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Bone Loss and the Progression of Abdominal Aortic Calcification Over a 25 Year Period: The Framingham Heart Study

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Abstract. Vascular calcification and osteoporosis are common age-related processes that are prominently displayed on routine lateral lumbar spine radiographs as dense calcium mineral deposits of the aorta that lie adjacent to osteopenic vertebrae. Using a population-based cohort of older men and women, we tested the hypothesis that the progression of vascular calcification of the abdominal aorta should be greatest in those individuals with the greatest amount of bone loss. From the original population-based Framingham Heart Study cohort, 364 women and 190 men had lateral lumbar spine and hand radiographs performed between 1966 and 1970 and repeated between 1992 and 1993. The lateral lumbar films were read for the presence of aortic calcification using a semiquantitative method, and the hand films were read for second metacarpal relative cortical area (MCA). Using multivariate regression techniques, the 25-year progression of the abdominal aortic calcification index was examined in relation to the change in the MCA, while adjusting for recognized risk factors for atherosclerotic cardiovascular disease. During the 25 years of follow-up, the MCA decreased by 22.4% in women (from 79.6 ± 7.8 (SD) to 61.8 ± 10.3) and by 13.3% in men (from 80.6 ± 6.9 to 69.9 ± 8.3). The aortic calcification score increased over eightfold in women (from 1.2 ± 2.7 (SD) to 9.9 ± 6.7) and sixfold in men (from 1.6 ± 2.8 to 9.6 ± 6.3). There was a significant association between percent change in MCA and change in aortic calcification index ($P = 0.01$) in women after controlling for all potential confounders. No association was observed in men ($P = 0.50$), including the 50% of men with the greatest bone loss. This is the first longitudinal study to show that women with the greatest magnitude of bone loss also demonstrate the most severe progression of abdominal aortic calcification, suggesting that the two processes may be related.

Key words: Osteoporosis — Pathologic calcification — Vascular disease — Cohort study — Aged

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Vascular calcification and osteoporosis are two common age-related processes that are prominently displayed on routine lateral lumbar spine radiographs as dense calcium mineral deposits of the aorta that lie adjacent to osteopenic vertebrae. Previous studies have demonstrated an association between these two phenomena [1–10], yet little is known about the natural evolution of these processes because there have been no longitudinal studies linking them. The two processes may represent independent age-related phenomena, or mobilization of calcium from the skeleton may contribute to deposition of calcium in developing atherosclerotic plaque. Some investigators have proposed that vascular compromise due to arterial calcification might, in itself, result in bone loss [8].

Atherosclerotic calcification has long been considered a late stage, unregulated sequelae of the atherosclerotic process; however, recent studies have shown that arterial calcification occurs early, progresses, and often precedes arterial narrowing [11, 12]. In fact, the process resembles bone formation [11, 13–15]. Furthermore, recent studies have implicated several possible metabolic linkages between vascular calcification and osteoporosis, including estrogen [16], vitamin K [17], vitamin D, lipid oxidation products [18, 19], and most recently, osteoprotegerin, a protein that regulates osteoclast activity and proliferation [20, 21]

Based on these observations, the hypothesis of this study was that the progression of vascular calcification of the abdominal aorta should be greatest in those individuals with the greatest amount of bone loss. This study was designed to investigate this hypothesis in a population-based cohort of older men and women enrolled in the Framingham Heart Study.

Material and Methods

Subjects

The Framingham Study began in 1948 with the primary goal of evaluating risk factors for heart disease in a population-based sample of the town of Framingham, Massachusetts. The original cohort consisted of 5209 participants (2873 women and 2336 men) who were 28–62 years old at the time of the first examination, from 1948 to 1951. These individuals have participated in followup examinations every 2 years since that time. At each examination, a medical history has been taken, and a physical examination and a series of laboratory tests have been performed [22].

Between 1966 and 1970, at the time of biennial examination 10–11, a postero-anterior radiograph of the right hand and a lateral lumbar spine radiograph was taken as part of an osteoporosis study. Of the 2408 women and 1791 men seen at either of those visits, 1394 and 1018, respectively, underwent postero-anterior hand radiography, and 1466 and 1049 had readable lateral lumbar X-rays taken. A total of 653 women and 633 men died during 25 years of followup. Of the surviving 1126 women and men, 576 returned to the Framingham clinic building to undergo both repeat hand X-rays and lateral lumbar spine X-rays (1992–1993). Of these, 364 women and 190 men had technically adequate films of both hand and spine at both times.

Assessment of Bone Mass

We used radiogrammetry to measure cortical bone mass of the second metacarpal on the right hand. We chose the second metacarpal because it is one of the largest bones of the hand, has a more constant shape than the other metacarpals [23], and is approximately circular at the midshaft, with the medullary cavity nearly centered in the tubular bone cylinder [23, 24].

Two readers, who were unaware of the status of the study participants with respect to aortic calcification, assessed cortical bone mass according to a standard protocol. Hand radiographs were placed flat on a lighted viewing box, and measurements of cortical external width (R) and medullary width (r) were made halfway up the second metacarpal with a digital caliper. Digital calipers were calibrated to the nearest 0.01 mm, and measurements were recorded to the nearest 0.1 mm. To assess intra-observer and inter-observer reliability in the measurement of cortical width, we gave 25 hand radiographs to each of the two readers twice for blinded readings. The intra-observer correlation coefficients for external and medullary width were 0.99 and 0.94, respectively; the corresponding inter-observer correlation coefficients were identical. We used the relative metacarpal cortical area, calculated as $100 \times (R^2 - r^2) \div R^2$, as an indicator of bone mass.

Assessment of Aortic Calcification

The semiquantitative method used to quantify the extent of aortic calcification in the abdominal aorta has been described in detail elsewhere [25]. Baseline lumbar spine radiographs from 1967 to 1970 and follow-up spine films from 1992 to 1993 were read without having knowledge of the metacarpal cortical area. In brief, calcific deposits in the abdominal aorta adjacent to each of the first four lumbar vertebrae were assessed separately for the posterior and anterior wall of the aorta using the midpoint of the intervertebral space above and below the vertebrae as the boundaries. Lesions were graded as follows: 0 = no aortic calcific deposits; 1 = small scattered calcific deposits filling less than one-third of the longitudinal wall of the aorta; 2 = one-third or more, but less than two-thirds of the longitudinal wall of the aorta calcified; 3 = two-thirds or more of the longitudinal wall of the aorta calcified. Individual level-specific severity scores for both the posterior and anterior walls were added to yield abdominal aortic calcification indexes ranging from 0 to 24. The inter-rater and intra-rater agreement (intra-class correlations) for this scoring system was 0.93 and 0.98, respectively, for baseline X-rays and 0.96 and 0.96, respectively, for follow-up X-rays.

Other Variables

Information was obtained on other atherosclerosis risk factors including age, body mass index (weight in kg/height in m^2), systolic blood pressure (measured by a physician using a standard sphygmomanometer, smoking (cigarettes per day), total and HDL cholesterol (measured on blood drawn from nonfasting subjects using nonenzymatic methods [26] until 1988 when enzymatic methods were used [27]), diabetes (casual glucose of more than 150 mg/dL on two or more visits, or treatment with insulin or oral hypoglycemic agents), previous history of coronary heart disease (CHD) (presence of myocardial infarction, angina pectoris, or coronary insufficiency) [28], and for women, estrogen use. These variables were available at the time of the first X-ray and the follow-up X-ray. In addition, since these variables were repeatedly assessed as part of the Framingham Study protocol, age, BMI, systolic blood pressure, smoking, presence of diabetes, and estrogen use were also available at every examination between the time of the baseline and follow- up X-rays. Total cholesterol and HDL cholesterol were available at two additional examinations (1978–1979 and 1988–1989). All aspects of this study were approved by the Boston University Institutional Review Board.

Statistical Analysis

Using analysis of variance for continuous variables and Chi-square tests for categorical variables, we compared the characteristics of the participants at the time of the baseline hand and lateral spine X-ray according to the quartile of change in relative metacarpal cortical area and the quartile of change in the aortic calcification index (Table 1). To obtain better control of age effects, subjects were classified according to age-specific quartiles using age groupings (<45 years, 45–49, 50–54, 55–59, 60–64, 65–69, and ≥70).

We constructed sex-specific regression models with *absolute change* in the aortic calcification index as the dependent variable and *percent change* in metacarpal cortical area as the independent variable. In order to evaluate a potential threshold effect, separate models were examined using the change in relative metacarpal cortical area as a categorical variable instead of a continuous variable. The cohort was divided into sex-specific quartiles of change in the metacarpal cortical area, and adjusted means were calculated for the change in the aortic calcification index across these quartiles using the least-squares means option of the PROC GLM program in SAS [29] (Fig. 2 and 3). To account for potential confounding, age, as well as the average values for body mass index, systolic blood pressure, cigarettes smoked, and total and HDL cholesterol measured repeatedly between the time of the baseline and follow-up X-rays were included in the models. For categorical variables diabetes and CHD, a subject was classified as having diabetes or a history of CHD if he or she was classified as having these diseases at any time from entry into the study to the time of the follow up X-ray examination. Estrogen use was modeled as the number of years that a woman reported estrogen use from the time of the baseline X-ray to the time of the follow-up X-ray.

We then repeated the models using change in aortic calcification as the dependent variable and baseline metacarpal cortical area as the independent variable, controlling for all of the covariates measured at the time of the baseline X-ray. Finally, cross-sectional analyses were performed at the time of the baseline X-ray measurements and at the time of the follow-up X-ray measurements (Fig. 3). For these cross-sectional analyses, covariates were concurrent with the time of the X-ray.

Results

The 554 subjects who survived from the time of the first

Table 1. Characteristics of the study participants by gender at the time of the baseline X-ray according to the age-specific quartile of change in abdominal aortic calcification index

Women	Age-specific quartile of change in aortic calcification index[a]			
Variable[b]	Lowest n = 86	2 n = 90	3 n = 94	Highest n = 94
Metacarpal cortical area	80.7 ± 8.2	79.1 ± 7.0	78.9 ± 8.5	79.7 ± 7.7
Age (years)	54.2 ± 5.0	53.9 ± 4.4	53.9 ± 4.7	54.1 ± 4.8
BMI (kg/m^2)	25.5 ± 4.2	25.3 ± 3.7	25.1 ± 3.1	24.7 ± 3.6
Total cholesterol (mg/dL)	230 ± 40	240 ± 43	248 ± 41	236 ± 35
HDL cholesterol (mg/dL)	62 ± 14	60 ± 16	60 ± 16	59 ± 17
Systolic BP (mmHg)	127 ± 18	128 ± 18	124 ± 20	131 ± 19
History previous CHD (%)	2.3	3.3	0	4.3
Currently smoking (%)[c]	22.4	26.7	39.4	44.6
Diabetes (%)	8.1	7.8	11.7	16.0
Current estrogen therapy (%)	17.9	21.1	16.3	13.2
Men	**Age-specific quartile of change in aortic calcification index[a]**			
Variable[b]	n = 45	n = 47	n = 53	n = 45
Metacarpal cortical area	81.3 ± 7.4	81.1 ± 6.3	79.9 ± 7.4	80.4 ± 6.6
Age (years)	53.8 ± 4.3	53.5 ± 4.5	53.9 ± 4.7	53.8 ± 4.6
BMI (kg/m^2)	26.2 ± 2.8	26.9 ± 3.1	26.9 ± 2.9	27.4 ± 3.1
Total cholesterol (mg/dL))	225 ± 36	223 ± 35	230 ± 39	221 ± 40
HDL cholesterol (mg/dL)	44 ± 12	46 ± 11	42 ± 11	47 ± 13
Systolic BP (mmHg)	124 ± 17	127 ± 14	132 ± 14	131 ± 16
History previous CHD (%)	2.2	2.1	0	11.1
Currently smoking (%)	28.9	36.2	34.6	38.6
Diabetes (%)[c]	2.2	14.9	17.0	24.4

[a] The "lowest" quartile represents subjects with the smallest increase in the lumbar aortic calcification index
[b] All means presented with their standard deviations
[c] $P < 0.05$ for trend

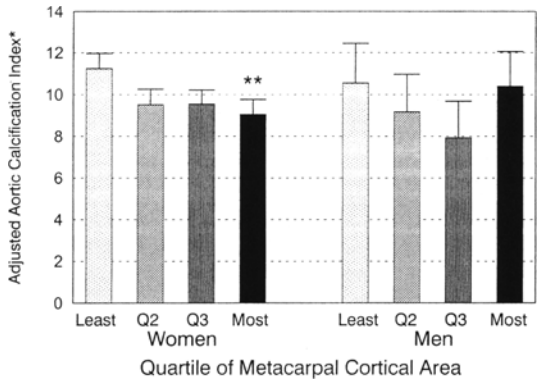


Fig. 1. Baseline and follow-up measurements of metacarpal cortical area and aortic calcification in men and women.

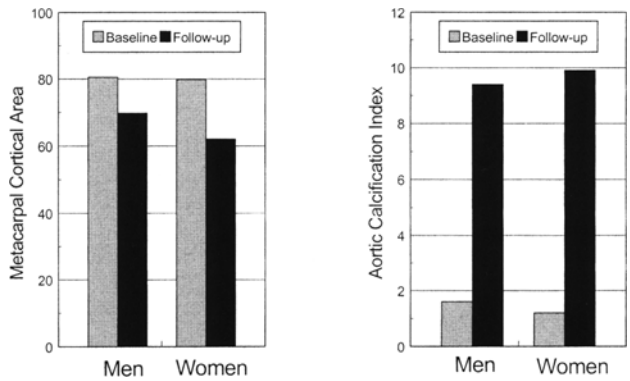
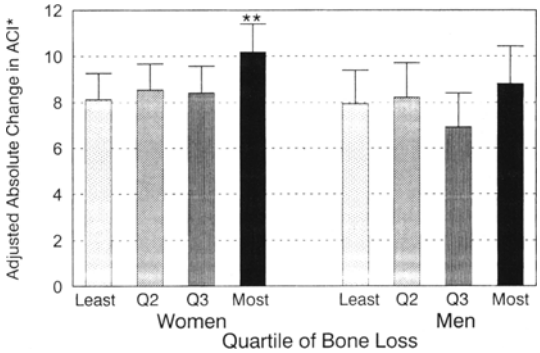


Fig. 2. Longitudinal results of absolute change (mean and 95% confidence interval) in abdominal aortic calcification index according to quartile of bone loss (absolute change in relative metacarpal cortical area) in women and men.

X-ray to the time of the follow-up X-ray and who had technically adequate films were 5 years younger at the time of the first X-ray (54.4 vs 59.2, $P < 0.0001$), were similar in gender (66% female, $P = 0.9$) and in body mass index (25.5 vs 25.7, $P = 0.3$), and had higher second metacarpal relative cortical area on their hand films at baseline (80.0 vs 76.1, $P < 0.0001$) than subjects who did not survive to have follow-up films. As shown in Figure 1, during the 25 years of follow-up, the relative metacarpal cortical area decreased by 22.4% in women (from 79.6±7.8(SD) to 61.8±10.3) and by 13.3% in men (from 80.6±6.9 to 69.9±8.3). The aortic calcification score increased over eight-fold in women (from 1.2±2.7 (SD) to 9.9±6.7) and six-fold in men (from 1.6±2.8 to 9.6±6.3).

Table 1 shows the characteristics of women and men at the time of the first X-ray according to the age-adjusted quartile of progression in aortic calcification index. In



* adjusted for age, BMI, sys BP, smoking, total chol, HDL chol, CHD, estrogen (women)
**$p<0.05$ compared with quartile 1

Fig. 3. Cross-sectional results of abdominal aortic calcification index (mean and 95% confidence interval) according to quartile of relative metacarpal cortical area in women and men at the time of the follow-up X-rays (1992–1993).

women, total cholesterol was different across quartiles of aortic calcification, although cholesterol values across quartiles did not follow consistently. In men, systolic blood pressure and prevalence of diabetes increased across quartiles of the aortic calcification index. No differences were observed in other baseline variables. There were no differences in any of the cardiovascular risk factors across age-adjusted quartiles of metacarpal cortical area.

When we looked at the association between percent change in metacarpal cortical area and change in aortic calcification index we observed that for each percent decline in metacarpal cortical area, the aortic calcification index increased 7.3%. ($P = 0.01$) in women after controlling for all potential confounders, including baseline aortic calcification index. In this model, the number of years of estrogen use was inversely associated with aortic calcification index although the P-value was 0.25. When the same analysis was repeated in men, for each percent decline in metacarpal cortical area, the aortic calcification index increased 3.3% ($P = 0.50$). Figure 2 shows the adjusted change in the aortic calcification index according to the quartile of change in metacarpal cortical area for women and men separately. In women, there was a significant association in that women in the highest quartile of bone loss had significantly greater adjusted increase in the aortic calcification score than women in the lowest quartile ($P = 0.02$). In men, no association was apparent between the progression of bone loss and the progression of aortic calcification. To determine if men with greater bone loss might experience a significant progression of vascular calcification, we repeated our analyses in the subgroup of men above the median value of bone loss and found no significant associations.

Similar analyses were undertaken to analyze the cross-sectional associations between metacarpal cortical area and aortic calcification. At the time of the baseline X-ray (1967–1970), there were no associations observed in either men or women (all P-values for models >0.23) between metacarpal cortical area and aortic calcification; however, at the time of the follow-up X-ray (1992–1993), women with the greatest bone mass had significantly lower aortic calcification scores than women with the lowest bone mass (Fig. 3). Again, no associations were observed for men. Finally, the analyses were repeated for the association between the baseline metacarpal cortical area and the change in the aortic calcification score, producing no significant findings in either men or women (data not shown).

Discussion

This is the first longitudinal study demonstrating that bone loss in women is related to the progression of vascular calcification. Women with the greatest magnitude of bone loss had the most severe progression of abdominal aortic calcification after controlling for age and other confounders, suggesting that the two processes may have a common etiology. The lack of an association in men raises the possibility that hormonal factors unique to women may play a role in the underlying pathophysiology, although due to smaller numbers of men, we had lower statistical power to find significant associations. In addition, men did not lose as much bone as women; however, even in the 50% of men with the highest rates of bone loss, there were no associations between bone loss and progression of vascular calcification.

Previous studies have examined the cross-sectional association of osteoporosis and vascular calcification using techniques such as photon and X-ray absorptiometry, quantitative computed tomography, radiogrammetry, and fracture history to measure osteoporosis, and radiographic criteria [1–10] and electron beam computed tomography of the heart [4] to measure vascular calcification. In contrast to all of the previous cross-sectional studies, the current study took advantage of 25-year longitudinal radiographic data to identify an association between the progression of aortic calcification and the progression of bone loss. In our cross-sectional analyses, we were unable to demonstrate an association in the baseline X-rays between abdominal aortic calcification and relative metacarpal cortical area, possibly because the prevalence of aortic calcification was so low in this age group (average age approximately 54 years). By the end of the 25-year follow-up period the prevalence of vascular calcification was greater, and there was a significant cross-sectional association between aortic calcification and metacarpal cortical area in women independent of age and other factors.

The positive findings in the current study, and those of Barengolts et al. [4] who used electron beam computed tomography, suggest the possibility that the different methods used to identify vascular calcification may partially explain conflicting findings in the literature. For example, in the current study, a radiographically based scale was created to grade the extent of aortic calcification, and in the study using electron beam computed tomography, the extent of

coronary calcification was quantified using a calcium score derived directly from the electron beam computed tomography scan [4]. The current aortic assessment methods create a range of calcification values. In contrast, in the largest study reported to date, no association was found between radiographic aortic calcification and bone mineral density after adjustment for age [9]. In that study, vascular calcification was graded as present or absent, and the severity of the lesions was not considered.

Several potentially important similarities in pathophysiology might explain the observed relation between vascular calcification and bone mineralization. Although a mechanistic link has not been proven, certain factors, such as parathyroid hormone, parathyroid hormone-related peptide, vitamin D, estrogen, and a variety of cytokines influence both vascular calcification and bone mineralization. In the process of bone mineralization, noncollagenous proteins (e.g., osteonectin, osteopontin, bone sialoprotein, osteocalcin, matrix gla protein) appear to be important for matrix organization and regulation of the mineralization process, and appear in newly forming bone in a precise temporal and spatial distribution. Many of these noncollagenous proteins have been found in calcification occurring in nonskeletal tissues such as heart valves and the vasculature [11, 30–33].

Genes may also be involved in both vascular calcification and bone metabolism. It has been recently reported that mice lacking the gene for matrix gla protein display prominent vascular (aorta and coronary arteries) and valvular calcification as well as osteopenia and fractures [17]. Similarly, mice lacking the gene for osteoprotegerin also display a phenotype of osteoporosis and vascular calcification [33], although the pattern of vascular calcification in the knockout mice for both matrix gla protein and osteoprotegerin does not appear to resemble that of the atherosclerotic plaque.

Any unifying etiology to our findings of an association between bone loss and aortic calcification must account for the observed differences between men and women. One potential candidate is estrogen. In our analyses, estrogen replacement therapy was significantly associated with less progression of vascular calcification in bivariate analyses ($r = -0.12$, $P = 0.02$), yet in the multivariate model, the P-value was no longer statistically significant. Studies in animals demonstrate that estrogen reduces the size of vascular lesions in carotid arteries and the aorta [34, 35] and accelerates endothelial cell growth *in vivo* [36]. These effects may also prevent the initiation of vascular calcification associated with atherosclerotic plaque formation. Preliminary evidence suggests that estrogen may have a negative effect on noncollagenous bone protein production within arterial plaques [16]. These noncollagenous proteins play an important role in bone mineralization, and are expressed in atherosclerotic plaque [11, 31, 32]. Although estrogen also plays an important role in skeletal homeostasis in adult men [37], the role of estrogen in vascular calcification in men is not known. Therefore, estrogen effects, including its metabolism and even expression of estrogen receptor genes, may still explain the differences we found between men and women.

There are several potential limitations of this study. First, the study sample includes only those men and women who survived to have the 25-year follow up X-rays. These individuals were younger and had greater metacarpal cortical area than those who did not survive. This unavoidable circumstance limits the generalizeability of these findings, and leaves open the possibility that there may be selective mortality in men and women with aortic calcifications and osteoporosis. Second, it is impossible to determine the direction of the association between vascular calcification and bone loss. One possibility is that factors contributing to bone resorption also play a role in vascular calcification. On the other hand, it is conceivable that vascular compromise due to calcified plaque could accelerate bone loss in tissues supplied by these diseased vessels. Another potential limitation of our study is the use of plain radiographs to assess both aortic calcification and bone mass. More sensitive techniques such as dual X-ray absorptiometry and CT scanning are available currently, but were obviously not used at the time of the initial assessment in our cohort. Thus our use of metacarpal cortical area was not a sensitive indicator of cancellous bone loss. Nevertheless, despite the use of these relatively crude imaging techniques, we were able to find important associations between loss of cortical thickness and the accumulation of aortic calcification in women. Finally, we were unable to support our longitudinal findings with a cross-sectional association between baseline vascular calcification and bone mass, nor an association between baseline aortic calcification and subsequent bone loss. While this might appear to weaken the overall validity of the study, it is quite possible that the prevalence of vascular calcification at the time of the initial X-ray was too low to be able to demonstrate an association.

This study supports the link between vascular calcification and bone loss in women. Although these two processes both accompany the aging process, our findings of a significant longitudinal association, after controlling for age and other confounders, suggest that the association is not based simply on age. Future studies of the biology underlying these degenerative conditions may uncover basic mechanisms that will lead to a greater understanding of their common etiology and perhaps treatments.

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