LOW CALCIDIOL LEVELS AND RISK OF PROGRESSION OF AORTIC CALCIFICATION

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MINI-ABSTRACT

In this observational study, we found a positive relationship between low calcidiol levels and the risk of aortic calcification progression. A 10 ng/mL increase of calcidiol was associated with a decrease in the risk of progression by 44%. This figure was higher than that observed if we increased age by 10 years.

Conflict of interest:

None

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ABSTRACT

Introduction: The aim of this study was to investigate the relationship between serum calcidiol levels and the onset and progression of aortic calcifications in a community-based sample of ambulatory subjects.

Methods: 302 men and women aged 50 and over underwent two lateral X-rays and were followed-up for 4 years. Abdominal aortic calcifications were classified as absent, mild-moderate, and severe. The biochemical measurements of serum calcium, phosphorus, PTH, total alkaline phosphatase, tartrate-resistant acid phosphatase, creatinine, calcidiol, calcitriol and osteocalcin were determined. Subjects who had received anti-osteoporotic treatments were excluded from the analysis.

Results: Subjects with progression of aortic calcifications had significantly lower serum calcidiol levels than those without progression. In the multivariate analysis, using the agreed upon serum levels for calcidiol (> 30 ng/mL) as the reference, those subjects with calcidiol levels between 10 to 20 ng/mL showed a higher risk of progression of aortic calcification (OR = 3.95; 95% CI = 1.16 to 13.40). An even higher OR was observed in subjects with calcidiol values < 10 ng/mL (OR = 4.10; 95%CI = 1.12 to 14.99). In addition, an increase by 1 ng/mL in osteocalcin levels was associated with a 17% reduction of the risk of aortic calcification progression.

Conclusions: An increase by 10 ng/mL of calcidiol was associated with a decrease in the risk of aortic calcifications progression by 44%. This figure was even higher than that observed if we increased age by 10 years. Levels of calcidiol higher than 30 ng/mL seem to be desirable to reduce the progression of aortic calcification and to maintain bone turnover.

INTRODUCTION

The vitamin D hormonal system consists of different metabolites which act upon the target organ receptors. The best indicator of vitamin D status is the concentration of 25-hydroxyvitamin D (25-OH D) or calcidiol. Several factors determine calcidiol levels, including those that affect the skin's synthesis of vitamin D through ultraviolet radiation, nutrition, and all factors which can modify vitamin D metabolism (1).

Adequate levels of calcidiol have been defined according age, serum PTH levels, calcium absorption, bone mass and fracture risk (2). A preliminary consensus based on the adequate monitoring of the previously mentioned parameters considered that $\geq$ 30 ng/ per millilitre of calcidiol can be considered as a reasonable good indicator of sufficient vitamin D replenishment (2-3).

Vitamin D deficiency causes musculoskeletal disorders, but it may also have an impact on the cardiovascular system (4-6), and on other several tissues and organs (7). Despite all these remarkable clinical observations, prospective data are still needed to better understand the impact of vitamin D deficiency on the cardiovascular system (8). Thus, we investigated the relationship between serum calcidiol levels and the onset and progression of aortic calcifications in a community-based sample of ambulatory subjects.

MATERIAL AND METHODS

A sample population of 302 ambulatory men and women aged between 50 and 89 was randomly selected from the Oviedo Municipal Register as part of the European Vertebral Osteoporosis Study (EVOS) (9). This cohort underwent a cross-sectional baseline evaluation consisting of an X-ray of dorsal and lumbar spine following a standard EVOS protocol (9). The X-ray evaluation was conducted again after four years. All X-rays were blindly evaluated for aortic calcifications and vertebral fractures by two independent observers.

Just as in a previous published study (10), aortic calcification on the lateral lumbar X-ray was defined and scored as follows: 0 (absent), 1 (mild-moderate), or 2 (severe). Isolated punctiform calcifications, a linear visible calcification less than two vertebral bodies, or one dense plaque were defined as mild-moderate aortic calcifications. Linear visible calcification along two or more vertebral bodies, and/or two or more dense plaques were defined as a severe aortic calcification (10). The intra-observer and inter-observer agreements on the X-rays analysed were 92% and 90% respectively, with Kappa coefficients of 0.78 and 0.73, indicating a good reproducibility.

The progression of aortic calcification was determined comparing the X-rays obtained at baseline with the X-rays repeated 4 years later. Progression of aortic calcification was defined under two circumstances: a) if a new aortic calcification appeared, or b) if the score of aortic calcifications increased in the second X-ray.

Bone mineral density (BMD) was measured by a Hologic® QDR-1000 (Hologic Inc., Waltham, MA) DXA densitometer. Both the antero-posterior lumbar spine (L2-L4) and the right femur (femoral neck) were analysed. For the lumbar BMD evaluation, subjects with marked degenerative osteoarthritis were excluded. Routine daily quality controls

ensured densitometer calibration, using lumbar spine phantom provided by the manufacturer. The coefficients of variation (CV) were 1.2% for the lumbar spine site and 1.9% for the femoral neck site.

A fasting blood sample was drawn in the morning from each subject for one year: 26% of the blood samples were collected in spring, 14% in summer, 36% in autumn, and 24% in winter. Once the serum was separated, it was kept frozen at 70ºC until the analyses were carried out. Serum calcium, phosphorus, creatinine, total alkaline phosphatase, and tartrate-resistant acid phosphatase (TRAP) were determined by using an autoanalyser (Hitachi Mod. 717, Ratigen, Germany). The serum 25-OH D (calcidiol) levels were determined by previous extraction with acetonitrile (IDS, Ltd., Bolton, UK), whose intra- and interassay coefficients of variation (CV) were 5.2% and 8.2%, respectively.

Serum 1,25-dihydroxyvitamin D (calcitriol) levels were measured using radioimmunoassay (IDS, Ltd.); intra- and interassay CV were 6.5% and 9%, respectively. The serum intact PTH and osteocalcin levels were measured using radio-immunoassay methods (Nichols Institute, San Juan Capistrano, CA); intra- and interassay CVs were 2.6% and 5.8%, respectively, for PTH and 4.5% and 5.1%, respectively, for osteocalcin. Biochemical markers were only measured when the second X-ray examination was conducted.

Subjects who had received anti-osteoporotic treatments - including any kind of vitamin D treatment -, during the period between the X-rays assessments performed at baseline and 4 years later were excluded from the analysis (n=34).

Statistical Analysis

The analysis of the data was carried out using SPSS version 17.0 for Windows. In both sexes, differences in the rate of progression of aortic calcifications were expressed in percentages with 95% confidence intervals and compared by a chi-square test. The progression of aortic calcifications was compared by using logistic regression analyses. The analyses were adjusted for all variables that showed any significant association in the univariate analysis and also for the season in which vitamin D levels were measured.

RESULTS

The characteristics of the population with or without aortic calcification progression are shown in table 1. In subjects exhibiting aortic calcification progression, age, waist/hip index and serum calcium were higher, whereas serum calcidiol and osteocalcin levels were lower. The percentage of current smokers was also higher in subjects with aortic calcification progression. However, the progression of aortic calcification was not associated with lumbar spine or femoral neck bone mineral density (BMD). In addition, no seasonal differences in the blood collection were found between subjects with and without progression of aortic calcifications

The progression of aortic calcifications was significantly higher in men than in women (52.7% vs. 34.8%, p<0.001). Similarly, in both sexes, the score of aortic calcifications increased in the second X-ray (28.0% in men and 18.9% in women, p<0.05) and it was slightly higher than the presence of new aortic calcifications (25.7 in men and 15.9 in women, p=0.082). No regression of calcification score was observed in either men or women.

In the subjects with aortic calcification progression, serum calcidiol levels were significantly lower than in those without progression (table 1). In fact, whereas only 26.1% of subjects with adequate calcidiol levels (> 30 ng/mL) showed aortic calcification progression, the percentage increased to 45.5% in those with calcidiol levels between 20 and 30 ng/mL , 45.6% in those with calcidiol levels between 10 and 20 ng/mL and rose to 47.7% in those with calcidiol deficiency (< 10 ng/mL) (figure 1). In contrast, none of the 6 subjects with calcidiol levels higher than 40 ng/mL showed aortic calcification progression.

Logistic regression analyses showed that a 10 ng/mL increase of calcidiol was associated with a 44% reduction in the risk of progression of aortic calcifications (table 2). This outcome was slightly higher than that obtained by increasing by 10 years the progression of aortic calcifications (34%, table 2). In addition, an increase of 1 ng/mL in the osteocalcin levels was associated with a 17% reduction of the risk of aortic calcification progression.

Using > 30 ng/mL as the reference and adequate calcidiol serum levels, the logistic regression analysis after adjusting by the same covariates used in table 2 showed that those subjects with serum calcidiol levels between 20 and 30 ng/mL did not feature a significant increase in the risk of aortic calcification progression, although a tendency was observed, odds ratio (OR) [OR = 3.31; 95% Confidence Interval (95% CI) = 0.92 to 11.95]. Those subjects with calcidiol levels between 10 and 20 ng/mL showed a higher OR (OR = 3.95; 95% CI = 1.16 to 13.40). An even higher OR was detected in subjects with calcidiol < 10 ng/mL (OR = 4.10; 95%CI = 1.12 to 14.99). Also, the multivariate analysis performed after adjusting by baseline aortic calcification values showed identical results.

DISCUSSION

During the last decade, it became clear in Western countries that insufficient and deficient serum vitamin D levels are highly prevalent, not only associated to some related diseases but also in the general population (7). Although calcitriol is the most active metabolite of the vitamin D hormonal system, the best indicator of vitamin D deposits or status is calcidiol concentration. Several studies have shown that the optimal levels of calcidiol to maintain not only an adequate bone metabolism but also other tissue functions, such as an appropriate muscular strength and cardiovascular health, should be greater than 30 ng/mL (2-3, 7, 11). Unfortunately, this figure is achieved in only 10% of our sample populations and others (8).

Our results indicate that an increased rate and aortic calcification progression are present not only when calcidiol levels are < 10 ng/mL, a figure which demonstrates a severe vitamin D deficit, but also with calcidiol levels in the 10-20 ng/mL range. In fact, the OR of aortic calcification onset or progression showed a stepwise increase which was inversely proportional to the serum calcidiol levels, reaching a value of 4.10 in those subjects with the most severe calcidiol deficit. Interestingly enough, each decrease by 10 ng/mL of calcidiol (almost equivalent to 1 standard deviation) was associated with 44% increase of the risk of aortic calcification progression; in our population, that value was slightly higher than the effect of increasing age by 10 years.

These findings are in agreement with the results of other cross-sectional studies performed in smaller cohorts that examined the association between vitamin D status and cardiovascular risk (4-6). Furthermore, recent prospective studies have also shown an association between vitamin D deficit and increased cardiovascular mortality (12-13).

As other authors have already pointed out (8), in order to explain the epidemiological results there are several mechanisms which help to understand the links between vitamin D deficiency and cardiovascular disease. Vitamin D deficiency leads to the stimulation of PTH gene transcription, and PTH increases, which in turn promotes myocyte hypertrophy and a vascular remodelling inflammatory mechanism which involves vascular smooth muscle cells releasing cytokines. The vitamin D hormonal system can also induce the suppression of inflammatory processes, which has been established as a key pathogenic mechanism in atherosclerosis and it can exerts an antiproliferative effect on myocardial cell hypertrophy and proliferation which underlies the pathogenesis of congestive heart failure. Finally, vitamin D also acts as a negative endocrine regulator for the renin-angiotensin system, which plays an important role in hypertension and cardiovascular health.

In or study, in addition to lower calcidiol levels, the progression of aortic calcifications was also independently associated with lower osteocalcin serum levels. Although this relationship is intriguing (14), a recent study of 328 people with diabetes in Japan demonstrated that higher osteocalcin serum levels were associated with a reduced vascular stiffness and lower carotid intimal medial thickness (15). All these findings are in agreement with recent studies which demonstrated an inverse relationship between the magnitude and progression of vascular calcification with the degree of bone mass and bone turnover, suggesting that vascular calcification may negatively influence bone turnover and bone mass (10, 16-17).

The association between aortic calcification progression and higher serum calcium values despite the presence of low calcidiol levels was an unexpected finding. However, this association may not be relevant, as in the univariate analysis serum calcium was associated with aortic calcification progression, mainly due to the effect of age

(bivariate correlation r=0.208, p<0.001). In fact, the relationship was no longer present in the multivariate analysis. Other authors have recently presented similar results (18).

No association was found between calcitriol levels and aortic calcification progression. Despite calcitriol being the most biologically active form of vitamin D, it is generally accepted that measurement of calcidiol serum levels does not provide any information on the vitamin D stores. Moreover, some previous studies have found no relationship (not even inverse) between plasma levels of calcidiol and calcitriol (19-21). These data suggest the correction of the calcidiol deficiency or insufficiency might reduce the risk of vascular calcification.

As other observational studies, we are aware of other likely confounders such as indication bias, confounding, and reverse causation. However, the direct measure of calcidiol status rather than relying on self-reported vitamin D intake or sunlight exposure, the participation of ambulatory-only subjects, together with presence of asymptomatic aortic calcifications, makes our study design less susceptible to have these kinds of bias and confounders.

Unfortunately, calcidiol levels were only measured during the follow-up. This important limitation prevents us from knowing whether exposure preceded aortic calcification progression. Other possible limitation of this study is the fact that aortic calcifications have been evaluated only by X-rays, instead of by more sensitive and sophisticated techniques such as electron beam or multidetector computed tomography imaging. However, the diagnoses of vascular calcification and arterial plaques has been recently compared with the above mentioned techniques, and it has been found that X-ray assessment is still a good marker of vascular calcifications and cardiovascular disease, even more useful than techniques such as measurement the thickness of the carotid intimae media (22-23).

Unfortunately, aortic calcification was not evaluated in the large EVOS cohort; thus the number of subjects who took part in our study prevented analyzing the main results separately by sex, and may limit the strength of the calcidiol concentration-dependent associations found. However, this same cohort has previously showed consistent and coherent results (10-11, 24).

In summary, severe calcidiol deficiency or insufficiency were associated with a higher risk of aortic calcification progression, which was up to 4 times higher than the risk observed in subjects with normal calcidiol levels. The progression of aortic calcification was associated to lower osteocalcin serum levels. Finally, calcidiol levels higher than 30 ng/mL seem to be desirable to reduce the aortic calcification progression and to maintain bone turnover.

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Legend

Figure1. Percentage of aortic calcification progression according to serum calcidiol levels.
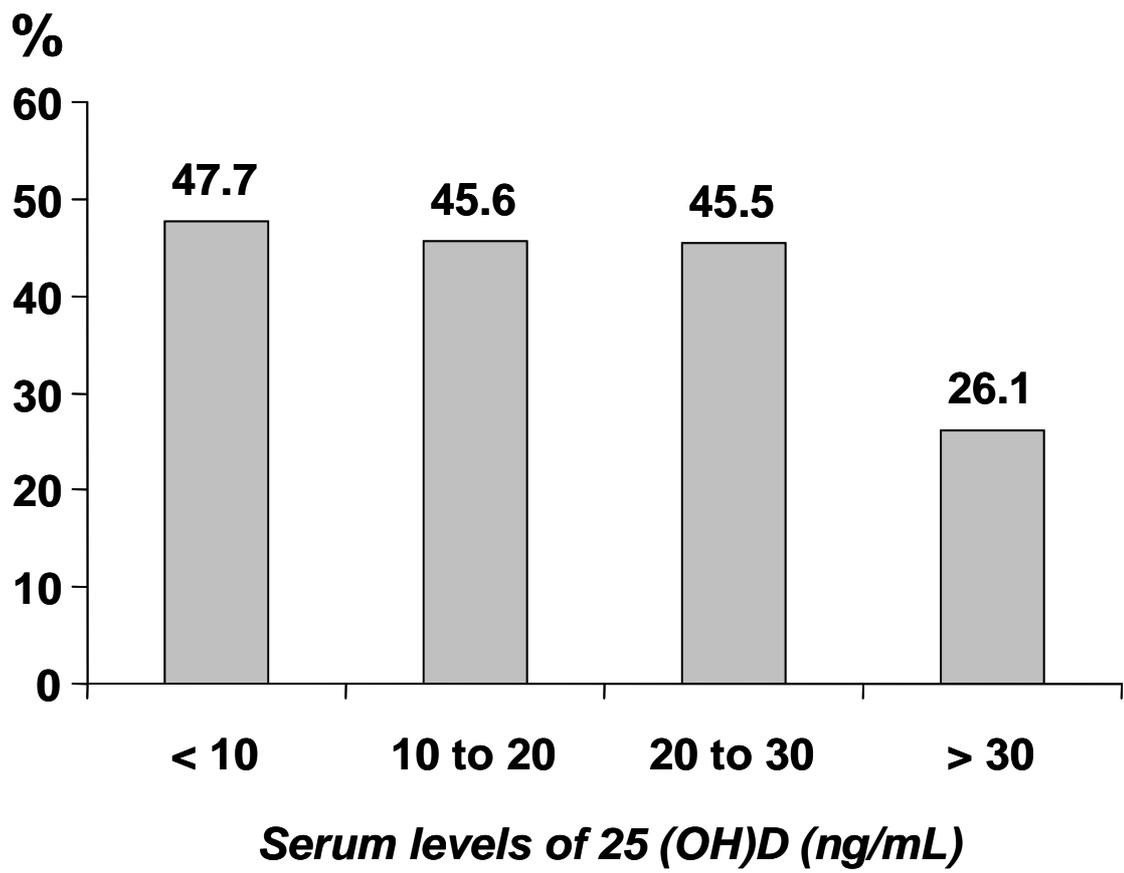
%

47.7	45.6	45.5	26.1
< 10	10 to 20	20 to 30	> 30

Serum levels of 25 (OH)D (ng/mL)

Table 1. Characteristics of the population with and without aortic calcification progression.

	Without progression (n=154)	With progression (n=127)	
	Mean±SD	Mean±SD	p value
Age (years)	67±9	70±8	**0.007**
BMI (kg/m^2)	28.4±4.1	28.0±3.9	0.425
Waist/hip index	0.91±0.11	0.95±0.10	**0.001**
Lumbar BMD (g/cm^2)	0.954±0.150	0.973±0.211	0.452
Neck BMD (g/cm^2)	0.756±0.114	0.758±0.146	0.889
Serum Ca (mg/dL)	9.35±0.34	9.44±0.31	**0.026**
Serum P (mg/dL)	3.45±0.48	3.46±0.45	0.891
Serum PTH (pg/mL)	51±20	55±27	0.203
Serum 25-OHD (ng/mL)	17.5±10.1	15.3±7.4	**0.048**
Serum 1,25-OHD (pg/mL)	40±15	44±17	0.062
Serum osteocalcin (ng/mL)	6.3±2.5	5.5±1.8	**0.005**
Clearance (mL/min)	68.8±16.4	65.4±17.3	0.100
	N (%)	N (%)	
Sex male	63 (42.3)	73 (61.3)	**0.002**
Vertebral fractures	17 (11.4)	13 (10.9)	0.904
Current smokers	17 (11.4)	26 (21.8)	**<0.001**

Table 2. Logistic regression analysis

Dependent variable	Independent variable	OR	95% CI	P value
Progression of aortic calcifications	Age (10 years)	1.34	1.01 – 1.70	0.049
	Current smokers (yes)	3.07	1.34 – 7.02	0.008
	25-OHD (each 10 ng/mL)	0.56	0.21 - 0.92	0.017
	Osteocalcin (each ng/mL)	0.83	0.72 – 0.96	0.011

The analysis was adjusted by age, sex, serum Ca, serum levels of 25-OHD, serum levels of osteocalcin, current smokers, season and waist/hip index.