

much worse than did the more medically challenged Dutch cohort.

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## Relation of Low Bone Mineral Density and Carotid Atherosclerosis in Postmenopausal Women

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**Due to the lack of convincing data about the association between atherosclerosis and osteoporosis, we evaluated the association between carotid atherosclerosis and bone mineral density in a sample of apparently healthy postmenopausal women who underwent health-screening in our hospital. We also evaluated a bone turnover marker, osteocalcin; we divided the population into 2 groups according to osteocalcin levels. We found a high prevalence of carotid atherosclerosis in subjects with high osteocalcin levels and low bone mineral density. ©2004 by Excerpta Medica, Inc.**

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**A**therosclerosis and osteoporosis are currently considered unrelated diseases, although both increase in frequency with advancing age, appear worse with estrogen deficiency, involve calcification, and share several common characteristics.<sup>1–3</sup> Several studies have suggested a relation between osteoporosis and atherosclerosis, showing a significant correlation between coronary artery calcium and bone mineral density in 45 postmenopausal women, as well as a significant correlation between carotid-plaque score and bone mineral density in 30 postmenopausal women.<sup>4,5</sup> Two other studies have indicated that progression of aortic atherosclerotic calcium was associated with increased bone loss in women during menopause.<sup>6,7</sup> Two clinical trials have not found such a relation. The rates of coronary events and stroke were

extremely low in both the placebo and active groups in a trial on osteoporosis.<sup>8</sup> The participants in another trial, recruited because they had coronary atherosclerosis, had a low incidence of fractures.<sup>9</sup> Therefore, the association between these 2 diseases remains to be established. The aim of the present study was to evaluate, in postmenopausal women, the relation between carotid atherosclerosis and bone mineral density and the potential role of bone turnover.

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We consecutively enrolled a sample of 157 postmenopausal women aged 45 to 75 years who underwent menopausal health-screening tests in our hospital clinic for the presence of  $\geq 1$  cardiovascular risk factors (hyperlipidemia, hypertension, diabetes, smoking). Postmenopausal status was defined as no natural menses for  $\geq 1$  year and serum follicle-stimulating hormone level  $>40$  IU/L. All the participants were Caucasian and underwent a medical history using a standardized questionnaire, administered to obtain information about current and past medication use; smoking habits; age at menopause; presence of cardiovascular disease; physical examination to evaluate body mass index calculated as weight (in kilograms) divided by square of height (in square meters); blood pressure measured in both arms; ultrasound densitometry to evaluate bone mineral density; vascular ultrasound to evaluate extracranial carotid atherosclerosis; and biochemical analyses, including osteocalcin assay. No patient had cardiovascular symptomatic disease on the basis of clinical history. The investigation conformed to the principles outlined in the Declaration of Helsinki. The local ethics committee approved the study, and all participants gave written informed consent for all procedures.

Venous blood was collected, after an overnight fast, into vacutainer tubes (Becton & Dickinson, Franklin

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Lakes, New Jersey) and centrifuged within 4 hours. Serum glucose, calcium, phosphorus, uric acid, albumin, total cholesterol, high-density lipoprotein cholesterol, and triglycerides were measured by standard laboratory techniques. Quality control was assessed daily for all determinations.

Osteocalcin, a biochemical marker of bone formation, was measured in plasma by immunoradiometric assay (IRMA, Adaltis Italia spa, Bologna, Italy). Intra- and interassay coefficients of variation were <10% and <15%, respectively. To avoid variation due to circadian rhythm, venous blood was collected between 8:00 and 9:00 A.M. and the samples were processed within 2 hours to prevent degradation. Because there is no agreement on osteocalcin normal values, we computed the median value and divided the population into 2 groups: those with osteocalcin levels below median (<3.76 nmol/L; <22.1 ng/ml) and subjects with osteocalcin levels above median (>3.75 nmol/L; >22 ng/ml).

The following criteria were used to define cardiovascular risk factors: diabetes (fasting blood glucose  $\geq 7$  mmol/L [126 mg/dl] or antidiabetic treatment), hyperlipidemia (total cholesterol >5.17 mmol/L [200 mg/dl] and/or triglycerides >2.26 mmol/L [200 mg/dl] or lipid-lowering drug use), hypertension (systolic blood pressure >130 mm Hg and/or diastolic blood pressure >80 mm Hg or antihypertensive treatment), smoking (present smokers), and obesity (body mass index  $\geq 30$  kg/m<sup>2</sup>).

Quantitative ultrasound (Osteospace, Medilink, Carnon, France) was used to measure the speed of sound (meters per second) and broadband ultrasound attenuation (decibels per megahertz) of both heels. In cases of a previous fracture within the lower extremity, only the opposite calcaneus was measured. T score was derived from the value of broadband ultrasound attenuation and expressed as the number of SDs from the mean value of a young, gender-matched population.

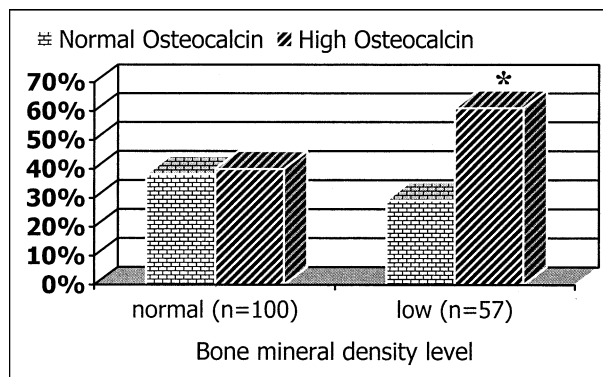
The device was calibrated daily in accordance with the manufacturer's recommendations. The same operator made all measurements. Short-term in vivo precision was established on the basis of repeated measurements in 10 healthy women. In each of them, 5 measurements with repositioning of the calcaneus were made. The coefficient of variation (CV%) was calculated using the following formula:  $CV\% = (SD/mean) \times 100$ ; percentage value was: 4.25% for broadband ultrasound attenuation and 1.28% for speed of sound. According to Lopez-Rodriguez et al,<sup>10</sup> we considered a subject as having low bone mineral density when they had a T score  $\leq -1.5$ .

The subjects underwent B-mode ultrasonography of the extracranial carotid arteries using a duplex system (a high resolution ultrasound instrument, ATL, HDI 5000, Bothell, Washington, with a 5- to 12-MHz linear array multifrequency transducer). All the examinations were performed by the same ultrasonographer, who was blinded to clinical information. The right and left common and internal carotid arteries (including bifurcations) were evaluated with the subjects in the supine position,

**TABLE 1** Clinical, Biochemical, and Ultrasound Characteristics (n = 157)

Variable	
Age, (yrs)	56 $\pm$ 8
Years of menopause, (yrs)	9 $\pm$ 8
Body mass index (kg/m <sup>2</sup> )	28.84 $\pm$ 5.67
Systolic blood pressure (mm Hg)	128 $\pm$ 17
Diastolic blood pressure (mm Hg)	77 $\pm$ 9
Glucose, (mmol/L)	0.55 $\pm$ 0.11
Creatinine, ( $\mu$ mol/L)	69 $\pm$ 8
Total cholesterol, (mmol/L)	5.76 $\pm$ 1.02
High-density lipoprotein-cholesterol, (mmol/L)	3.66 $\pm$ 0.94
Triglycerides (mmol/L)	1.54 $\pm$ 0.39
Calcium (mmol/L)	1.51 $\pm$ 1.56
Phosphorus (mmol/L)	2.33 $\pm$ 0.11
Uric acid ( $\mu$ mol/L)	1.20 $\pm$ 0.16
Intimal medial thickness (mm)	0.68 $\pm$ 0.14
T score	-1.28 $\pm$ 1.22
Diabetes mellitus	9 (6%)
Systemic hypertension	61 (39%)
Hyperlipidemia	69 (44%)
Obesity	55 (35%)
Smoker	34 (22%)

Data are presented as mean  $\pm$  SD or numbers (percentages).



**FIGURE 1.** Prevalence of atherosclerosis according to bone mineral density and osteocalcin levels. \*p <0.05.

with the head turned away from the sonographer and the neck extended with mild rotation. The intima-media thickness, defined as the distance between the intimal-luminal interface and the medial-adventitial interface, was measured as previously described.<sup>11</sup> Briefly, in the posterior approach and with the sound beam set perpendicular to the arterial surface, 1 cm from the bifurcation, 3 longitudinal measurements of intima-media thickness were completed in the right and left common carotid arteries far walls, at sites free of any discrete plaques. The mean of the 3 right and left longitudinal measurements was then calculated. Plaque, detected in longitudinal and transverse planes with anterior, lateral and posterior approaches, was defined as an echogenic focal structure encroaching on the vessel lumen with a distinct area 50% greater than the intima-media thickness of neighboring sites. Stenosis was defined as a peak systolic velocity of >120 cm/s, and occlusion was defined as absence of a Doppler signal. According to these criteria, subjects were considered normal if no lesion was

**TABLE 2** Clinical, Biochemical and Ultrasound Characteristics in the Low Bone Mineral Density Group According to Osteocalcin Level

Characteristic	Osteocalcin Level	
	Normal (n = 24)	High (n = 33)
Age, (yrs)	58 ± 7	58 ± 8
Years of menopause	11 ± 7	11 ± 9
Body mass index, (kg/m <sup>2</sup> )	27.63 ± 4.93	30.02 ± 6.64
Systolic blood pressure, (mm Hg)	129.79 ± 18.65	130.81 ± 14.72
Diastolic blood pressure (mm Hg)	76.63 ± 8.82	80.63 ± 11.17
Glucose (mmol/L)	5.21 ± 0.64	5.47 ± 0.98
Creatinine, (μmol/L)	68.61 ± 8.39	68.04 ± 9.22
Total cholesterol (mmol/L)	5.87 ± 0.84	5.74 ± 1.05
Low-density lipoprotein cholesterol (mmol/L)	3.53 ± 0.99	3.50 ± 0.77
High-density lipoprotein-cholesterol (mmol/L)	1.60 ± 0.47	1.55 ± 0.38
Triglycerides (mmol/L)	1.51 ± 0.64	1.52 ± 2.04
Calcium (mmol/L)	2.36 ± 0.11	2.34 ± 0.11
Phosphorus (mmol/L)	1.16 ± 0.17	1.18 ± 0.13
Uric acid (μmol/L)	252.4 ± 76.34	239.9 ± 59.55
Intimal media thickness (mm)	0.68 ± 0.14	0.69 ± 0.16
T score	-2.50 ± 0.97	-2.52 ± 0.88
Diabetes mellitus	2 (8%)	1 (3%)
Systemic hypertension	8 (33%)	14 (42%)
Hyperlipidemia	12 (50%)	13 (39%)
Obesity	5 (21%)	15 (45%)
Smoking	3 (12%)	7 (21%)

Data are presented as mean ± SD or numbers (percentages).

**TABLE 3** Multivariate Logistic-Regression Analysis of Predictors of Atherosclerosis in the Low Bone Mineral Density Group

Variables	Odds Ratio (95% CI)	p Value
Model 1		
High osteocalcin	6.5 (1.36–31.4)	0.019
Age	1.24 (1.07–1.42)	0.003
Systolic blood pressure	1.02 (0.97–1.07)	0.4
Model 2		
High osteocalcin	14.61 (1.7–126)	0.015
Age	1.30 (1.09–1.54)	0.003
Systemic hypertension	1.75 (0.27–11.4)	0.56
Hyperlipidemia	6.89 (0.96–49.3)	0.05
Diabetes mellitus	7.3 (0.10–523)	0.36
Obesity	0.74 (0.08–6.77)	0.79
Smoker	8.4 (0.8–85.2)	0.07

CI = confidence interval.

detected, or having carotid atherosclerosis when a plaque, stenosis, or occlusion was detected in ≥1 segment of the carotid tree. The coefficient variation of the methods was 3.3%.

Unpaired Student's *t* test and 1-way analysis of variance were used to compare means between the groups. Data are reported as mean ± SD. The chi-square test was used to compare the prevalence of variables among the groups. Univariate regression analysis was performed to identify risk factors for carotid atherosclerosis. Multivariate logistic-regression analysis was performed to test for confounding variables between high osteocalcin and carotid atherosclerosis. The results are expressed as odds ratios. Ninety-five percent confidence intervals are reported. We used 2 different models; in the first, the result was adjusted for systolic blood pressure and age, the only variables significantly correlated with carotid athero-

sclerosis in univariate analysis. In the second, the result was adjusted for the classic cardiovascular risk factors. All reported *p* values are 2-sided. Significant differences were assumed to be present at *p* < 0.05. All comparisons were performed using the statistical package SPSS 11.0 for Windows (SPSS Inc, Chicago, Illinois).

All patients completed the study protocol. Demographic, clinical, biochemical, ultrasound characteristics and prevalence of cardiovascular risk factors of the study population are shown in Table 1. The prevalence of carotid atherosclerosis was 42% in the whole population. This result is very similar to that obtained by Lassila et al,<sup>12</sup> who showed that 50% of postmenopausal women with a mean age of 57 years had at least a focal plaque in the carotid artery tree.

There were no significant differences among the group with low or normal bone mineral density in terms of demographic, clinical, and biochemical variables, or in the prevalence of risk factors, except for age (data not shown). The prevalence of carotid atherosclerosis was 47% and 39% (*p* = NS) in the low and normal bone mineral density groups, respectively. It was 35% and 49% in the groups with osteocalcin below and above the median, respectively (*p* = NS). This difference was entirely explained by the difference present in the low bone mineral density group (Figure 1). Among the women with low bone mineral density, the prevalence of carotid atherosclerosis was 61% in subjects with osteocalcin above the median compared with

29% in subjects with osteocalcin below the median (*p* < 0.05) (Figure 1). These latter subgroups were similar in demographic, clinical, biochemical, and ultrasound characteristics as well as the prevalence of risk factors (Table 2) and drug use (data not shown). Therefore, the osteocalcin levels above the median are not a marker for another major cardiovascular risk factor, which is also consistent with the known lack of association between osteocalcin and cardiovascular risk factors.

Because statins can increase osteocalcin levels,<sup>13</sup> we performed an analysis after excluding patients who received statin treatment, and the carotid atherosclerosis prevalence remained significantly higher in subjects with low bone mineral density and osteocalcin above the median (57% vs 16% in subjects with osteocalcin below the median; *p* < 0.05).

A multivariate logistic regression analysis was per-

formed to adjust for confounding variables and the odds ratio of carotid atherosclerosis in subjects with low bone mineral density and osteocalcin above the median, compared with women with low bone mineral density and osteocalcin below the median. Results were: 6.5 ( $p = 0.019$ ) in the first model (after correction for age and systolic pressure) and 14.6 ( $p = 0.015$ ) in the second model (after correction for classic cardiovascular risk factors) (Table 3).

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The main result of our study is the increased prevalence of carotid atherosclerosis among postmenopausal women with osteocalcin levels above median and low bone mineral density. Because it has been demonstrated that carotid atherosclerosis is correlated with increased cardiovascular mortality,<sup>14,15</sup> this group should be regarded as having a high cardiovascular risk.

In the present study, the prevalence of carotid atherosclerosis was not increased in women with low bone mineral density, although they were older and therefore more prone to atherosclerosis. This result is consistent with the 2 cited trials,<sup>8,9</sup> in which there was no apparent association between atherosclerosis and low bone mineral density. Only women with low bone mineral density had osteocalcin levels above the median associated with carotid atherosclerosis, suggesting that this additional factor may be of importance. Biochemical markers of bone metabolism are used to assess skeletal turnover, but the variability of marker assays is still an issue of practical concern. However, osteocalcin (also known as bone Gla protein) has been used as a marker of high bone turnover because it is the most abundant noncollagenous protein (15%) found in bone and it is produced by osteoblasts in the course of bone remodeling.<sup>16</sup> We use the osteocalcin median level as a cutoff because there is no agreement on normal values; there is a large variation of this level between different laboratories. Finally, using this cutoff, Bauer et al<sup>17</sup> found a significantly faster rate of bone loss in women with osteocalcin levels above median. However, the nature of the mechanism responsible for the putative linkage between bone turnover and atherosclerosis is currently unclear. In patients affected by severe atherosclerosis, circulating matrix Gla proteins, including osteocalcin, were significantly increased.<sup>18</sup> However, in our population this was not the case (data not shown). Another investigation showed that during atherogenesis bone matrix

regulatory proteins, such as osteocalcin, had a regulatory role not only in osteoclastogenesis but also in atherosclerotic calcium.<sup>19</sup>

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