Original article

Effect of vitamin D_3 and calcium carbonate supplementation on muscle strength in postmenopausal women living with HIV

Michael T Yin[1], Mariana Bucovsky[1], John Williams[1], Danielle Brunjes[1], Arindam RoyChoudhury[2], Ivelisse Colon[1], David C Ferris[3,4], Susan Olender[1], P Christian Schulze[5], Anjali Sharma[6], Cosmina Zeana[4], Barry Zingman[6], Elizabeth Shane[1]*

[1]Columbia University Irving Medical Center, New York, NY, USA
[2]Division of Biostatistics and Epidemiology, Department of Healthcare Policy and Research, Weill Cornell Medical College, Cornell University, New York, NY, USA
[3]Mt Sinai St Luke's and Mt Sinai West, New York, NY, USA
[4]BronxCare Health System, Bronx, NY, USA
[5]University Hospital Jena, Jena, Germany
[6]Albert Einstein College of Medicine and Montefiore Medical Center, Bronx, NY, USA

*Corresponding author e-mail: mty4@cumc.columbia.edu

Background: Both falls and fractures are increased in older persons living with HIV (PLWH). Low serum total 25-hydroxyvitamin D (25-OHD) levels have been associated with falls, fractures and poor muscle strength. We hypothesized that vitamin D (VitD) supplementation would improve muscle strength in postmenopausal PLWH.
Methods: In a 12-month prospective, randomized, double-blind, study of 69 African American and Hispanic postmenopausal PLWH on antiretroviral therapy with 25-OHD ≥10 ng/ml and ≤32 ng/ml, we investigated the effects of daily low (1,000 IU; *n*=31) and moderate (3,000 IU; *n*=38) cholecalciferol doses on lean mass and strength. Change in lean body mass was assessed by dual-energy X-ray absorptiometry (DXA), and isometric and isokinetic muscle strength in the dominant lower extremity was assessed using the Biodex System 4 Pro.
Results: Mean age was 56 ±5 years, median $CD4^+$ T-cell count 722 cells/mm^3 and 74% had HIV RNA≤50 copies/ml. Serum 25-OHD did not differ at baseline, but was higher in the moderate than low VitD group at 6 and 12 months. In both groups, there were significant increases in lower extremity isokinetic torque, work and power at 12 months, with no change in lean mass.
Conclusions: VitD supplementation was associated with a modest increase in lower extremity strength in postmenopausal PLWH, without a concomitant increase in muscle mass. Magnitude of increase in strength were similar with 3,000 IU and 1,000 IU daily. Future larger studies will be required to determine the optimal dose of VitD to improve muscle strength and to determine whether supplementation reduces the risk of falls and fractures in PLWH.

Introduction

Prevalence of frailty is higher among persons living with HIV (PLWH) than age-matched controls [1–3] and is associated with increased fall rates [4,5]. In addition to its skeletal effects, low 25-hydroxyvitamin D (25-OHD), particularly levels <20 ng/ml, has been associated with impaired muscle strength, function and balance in the general population, which has important implications for falls and fracture risk [6–8]. Vitamin D receptor (VDR) is expressed abundantly in skeletal muscle cells. Vitamin D (VitD) deficiency results in atrophy of these muscle fibres, fat infiltration and resultant fibrosis [8]. In a randomized, placebo-controlled study of older, mobility-limited women with moderately low 25-OHD levels, supplementation with 4,000 IU $VitD_3$ resulted in a 30% increase in intramyonuclear VDR protein and a 10% increase in total (type I and II) muscle fibre size over a 4-month period [9].

Few studies have specifically evaluated impact of VitD supplementation on neuromuscular outcomes among PLWH [10]. A recent randomized clinical trial

of 56 children and young adults living with HIV compared the effects of 7,000 IU of VitD daily versus placebo over 12 months and found a mild increase in test scores for fine and gross motor skills, but no change in other parameters. There are no published studies on the effect of VitD supplementation in older PLWH who may benefit most.

Provision of adequate calcium and VitD is the cornerstone of effective prevention of osteoporosis and its consequences, as VitD in particular has been shown to reduce bone loss and falls, and lower the risk of hip fracture in older individuals [11–13]. A comprehensive meta-analysis of randomized controlled trials found that among adults with baseline 25-OHD <10 ng/ml, VitD supplementation resulted in increased handgrip strength [14] and that improvements in muscle function were associated with increased lean body mass [15].

We have previously published the results of a randomized clinical trial of two doses of $VitD_3$ repletion (3,000 IU versus 1,000 IU) on bone turnover and change in areal and volumetric bone mass and microarchitecture in minority postmenopausal women living with HIV [16]. We found changes in bone mineral density and bone microarchitecture did not differ between moderate and low VitD treatment groups. We now report on the muscle mass and muscle strength data for this study.

Methods

Study population

Postmenopausal African American or Hispanic women living with HIV between ages 40–70 were recruited from four sites in New York City (Columbia University Irving Medical Center, Mt Sinai St Luke's and Mt Sinai West, Bronx Lebanon Hospital Center, and Montefiore Medical Center, New York, NY, USA). All participants had serum 25-OHD ≥10 ng/ml and ≤32 ng/ml and were on stable antiretroviral therapy (ART) for at least 2 years with 2 or more HIV RNA levels <400 copies/ml within the past year prior to enrolment. Exclusion criteria included: metabolic bone diseases, renal insufficiency (serum creatinine >1.5 mg/dl or estimated glomerular filtration rate <45 ml/min/1.73^2), current use of glucocorticoids or hormone replacement therapy, current or past osteoporosis therapy, history of fragility fracture or T-score <-3.0, history of hypercalcaemia or calcium-containing kidney stones, and weight over 300 lbs.

Participants were randomized to receive 3,000 versus 1,000 IU cholecalciferol (vitamin D_3) daily (Tishcon Corporation, Westbury, NY, USA) plus 500 mg calcium carbonate twice daily and counselled to take with food to facilitate absorption. Participants were randomized in a 1:1 ratio using permuted blocks stratified by screening serum 25-OHD (≤20 and >20 ng/ml). Sample size for the main study was based upon the primary bone mineral density outcomes which have been reported [16]. Adherence to VitD and calcium was monitored by counts of returned pills at each 3-month study visit. The Institutional Review Boards of all participating sites approved the study. Secondary outcomes include change in appendicular lean mass by dual-energy X-ray absorptiometry (DXA), muscle function and frailty measures from baseline to 12 months. Changes in plasma 25-OHD, parathyroid hormone (PTH) over 12 months were also examined in relation to the skeletal muscle measures. All DXA scans and muscle function measures were performed at Columbia University Irving Medical Center (CUIMC; New York, NY, USA) using a standardized protocol.

Lean body mass measures

Body composition was measured by DXA on a QDR 4500 bone densitometer (Hologic, Inc., Bedford, MA, USA) at CUIMC and analysed using established protocols. Height and weight were measured by Harpenden stadiometer and balance beam scale, respectively. Non-fat and non-bone tissue was defined as lean body mass.

Muscle function testing

Muscle functional testing was performed by trained staff under the supervision of a physician. Biodex System 4 Pro (Biodex Medical Systems, Shirley, NY, USA) was used to measure both isometric and isokinetic movements. The dominant knee was tested first, followed by the dominant elbow.

For isometric contractions, participants contracted against a constant 60-degree angle at the knee and a constant 30-degree angle at the elbow for 6 s. Participants alternated between flexion and extension, three repetitions each with a 30 s rest period between each. Data were collected to determine peak torque to body weight (PKTQ/BW).

For isokinetic contractions, participants performed flexion and extension movements at the knee and elbow for five repetitions at a speed of 60 degrees/s. This was followed by a rest period of 2.5 min. For the second isokinetic protocol, participants performed 25 repetitions of flexion and extension movements at the knee and elbow at a speed of 180 degrees/s. Data were collected to determine PKTQ/BW, average power, work to body weight (WK/BW), fatiguability, and time to peak torque. Normalization of the data to body weight allowed for a more meaningful comparison of measurements among participants.

Frailty was defined as the presence of ≥3 of the following criteria: unintentional weight loss, self-reported exhaustion, weakness, slow walking speed, and low

physical activity, using the same cut points developed by Fried *et al*. [17] in the Cardiovascular Health Study (CHS) study.

Biomarker assays

Serum samples were stored at -70°C until batched analysis at the Irving Institute Biomarkers Core at Columbia University Medical Center. We measured 25-OHD$_2$ and D$_3$ by liquid chromatography tandem mass spectrometry, and the total reported as the sum of 25-OHD$_2$ and D$_3$; calibration standards for both 25-OHD$_2$ and D$_3$ were validated against National Institute of Standard and Technology standard, SRM972a. We also measured intact PTH by radioimmunoassay (Scantibodies, Santee, CA, USA), interleukin (IL)-6 (ELISA; R&D Systems, Minneapolis, MN, USA), soluble receptors of tumour necrosis factor (TNF)-α (soluble [s]TNFr-I and -II; ELISA; R&D Systems), and soluble CD14 (sCD14; ELISA; R&D Systems). Except for 25-OHD, biomarkers were measured in duplicate and values averaged for analysis.

Statistical analysis

A complete case approach was utilized for all lean mass, strength and frailty analyses in participants at 48 weeks (n=69). Stratified Wilcoxon rank-sum tests were used to test for distribution shifts between the treatment groups. Fisher's exact tests and Wilcoxon rank-sum tests were used to evaluate for differences between groups for categorical and continuous secondary outcomes, respectively. Wilcoxon signed-rank test was used to evaluate within treatment group change. All statistical tests were two-sided and interpreted at the 5% level of significance without adjustment for multiple comparisons. Analyses were performed using R 3.4.0.

Results

Between 2011 and 2016, we enrolled and randomized 85 participants who were included in the intent-to-treat analysis. Among all participants, 82 received at least one dose of the allocated intervention (41 in low VitD and 41 moderate VitD). Overall, 69 participants (31 in the low VitD and 38 in the moderate VitD groups) completed the 12-month study with complete muscle function and interpretable DXA data and were included in the complete-case analysis [16].

Baseline demographic, immunological and other parameters are summarized in Table 1 for the 69 participants in the complete-case analysis. Overall, the mean age was 56.6 years, 42% were African American, 26% Hispanic, median CD4$^+$ T-cell count 738 cells/mm^3 and 71% had HIV RNA≤50 copies/ml. Age, race/ethnicity, proportion with diabetes and HIV-specific parameters such as current/nadir CD4$^+$ T-cell count, history of AIDS-defining illness, ART class and proportion with HIV RNA levels <50 copies/ml were similar between groups. However, at baseline, the moderate VitD group had lower BMI, total body fat and trunk fat (Table 1). Mean 25-OHD and PTH did not differ between treatment groups; 52.6% and 51.6% had VitD deficiency with 25-OHD <20 ng/ml in the moderate VitD and low VitD groups, respectively (P=0.934). Serum levels of inflammatory biomarkers (TNF-α, IL-6 and sCD14) did not differ between treatment groups.

Change in 25-OHD and PTH

Serum levels of total 25-OHD were higher in the moderate than low VitD group at 6 months (33.1 ±10.6 versus 28.6 ±8.2 ng/ml, P=0.031) and 12 months (30.2 ±9.8 versus 24.8 ±8.0 ng/ml, P=0.014). Similarly, percentage change from baseline in serum 25-OHD levels were greater in the moderate than low VitD groups at 6 months (67 ±78% versus 45 ±44%, P=0.041) and 12 months (51 ±69% versus 28 ±42%, P=0.031). Overall, PTH levels decreased in both groups, but the decrease did not differ between moderate and low VitD groups (data not shown).

Change in lean mass by DXA at 12 months

At baseline, mean upper and lower extremity lean mass at the dominant side were similar between two the treatment groups (Table 1). Weight was relatively stable over the 12-month course of study, and change in weight did not differ between the low VitD versus moderate VitD groups, respectively: +0.31 kg versus -0.40 kg (P=0.46). Similarly, there was no significant within-subject change in lean mass in either the upper or lower extremity in either treatment group (Tables 2 and 3). Among participants with 25-OHD <20 ng/ml at baseline, there were no significant differences in lower extremity or upper extremity lean mass at baseline or after 12 months between low and moderate VitD treatment groups.

Change muscle strength at 12 months

Skeletal muscle strength parameters are shown for the dominant limb in the upper extremity in Table 2 and in the lower extremity in Table 3. At baseline, there were no significant differences between groups in isometric and isokinetic muscle strength in either the upper or lower extremities.

In the low VitD group, there were significant within-group increases in upper extremity isometric muscle strength, peak torque (extension) from baseline to 12 months, but no increases in other muscle groups. There were no apparent increases in isokinetic muscle strength (5 repetitions) from baseline to 12 months in the upper extremities (Table 2), but there were significant

Table 1. Baseline characteristics of participants who received allocated intervention and completed 12-month study evaluation[a]

	Low VitD group A (*n*=31)	Moderate VitD group B (*n*=38)	*P*-value
Patient characteristic			
Age	56.6 ±4.6	56.7 ±5.8	0.932
Race/ethnicity			0.497
African American, *n* (%)	11 (35%)	18 (47%)	
Caucasian, *n* (%)	12 (39%)	10 (26%)	
Hispanic, *n* (%)	8 (26%)	10 (26%)	
Height, cm	158.5 ±8.2	161.5 ±7.4	0.116
Weight, kg	80.6 ±14.4	76.0 ±15.2	0.204
Body mass index, kg/cm^2	32.3 ±6.9	29.1 ±5.3	0.033
Current smoking, *n* (%)	11 (30%)	16 (41%)	0.43
Diabetes, *n* (%)	5 (14%)	3 (8%)	0.47
HIV parameter			
Current CD4$^+$ T-cell count, cells/mm^3	664 ±286	769 ±419	0.227
Nadir CD4$^+$ T-cell count, cells/mm^3	187 ±149	264 ±229	0.102
History of AIDS, *n* (%)	17 (54%)	18 (47%)	0.707
Current HIV VL≤50, *n* (%)	23 (74%)	26 (68%)	0.796
Current HIV VL<400 copies/μl, *n* (%)	31 (100%)	38 (100%)	NA
ART class			0.29
NNRTI-based, *n* (%)	18 (45%)	20 (49%)	
PI-based, *n* (%)	14 (35%)	18 (44%)	
Integrase-based, *n* (%)	5 (13%)	3 (7%)	
Tenofovir-containing regimen, *n* (%)	29 (67%)	30 (71%)	
Baseline DXA and body composition			
Total body fat, kg	33.2 ±9.6	28.4 ±9.7	0.044
Trunk fat, kg	16.4 ±5.7	13.8 ±4.7	0.036
Total body lean mass, kg	47.0 ±6.4	46.6 ±8.0	0.838
Total body lean subtotal, kg	43.3 ±6.3	42.9 ±7.8	0.815
Upper extremity lean mass, kg	4.9 ±0.8	4.8 ±1.0	0.600
Lower extremity lean mass, kg	15.4 ±2.4	15.1 ±2.9	0.740
Biochemical marker			
25-hydroxyvitamin D, ng/ml	19.4 ±5.9	19.8 ±6.5	0.751
Parathyroid hormone, ng/ml	53.5 ±25.9	60.5 ±37.7	0.357
Tumour necrosis factor α, pg/ml	10.0 ±6.4	8.5 ±4.0	0.268
Interleukin-6, pg/ml	3.5 ±2.3	3.2 ±2.0	0.650
Soluble CD14, ng/ml	258 ±568	414 ±870	0.371

[a]*n*=69. Data are mean ±SD unless stated otherwise. ART, antiretroviral therapy; DXA, dual-energy X-ray absorptiometry; NA, not applicable; NNRTI, non-nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; VitD, vitamin D; VL, viral load.

within-subject changes in peak torque (flexion), total work (flexion) and average power (flexion) in the lower extremities (Table 3). Similarly, there were no apparent increases in isokinetic muscle strength (25 repetitions) from baseline to 12 months in the upper extremities (Table 2), but significant within-subject change in total work (flexion) and trends for peak torque (flexion) and average power (flexion) in the lower extremities (Table 3).

In the moderate VitD group, there were no significant within-group increases in upper extremity isometric muscle strength. There were no apparent increases in isokinetic muscle strength (5 repetitions) from baseline to 12 months in either the upper extremities (Table 2), but there were significant within-subject change in peak torque (flexion), total work (flexion) and average power (flexion and extension) in the lower extremities (Table 3). There were no apparent increases in isokinetic muscle strength (25 repetitions) from baseline to 12 months in either upper or lower extremities (Tables 2 and 3).

Greater increase in muscle strength was apparent in the moderate versus low VitD group in lower extremity isokinetic strength, 5 repetitions, only for peak torque (flexion) (26.1 ±11.5% versus 16.3 ±6.4%, $P<0.05$; Figure 1). Adjustment for BMI attenuated the between-group difference ($P=0.372$). None of the other between-group comparisons in change in muscle strength (isometric, isokinetic 5 repetitions and isokinetic 25 repetitions) were statistically significant (data not shown).

Table 2. Upper extremity muscle mass and strength parameters at baseline and 12 months by treatment arm

Parameter	Low VitD			Moderate VitD		
	Baseline	12 months	*P*-value	Baseline	12 months	*P*-value
UE lean mass, kg	4.9 ±0.8	4.8 ±1.0	0.634	4.8 ±1.0	4.7 ±1.1	0.547
Isometric						
Peak torque/BW, extension, %	36.3 ±10.8	40.6 ±11.8	0.038	40.4 ±12.4	42.6 ±14.1	0.577
Peak torque/BW, flexion, %	45.7 ±11.8	48.6 ±11.6	0.176	47.2 ±12.3	47.7 ±10.7	0.864
Isokinetic 5 repetitions						
Peak torque/BW, extension, %	28.4 ±8.6	31.0 ±8.2	0.191	31.4 ±9.6	32.3 ±8.9	0.731
Peak torque/BW, flexion, %	37.32 ±10.6	40.2 ±7.6	0.284	38.7 ±11.2	41.2 ±9.6	0.659
Total work/BW, extension, %	105.3 ±36.4	111.9 ±44.7	0.830	111.3 ±42.6	113.4 ±34.0	0.699
Total work/BW, flexion, %	150.5 ±53.9	159.7 ±45.4	0.473	150.6 ±54.0	165.5 ±37.3	0.170
Average power, extension, J	13.3 ±5.2	14.7 ±5.9	0.433	14.1 ±5.6	14.4 ±4.4	0.740
Average power, flexion, J	18.7 ±7.9	20.6 ±6.1	0.203	18.7 ±6.7	20.7 ±5.0	0.246
Isokinetic 25 repetitions						
Peak torque/BW, extension, %	25.5 ±7.2	25.3 ±5.9	0.807	26.9 ±8.0	25.3 ±6.8	0.332
Peak torque/BW, flexion, %	38.9 ±10.0	40.1 ±7.4	0.584	37.7 ±11.8	38.6 ±8.7	0.577
Total work/BW, extension, %	340.4 ±143.9	361.4 ±159.9	0.679	335.0 ±137.0	349.5 ±109.0	0.646
Total work/BW, flexion, %	621.2 ±208.9	639.9 ±173.5	0.432	580.3 ±186.0	641.9 ±152.4	0.087
Average power, extension, J	20.6 ±9.5	22.6 ±10.6	0.535	20.5 ± 9.1	21.5 ±6.6	0.677
Average power, flexion, J	34.4 ±14.3	36.7 ±12.3	0.399	32.7 ±11.9	35.6 ±8.9	0.185

Data are mean ±SD unless stated otherwise. BW, body weight; UE, upper extremity; VitD, vitamin D.

Table 3. Lower extremity muscle mass and strength parameters at baseline and 12 months by treatment arm

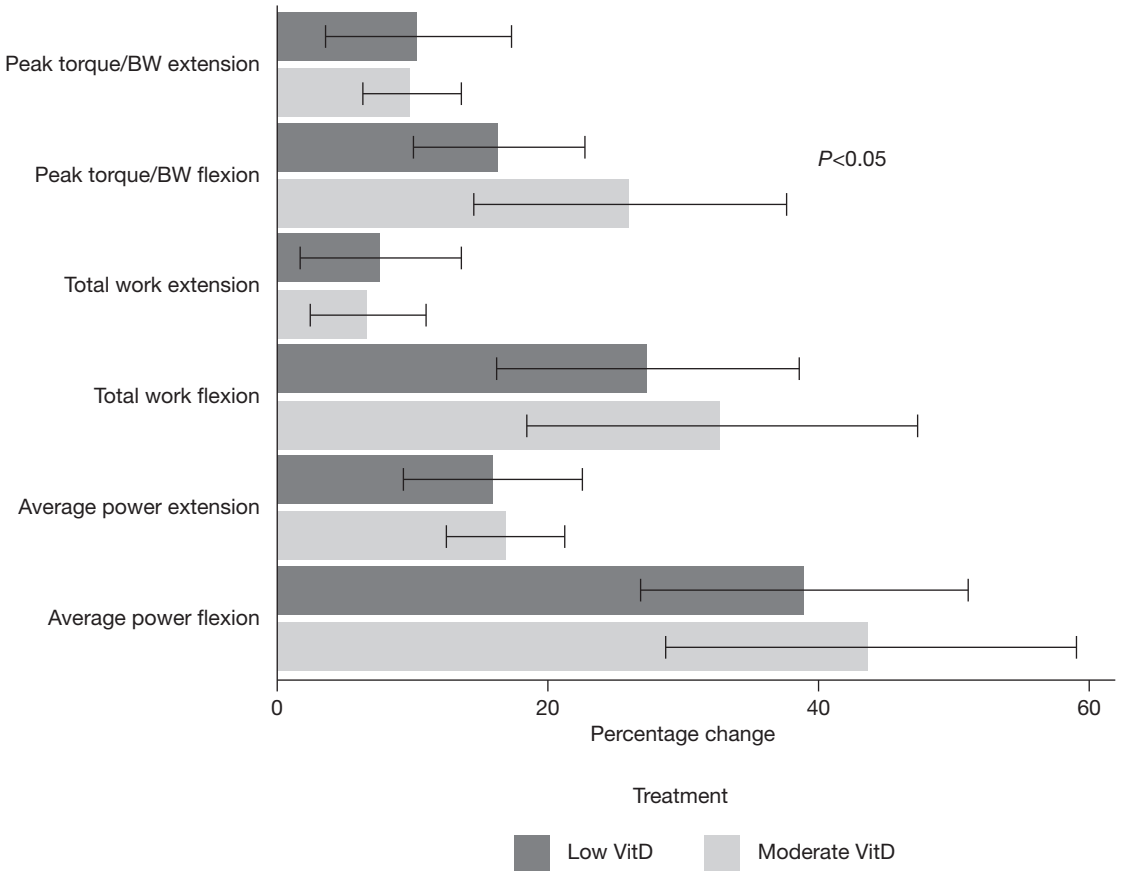
Parameter	Low VitD			Moderate VitD		
	Baseline	12 months	*P*-value	Baseline	12 months	*P*-value
LE lean mass, kg	15.4 ±2.4	14.8 ±2.8	0.355	15.1 ±2.9	14.5 ±2.4	0.322
Isometric						
Peak torque/BW, extension, %	127.06 ±43.0	130.6 ±38.1	0.585	129.3 ±42.1	138.1 ±38.0	0.548
Peak torque/BW, flexion, %	59.8 ±20.3	66.0 ±19.0	0.225	63.2 ±21.2	69.7 ±22.6	0.377
Isokinetic 5 repetitions						
Peak torque/BW, extension, %	106.9 ±32.9	117.9 ±37.0	0.193	111.1 ±37.5	122.1 ±37.8	0.258
Peak torque/BW, flexion, %	49.0 ±14.1	56.9 ±16.5	0.046	49.8 ±22.0	62.7 ±21.6	0.021
Total work/BW, extension, %	342.2 ±112.8	368.1 ±141.7	0.554	346.8 ±104.1	370.0 ±96.0	0.447
Total work/BW, flexion, %	157.3 ±65.0	200.4 ±82.6	0.023	151.4 ±82.3	205.4 ±75.3	0.010
Average power, extension, J	45.6 ±13.5	52.9 ±20.6	0.228	45.6 ±14.3	53.3 ±15.0	0.041
Average power, flexion, J	20.0 ±8.4	27.8 ±11.9	0.007	19.1 ±11.4	27.9 ±10.8	0.002
Isokinetic 25 repetitions						
Peak torque/BW, extension, %	60.1 ±18.2	64.2 + 20.3	0.341	58.0 ± 15.7	62.5 ±15.5	0.244
Peak torque/BW, flexion, %	35.3 ±10.4	40.1 + 10.1	0.063	35.3 ± 11.3	39.3 ±11.2	0.150
Total work/BW, extension, %	1,149.7 ±365.0	1,195.0 ±399.7	0.608	1,118.0 ±350.7	1,185.4 ±272.4	0.403
Total work/BW, flexion, %	429.3 ±271.2	575.2 ±298.5	0.048	431.6 ±308.2	561.4 ±257.7	0.072
Average power, extension, J	78.9 ±27.3	83.9 ±30.8	0.560	75.7 ±25.0	84.4 ±20.6	0.189
Average power, flexion, J	27.7 ±18.1	38.1 ± 21.0	0.057	27.8 ±20.5	37.4 ±17.8	0.043

Data are mean ±SD unless stated otherwise. BW, body weight; LE, lower extremity; VitD, vitamin D.

Change in functional measures after 12 months

At baseline, there were no significant differences between groups in grip strength, 4-metre walk speed or time to complete 10 chair stands. There were significant within-group improvements in chair stand time in both the moderate VitD (31.5 ±8.5 to 26.2 ±7.9 s, $P<0.0001$) and low VitD (29.6 ±9.7 to 24.6 ±7.3 s, $P=0.009$) after 12 months; however, there were no significant differences between treatment groups. There were no significant within-group improvements in any other muscle function test in either treatment group. Overall, there was a very low prevalence of frailty, with only 1/38

Figure 1. Isokinetic strength in lower extremities[a]



[a]Five repetitions: percentage increase in muscle strength over 12 months in women receiving low versus moderate vitamin D (VitD) supplementation. BW, body weight.

(3%) of the moderate VitD and 2/31 (6%) of the low VitD group meeting frailty criteria.

Discussion

In this study, we found that VitD supplementation at 3,000 IU daily increased mean total 25-OHD levels to ≥30 ng/ml in minority postmenopausal women living with HIV, with 53% achieving 25-OHD levels >30 ng/ml at 12 months. Improvements in muscle function were apparent in both groups receiving VitD (3,000 IU and 1,000 IU). Greater improvements were apparent in isokinetic muscle function for knee flexion (peak torque) with moderate (3,000 IU daily) versus low (1,000 IU daily) VitD supplementation, but did not remain significant after adjustment for BMI. Improvements in muscle strength were not associated with increase in muscle mass or weight.

Provision of adequate calcium and VitD is the cornerstone of effective prevention of osteoporosis and its consequences, as VitD in particular has been shown to reduce bone loss and falls and lower the risk of hip fracture in older individuals [11–13]. A comprehensive meta-analysis of randomized controlled trials in the general population found that among adults with baseline 25-OHD <10 ng/ml, VitD supplementation resulted in increased handgrip strength [14] and that improvements in muscle function were associated with increased lean body mass [15]. From existing studies of VitD supplementation in postmenopausal women, doses of 1,000 IU per day or greater have resulted in improvement in physical measures in randomized clinical trials. In a study of 70–90-year-old postmenopausal women (*n*=302), Zhu *et al.* [18] found that VitD 1,000 IU plus calcium citrate once daily for a year resulted in improved muscle strength and mobility as measured by strain gauge and get up and go for women in the lowest tertile of muscle function compared with placebo. In a study of 50–65-year-old postmenopausal women (*n*=160), Cangussu *et al.* [19] found that VitD

1,000 IU/day with calcium supplementation taken for 9 months improved chair rising test and maintained lean body mass by DXA in comparison to the placebo.

Several studies suggest that frailty is more common among PLWH than uninfected controls and that the presence of frailty is associated with future falls [5,20]. Prevalence of frailty using Fried's frailty phenotype was 11.3% among women aged 70–79 in the Women's Health and Aging Studies (WHAS) [21] and 11.0% among minority women aged 65–70 in the CHS [17]. In a prior study of postmenopausal Hispanic and African American women with HIV in New York City with a mean age of 58, we found a 11% prevalence of frailty using the same criteria [1].

Two recent studies evaluated risk factors for falls among PLWH in comparison to control. The first, found that among PLWH, smoking, higher number of medications and imbalance symptoms were positively associated with falls, whereas protease inhibitor use appeared to be protective [22]. In the second study, factors independently associated with falls included factors affecting cognition and balance such as age, marijuana use, number of central nervous system active agents, subjective cognitive complaints, depressive symptoms and neuropathy [20]. Neither study evaluated associations between falls and VitD levels. In this study sample of postmenopausal women with a mean age of 56, we had a much lower prevalence of frailty (4.3%) than expected and none of the participants had falls or fractures during the course of the study. The lower prevalence of frailty and lack of falls in our sample, may have accounted for the modest observed effect with the VitD supplementation.

The major focus of VitD supplementation in PLWH has been to address skeletal complications, such as bone loss and fractures. Although there has also been interest in assessing the effects of VitD supplementation on muscle function, immune modulation and prevention of HIV- and non-AIDS-related outcomes, only one randomized clinical study has evaluated the potential impact of VitD supplementation on neuromuscular outcomes in PLWH [10]. This study randomized 56 children and young adults living with HIV to receive 7,000 IU of VitD daily versus placebo over 12 months and measured a battery of neuromuscular motor skills including the Bruininks–Oseretsky test of motor performance, jump power and energy, and muscular force and strength. The authors observed a mild increase in Bruininks–Oseretsky test score (β=1.14; *P*=0.041), a measure of gross and fine motor skills, but no change was observed in other parameters. There are no studies of VitD supplementation and muscle function in older PLWH.

In our study, median 25-OHD levels were ≥30 ng/ml at both the 6- and 12-month time points in the moderate and low VitD treatment arm, with over 56% and 53% of women achieving levels >30 ng/ml at 6 and 12 months, respectively. We observed differences between treatment groups only in isokinetic knee flexion, but not in other strength parameters. However, these improvements were modest and were not significant after adjustment for between-group differences in BMI. Furthermore, without a true placebo group, we are limited in our interpretation of these changes, since there is a well-recognized 'training effect' with the Biodex and with muscle function tests.

The most comprehensive Biodex data in older adults come from a study of Flemish Caucasian adults (19–73 years; 381 men and 210 women) that evaluated baseline and 10-year change muscle strength using the Biodex Medical Systems 3 dynamometer [23,24]. Among women age 40–60, at baseline the mean isometric peak torque leg extension measure (122.2 ±25.8 nM) is very similar to our measure. Their isokinetic measures differ from ours and therefore are difficult to compare. Among women between ages 40–60, the percentage change in isometric peak torque leg extension strength was -1.09 ±2.11% per year and isokinetic isometric peak torque was -0.81 ±2.29% [23]. Decrease in strength was even more profound in women over age 60, with a mean change of -1.92 ±1.74% per year in isometric and -1.5 ±2.67% change in isokinetic leg extension strength [23]. The mean age of our study population was 56; therefore, it is possible that both our VitD supplementation regimens had a positive effect on muscle function and we would have been able to detect this difference if we had included a VitD placebo group.

Our study had several limitations. At the time the study was conceived, a large amount of observational data suggested that VitD supplementation was beneficial in multiple domains, and there were ethical concerns about randomizing severely VitD deficient postmenopausal women to placebo for 12 months. Therefore, for ethical reasons, we chose to compare a lower (1,000 IU/day) dose with a higher (3,000 IU/day) dose that was below the 4,000 IU set by the Institute of Medicine guidelines as the upper limit of daily VitD supplementation [25], rather than conducting a placebo-controlled trial. Also, our modest sample size and higher rate of discontinuation than anticipated may have limited our ability to detect more consistent benefits of therapy. The 25-OHD levels at baseline may not have been sufficiently low to be able to result in large improvement in muscle function with VitD supplementation. DXA does not distinguish between specific muscles in the arms and legs; therefore, correlations between appendicular lean mass and muscle strength may underestimate stronger correlations between specific muscle groups and strength. Another limitation is that there are no established cut points for Biodex that can be related

to clinical outcomes, such as physical disability and functional impairment, unlike skeletal muscle mass and skeletal muscle index [26]; therefore, the clinical significance of Biodex findings are less clear.

In conclusion, our study found modest improvements in some parameters of lower extremity muscle function in both VitD treatment groups, without concomitant increase in lean mass. We believe that higher doses of VitD could be used in future studies, but the greatest impact is likely to occur in more compromised subgroups, such as in patients with frailty or older patients. Additional studies are required to determine the optimal dose of VitD to improve muscle strength, which groups of PLWH will benefit from supplementation, and whether supplementation reduces clinically meaningful outcomes such as decreasing falls and improving physical activity.

Acknowledgements

This work was supported by the National Institutes of Health through grants AI1065200 (ES), AI059884 (MTY), AR06199301 (AS) and the National Center for Advancing Translational Sciences, through grant number UL1TR001873. This content does not represent official views of the NIH.

Disclosure statement

The authors declare no competing interests.

References

1. Young P, Shah J, Zhang C, *et al*. Frailty in postmenopausal African American and Hispanic HIV-infected women. *J Frailty Aging* 2016; **5:**242–246.
2. Desquilbet L, Jacobson LP, Fried LP, *et al*. HIV-1 infection is associated with an earlier occurrence of a phenotype related to frailty. *J Gerontol A Biol Sci Med Sci* 2007; **62:**1279–1286.
3. Gustafson DR, Shi Q, Thurn M, *et al*. Frailty and constellations of factors in aging HIV-infected and uninfected women--The Women's Interagency HIV Study. *J Frailty Aging* 2016; **5:**43–48.
4. Tassiopoulos K, Abdo M, Wu K, *et al*. Frailty is strongly associated with increased risk of recurrent falls among older HIV-infected adults. *AIDS* 2017; **31:**2287–2294.
5. Sharma A, Hoover DR, Shi Q, *et al*. Frailty as a predictor of falls in HIV-infected and uninfected women. *Antivir Ther* 2019; **24:**51–61.
6. Murad MH, Elamin KB, Abu Elnour NO, *et al*. Clinical review: the effect of vitamin D on falls: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2011; **96:**2997–3006.
7. Dhaliwal R, Aloia JF. Effect of vitamin D on falls and physical performance. *Endocrinol Metab Clin North Am* 2017; **46:**919–933.
8. Koundourakis NE, Avgoustinaki PD, Malliaraki N, Margioris AN. Muscular effects of vitamin D in young athletes and non-athletes and in the elderly. *Hormones (Athens)* 2016; **15:**471–488.
9. Ceglia L, Niramitmahapanya S, da Silva Morais M, *et al*. A randomized study on the effect of vitamin D(3) supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. *J Clin Endocrinol Metab* 2013; **98:**E1927–E1935.
10. Brown JC, Schall JI, Rutstein RM, Leonard MB, Zemel BS, Stallings VA. The impact of vitamin D3 supplementation on muscle function among HIV-infected children and young adults: a randomized controlled trial. *J Musculoskelet Neuronal Interact* 2015; **15:**145–153.
11. Bischoff-Ferrari HA, Dawson-Hughes B, Willett WC, *et al*. Effect of vitamin D on falls: a meta-analysis. *JAMA* 2004; **291:**1999–2006.
12. Bischoff-Ferrari HA, Willett WC, Wong JB, Giovannucci E, Dietrich T, Dawson-Hughes B. Fracture prevention with vitamin D supplementation: a meta-analysis of randomized controlled trials. *JAMA* 2005; **293:**2257–2264.
13. Dawson-Hughes B, Bischoff-Ferrari HA. Therapy of osteoporosis with calcium and vitamin D. *J Bone Miner Res* 2007; **22 Suppl 2:**V59–V63.
14. Beaudart C, Buckinx F, Rabenda V, *et al*. The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. *J Clin Endocrinol Metab* 2014; **99:**4336–4345.
15. El-Hajj Fuleihan G, Nabulsi M, Tamim H, *et al*. Effect of vitamin D replacement on musculoskeletal parameters in school children: a randomized controlled trial. *J Clin Endocrinol Metab* 2006; **91:**405–412.
16. Yin MT, RoyChoudhury A, Bucovsky M, *et al*. A randomized placebo-controlled trial of low- versus moderate-dose vitamin D3 supplementation on bone mineral density in postmenopausal women with HIV. *J Acquir Immune Defic Syndr* 2019; **80:**342–349.
17. Fried LP, Tangen CM, Walston J, *et al*. Frailty in older adults: evidence for a phenotype. *J Gerontol A Biol Sci Med Sci* 2001; **56:**M146–M156.
18. Zhu K, Austin N, Devine A, Bruce D, Prince RL. A randomized controlled trial of the effects of vitamin D on muscle strength and mobility in older women with vitamin D insufficiency. *J Am Geriatr Soc* 2010; **58:**2063–2068.
19. Cangussu LM, Nahas-Neto J, Orsatti CL, Bueloni-Dias FN, Nahas EA. Effect of vitamin D supplementation alone on muscle function in postmenopausal women: a randomized, double-blind, placebo-controlled clinical trial. *Osteoporosis Int* 2015; **26:**2413–2421.
20. Sharma A, Hoover DR, Shi Q, *et al*. Falls among middle-aged women in the Women's Interagency HIV Study. *Antivir ther* 2016; **21:**697–706.
21. Bandeen-Roche K, Xue QL, Ferrucci L, *et al*. Phenotype of frailty: characterization in the women's health and aging studies. *J Gerontol A Biol Sci Med Sci* 2006; **61:**262–266.
22. Erlandson KM, Plankey MW, Springer G, *et al*. Fall frequency and associated factors among men and women with or at risk for HIV infection. *HIV Med* 2016; **17:**740–748.
23. Charlier R, Knaeps S, Mertens E, *et al*. Age-related decline in muscle mass and muscle function in Flemish Caucasians: a 10-year follow-up. *Age (Dordr)* 2016; **38:**36.
24. Charlier R, Mertens E, Lefevre J, Thomis M. Muscle mass and muscle function over the adult life span: a cross-sectional study in Flemish adults. *Arch Gerontol Geriatr* 2015; **61:**161–167.
25. Ross AC, Manson JE, Abrams SA, *et al*. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011; **96:**53–58.
26. Janssen I, Baumgartner RN, Ross R, Rosenberg IH, Roubenoff R. Skeletal muscle cutpoints associated with elevated physical disability risk in older men and women. *Am J Epidemiol* 2004; **159:**413–421.

Accepted 17 January 2021; published online 11 March 2021