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Abstract—Atherosclerosis and osteoporosis are major causes of morbidity and mortality in postmenopausal women and have been suggested to be associated. No study has examined whether progression of atherosclerotic calcification is associated with bone loss. In the present study, we examined progression of aortic calcification, diagnosed by radiographic detection of calcified deposits in the abdominal aorta, in relation to metacarpal bone loss, as assessed by metacarpal radiogrammetry, during menopause. Initially premenopausal women (n=236), aged 45 to 57 years at baseline, were followed for 9 years. We additionally assessed the cross-sectional association between the extent of aortic calcification and metacarpal bone mass and density in 720 postmenopausal women. Twenty-five percent of women going through menopause showed progression of aortic calcification. The average loss of metacarpal bone mass among women with progression of aortic calcification was 3.2 mm^2, and their loss of metacarpal bone density was 7.2 mm^2 %, whereas in women without progression of aortic calcification, these losses were 2.0 mm^2 and 5.6 mm^2 %, respectively, adjusted for age and years of follow-up ($P<0.05$). Additional adjustment for age at menopause, body mass index, blood pressure, smoking, diabetes mellitus, and use of hormone replacement therapy, thiazide, and loop diuretics did not influence these results. In postmenopausal women, a graded inverse cross-sectional association between the extent of aortic calcification and metacarpal bone mass and density was found. In conclusion, our results indicate that progression of atherosclerotic calcification is associated with increased bone loss in women during menopause. **(*Arterioscler Thromb Vasc Biol*. 2000;20:1926-1931.)**

Key Words: atherosclerosis ■ vascular calcification ■ osteoporosis ■ menopause

Cardiovascular disease and osteoporosis are major causes of morbidity and mortality in postmenopausal women[1,2] and are generally considered unrelated. Several studies, however, indicate that atherosclerosis and osteoporosis are associated.[3–10] Calcification is a common feature of atherosclerotic plaques and is regulated in a way similar to bone mineralization.[11–16] The relation of vascular calcification to the pathogenesis of atherosclerosis and plaque rupture is not clear yet, but data indicate that moderate calcification of plaques contributes to vascular morbidity and mortality.[17–20]

Several cross-sectional studies have been conducted on the association between atherosclerotic calcification and osteoporosis among elderly women.[3–8,21–23] Most of these studies found an association,[3–8] although some did not.[21–23] Potential confounding factors other than age have not been taken into account in most of these studies.[3–5,8,21,22] No study examined whether progression of atherosclerotic calcification is associated with bone loss. Because the prevalence of atherosclerosis and osteoporosis increases from menopause onward,[24,25] the change from the premenopausal to the postmenopausal state may be an appropriate period to study this association longitudinally.

In the present population-based study, we examined the association between progression of aortic calcification and metacarpal bone loss during menopause in 236 women. In addition, we studied the cross-sectional association between the extent of aortic calcification and metacarpal bone mass and density in 720 postmenopausal women.

Methods

Population

Between 1975 and 1978, a population-based study on risk factors for chronic diseases was conducted in the Dutch town of Zoetermeer. Inhabitants of 2 districts were invited for a medical examination. In 1985, all female participants aged 45 to 64 years at baseline were invited for a follow-up examination. Details of that study have been previously published.[25,26] The response rate of the women at baseline was 77%. Of 1167 women invited for the follow-up study, 71 had died and 87 had moved away. Of the remaining women, 855 (85%) were reexamined.

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From the Department of Epidemiology & Biostatistics (A.E.H., H.A.P.P., A.H., J.C.M.W.) and the Department of Internal Medicine (A.E.H., H.A.P.P.), Erasmus University Medical School, Rotterdam, the Netherlands, and the Department of Clinical Epidemiology (A.M.v.H.), Leiden University Medical Centre, Leiden, the Netherlands.

Correspondence to Dr J.C.M. Witteman, Department of Epidemiology & Biostatistics, Erasmus University Medical School, PO Box 1738, 3000 DR Rotterdam, The Netherlands. E-mail witteman@epib.fgg.eur.nl

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Measurement of Aortic Calcification

Aortic calcification was diagnosed by radiographic detection of calcified deposits in the abdominal aorta.[26] At baseline and at follow-up, lateral abdominal films (T12-S1) were made from a fixed distance while the subject was seated. Aortic calcifications were considered present when linear densities were seen in an area parallel and anterior to the lumbar spine (L1-L4). Baseline and follow-up values for the extent of calcification were scored according to the length of the involved area ($\leq$1 cm, 2 to 5 cm, 6 to 10 cm, and $>$10 cm). In the analyses, we considered the first 2 classes as mild calcification and the third and fourth classes as advanced calcification.

Progression of calcification was defined as the occurrence of new calcifications or enlargement of the calcified area present at baseline. Baseline and follow-up films were examined in pairs. The extent of progression was graded, but because of the relatively small numbers in the categories, we combined severity grades into 2 groups: progression absent and progression present. No subject showed a decrease in the extent of aortic calcification.

All films were examined by 2 independent observers without knowledge of the metacarpal bone mass and density of the subjects. Before the scoring, a sample of the films was read by the 2 observers simultaneously so as to reach agreement on interpretation of the scoring protocol. Observers were aware of the date of the radiographs. If there were differences between observers regarding readings, films were reviewed by both observers simultaneously so as to reach consensus. The score that was agreed upon by both observers was recorded. The percentage of agreement for absence versus presence of progression was 88%, and the κ statistic was 0.74.

The validity of radiographic assessment of aortic intimal calcification was studied by comparisons made on necropsy material. The method was shown to be highly specific, and in most cases, visible calcification represented advanced atherosclerosis.[27] A comparison study with computed tomography in 56 unselected elderly subjects showed that calcifications that were detected on the abdominal x-ray in 32 subjects were independently shown to be located in the aorta on the corresponding CT images in all but 1 subject.[26] Moreover, aortic calcification is known to be associated with cardiovascular disease risk factors[26,28] and atherosclerosis at other sites[29] and to predict cardiovascular morbidity and mortality.[18,19] Comparison of roentgenographic aortic calcification with coronary artery calcium as detected by electron beam tomography within 457 subjects showed that aortic calcification was present in 3.9%, 13.7%, and 31.5% of the subjects within the lowest, the middle, and the highest tertile of coronary artery calcium, respectively (P for trend$<$0.001, adjusted for age and sex). These results indicate that aortic calcification is strongly related to coronary calcification.

Metacarpal Radiogrammetry

Anteroposterior radiographs of the hands were used for measurements of the cortical thickness of metacarpals II, III, and IV of both hands. At baseline and at follow-up, measurements of the outer diameter (D) and the medullar diameter (d) of the metacarpal bones were conducted at the midshaft with the use of a $\times$7 magnifying loupe with an accuracy of 0.01 mm. The metacarpal cortical area (MCA) was calculated as the mean value of D^2-d^2 for 6 metacarpals. As a standardization for differences in body size, the relative cortical area (RCA) was calculated. This was achieved by expressing the MCA as a percentage of the size of the metacarpal bone: $100\%\times(D^2-d^2)/D^2$ for each metacarpal bone.[30,31] The mean value of the 6 metacarpals was used for the analyses. The MCA and RCA can be interpreted as indicators of bone mass and bone density, respectively. For the MCA and the RCA, the total loss during follow-up was calculated by subtracting the baseline measurements from those at follow-up. The observers measuring the metacarpal bone mass and density were unaware of the aortic calcification score of the subjects.

We estimated the measurement precision of metacarpal radiogrammetry in 100 duplicate measurements. The mean intraindividual standard deviation of a duplicate measurement was 1.9 mm^2 (4% of the initial mean value) for MCA and 2.5 mm^2 % (3% of the initial mean value) for RCA, which is sufficient to allow inferences concerning bone loss after a 9-year period. In women, the mineral content of the metacarpals correlates well with that at other peripheral skeletal bone sites (r ranges from 0.75 to 0.96).[32] The accuracy of the measurement was demonstrated by Exton-Smith et al,[33] who found a correlation of 0.85 between the mineral content of the metacarpal cortical area and the ash mineral content of the metacarpal bones.

Menopausal State

Menopausal state was assessed by a self-administered questionnaire that asked whether the menses had stopped and, if so, at what age and the reason for their cessation (natural or artificial). The type of artificial menopause was ascertained during an interview by a doctor. Postmenopausal state was defined as no menstruation for at least 1 year.

Assessment of Covariates

Assessment of covariates was similar at baseline and at follow-up. Height and weight were measured without shoes and with indoor clothing. Body mass index was calculated (weight/height^2). Blood pressure was measured with a random zero sphygmomanometer with the subject seated. The mean of 2 readings was reported. Serum total cholesterol at baseline was measured by an automatic enzymatic method. During follow-up, a modified reagent was used (CHOD/PAP High Performance, Boehringer-Mannheim). Information on smoking habits and medical history was obtained by a self-administered questionnaire, which was checked during an interview by the study physician. Diabetes mellitus was considered present when it was reported in the questionnaire and confirmed during the interview with the physician. Subjects were asked to bring their current medication to the research center, where treatments were noted.

Population for Analyses

Of the 855 women examined at follow-up, menstruation had ceased for $<$1 year in 7 women, and for 11 women, information on menopausal state was missing. Because films were missing or not readable, information on aortic calcification and/or metacarpal bone density was missing in 45 women, leaving 792 postmenopausal women. Of these women, 282 were premenopausal at baseline. Data on progression of aortic calcification or bone loss were missing in 27 women. Age at menopause could not be ascertained for 19 women, leaving 236 women for the analyses of the association between progression of aortic calcification and bone loss. The mean duration of follow-up for these women was 8.9 ± 0.8 years. For the cross-sectional analyses in postmenopausal women at follow-up, we excluded women with missing information on age at menopause only if their age at follow-up was $<$60 years (n=72), because we assumed elderly women to be postmenopausal. This left 720 postmenopausal women for the cross-sectional analyses at follow-up.

Data Analysis

Initially, we compared continuous baseline characteristics between premenopausal women with and without progression of aortic calcification during follow-up by use of a general linear model, adjusted for age. Dichotomous variables were compared by a χ^2 test.

We used a general linear model to compute and compare adjusted mean values of metacarpal bone loss in categories of progression of aortic calcification. The cross-sectional association between aortic calcification and metacarpal bone mass and density in all postmenopausal women at follow-up was assessed by linear regression analysis with MCA and RCA as dependent variables and the variable indicating the extent of aortic calcification (no, mild, or advanced) as an independent variable. A test of significance for the coefficient of this ordinal variable was considered to be a test for trend. Adjusted mean values of bone mass and density in categories of aortic calcification were computed by use of a general linear model.

Statistical significance was considered to be present at $P<0.05$. SPSS 8.0 for Windows was used for analyses.

Results

The characteristics of the study population are shown in Table 1. The age of premenopausal women at baseline ranged

TABLE 1. Baseline and Follow-Up Characteristics of the Study Population

	Premenopausal at Baseline and Postmenopausal at Follow-Up		All Postmenopausal at Follow-Up (n=720)
	Baseline (n=236)	Follow-Up (n=236)	
Age, y	49.0±2.5	57.9±2.6	62.9±5.6
Height, m	1.64±0.06	1.63±0.06	1.62±0.06
Weight, kg	67.2±9.7	69.2±11.3	69.0±10.4
Body mass index, kg/m^2	25.1±3.4	26.1±4.2	26.3±3.9
Systolic blood pressure, mm Hg	132±19	141±21	145±21
Diastolic blood pressure, mm Hg	82±11	83±9	82±10
Serum cholesterol, mmol/L	5.8±0.9	7.0±1.2	7.2±1.3
Current smokers, %	37	28	24
Former smokers, %	27	36	31
Diabetes mellitus, %	1	4	6
Use of hormone replacement therapy, %	0.4	3	1
Use of thiazide diuretics, %	13	14	15
Use of loop diuretics, %	0.4	2	4
Cardiovascular disease history, %	1.7	3.4	3.5
Mild aortic calcification, %	11	16	23
Advanced aortic calcification, %	0.4	11	20
MCA, mm^2	51.5±6.6	49.2±6.5	47.9±6.5
RCA, mm^2, %	81.2±6.8	75.2±6.5	71.7±8.0

Values are mean±SD or percentages.

from 45.0 to 56.8 years. Mild aortic calcification was present in 25 premenopausal women at baseline, whereas only 1 woman showed advanced aortic calcification. Metacarpal bone mass (MCA) and density (RCA) decreased during follow-up, by 4.5% and 7.4%, respectively. The age of all postmenopausal women at follow-up ranged from 53.5 to 76.2 years.

During follow-up, progression of aortic calcification was observed in 59 women going through menopause (25%). No subject showed a decrease in the extent of aortic calcification. Compared with premenopausal women without progression of aortic calcification during follow-up, women with progression of aortic calcification had a higher systolic blood pressure (136 versus 130 mm Hg, respectively; $P=0.03$), a higher serum cholesterol (6.2 versus 5.7 mmol/L, respectively; $P<0.001$), both adjusted for age, and smoked more (56% versus 31%, respectively; $P=0.001$) at baseline. No significant differences were seen in other cardiovascular disease risk factors.

Among women with progression of aortic calcification, the average loss of initial metacarpal bone mass was 6.1%; their average loss of initial metacarpal bone density was 8.9%. In women without progression of aortic calcification, these losses were 3.9% and 6.9%, respectively. Additional adjustment for potential confounding factors did not influence these results (Table 2), nor did additional adjustment for cardiovascular disease history (data not shown). In women already postmenopausal at baseline, there was no association between progression of aortic calcification and metacarpal bone loss during follow-up (data not shown).

We detected an inverse, graded, cross-sectional association between extent of aortic calcification and metacarpal bone mass and density in all postmenopausal women at follow-up, adjusted for age (Table 3). Again, additional adjustment for potential confounders did not influence the results (Table 3), nor did additional adjustment for cardiovascular disease history (data not shown).

TABLE 2. Bone Loss According to Progression of Aortic Calcification of 236 Women Premenopausal at Baseline and Going Through Menopause During Follow-Up

	Aortic Calcification		
Bone Loss	Progression (n=59)	No Progression (n=177)	*P*
Change in MCA,* mm^2	−3.2±0.4	−2.0±0.2	0.01
Change in MCA,† mm^2 %	−3.5±0.4	−2.0±0.2	<0.01
Change in RCA,* mm^2	−7.2±0.6	−5.6±0.3	0.02
Change in RCA,† mm^2 %	−7.5±0.6	−5.5±0.3	<0.01

Values are mean±SE.

*Adjusted for age and years of follow-up.

†Adjusted for age, years of follow-up, age at menopause, body mass index at baseline, change in body mass index during follow-up, systolic blood pressure at baseline, change in systolic blood pressure during follow-up, smoking at baseline (never, former, or current), stopping and starting of smoking during follow-up, diabetes mellitus at baseline, diabetes mellitus developed during follow-up, and use of hormone replacement therapy, thiazide, and loop diuretics at baseline and at follow-up. Because of missing values, the number of subjects is not exactly the same.

TABLE 3. Bone Mass and Density According to Aortic Calcification in 720 Postmenopausal Women at Follow-Up

	Aortic Calcification			
Bone Measure	No (n=409)	Mild (n=167)	Advanced (n=144)	*P* Trend
MCA,* mm^2	48.1±0.3	48.4±0.5	46.4±0.5	0.02
MCA,† mm^2 %	48.2±0.3	48.5±0.5	46.5±0.5	0.04
RCA,* mm^2	72.1±0.4	71.5±0.6	70.8±0.6	0.06
RCA,† mm^2 %	72.2±0.4	71.7±0.6	71.1±0.6	0.15

Values are mean±SE.

*Adjusted for age.

†Adjusted for age, body mass index, systolic blood pressure, smoking (never, former, or current), diabetes mellitus, and use of hormone replacement therapy, thiazide, and loop diuretics at follow-up. Because of missing values, the number of subjects is not exactly the same.

Discussion

Our results show that during menopause, women with progression of aortic calcification lose more metacarpal bone than women without progression of aortic calcification. In postmenopausal women, a higher degree of aortic calcification is associated with a lower metacarpal bone mass and density.

When interpreting our results, some methodological issues should be taken into account. An advantage of the present study is the fact that the association between progression of aortic calcification and bone loss was studied during menopause, the period from which the prevalence of atherosclerosis and osteoporosis increases.[24,25] The prevalence of hormone replacement therapy use in our population was low, which was common in the Netherlands during the period the present study was conducted.[34] We measured aortic calcification radiographically. We assume that this is intimal calcification, which is clearly distinguishable from medial calcification.[35] A limitation of our measurement of aortic calcification is the fact that it detected progression in a linear manner, whereas in fact it may have been circumferential. However, we assume that errors in the measurement of progression of aortic calcification and bone loss occurred randomly, which means that, if anything, we underestimated the association between progression of aortic calcification and bone loss. Although the density of calcification may be relevant with respect to plaque vulnerability and the subsequent onset of acute coronary events, the present study does not provide data on the density of calcification. No woman showed a decrease in the extent of aortic calcification. However, the fact that readers were aware of dates of the radiographs could have biased them against the detection of decreased calcification. Lack of information contributed to loss of data. We assume that the association between progression of aortic calcification and metacarpal bone loss will not differ between subjects with or without complete availability of data, making selection bias unlikely.

We are the first to describe an association between progression of atherosclerotic calcification and bone loss in women during menopause. The results of the present study are in line with those previous studies that showed a cross-sectional association between bone mineral density and aortic calcification,[3–6] carotid plaques,[7] and coronary calcification[8] among elderly women. Most of the reported studies, however, did not adjust for potential confounding factors apart from age.[3–5,8,21,22] Vogt et al[23] found an association between aortic calcification and bone mineral density at 2 of the 5 measured sites, which remained after adjustment for potential confounders. Two studies in elderly women found an adjusted association between bone mass and density at baseline and cardiovascular death[9] and mortality due to stroke[10] during follow-up.

Atherosclerotic calcification and bone mineralization show similarities. The mineral within calcified atherosclerotic plaques is hydroxyapatite, the same mineral found in bone,[11] and matrix vesicles, the initial nucleation sites for hydroxyapatite mineral in bone, are found in atherosclerotic lesions.[12] Calcifying vascular cells appear in many ways similar to osteoblasts,[13] and specific factors and proteins crucial to bone formation are also present within atherosclerotic lesions. The bone differentiation factor bone morphogenetic protein-2a has been found in atherosclerotic lesions,[14] and arterial calcification involves a variety of bone matrix proteins, such as type-I collagen,[15] and the noncollagenous proteins osteopontin[11] and osteocalcin.[16]

The association between progression of aortic calcification and bone loss during menopause may result from a common etiologic factor, such as estrogen deficiency. Epidemiological data suggest that estrogen deficiency is a risk factor for cardiovascular disease and osteoporosis.[36,37] Arteries and bone are target organs for estrogen. Estrogen receptors have been demonstrated on vascular endothelial and smooth muscle cells,[38] osteoblasts,[39] and osteoclasts,[40] suggesting a direct effect of estrogen on vascular and bone cells. Whereas all subjects went through menopause, women with progression of aortic calcification had more bone loss than women without progression of aortic calcification, suggesting that there could be a difference in estrogen loss between subjects. On the other hand, it may not be estrogen deficiency per se, but sensitivity to estrogen deficiency (eg, due to variability of the estrogen receptor gene)[41] that is the common etiologic factor.

Calcium-regulating hormones may be involved in the association between vascular calcification and osteoporosis. Parathyroid hormone levels increase with aging.[42] Concurrently, estrogen deficiency is suggested to increase the sensitivity of the skeleton to parathyroid hormone[43] and to reduce intestinal calcium absorption.[44] Hyperparathyroidism, which can also be induced in the elderly by vitamin D deficiency, can on the one hand contribute to bone loss[45] and on the other hand add to soft tissue calcium deposition, in particular, vascular calcification.

Alternatively, it may not be calcification itself but progression of the underlying process of atherosclerosis that is associated with bone loss. Estrogen deficiency may have indirect effects on arteries and bone by the production of inflammatory agents, such as interleukin-1 and -6 and tumor necrosis factor,[46] which are involved in atherogenesis[47] and contribute to bone resorption.[48–50] Another common factor to explain the apparent association between atherosclerosis and bone loss may be the presence of oxidized lipids, which

promote atherogenesis[51] and inhibit differentiation and mineralization of bone cells.[52] Plasma homocysteine is a cardiovascular risk factor that increases after menopause,[53] and osteoporosis is a common feature in patients with homocystinuria.[54] Although no association between homocysteine and bone density was found in a small group of postmenopausal women,[55] hyperhomocysteinemia might be involved in the association between atherosclerosis and osteoporosis.

In summary, our results indicate that progression of atherosclerotic calcification is associated with bone loss in women during menopause, suggesting a common etiologic factor.

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