Calcidiol levels and muscle function maintenance, functional capacity and bone mineral bone density in non-selected Spanish population

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Summary

Introduction: Vitamin D offers beneficial effects that reportedly help maintain musculoskeletal function.
Aim: To analyze the effect of calcidiol levels on muscle function in both hands, on activities of daily life and on changes in bone mineral density (BMD) in an unselected population.
Material and methods: The EVOS study cohort was used, which carried out, among others, measures of muscular strength of grip in both hands, questions related to difficulty in performing daily activities, densitometric study in the lumbar and hip spine, and biochemistry to determine the levels of calcidiol.
Results: Calcidiol values ≥20 ng/mL were associated with greater grip strength in both hands. After adjusting for age, sex, BMI and seasonality, calcidiol levels <20 ng/mL were independently associated with lower grip strength only in the left hand (OR=2.35; 95% CI: 1.03-5.38). Likewise, the inability or difficulty to "pick up a book or object from a high shelf" and "get up from the bed" were significantly associated with calcidiol levels <20 ng/mL. Levels of calcidiol <20 ng/mL were associated with greater BMD losses in the femoral neck and total hip. These associations were maintained in the multivariate analysis.
Conclusions: Maintaining levels of calcidiol ≥20 ng/mL was associated with greater muscular strength of grip in the hands, maintenance of daily activities and lower BMD losses in the hip. This study corroborates the utility of maintaining adequate levels of vitamin D to maintain musculoskeletal function.

Key words: calcidiol, muscle strength, functional capacity, bone mineral density.

Introduction

The aging process is associated with a loss of muscle mass and strength, as well as a decrease in bone mineral density (BMD), which can lead to reduced mobility, greater risk of falls and the appearance of fractures[1,2]. In recent years, special emphasis has been placed on maintaining an adequate vitamin D status to optimize muscle strength and BMD in order to reduce falls and fractures[3-5]. Although a recent meta-analysis questions the usefulness of vitamin D supplements to reduce the risk of falls, BMD decrease and fractures[6], there are sufficient arguments that demonstrate the importance of vitamin D on muscle and bone health. Vitamin D stimulates the absorption of calcium from the intestine and maintains the serum calcium levels that are required for normal bone mineralization and for the maintenance of muscle function[7]. Several *in vivo* studies suggest vitamin D's role in regulating muscle mass and its function. Observational studies show that vitamin D deficiency in the elderly is associated with reduced muscle mass and strength[8-10], lower physical performance[8,11], and increased risk of falls[12]. In addition, a meta-analysis of 17 clinical trials showed that supplementation with vitamin D in subjects with basal calcidiol levels below 10 ng/mL had a positive effect on hip muscle strength[13]. These studies suggest that vitamin D can affect muscle mass and function. However, it is not clear whether vitamin D plays a direct or indirect role. In recent years, the local conversion of calcidiol to calcitriol, the most active vitamin D metabolite, which is synthesized mainly in the kidney through its precursor calcidiol, has been increasingly important[7]. This local synthesis has been reported in several other cell types, such as in oste-

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oblasts[14-17], prostate cells[18] and monocytes[19], which reinforces the importance of reaching adequate levels of calcidiol in the body.

Therefore, the aim of our study was to analyze in an unselected population the effect of calcidiol (25-OHD) levels on muscle strength in both hands, activities of daily life related to the functional capacity of the individual and the changes in the BMD.

Material and methods

The initial study protocol was designed to ascertain the prevalence of vertebral fracture. To do this, 624 men and women over 50 years of age were randomly selected from the municipal registry of Oviedo, Spain. The protocol consisted of all subjects completing a questionnaire on risk factors related to osteoporosis. This questionnaire was designed for the EVOS study, translated into several languages, and had an adequate reproducibility index[20, 21]. Similarly, the entire cohort underwent two lateral radiographs (this radiographic study was not completed in only two cases), the collection of anthropometric measurements such as height and weight to determine the body mass index (BMI), and a densitometric study. All subjects had sufficient ambulatory capacity to climb two floors without a lift and 99% lived in their own home.

After the prevalence study, this cohort was followed prospectively for 4 years by means of 2 postal questionnaires to find out the incidence of non-vertebral osteoporotic fracture. In the fourth year of the follow-up period (between the second and the third postal questionnaire), participants who had answered at least one of the two previous questionnaires were invited to repeat the same tests performed in the prevalence study, to which measures of muscular strength of grip in both hands were added to him with a dynamometer that owns a scale that goes from the minimum 0 to the maximum of 300 mm of Hg, a survey with 12 items on the difficulty or not to carry out daily life activities, as well as a biochemical study of general markers and bone and mineral metabolism. In this second cross-sectional study, 404 subjects participated (212 women and 192 men), of which 322 agreed to take part in the biochemical study. A total of 32 subjects (9.9%) were excluded from the analysis as they had undergone osteoporotic treatment, including treatment with vitamin D. From a total of 290 subjects, we had all the data in both cross sections.

Densitometric evaluation

The BMD was measured with a Hologic® QDR-1000 DXA densitometer (Hologic Inc., Waltham, Massachusetts, USA). In all cases, the anterior-posterior lumbar spine (L2-L4) and the density of the right femur were analyzed. For the evaluation of lumbar BMD, 4 subjects with marked degenerative osteoarthritis were excluded. The coefficients of variation (CV) were 1.2% and 1.9%, respectively[22]. The long-term daily quality control was followed by a *phantom* of the lumbar spine, with CV=0.0±0.1%[20]. In the fourth year of the follow-up period, BMD was also determined in the same areas as those measured in the first cross-sectional study, using the rate of change in BMD between both cross-sectional studies as a method to evaluate BMD development over time.

Biochemical analysis

In the fourth year of follow-up and over 1 year, a sample of blood and urine was taken in fasting from each subject: 33% of the blood samples were taken in the spring, 12% in the summer, 32% in the autumn and 23% in winter. Once the serum was separated, it was stored frozen together with the urine at -80ºC until the analyzes were carried out. Serum levels of calcium, creatinine, total alkaline phosphatase and resistant tartrate acid phosphatase were determined using an autoanalyzer (Hitachi Mod. 717, Ratigen, Germany). The serum levels of calcidiol (25OHD) were determined by previous extraction with acetonitrile (IDS, Ltd., Bolton, United Kingdom), whose intra- and interassay coefficients of variation (CV) were, respectively, 5.2% and 8.2% respectively.

Levels of 1,25-dihydroxyvitamin D were measured by radio-immunoassay (IDS, Ltd.); the intra- and interassay CVs were 6.5% and 9%, respectively. Intact levels of PTH were measured using radio-immunoassay methods (Nichols Institute, San Juan de Capistrano, California, USA); the intra- and interassay CV values were 2.6% and 5.8%, respectively.

All the studies carried out followed the principles set out in the Helsinki Declaration and were formally approved by the Clinical Trials Committee of the Principality of Asturias.

Statistic analysis

The analysis of the data was carried out using version 17.0 of SPSS for Windows. The quantitative variables were analyzed by Student's t test. The qualitative variables analyzed by chi square.

To analyze at multivariate level the effect of calcidiol levels on muscle strength, the muscular strength of grip in both hands was categorized as 0 for values equal to 300 mm Hg (maximum pressure of the dynamometer) and 1 for values <300 mm of Hg. The logistic regression analysis was adjusted for age, sex, BMI and seasonality (season of the year in which blood extraction was carried out).

To study the association between the performance of daily life activities with serum levels of calcidiol, a logistic regression analysis was carried out after adjusting for age, sex, BMI and seasonality.

When statistically significant associations were found between the levels of calcidiol and the rate of change in BMD in the univariate analysis, a linear regression was performed adjusted for age, sex, BMI and seasonality.

Results

Table 1 shows sociodemographic, anthropometric, clinical variables and biochemical markers of the cohort analyzed as a function of serum levels of calcidiol. In those with calcidiol levels of ≥20 ng/mL, there was a predominance of men, younger age, higher BMD values in all the skeletal segments analyzed, lower frequency of previous fractures, higher levels of calcitriol and lower levels of PTH and total alkaline phosphatase.

Calcidiol values ≥20 ng/mL (28.6% of the cohort) were associated with greater grip strength in both hands compared to levels <20 ng/mL (Figure 1). After adjusting for age, sex, BMI and seasonality, calcidiol levels <20 ng/mL alone were associated independently with decreases in grip strength (OR=2.35; 95% CI: 1.03-5.38). On the other hand, that association was lost in the right hand (OR=1.91; 95% CI: 0.92-3.98).

The daily life activities as a function of calcidiol levels are reflected in table 2. Of the 12 activities analyzed, the inability or difficulty to "lean to catch a soil object" was significantly associated with lower levels of calcidiol (p=0.009, table 2).

Table 1. Demographic, anthropometric characteristics, clinical variables and biochemical markers as a function of serum levels of calcidiol

	Calcidiol <20/mL	Calcidiol ≥20 ng/mL	Value of p
Sex man (n)	95 (45.4%)	53 (63.9%)	0.005
Age (years)	69.0 ± 8.4 (n=207)	65.7 ± 8.2 (n=83)	0.002
BMI (kg/cm²)	28.3 ± 84.2 (n=207)	27.7 ± 3.3 (n=83)	0.249
BMD lumbar spine (g/cm²)	0.932 ± 0.179 (n=153)	1.003 ± 0,158 (n=61)	0.007
BMD femoral neck (g/cm²)	0.743 ± 0.129 (n=207)	0.788 ± 0.130 (n=83)	0.008
Total hip DMO (g/cm²)	0.858 ± 0.147 (n=207)	0.910 ± 0.149 (n=83)	0.007
Vertebral fracture according to Genant (n)	36 (17.4%)	11 (13.3%)	0.362
Previous fracture (n)	53 (25.6%)	11 (13.3%)	0.032
Falls (n)	49 (23.7%)	27 (20.5%)	0.532
Calcium (mg/dL)	9.4 ± 0.3 (n=207)	9.4 ± 0.4 (N=83)	0.547
Calcidiol (ng/mL)	11.7 ± 4.2 (n=207)	27.7 ± 7.4 (n=83)	<0.001
Calcitriol (pg/mL)	38.7 ± 14.4 (n=207)	47.8 ± 18.2 (n=83)	<0.001
PTH (pg/mL)	55.4 ± 24.5 (n=207)	45.6 ± 18.7 (n=83)	<0.001
Total alkaline phosphatase (UI/L)	183 ± 76 (n=207)	162 ± 54 (n=83)	0.025
Creatinine (mg/dL)	1.06 ± 0.16 (n=207)	1.01 ± 0.19 (n=83)	0.051
FATR (U/L)	2.1 ± 0.7 (n=207)	2.0 ± 0.5 (n=83)	0.547

Likewise, the difficulty or inability to: "get out of bed" was associated with lower levels of calcidiol; "Pick up a book or object from a high shelf"; "Leaning from a chair to take object from the floor"; "Remove the stockings or socks" and "run 100 meters without stopping" (Table 2). Only "bending down to get an object from the floor" and "getting up from the bed" were significantly associated with calcidiol levels after multivariate adjustment for age, sex, BMI and seasonality. Thus, increments of 10 ng/mL of calcidiol were associated with a decrease of 30% and 58%, respectively, in the difficulty or inability to "bend over to pick up an object from the floor" or to "get up from bed".

The stratification of calcidiol levels showed that, in the multivariate adjustment, the presence of calcidiol deficiency (<10 ng/mL) not only significantly increased the inability or difficulty to "get out of bed: (OR=2.14; 95% CI: 1.21-3.77)" but also to "take a book or object from a high bookshelf: (OR=2.02; 95% CI: 1.09-3.73)", "lean from a chair to take object from the floor: (OR=1.78; 95% CI: 1.03-3.07)" and "remain seated in a hard chair for 1 hour: (OR=1.78; 95% CI: 1.03-3.07)".

The percentage of change in BMD at the level of the lumbar spine, femoral neck and total hip as a function of the serum levels of calcidiol is shown in table 3. The presence of calcidiol levels <20 ng/mL was associated with greater losses of BMD, both at the femoral neck and total hip level, with no significant differences at the lumbar level. After a multivariate analysis, changes in BMD at femoral neck and total hip level were independently associated with calcidiol levels <20 ng/mL (typified beta coefficient=0.130, p=0.041 and typified beta coefficient=0.142, p=0.033, respectively).

Discussion

In this study, low levels of calcidiol (<20 ng/mL) have been found to contribute to a lower muscular strength of grip in the hands, to more difficulties to perform certain activities of daily life, as well as to greater losses of BMD in the hip.

There are both basic and clinical evidences that support the participation of vitamin D in skeletal muscle function[23]. In recent work in people with spinal cord injuries requiring rehabilitation, low levels of calcidiol were independent predictors of decreased physical function[24]. The lower muscle strength of grip in relation to the low levels of calcidiol found in our study has also been reported by other authors[25]. Thus, in a longitudinal study of Dutch adults, aged between 55 and 85 years, serum levels of calcidiol below 10 ng/mL were associated with a 40% loss in grip strength compared to baseline[26].

Figure 1. Grip strength measurements (mm Hg) in hand A) left; B) right according to the serum levels of calcidiol. *p<0.05 for calcidiol <10 ng/mL and calcidiol between 10-20 ng/mL

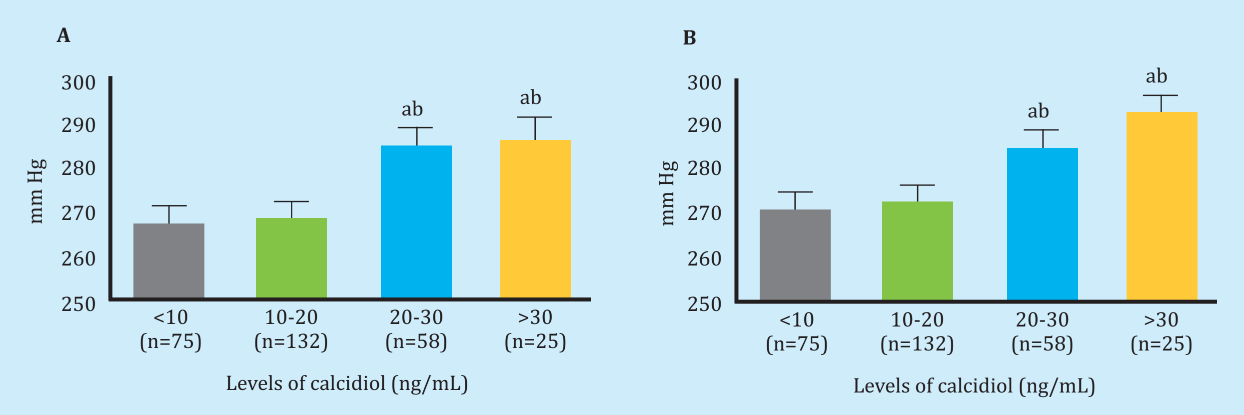

Table 2. Levels of calcidiol (ng/mL) depending on the difficulty or not to perform certain activities of daily life

	Without difficulty	Unable to do it or with difficulties	Value of p
Take a book or object from a high shelf	16.9 ± 8.9 (n=232)	13.5 ± 8.4 (n=58)	0.009
Carry an object of 10 kg for 10 meters	16.8 ± 8.6 (n=169)	15.4 ± 9.4 (n=120)	0.186
Wash and dry yourself	16.3 ± 8.8 (n=257)	15.7 ± 10.4 (n=33)	0.718
Lean forward to pick up an object from the ground	17.4 ± 9.2 (n=174)	14.6 ± 8.3 (n=116)	0.009
Wash your hair in a sink	16.3 ± 8.8 (n=251)	15.8 ± 9.7 (n=39)	0.728
Sit one hour in a hard chair	16.7 ± 8.6 (n=203)	15.2 ± 9.7 (n=85)	0.179
Standing in a queue for 30 minutes	17.0 ± 9.2 (n=157)	15.4 ± 8.6 (n=133)	0.137
Get up in bed	17.4 ± 9.2 (n=214)	13.1 ± 7.2 (n=76)	0.000
Remove socks or similar clothes from the feet	17.0 ± 9.0 (n=193)	14.7 ± 8.7 (n=97)	0.040
Leaning from a chair to pick up an object from the floor	17.4 ± 9.0 (n=183)	14.4 ± 8.4 (n=107)	0.006
Raise a box of 6 full bottles and place them on a table	16.8 ± 8.5 (n=176)	15.5 ± 9.5 (n=114)	0.226
Running 100 meters without stopping	17.4 ± 9.2 (n=167)	14.8 ± 8.4 (n=123)	0.015

Table 3. Percentage of change in BMD at the level of the lumbar spine, femoral neck and total hip between the two cross-sectional studies as a function of serum levels of calcidiol

	Calcidiol <20/mL	Calcidiol ≥20 ng/mL	Valor de p
% change in BMD at the lumbar level	-0.59 ± 4.14 (n=153)	0.39 ± 5.29 (n=61)	0.193
% change in BMD at femoral neck level	-1.49 ± 5.29 (n=160)	-0.10 ± 5.09 (n=66)	0.036
% change in BMD at the total hip level	-0.30 ± 3.61 (n=160)	1.08 ± 4.54 (n=66)	0.017

In our study, some activities of daily life were found to be compromised by the low levels of calcidiol, this effect being more marked in the presence of calcidiol deficiency (<10 ng/mL). The activities that were most affected were those that had more to do with the functional capacity of the organism than those dependent on greater muscular strength such as "transporting an object of 10 kg for 10 meters" or "lifting a box with 6 full bottles and place them on a table. " Other recent studies have also associated low levels of vitamin D with the greatest difficulty in performing activities of daily living. Thus, in a recent study by Arbex Borim et al., the reduction of muscle strength or dynapenia combined with low levels of calcidiol were found to be a risk factor that conditioned the development of daily life activities in a sample of 4,630 people over 50 free of disability at the outset of the study, followed for 2 years[27]. Similarly, Wicherts et al., analyzing a study of men and women between 65 and 88 years old, found that those with calcidiol levels below 20 ng/mL had a worse state and physical performance at both baseline and 3 years of follow-up compared to those with levels higher than 30 ng/mL[11]. Another Dutch prospective study showed that vitamin D levels were associated with functional limitations in the age stratum between 55 to 65 years and in those over 65 years[28]. However, others authors have not found any association between calcidiol levels below 10 ng/mL and lower hip flexion, knee extension force, grip strength, gait speed or disability in activities related to mobility of the upper extremities. This last study was carried out in 628 women over 65 years of age followed for 3 years and who presented a moderate to severe disability at the beginning of the study. The existence of very few participants with low calcidiol values may have limited the possibility of obtaining differences[29].

The association between levels of calcidiol and BMD revealed in a recent meta-analysis is more contradictory[6]. Epidemiological evidence indicates that the highest levels of calcidiol are associated with higher BMD in both the young and aging population, maintaining a linear relationship to levels of 30 ng/mL, an association that does not seem so clear and solid in black populations. or Hispanics of North America[30]. Our data indicate a direct association between calcidiol levels <20 ng/mL and BMD at femoral neck and total hip level. A 2014 meta-analysis concluded that there was very little evidence that vitamin D influenced BMD, since there was no consistent relationship between vitamin D supplementation and BMD in most of the anatomical sites analyzed (lumbar spine, total hip, trochanter, whole body or forearm), although a positive association was observed in the femoral neck, as was observed in our study[31]. Similarly, a recent article shows that patients with hip fractures have lower levels of calcidiol, lower bone mass, decreased bone quality and an increased risk of fracture[32]. It is important to highlight that in our study all subjects who were receiving treatment for osteoporosis, including supplements with vitamin D, were eliminated, which does not allow us to assess the possible effect of vitamin D supplementation on bone mass.

Our study has limitations, but also strengths. Regarding the former, the fact of having a single biochemical determination (after 4 years of follow-up) without knowing the values at the beginning of the study limits the associations found. On the other hand, the questionnaire on difficulties to carry out activities of daily life was not self-administered but administered by an interviewer, which could have biased the responses of the participants, especially in those questions that referred to personal hygiene difficulties. As strengths, the analyzed cohort participated in the EVOS-EPOS study, being one of the few groups that completed all the study guidelines. The participation percentages of more than 80% in the four postal follow-ups carried out during 8 years guarantee the representativeness of the sample analyzed. In addition to the articles published with data from the full cohort of the EVOS-EPOS study, the cohort of the city of Oviedo, which has been used for this study, has contributed individually to the publication of several original articles in high-impact journals[33-39].

To sum up, calcidiol levels above 20 ng/mL are associated with greater muscle grip strength in the hands, better performance in activities of daily life such as "bending over to pick up an object from the ground" and "Get up from the bed" and with a greater BMD in total hip and femoral neck, suggesting that maintaining calcidiol levels above 20 ng/mL would favor an adequate musculoskeletal function.

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