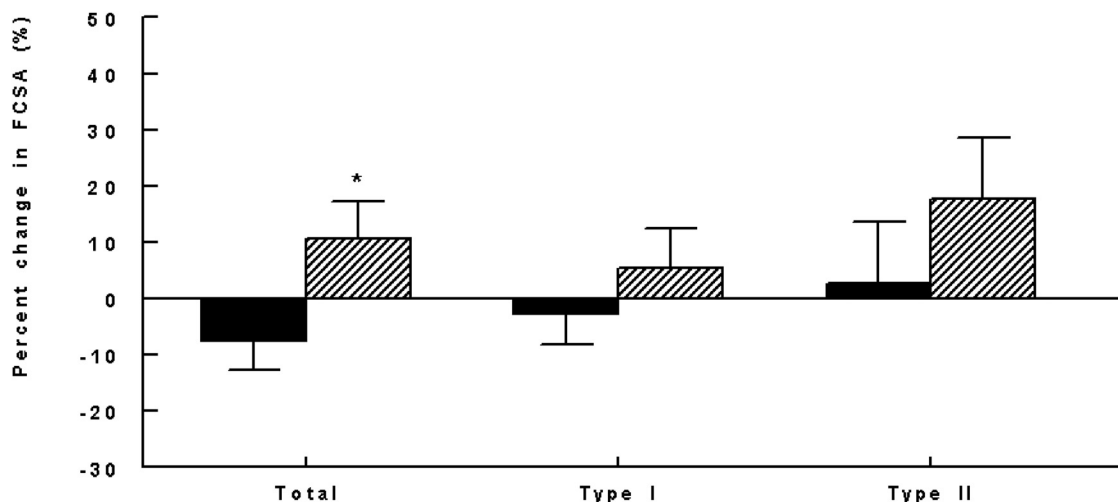


Figure 1. Four-month percent changes in FCSA by fiber type and group ($P = .048$ [total], .363 [type I], and .356 [type II]). Black bars represent placebo; hatched bars represent vitamin D. *, $P < .05$.

Table 3. Baseline and Final Ratio of VDR-Positive Myonuclei to Total Myonuclei in the 2 Groups (n = 8 in Placebo; n = 6 in Vitamin D Group)

	Baseline Ratio Mean \pm SD	Final Ratio Mean \pm SD
Total (type I + II fibers)		
Placebo	0.60 \pm 0.03	0.65 \pm 0.11
Vitamin D ₃	0.61 \pm 0.04	0.79 \pm 0.03
P value	.581	.012
Type I fibers		
Placebo	0.65 \pm 0.07	0.69 \pm 0.15
Vitamin D ₃ ^a	0.66 \pm 0.12	0.80 \pm 0.05
P value	.816	.149
Type II fibers		
Placebo	0.56 \pm 0.07	0.56 \pm 0.14
Vitamin D ₃ ^a	0.52 \pm 0.04	0.73 \pm 0.09
P value	.380	.030

^a One subject did not have adequate $\times 400$ digital images with fiber type staining and was not included.

VDR protein concentration and a 10% increase in total (type I and II) muscle fiber size over a 4-month period. These findings, along with a trend toward lower UNi excretion, are consistent with the concept that vitamin D may promote muscle mass in this population at high risk for disability.

Intramyonuclear VDR concentration

The proportion of VDR-positive myonuclei at baseline in these older women was approximately 60%, which is consistent with a previous report (25) despite some differences in study design and a different VDR monoclonal antibody used for immunostaining. The previous cross-sectional study by Bischoff-Ferrari et al (25) examined the

percentage of VDR-positive myonuclei in women with a broad baseline range in age (20–100 years) and serum 25OHD level (11–107 nmol/L) and who were undergoing either spine or hip surgery.

Our study is the first to show increased intramyonuclear VDR concentration after vitamin D supplementation in human muscle tissue samples. Recent data in C2C12 myoblasts also revealed significant increases in intramyonuclear staining of VDR protein occurring several days after a single administration of either 1,25-dihydroxyvitamin D₃ or 25OHD₃ (11). Previous studies in cell culture and animals (26, 27) have indicated that the content of VDR in target tissues is positively associated with the level of biological activity in response to vitamin D administration. Therefore, an increase in VDR content in myocytes after 4 months of vitamin D supplementation supports the concept that there may be sustained clinical effects of vitamin D supplements on muscle metabolism and/or function. Fiber type-specific analyses indicate that the pattern of changes in VDR concentration in type I and II fibers may be similar, but a larger study is needed to confirm this preliminary observation.

The presence of the VDR in human skeletal muscle cells has been a subject of debate mainly due to the absence of the VDR in a muscle tissue section from a subject using the D-6-specific VDR antibody (28). However, a previous study indicated that VDR signal was detectable in 8 fresh-frozen human muscle tissue sections using the VDR/NR111 monoclonal antibody, the D-6-specific VDR monoclonal antibody, and a third commercial antibody (21). Based on these results, we conclude that the VDR/

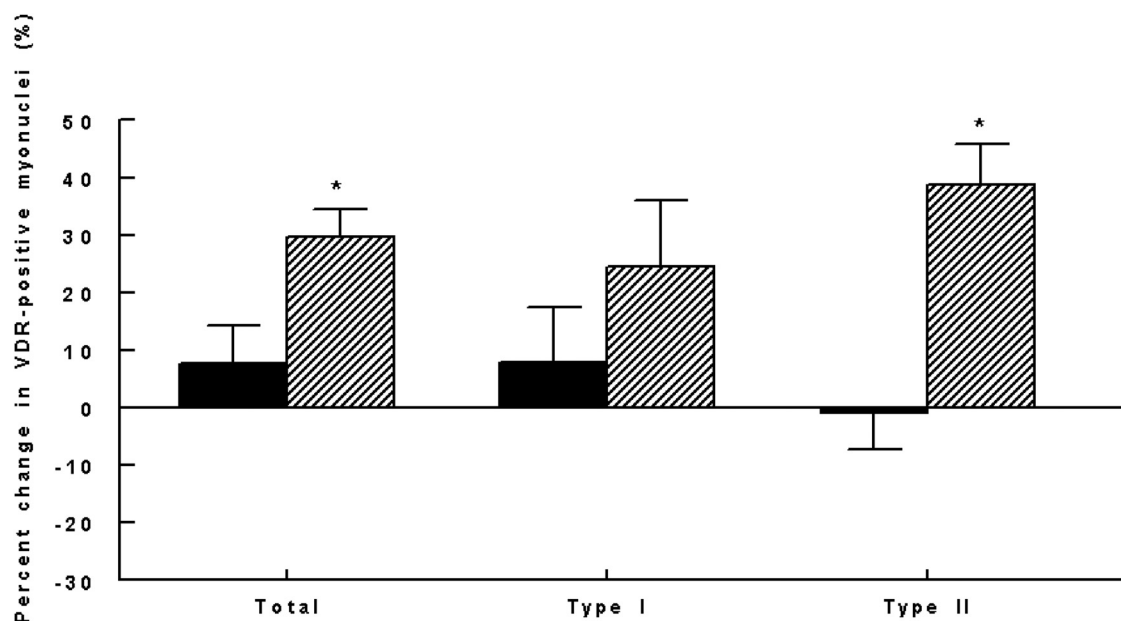


Figure 2. Four-month percent changes in intramyonuclear VDR concentration by fiber type and group ($P = .025$ [total], $.301$ [type I], and $.002$ [type II]). Black bars represent placebo; hatched bars represent vitamin D. *, $P < .05$.

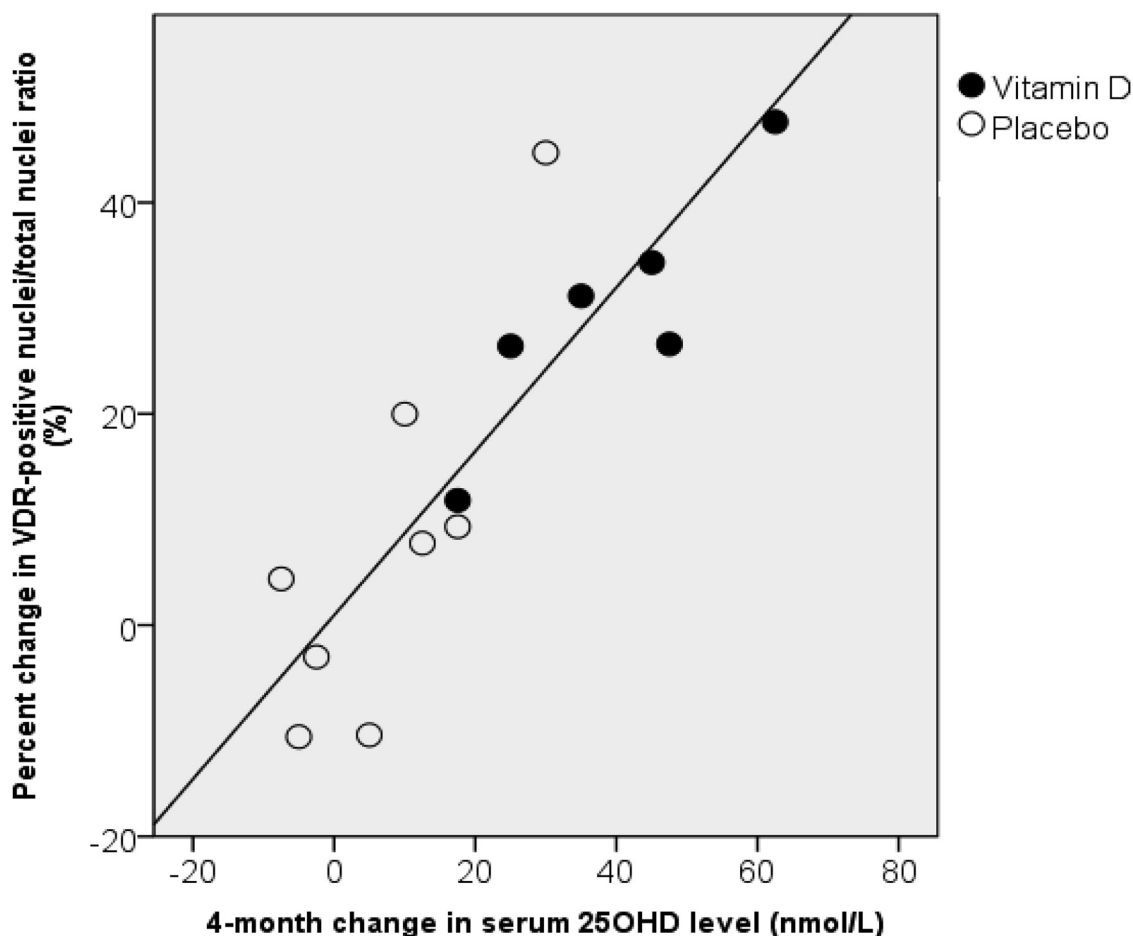


Figure 3. Association between change in 25OHD (nanomoles per liter) and the percent change in ratio of VDR-positive to total nuclei ($r = 0.87$, $P < .001$). ○, placebo; ●, vitamin D.

NR1H1 is a reliable monoclonal antibody to use to detect VDR in fresh-frozen human muscle samples.

Muscle morphology

When comparing change in total (type I and II) muscle fiber area, the vitamin D group had greater gains in fiber size compared with placebo on average. There were similar trends in the fiber-specific analyses, yet these results were not statistically significant. To date, there are limited data on the impact of vitamin D on muscle fiber size. More than 3 decades ago, an uncontrolled study obtained muscle biopsies from 11 older osteoporotic women with profound vitamin D deficiency before and after treatment with 1α -hydroxyvitamin D and calcium for 3 to 6 months. Muscle cross-sections showed an increase in type IIa muscle FCSA (24). A more recent study found that treatment of older Japanese female stroke survivors with 1000 IU vitamin D₂ daily increased mean type II muscle fiber diameter by >90% over a 2-year period in the nonparetic limb compared with placebo (29). In these rehabilitated women whose baseline 25OHD levels were <25 nmol/L, there was also a correlation between serum 25(OH)D level and type II muscle fiber diameter both at baseline and after

2 years of follow-up. Our study did not confirm these previous reports (24, 29) that also suggested an effect of vitamin D on relative proportion of type II muscle fibers, nor did it note significant differences in knee extension power and physical function as measured by the SPPB test. A larger sample size may be needed to detect significant differences in muscle strength and physical function.

Strengths and limitations

This pilot study had some important strengths, including the fact that the dose of vitamin D₃ effectively and safely increased serum 25OHD levels to current optimal levels for bone outcomes in older adults (30). Our subjects' adherence to the intervention was high as demonstrated by the large group difference in 25OHD levels, and their dropout rate was low. We chose a parallel-arm, blinded, placebo-controlled design to study the vitamin D₃ effects. The primary limitation of this pilot study was the small sample size, which likely prevented us from detecting some clinically meaningful effects, such as changes in muscle performance, and resulted in imbalances in calcium intake and body weight between the groups. However, these baseline differing factors (ie, calcium and weight) were not

significantly correlated to baseline or changes in FCSA or VDR concentration in our sample. Our findings pertain to older, mobility-limited women with moderately low vitamin D status at baseline. We, therefore, cannot comment on the degree to which these findings may vary in men or premenopausal women. Finally, we cannot comment on whether the relatively high dose of 4000 IU per day was needed or whether a lower dose would have had similar effects on muscle fiber size and VDR concentration.

In conclusion, supplementation with vitamin D₃ for 4 months in older mobility-limited women with moderately low vitamin D status increased intramyonuclear VDR concentration and muscle fiber size. Further work is needed to confirm these findings in a larger sample and to determine whether our two main findings are related, that is, whether vitamin D increases muscle fiber size by activating the VDR. Additionally, it will be important to identify the signaling pathways involved.

Acknowledgments

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gator-initiated funding from Pfizer and is on the scientific advisory board at Pfizer and Cytochroma.

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