Relationship Between Brachial Arterial Endothelial Function and Lumbar Spine Bone Mineral Density in Postmenopausal Women

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Background Osteoporosis and endothelial dysfunction have been associated with atherosclerosis. The correlation between brachial arterial endothelial function and lumbar spine bone mineral density (BMD) in postmenopausal women will be investigated.

Methods and Results The endothelial function in 85 postmenopausal women, including 28 women with normal spinal BMD, 27 women with osteopenia, and 30 women with osteoporosis were studied. Brachial arterial flow-mediated vasodilatation (FMD) after reactive hyperemia was assessed by ultrasonography. The BMD at the lumbar spine (lumbar 2 to 4 vertebrae) was measured by dual-energy X-ray absorptiometry. Age, years since menopause, and FMD were significantly greater in the osteoporosis group than in the normal BMD group ($p < 0.01$, $p < 0.05$, and $p < 0.05$, respectively). The BMD was significantly lower in the osteoporosis group than in the osteoporosis or normal BMD group (both $p < 0.01$). After adjusting for age and years since menopause, women with osteoporosis had significantly lesser FMD than those with normal BMD ($p < 0.05$). The univariate linear regression analysis revealed that brachial arterial FMD was significantly positively correlated with BMD ($r = 0.31$, $p < 0.01$), but showed no significant association with other clinical variables. In multivariate regression analysis, the FMD was significantly positively correlated with BMD ($p < 0.01$), but not with other variables.

Conclusions Postmenopausal women with osteoporosis might have impaired brachial arterial endothelial function, suggesting that brachial artery endothelial function might be associated with lumbar spine bone mass in postmenopausal women. (Circ J 2007; 71: 1555–1559)

Key Words: Bone mass; Endothelial function; Menopause; Women

Cardiovascular disease and osteoporosis are known to be major causes of morbidity and mortality in postmenopausal women.^{1,2} In recent years, several studies have examined the association between atherosclerosis at different sites and osteoporosis or low bone mineral density (BMD) in women, and suggested that the development of osteoporosis is a risk for advanced atherosclerosis after menopause.^{3–7} Endothelial dysfunction is thought to represent one of the initial stages in the development of atherosclerosis. The endothelium plays a major role in determining the vascular tone by producing and releasing vasodilators, such as nitric oxide (NO), which helps in preventing atherosclerosis by maintaining vasodilatation and inhibiting platelet aggregation, leukocyte adhesion, and

proliferation of the vascular smooth muscle cells.^{8,9} A recent study reported that postmenopausal women with osteoporosis have a lower endothelial function than those with normal BMD.⁵ However, the relationship between brachial arterial endothelial function and lumbar spine BMD in postmenopausal women remains unknown.

We compared the brachial arterial endothelial function among postmenopausal women with normal lumbar spine BMD, osteopenia, and osteoporosis. In addition, we evaluated the correlation between brachial arterial endothelial function and lumbar spine BMD in postmenopausal women.

Methods

Subjects

Of 322 consecutive Japanese postmenopausal women who came our clinic to assess the presence of osteoporosis, 85 subjects (mean age: 57.9 ± 8.3 years; range: 45 ± 75 years) participated in this study. At least 1 year had passed since the last menstrual period in each of the subjects. The menopausal status was confirmed by a serum estradiol concentration of < 20 pg/ml and a serum follicle-stimulating hormone (FSH) concentration of > 40 mIU/ml. Patients with a history of tobacco, alcohol or caffeine use, fractures, diabetes mellitus, hypertension, myocardial infarction, liver disorders, or a family history of osteoporotic fractures or premature myocardial infarction were excluded from the study. None

(Received April 3, 2007; revised manuscript received May 30, 2007; accepted June 12, 2007)

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Table 1 Clinical and Laboratory Characteristics of the Normal BMD, Osteopenia, and Osteoporosis Groups

	Normal BMD	Osteopenia	Osteoporosis
No.	28	27	30
Age (years)	54.4±7.1	55.6±6.9	63.2±7.8**†
Years since menopause (years)	4.8±6.9	5.4±6.0	13.2±7.7**
Body mass index (kg/m ²)	22.6±2.7	22.3±2.1	21.5±3.8
Follicle-stimulating hormone (mIU/ml)	65.5±14.3	64.4±11.4	65.7±19.2
Estradiol (pg/ml)	12.3±2.1	11.3±2.6	12.0±2.9
Systolic blood pressure (mmHg)	121.6±14.0	122.2±16.9	123.3±13.3
Diastolic blood pressure (mmHg)	76.9±7.4	75.8±10.0	75.5±8.1
Heart rate (beats/min)	64.4±5.2	65.3±4.4	65.5±5.8
Total cholesterol (mg/dl)	206.8±28.8	211.6±33.2	204.8±32.3
HDL-cholesterol (mg/dl)	60.1±12.4	57.0±12.0	58.8±13.0
Triglyceride (mg/dl)	93.3±43.1	105.3±48.0	88.5±41.1
LDL-cholesterol (mg/dl)	128.0±26.0	133.6±33.3	128.3±28.0
Fasting plasma glucose (mg/dl)	103.2±16.1	96.5±10.1	96.8±6.9
Creatinine (mg/dl)	0.68±0.10	0.65±0.09	0.66±0.10
Calcium (mg/dl)	9.1±0.4	9.2±0.3	9.2±0.4
Phosphorus (mg/dl)	3.6±0.4	3.4±0.3	3.6±0.4
Osteocalcin (ng/ml)	7.7±2.4	8.0±2.0	9.6±2.7*
Basal arterial diameter (mm)	4.0±0.5	4.1±0.6	4.0±0.6
Basal blood flow (ml/min)	187±78	168±52	183±89
Hyperemic blood flow (%)	749±777	631±718	451±800
Flow-mediated vasodilatation (%)	9.4±5.7	7.6±2.8	6.2±3.8*
Nitroglycerin-induced vasodilatation (%)	17.3±8.0	17.1±9.0	17.4±16.1
BMD (g/cm ²)	1.04±0.06	0.84±0.06**	0.64±0.05**†

BMD, bone mineral density; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

All the results are presented as means ± SD.

* $p < 0.05$, ** $p < 0.01$ vs normal BMD, † $p < 0.01$ vs osteopenia.

of the subjects had received hormone replacement therapy or had taken any steroids or medications known to influence lipoprotein metabolism, bone metabolism or blood pressure. Written informed consent was obtained from each of the participants prior to admission into the study, and the study protocol was approved by the Ethics Committee of the Cardiovascular Hospital of Central Japan.

The study subjects were assigned to 1 of 3 groups according to the BMD at the lumbar spine: control group (normal BMD; 28 women), osteopenia group (BMD 1–2.5 SD below the mean value for young adults; 27 women), and osteoporosis group (BMD more than 2.5 SD below the mean value for young adults; 30 women). This classification system, which was based on the BMD, was established by an expert panel from the World Health Organization!⁰

Physical Examination

The height and weight of the subjects were measured, and the body mass index was calculated. The blood pressure was measured in the morning after the patient had fasted overnight (12h). The measurement was obtained by the same investigator with a sphygmomanometer using the right arm of the subject, after she had rested for 10 min in the supine position.

Measurement of BMD

The validity of this method has been demonstrated in previous study!¹¹ The BMD was evaluated at the lumbar spine (using the central portion of a lateral scout view of the lumbar 2 to 4 vertebrae) by dual-energy X-ray absorptiometry (DXA; QDR-1000W, Hologic, Waltham, MA, USA), and expressed as grams per square centimeter. The Z-score is the number of standard deviations (SD) by which a given measurement differs from the mean for a sex-, age-, and race-matched reference population. The T-score is the number of SDs by which a given measurement

differs from the mean for a normal young adult reference population. All the measurements were performed by 2 operators, and 1 technician analyzed all of the scans. The inter- and intra-operator coefficients of variation were less than 1%.

Measurement of Brachial Arterial Endothelial Function

The validity of this method has been demonstrated in previous studies!^{12–14} In brief, after the subjects had rested in the supine position for 10 min, imaging of the right brachial artery and measurement of the vasodilatory responses were conducted using a high-resolution Doppler ultrasonography equipment with a 7.5-MHz transducer (SSH-160A, Toshiba Medical Systems Corporation, Tokyo, Japan). A non-tortuous segment of the brachial artery was scanned longitudinally approximately 4–5 cm above the elbow, where the clearest images could be obtained. After determining the optimum transducer position, the skin was marked and the arm was kept in the same position throughout the study. After baseline images of the brachial artery were obtained and the arterial flow velocity was determined, a blood pressure cuff tied around the proximal portion of the forearm, distal to the antecubital fossa, was inflated to 250 mmHg for 5 min, followed by quick deflation. Flow velocity in the brachial artery was determined immediately and 1 min after the quick cuff deflation. After allowing a 10 min rest period, a new baseline image was obtained, the patient was given sublingual nitroglycerin (0.3 mg), and the brachial artery imaging was conducted again for the next 4 min. All the scans were recorded on VHS videotape for later analysis, along with blood pressure and heart rate recordings during each stage of the investigation. The diameter of the brachial artery was measured from the anterior to posterior interface between the media and adventitia (“m” line) at the end of diastole, which was defined by the R wave on a continuously recorded electrocardiogram. The vessel diameter was

Table 2 Osteocalcin, Brachial Arterial Flow-Mediated Vasodilatation, and Lumbar Spine BMD of the Normal BMD, Osteopenia, and Osteoporosis Groups (Analysis of Covariance, Adjusted for Age and Years Since Menopause)

	Normal BMD	Osteopenia	Osteoporosis
Osteocalcin (ng/ml)	7.7±2.5	8.1±2.5	9.5±2.7*
Flow-mediated vasodilatation (%)	9.6±4.4	7.9±4.4	5.8±4.7*
BMD (g/cm ²)	1.04±0.06	0.84±0.06**	0.64±0.07**†

Abbreviation see in Table 1.

All the results are presented as means ± SD.

* $p < 0.05$, ** $p < 0.01$ vs normal BMD, † $p < 0.01$ vs osteopenia.

Table 3 Correlations of Brachial Arterial Flow-Mediated Vasodilatation With Clinical Variables by Pearson's Correlation

	<i>r</i>	<i>p</i> value
Age	−0.04	0.74
Years since menopause	−0.02	0.87
Body mass index	−0.15	0.17
Follicle-stimulating hormone	0.12	0.28
Estradiol	0.02	0.85
Systolic blood pressure	0.03	0.81
Diastolic blood pressure	−0.02	0.83
Heart rate	0.13	0.22
Total cholesterol	0.05	0.65
HDL-cholesterol	0.06	0.57
Triglyceride	−0.19	0.08
LDL-cholesterol	0.09	0.44
Fasting plasma glucose	0.02	0.84
Osteocalcin	−0.08	0.48
Lumbar spine BMD	0.31	<0.01

Abbreviations see in Table 1.

measured over 4 cardiac cycles and the measurements were averaged, without previous knowledge of the subject's treatment group. Flow-mediated vasodilatation (FMD) was calculated as the percent increase in arterial diameter during hyperemia, and represents an index of endothelium-dependent vasodilatation. The percent dilatation induced by nitroglycerin, which represents an index of endothelium-independent vasodilatation, was also calculated in a similar manner. The inter- and intra-observer variabilities for the repeated measurements of the arterial diameter at rest were 0.06 ± 0.03 and 0.04 ± 0.03 mm, respectively. The variability of FMD determined on 2 separate days was $2.7 \pm 1.2\%$.

Assays

Blood samples were drawn from the antecubital vein in the morning after the subject had fasted for 12 h. The subjects were allowed to rest in the supine position for at least 10 min prior to the sample collection. The fasting blood samples were centrifuged at 4°C for 15 min at 3,000G, within 1 h of collection. The serum concentrations of FSH and estradiol were measured by radioimmunoassay. The serum total cholesterol, high-density lipoprotein-cholesterol, triglyceride, creatinine, calcium, and phosphorus concentrations were determined by using standard laboratory techniques (Medca Japan, Konosu, Japan). The concentrations of low-density lipoprotein-cholesterol were calculated using the Friedwald formula. Plasma glucose was measured in duplicate with an automatic analyzer by the glucose oxidase method (Medca Japan, Konosu, Japan). The serum osteocalcin was measured by using the immunoradiometric assay. The intra- and inter-assay coefficients of variation were <7% for FSH, and <5% for estradiol, <6% for lipids,

Fig 1. Correlation between brachial arterial flow-mediated vasodilatation (FMD) and lumbar spine bone mineral density (BMD) in postmenopausal women.

<7% for plasma glucose, and <8% for osteocalcin.

Statistical Analysis

Data are expressed as mean ± SD. One-way analysis of variance (ANOVA) was used to compare the clinical characteristics among the 3 groups. The laboratory data for the 3 groups were compared by using analysis of covariance (ANCOVA), after adjusting for age, body mass index, and years since menopause. Pearson's correlation coefficient analyses were used to examine the relationships between the values of FMD and the values of BMD and other clinical variables. Furthermore, multiple regression analysis was performed among the values of FMD and the values of BMD and other clinical variables. All the probability values were 2-tailed. The p values <0.05 were considered to denote statistical significance. All statistical analyses were performed using the SPSS software (v11.0, Michigan, IL, USA).

Results

Age was significantly greater in the osteoporosis group than in the normal BMD ($p < 0.01$) and osteopenia groups ($p < 0.01$). Years since menopause, osteocalcin concentrations, and FMD in the osteoporotic group were also significantly greater than those in the normal BMD group ($p < 0.01$, $p < 0.01$, and $p < 0.05$, respectively). No significant

Table 4 Independent Predictors of Brachial Arterial Flow-Mediated Vasodilatation With Clinical Variables by Multiple Regression Analysis

		<i>p</i> value
Age	-0.12	0.83
Years since menopause	0.25	0.64
Body mass index	-0.22	0.09
Follicle-stimulating hormone	0.10	0.44
Estradiol	0.03	0.83
Systolic blood pressure	-0.18	0.39
Diastolic blood pressure	0.12	0.54
Total cholesterol	0.08	0.48
HDL-cholesterol	-0.10	0.44
Triglyceride	-0.19	0.17
LDL-cholesterol	0.07	0.57
Fasting plasma glucose	-0.01	0.93
Osteocalcin	0.03	0.83
Lumbar spine BMD	0.40	<0.01

Abbreviations see in Table 1.

differences in nitroglycerin-induced vasodilatation were seen among the 3 groups. BMD was significantly lower in the osteoporosis group than in the osteoporosis or normal BMD group ($p<0.01$ for both) and in the osteopenia group than in the normal BMD group ($p<0.01$). However, there were no significant differences in other characteristics among the 3 groups (Table 1).

After adjusting for age and years since menopause, serum osteocalcin concentrations were found to be significantly higher in the osteoporosis group than in the normal BMD group (9.5 ± 2.7 vs 7.7 ± 2.5 ng/ml; $p<0.05$). Women with osteoporosis were found to show significantly lower FMD than those with normal BMD (5.8 ± 4.7 vs $9.6\pm 4.4\%$; $p<0.05$). Furthermore, the BMD was significantly lower in the osteoporosis group than in the osteoporosis or normal BMD group ($p<0.01$ for both) and also in the osteopenia group than in the normal BMD group ($p<0.01$; Table 2).

The results of the univariate regression analysis revealed that brachial arterial FMD was significantly positively correlated with BMD ($r=0.34$, $p<0.01$), but showed no significant association with other clinical variables (Table 3, Fig 1). In addition, multivariate regression analysis revealed that the FMD was significantly positively correlated with BMD ($p<0.01$), but showed no significant association with other clinical variables (Table 4).

Discussion

The present study showed that brachial arterial FMD was more impaired in postmenopausal women with osteoporosis than in those with normal bone mass. Brachial arterial FMD was significantly positively associated with lumbar spine BMD.

The association between brachial arterial endothelial function and lumbar spine BMD has been shown in a few studies.⁵ Sanada et al reported that postmenopausal women with a lower lumbar spine BMD, as measured by DXA, have impaired forearm endothelial function as measured by strain-gauge plethysmography.⁵ The present study also revealed that postmenopausal women with low lumbar spine BMD, as measured by DXA, have impaired brachial arterial endothelial function as measured by FMD. Although there were differences in the methods used to assess the brachial arterial endothelial function between the aforementioned study and the present study, the findings of the

2 studies were consistent. In addition, the present study found a positive association of brachial arterial FMD with lumbar spine BMD in postmenopausal women. In our previous study,¹⁵ brachial-ankle pulse wave velocity, which has been regarded as a marker reflecting vascular damage,¹⁶ was significantly negatively correlated with lumbar spine BMD in postmenopausal women. This study supports the results of the present study. Accordingly, it is likely that postmenopausal women with osteoporosis might have more impaired brachial arterial endothelial function than postmenopausal women with normal bone mass.

The nature of the association between brachial arterial endothelial function and BMD is not yet clearly understood. However, we can speculate on several possible explanations. Epidemiological data have suggested that estrogen deficiency is a risk factor for cardiovascular disease and osteoporosis.^{17,18} Bone and arteries are target organs for estrogen actions. Estrogen receptors have been demonstrated on osteoblasts,¹⁹ osteoclasts,²⁰ and vascular endothelial and smooth muscle cells,²¹ suggesting that there are direct effects of estrogen on vascular endothelial cells and bone cells. Hormone replacement therapy in postmenopausal women improves brachial arterial endothelial function¹² and increases BMD.²² Estrogen might be one of the important factors explaining the relationship between brachial arterial endothelial function and bone mass. However, in the present study, the serum estradiol levels showed no relation to brachial arterial FMD or lumbar spine BMD; these results might be reflected by a small group of early postmenopausal women.

There are other possible explanations for the present study findings. NO is a free radical involved in vascular relaxation.²³ In humans, dilatation of conduit arteries in response to reactive hyperemia is reduced by inhibitors of NO synthesis, suggesting an important role for NO in FMD.²⁴ Impairment of NO production might cause a fall in radial blood flow in healthy volunteers.²⁴ In dogs, bone blood flow facilitates bone formation and mineralization.²⁵ Because the decreased NO might cause bone loss, NO might play an important role in the pathogenesis of both endothelial dysfunction and osteoporosis. In addition, osteocalcin, known as bone Gla protein, has been used as a marker of high bone turnover because it is the most abundant non-collagenous protein found in bone and it is produced by osteoblasts in the course of bone remodeling.²⁶ In patients with severe atherosclerosis, circulating osteocalcin levels were increased.²⁷ Another study showed that during atherogenesis, osteocalcin had a regulatory role not only in atherosclerotic calcium but also in osteoclastogenesis.²⁸ Although osteocalcin might be one of the important factors explaining the relationship between endothelial function and bone mass, the present study found that brachial arterial FMD showed no significant association with osteocalcin concentrations. Moreover, secondary hyperparathyroidism, which can be induced by vitamin D deficiency in the elderly, is known to be associated with soft-tissue calcium deposition as well as bone loss.²⁹ Oxidized lipids also promote atherogenesis³⁰ and inhibit differentiation and mineralization of bone cells.³¹ Several bone-associated proteins, including osteopontin and osteoprotegerin, have been reported to be expressed in atherosclerotic lesions.^{32,33} Unfortunately, we had no data about these factors in our study subjects.

In conclusion, postmenopausal women with osteoporosis might have impaired brachial arterial endothelial function,

suggesting that brachial arterial endothelial function might be associated with lumbar spine bone mass in postmenopausal women. The number of subjects in the present study was low and many other risk factors for atherosclerosis and osteoporosis were not measured. Further study is needed to explore the relationship between brachial arterial endothelial function and bone mass in postmenopausal women.

Acknowledgments

We are grateful to Naoaki Tsunoda, Hiroyuki Takada, Hiromitsu Takahashi and Hiroe Hagiwara for their technical assistance, and we thank Kazuo Sakaguchi, Miki Shirouzu, Tomoko Sakurai, Mina Aoki, Yuko Masuda, Kanae Kodaira, Setsuko Kobayashi and Masumi Tanimoto for their assistance with the clinical coordination.

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