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Bone health and coronary artery calcification: The Rotterdam Study

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ABSTRACT

Objectives: Vascular calcification has been associated inconsistently to low bone mineral density and fractures. The aims of the present study were to investigate the associations between coronary artery calcification (CAC) and BMD change, BMD and fracture risk in elderly subjects of the population-based Rotterdam Study.

Methods: BMD was assessed through dual-energy X-ray absorptiometry and CAC through Electron-Beam Computed Tomography in 582 men and 694 women. We investigated the associations between BMD change (6.4 years follow-up) and CAC at follow-up and between BMD and CAC (measured simultaneously). In sensitivity analyses we stratified analyses for estradiol levels in women. The association between CAC and fracture risk (9 years follow-up) was tested through competing-risks models. Models were sex-stratified and adjusted for age, body mass index, smoking, bisphosphonate use and age at menopause.

Results: There was no association between BMD change and CAC in men. In women, each 1% increase in annual BMD loss was significantly associated with higher follow-up CAC [β = 0.22 (0.06–0.38), p = 0.006; prevalence ratio: 4%]. Stratified analyses showed significant associations between BMD loss and follow-up CAC only in women with lower estradiol levels. We found no association between CAC and fracture risk and no association between BMD and CAC cross-sectionally.

Conclusions: BMD loss was associated with higher follow-up CAC in women, which might be related to low estrogen levels. No association between CAC and BMD or fracture risk was found. Further studies are required to elucidate the mechanisms that might underlie the association between BMD change and coronary calcification in women.

1. Introduction

Osteoporosis and cardiovascular disease (CVD) are common age-related diseases that have an increased co-existence independent of shared risk factors such as increased age, menopause, physical inactivity, alcohol intake and vitamin D deficiency [1]. Common pathophysiological mechanisms have been proposed such as inflammatory cytokines, oxidized lipids, increased homocysteine levels and decreased estrogen levels [1].

Vascular calcification is defined as the abnormal deposition of calcium in the vascular system [2]. Formerly considered a passive consequence of atherosclerosis, it is nowadays recognized as a highly active process associated with an increased risk of cardiovascular events independently of other traditional risk factors [3]. The resemblance that ectopic calcification shares with the normal calcification process of bone is remarkable and several studies [4,5] have verified the observation made by Virchow in 1863 that cardiovascular calcification is “an ossification, not a mere calcification” [6].

The increased co-existence of vascular calcification with osteoporosis [7] is called the *calcification paradox*. It has motivated several investigators to evaluate whether bone mineral density (BMD) and vascular calcification (VC) in several vascular beds are

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associated beyond the aging process and independent of potential confounders [8–14]. Among studies with a cross-sectional design, an inverse relation between aortic or coronary artery calcification (CAC) and BMD has been reported by some [8,9] but not others [10,11]. In contrast, longitudinal studies have consistently shown that increased BMD loss is associated with increased aortic vascular calcification assessed through different imaging modalities, such as X-rays and radiogrammetry [12,13] as well as through computed tomography [14], this relation has not been explained by aging and other shared risk factors and has been found mainly in women. Longitudinal studies evaluating the association between bone turnover and CAC have been performed mainly in subjects with chronic kidney disease, and results have been inconsistent; while some studies have shown that low bone turnover is associated with increased risk of CAC [15] others have not replicated such findings [16].

Studies addressing the association between vascular calcification and fracture risk have focused mainly on aortic calcification, and the results have been conflicting. While some of them have reported an increased fracture risk with increased vascular calcification [14,17], other studies have not found such results [11,18].

Since previous studies found an association in women between BMD loss and aortic vascular calcification we aimed to investigate whether in the prospective population based Rotterdam study changes in BMD are associated with vascular calcification measured in the coronary arteries (CAC) in either sex and whether CAC is associated with incidental fractures and BMD. We also studied whether findings can be explained by hormonal status or bone turnover.

2. Materials and methods

2.1. Study population

The Rotterdam Study is a prospective cohort study of elderly men and women designed to investigate the incidence and determinants of chronic disabling diseases. Rationale and design have been described elsewhere [19]. The Rotterdam Study I cohort (RS-I) was initiated in 1990 and consisted of 7983 participants. All subjects were >55 years at recruitment and reside in Ommoord, a district in Rotterdam and they have been assessed at baseline and through four follow-up visits. BMD was measured in all follow-up evaluations of the participants, and CAC scores were measured at RS-I-3 visit (third evaluation of the RS-I cohort). In total, 1276 subjects had available information on CAC levels, previous BMD measurements and incident fracture data (Fig. 1). The Rotterdam Study was approved by the Medical Ethics Committee of Erasmus MC.

2.2. DXA scanning

BMD was assessed using dual-energy X-ray absorptiometry (DXA). Trained radiographic technicians performed BMD measurements for participants at the first visit (1990–1993) and the third visit (1997–1999) with a GE Lunar DPX-L densitometer. For the longitudinal analysis of BMD change and its association with follow-up CAC, absolute annual percent BMD change at the femoral neck was calculated with the formula [100*($BMD_{RS\text{-}I\text{-}1} - BMD_{RS\text{-}I\text{-}3}$)/($BMD_{RS\text{-}I\text{-}1}$)*time length between measurements] [20], with a positive value reflecting BMD loss. Results are expressed per 1% increase in annual femoral neck BMD loss. Femoral neck BMD (from henceforth referred to simply as BMD) was chosen, as it is not affected by degenerative changes seen with age as lumbar spine BMD and has been proposed for defining osteoporosis in

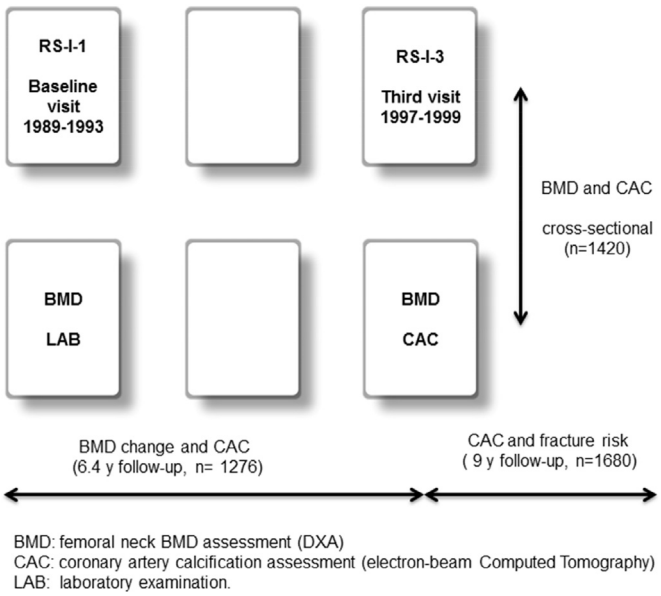

Fig. 1. Flowchart for time line, design and sample size for the analyses.

epidemiologic studies [21]. For the cross-sectional analyses of BMD and CAC, BMD is expressed in sex-specific standard deviations (SD).

2.3. Coronary artery calcification assessment

At the third visit of the Rotterdam Study all participants who completed the third phase of the Rotterdam Study were invited to participate in the Rotterdam Coronary Calcification Study [22]. Epicardial coronary arteries calcification was detected by electron-beam Computed Tomography (EBT; C-150 Imatron Scanner, GE Healthcase, South San Francisco, CA). Before the subjects were scanned, they performed adequate breath-holding exercises. From the level of the root of the aorta through the heart, 38 images were obtained with a 100-ms scan time and a 3-mm slice thickness. During one breath hold, images were acquired at 80% of the cardiac cycle by using echocardiographic triggering. Quantification of coronary calcification was performed with AccuImage software (AccuImage Diagnostics Corporation, South San Francisco, CA) displaying all pixels with a density >130 Hounsfield Units (HU). The presence of calcification was defined as a *minimum* of 2 adjacent pixels (area = 0.65 mm^2) with a density >130 HU. Calcium scores were calculated by multiplying the area in mm^2 of individual calcified lesions with a factor based on the peak density of the lesion. The total calcification score for the entire epicardial coronary vascular system comprised the sum of the scores for all individual lesions.

2.4. Fracture assessment

Fracture events were obtained from computerized records of general practitioners (GPs) in the research area (covering 80% of the cohort); additionally research physicians regularly followed participant information in the GP's records outside the research area. All reported events were verified by two trained research physicians, who independently reviewed and coded the information. Finally, all coded events were reviewed by a medical expert for final classification according to the International Classification of Diseases, tenth revision (ICD-10) [23]. Participants were followed from the date of the CAC scan until January 1, 2007, or until a first fracture or death occurred.

2.5. Covariates

Several covariates known to influence both BMD and coronary artery calcification scores (CACs) [4,24–26] were included in the regression models, particularly age, smoking, body mass index (BMI) and medication use (missingness <2%). BMI was calculated in kg/m^2, from height and weight measured in standing position without shoes. BMI change was calculated as the absolute difference between measurements in the first and third visit of the Rotterdam Study. Smoking status was assessed by interview and coded as never-, former- and current smokers. Cigarette pack-years (for former and current smokers) were calculated as duration of smoking (in years) multiplied by the number of smoked cigarettes, divided by 20. Regarding medication use information, more than 99% of participants collected their drug prescriptions at seven regional pharmacies, which are fully computerized. Complete drug use information is available as of January 1st, 1991. The pharmacy data include the Anatomical Therapeutical Chemical (ATC) code from the World Health Organization (WHO) Collaboration Centre for Drug Statistics Methodology, the collection dates, total amount of drug units and product names of the drugs. Adjustments in our analyses were done for bisphosphonate [2] and hormone replacement therapy (HRT) use [27] due to the fact that both medication types have potential beneficial effects on vascular calcification. Bisphosphonate use was defined as exposure to the antiresorptive medication of at least 365 cumulative days before the date of the CAC scan. Further adjustments were done for serum lipid reducing therapy (mainly statins) and diuretic use, due to its effects on BMD and potential influence in coronary artery calcification [28].

Baseline comorbidity status was included in several models, namely prevalent diabetes mellitus, heart failure, peripheral artery disease and myocardial infarction; definition of such cases has been previously described elsewhere [29–32].

Laboratory covariates included in the analyses were 17β-estradiol (pmol/L) and alkaline phosphatase (missingness of 78% and 21%, respectively). For these measurements, non-fasting blood samples were drawn by venipuncture at the baseline visit between 0830 and 16 h. Platelets were removed by centrifugation and samples were stored at −80 C until measurements. 17β-estradiol (E2) was measured by direct immunoassay, and alkaline phosphatase (AP) was measured through an enzymatic colorimetric method. Other covariates included for further adjustments were total cholesterol, creatinine, 25-hydroxyvitamin D, serum calcium and phosphate levels, measured from blood samples obtained at baseline as previously described [19]. Intake of dietary calcium and Vitamin D was assessed by interview at baseline for food intake assessment using an extensive semi quantitative food frequency questionnaire (FFQ) at the study center by a trained dietician [19].

Additionally, analyses done for women were adjusted for age at menopause, collected by interview in the first visit.

2.6. Statistical analysis

Due to high skewness of the CAC measurements distribution that could not be completely corrected after log transformation, the association between BMD or BMD change and CAC scores was tested through generalized linear models, allowing Gaussian but also non-normal distributions for continuous variables. Log-transformed CAC scores (Ln(CAC + 1)) were set as the dependent variable, with either BMD or BMD change as independent variables, adjusted for potential confounders. Fitness of different models was compared through the Akaike Information Criteria – AIC [33], models with lower values corresponding to a better fit. For assessment of the CAC score status in a binary fashion (yes/no), prevalence ratios were obtained with a *log* link instead of *logit* link, due to the fact that odds ratios overestimate the relative risks when the outcome is highly prevalent [34]. Assessments were made for BMD change and prevalent CAC at the third visit of the Rotterdam Study, between CAC and subsequent fractures, and cross-sectionally for BMD and prevalent CAC both measured during the third visit (see Fig. 1).

As part of sensitivity analyses, we tested the significance of the interaction terms between BMD change with 17β-estradiol and alkaline phosphatase levels in those subsets with these measurements available (n = 161 and n = 556 with 17β-estradiol and alkaline phosphatase levels available, respectively) and performed stratified analysis according to 17β-estradiol (pmol/L) and alkaline phosphatase (U/L) levels, setting the cut-off point at the median value. Furthermore, analyses were performed after exclusion of participants with prevalent cardiovascular disease.

The association between CAC scores (at third visit) and incident fractures during follow-up was tested using competing-risks regression models which yield hazard ratio estimates and allow for informative censoring [35]. In this setting, the outcome of a fracture might not be seen because death occurs first, mainly because important risk factors for fracture incidence are shared for all-cause mortality [36]. For this analysis, the beginning of the follow-up period was set as the date of the CAC scan. The proportionality assumption was tested building interaction terms with time.

Analyses were performed with subjects with complete information on covariates, exposure and outcome.

SPSS (version 21.0, Armonk, NY: IBM Corp) and Stata (version 12, College Station TX: Stata Corp LP) were used for analyses. Statistical significance was defined as $p < 0.05$.

3. Results

General characteristics of the population with information available on BMD change and CAC are displayed in Table 1. Age and BMI were similar between men and women. Men had higher BMD, lower BMD loss rate, heavier smoking habits, and almost six-times higher CAC scores than women. CAC prevalence was high in both men and women (more than 85%).

The association between BMD change at the femoral neck (between baseline and third visit over an average of 6.4 y period) and

Table 1
General characteristics of the study population of 1276 men and women with information available on both BMD change and CAC.

	Men (n = 582)		Women (n = 694)	
	Visit 1	Visit 3	Visit 1	Visit 3
Age (y)[a]	64.1 (59.9–68.2)	70.5 (66.4–74.9)	63.7 (59.8–68.2)	70.2 (66.3–74.7)
BMI (kg/m^2)[a]	25.9 (24.2–27.9)	26.2 (24.4–28.4)	25.9 (23.6–29.0)	26.8 (24.1–30.0)
BMD (g/cm^2)[b]	0.93 (0.13)	0.91 (0.13)	0.86 (0.13)	0.81 (0.13)
Annual FN BMD change (%)[a]	–	0.37 (−0.18–0.86)	–	0.78 (0.22–1.37)
Prevalent CAC (%)	n/a	569 (98%)	n/a	591 (85%)
CAC score[a]	n/a	271.5 (58.3–925.8)	n/a	48.7 (4.4–289.8)
Age at menopause[a] (y)	n/a	n/a	50.0 (46–52)	
Smoking (%)[c]	544 (93%)	535 (92%)	372 (54%)	369 (53%)
Prevalent CV disease[d] (%)	121 (21%)	–	94 (13%)	–

[a] Median and interquantile range.
[b] Mean and standard deviation.
[c] Current and former smokers.
[d] Prevalent cardiovascular disease, defined as prevalent myocardial infarction, heart failure or peripheral artery disease.

follow-up CAC is depicted in Table 2. We found no significant associations in men [$\beta = -0.02$ (95%CI: −0.20–0.17), p = 0.85); CAC prevalence ratio of 1%, p = 0.16]. In women, we found that each 1% increase in annual BMD loss was significantly associated with higher CAC score on follow-up [$\beta = 0.22$ (0.06–0.38), p = 0.006] and with higher CAC prevalence ratio of 4% (p = 0.007). Adjustment for bisphosphonate use (n = 48 users among a total of 1276 analyzed subjects) did not essentially change results. Additionally, adjustments for prevalent diabetes mellitus status, lipid lowering therapy (mainly statins) use, diuretic use, and levels of 25 hydroxyvitamin D, calcium, phosphate, creatinine and total cholesterol and dietary intake of calcium and vitamin D at baseline yielded similar results (data not shown). These "full-model" analyses were performed in a smaller subset of participants with available information in all mentioned covariates (n = 235 men and n = 290 women).

We investigated a potential relation between CAC scores and any type of fracture (total number of events = 254; Table 3). We found no associations for any type of fracture incidence in either sex (Table 3).

We performed a cross-sectional analysis of BMD and CAC scores at the third visit (see Fig. 1), and found no association for either sex (men: $\beta = -0.03$ (−0.20–0.13), p = 0.68; women: $\beta = 0.01$ (−0.16–0.19), p = 0.89). Likewise, BMD was not associated with CAC prevalence in either sex in this cross-sectional analysis (Supplementary Table 1).

3.1. Sensitivity analysis

To further explore the association between BMD loss and follow-up CAC, we built interaction terms between BMD loss and two categories of 17β-estradiol (E2) and alkaline phosphatase (AP) stratified by the median values (n = 161 and n = 556 women with E2 and AP measurements available, respectively). The *p* value results for both interaction terms were suggestive ($p = 0.13$); therefore we proceeded to stratify the analysis of BMD loss and CAC by median level of E2 and AP. Table 4 shows that the associations between BMD loss and CAC seems to be confined to women with E2 levels below the median [$\beta = 0.55$ (0.08–1.03), $p = 0.02$] and to women with AP levels above the median [$\beta = 0.36$ (0.12–0.60), $p = 0.003$].

In addition, we investigated the influence of HRT use (n = 119 HRT users) and prevalent CVD (n = 96 women) on the relationship between BMD change and follow-up CAC in women, and the results remained robust after these additional analyses (data not shown).

Table 2
Annual percent BMD change at femoral neck and CAC scores in RS-I-3.

	Model I			Model II		
CAC as continuous variable						
	n	**β (95% CI)**[a]	***p***	**n**	**β (95% CI)**[a]	***p***
Men	582	−0.02 (−0.20–0.17)	0.85	582	−0.02 (−0.21–0.17)	0.83
Women	694	**0.22** (0.06–0.38)	0.006	694	**0.23** (0.07–0.39)	0.005
CAC as binary variable[b]						
	n	**PR (95% CI)**[c]	***p***	**n**	**PR (95% CI)**[c]	***p***
Men	582	1.01 (0.99–1.02)	0.16	582	1.01 (0.99–1.02)	0.16
Women	694	**1.04** (1.01–1.07)	0.007	694	**1.04** (1.01–1.07)	0.007

Statistically significant results are highlighted in bold.
Model I: adjusted for age, BMI, delta BMI, smoking; in women also age at menopause.
Model II: adjusted for covariates in Model I + bisphosphonate use before the date of the scan.
[a] β from linear regression for log CAC scores for 1% annual increase in BMD loss ($100*[BMD_{RS\text{-}I\text{-}1} - BMD_{RS\text{-}I\text{-}3}]/[BMD_{RS\text{-}I\text{-}1}]$*time length between measurements).
[b] CAC binary refers to presence/absence of CAC. Present CAC is defined as a CAC score above 0.
[c] Prevalence ratio of CAC for 1% annual increase in BMD loss.

Table 3
Risk of incidence of all types of fracture as a function of CAC scores at RS-I-3 (third visit).

	Model I			Model II		
	no. of fxs	HR (95% CI)[a]	*p*	no. of fxs	HR (95% CI)[a]	*p*
All-fracture incidence						
Men	83/808	1.01 (0.90–1.14)	0.80	64/615	0.96 (0.86–1.08)	0.48
Women	171/872	1.02 (0.95–1.10)	0.48	124/655	0.99 (0.91–1.07)	0.75

Hazard ratios derived from competing-risks regression models.
Model I. Adjusted for age, BMI and smoking at RS-I-3.
Model II. Adjusted for covariates in Model I + BMD at RS-I-3.
[a] Hazard ratios expressed per increase in log CAC.

4. Discussion

Overall we found that BMD loss (within an average period of 6.4 years follow-up) was significantly associated with higher follow-up CAC scores in women persisting after adjusting for multiple factors. This relationship was not observed for men, and we found no association of CAC scores with subsequent fractures in either sex.

Our results are in line with three previous longitudinal studies that reported a significant association between BMD loss and vascular calcifications in the aorta in women [12–14] but associations of BMD change with CAC have not been reported in the general population to the best of our knowledge. We hereby describe for the first time an association with CAC in a general population setting of elderly (aged over 55 years). The association we found was not confounded by age, smoking, changes in BMI or bisphosphonate treatment.

The fact that BMD loss was associated with CAC among women only might suggest involvement of underlying hormonal factors as potential mechanisms. Exploratory stratified analyses showed that the association of BMD loss with CAC scores was observed in those women with lower baseline estradiol suggesting that low E2 levels could be involved in the development of both coronary calcification and BMD loss.

We found a significant association in the subgroup of women with higher AP levels, which may reflect higher bone turnover status induced by estradiol deficiency in the postmenopausal state [37]. AP induces the degradation of pyrophosphate (Pi), that plays a key role in ectopic calcification inhibition [38] that otherwise would occur in most tissues due to the fact that collagen, ubiquitously present, acts as a potent nucleating agent for the deposition of hydroxyapatite crystals [39]. The increased AP levels in the postmenopausal state [40] may lead to lower Pi levels and therefore loss of inhibition of vascular calcification.

Vascular Smooth Muscle Cells (VSMC) can undergo differentiation towards an "osteoblast-like" phenotype, changing from a

Table 4
Annual percent BMD change at femoral neck and CAC scores in RS-I-3 (third visit) in women stratified by baseline 17β-estradiol (E2) and alkaline phosphatase (AP) levels.

	n	β (95% CI)[a]	*p*	n	β (95% CI)[a]	*p*
	E2 > 16.4 pmol/L[b]			E2 < 16.4 pmol/L[b]		
Women	81	−0.03 (−0.49–0.42)	0.88	80	0.55 (0.08–1.03)	0.02
	AP < 76 U/L[c]			AP > 76 U/L[c]		
Women	278	0.06 (−0.22–0.34)	0.68	278	0.36 (0.12–0.60)	0.003

Models adjusted for age, BMI, delta BMI, and smoking.
[a] β from linear regression for log CAC scores for 1% annual increase in BMD loss ($100*[BMD_{RS\text{-}I\text{-}1} - BMD_{RS\text{-}I\text{-}3}]/[BMD_{RS\text{-}I\text{-}1}]$*time length between measurements).
[b] E2 corresponds to baseline 17β-estradiol levels. Cut-off point was set at median value.
[c] AP corresponds to baseline alkaline phosphatase levels. Cut-off point was set at median value.

contractile to a synthetic state with subsequent secretion of extracellular matrix that eventually gets calcified [4,41]. There are several pathophysiological mechanisms that could explain the role that E2 plays in vascular calcification inhibition. In the first place, E2 prevents atherosclerotic plaque development [42], the only type of lesion that can get calcified in the coronary arteries as Mönckeberg's medial calcification does not occur in this vascular bed [43]. It has been previously shown that the administration of E2 decreases VSMC proliferation in animal and human models, through activation of nitric oxide synthase [44] and through decreased mitogen-induced VSMC proliferation. In second place, VSMC and endothelial cells express RANK, RANKL and OPG, and therefore can respond to RANKL stimulation. RANKL induces VC through an increase in bone morphogenetic protein 2 (BMP-2, the main stimuli of AP) and a decrease in matrix Gla protein (MGP), an inhibitor of VC. Importantly, E2 is able to attenuate RANKL-induced VC [42]. Therefore, through differential actions in the expression of key proteins, E2 preserves the original contractile VSMC features, decreasing trans-differentiation towards a calcifying phenotype [44].

The beneficial effects of E2 on the coronary bed have been reported only in women [45]. This observation may explain the absence of a significant association between BMD loss and CAC in men in our study, despite the fact that BMD loss in the aging men is also associated with estradiol deficiency [46]. Consistent with our results, Kiel and colleagues previously described a lack of association between BMD loss and aortic calcification in men from the Framingham cohort [12].

We observed no different association between BMD loss and CAC regarding previous HRT use, suggesting that perhaps exogenous estradiol administration did not counterbalance the loss of atheroprotective effects associated with menopausal-related endogenous 17β-estradiol decrease. However, it should be emphasized that the age of HRT initiation or its duration in women from our cohort might not have been appropriate or long enough respectively to achieve a protective effect against coronary calcification, as the majority of HRT users reported a treatment length of less than 5 years and a previous RCT showed a beneficial effect of HRT after an average of 8.7 years of treatment in women aged 50–59 y at enrollment [27]. Nevertheless, it is important to mention that the effects of estrogen in the vascular system are complex and robust evidence has proven that in general HRT lacks sufficient beneficial effects on cardiovascular disease in both primary and secondary prevention settings in postmenopausal women [47].

Similar to other prospective studies performed in aortic calcification [11,18], we found no significant association between CAC and all-fracture risk in either sex during a mean follow-up of 9 years. This analysis takes risk of death into account. Of note, significant associations between aortic calcification and fractures have been previously [17] reported in studies with cross-sectional designs or with utilization of odds ratios as estimates of relative risks precluding determination of causality for the calcification process on fracture risk. Furthermore, different devices in assessing bone mineral density, diversity in covariates adjusted for or different cohort characteristics might limit the comparability of results from multiple studies. We used electron-beam CT, which is a high sensible device to identify calcification and is superior to fluoroscopic measures; this is one of the strengths of our study.

Further strengths of our study include the prospective design in BMD change and fracture assessment with high completeness of follow-up [48] (more than 95%) that allows a better determination of how the natural history of disease might occur. The availability of several important confounders aid to decrease the bias introduced by risk factors that influence BMD loss and CAC. The assessment of longitudinal measurements of BMD using the same device avoided the need for calibration. The stratified analyses according to 17β-estradiol, AP levels and HRT use provide a deeper insight into the mechanisms and suggest that low estradiol levels may underlie both BMD loss and higher CAC but since these results derive from small subgroup analyses they require replication in larger (cohort) studies. There are other limitations. The analyses were performed in a subsample of the Rotterdam Study with available data on CAC measurement. However, despite some minor differences, characteristics of the responders to the Rotterdam Coronary Calcification Study were highly similar to those of the nonresponders [22]. Another limitation of the study is the lack of availability of PTH and FGF23 serum levels. Nevertheless, an association between long-term exposure of high PTH and vascular calcification has been demonstrated mainly in patients with renal dysfunction [49] and FGF23 does not seem to induce vascular calcification [50]. Despite multiple adjustments, residual confounding cannot be discarded. The fact that the entire cohort is composed of European Caucasians limits the generalizability of our findings to other populations or ethnic groups. Besides, the relatively short follow-up time available for the incidental fracture analysis might have limited our ability to detect an association between CAC and fracture risk. Furthermore, the stratified analysis according to E2 and AP levels were performed only in a small subset of women with such information available.

In conclusion, we found that BMD loss is significantly associated with higher CAC scores on follow-up in women only and we found no association between CAC levels and subsequent fractures. Our findings suggest that endogenous estradiol deficiency might underlie both pathological processes and thus be a shared risk factor for BMD loss and CAC but further studies are required to replicate these findings. Further research is warranted to explain the mechanisms that might underlie the association between BMD loss and CAC in women.

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The funding sources had no role in the study design, collection, analysis or interpretation of data, in the writing of the report or in the decision to submit the article for publication.

Disclosures

Authors have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2015.02.013.

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