Table 5
Multiple regression analysis of factors independently associated with PWV

Independent variable	hc PWV		hf PWV		hb PWV		fa PWV	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Age	0.582‡	0.570‡	0.411§	0.406§	0.422§	0.411§	0.357§	0.334§
Menopause	0.001	0.036	0.019	−0.018	−0.039	−0.040	−0.028	−0.082
Smoking index	0.075	0.067	0.057	0.058	0.059	0.054	−0.017	−0.016
Systolic BP	0.228§	0.241†	0.323‡	0.334‡	0.354‡	0.375‡	0.431‡	0.455‡
LDL cholesterol	−0.014	−0.040	−0.005	0.009	0.005	10^{-4}	0.037	0.065
Physical functioning score (SF-36)	−0.070	−0.066	−0.054	−0.046	−0.143§	−0.156§	−0.167§	−0.186§
Lumbar spine BMD	0.052	–	0.060	–	0.022	–	0.025	–
Calcaneus OSI	–	0.094	–	−0.047	–	0.009	–	−0.088
R^2	0.509‡	0.514‡	0.410‡	0.426‡	0.460‡	0.475‡	0.512‡	0.536‡

Values are standard regression coefficients (s). R^2: multiple coefficient of determination. BP: blood pressure, LDL: low-density lipoprotein, BMD: bone mineral density, OSI: osteo-sono-assessment index, hc PWV: heart–carotid pulse wave velocity, hf: heart–femoral, hb: heart–brachial, fa: femoral–ankle.
‡ $p<0.0001$.
§ $p<0.05$.
† $p<0.001$.

FA IMT to evaluate their independent association with CA and FA IMT. In models which included age, menopause, smoking index, systolic BP, serum LDL cholesterol in addition to lumbar spine BMD or calcaneus OSI, neither bone condition failed to emerge as an independent factors significantly associated with CA IMT. In models to evaluate the association with FA IMT, calcaneus OSI but not lumbar spine BMD was significantly associated in a negative manner with FA IMT. Although we performed multiple regression analysis including HDL cholesterol and triglyceride in place of LDL cholesterol in Table 4, these factors failed to emerge as a significant factor independently associated with any of IMT (data not shown).

3.5. Multiple regression analysis of factors independently associated with PWV

Table 5 represents the results of multiple regression analysis of various clinical variables, which correlated significantly in a simple regression analyses, used to evaluate their association with PWV. Model 1 included age, menopause, smoking index, systolic BP, serum LDL cholesterol, physical functioning score and lumbar spine BMD, and model 2 included calcaneus OSI in place of lumbar spine BMD. Age and systolic BP were independent factors significantly associated with PWV of all segments in both models 1 and 2. Lumbar spine BMD and calcaneus OSI failed to emerge as a significant factor associated with PWV of any segment. Physical functioning score associated significantly with hb and fa but not hc and hf PWV, suggesting the preferential association with arterial stiffening in peripheral segment. Although we performed multiple regression analysis including HDL cholesterol and triglyceride in place of LDL cholesterol in Table 5, these factors failed to emerge as a significant factor independently associated with any of PWV (data not shown).

4. Discussion

The present study demonstrated: (i) a significant and negative association of physical functioning score with stiffening of peripheral artery and (ii) a significant and negative association of calcaneus OSI with arterial thickening in femoral artery.

Physical activity is known to suppress increased aortic PWV in the general public [35–39], the mechanism by which is partly explained by the data that regular aerobic exercise augments endothelium-dependent vascular relaxation induced by nitric oxide [40]. Therefore, it is reasonable that the physical functioning domain of SF-36 scores showed a significant and positive correlation with PWV in all segments. In multiple regression analysis, physical functioning score showed an independent association with peripheral segment of PWV, hb and fa PWV, but not with central segment of hc and hf PWV. It was previously reported that, as muscle mass decreases in the paretic lower limb of hemiparetic patients, vascular resistance becomes greater [41]. Increased shear stress due to increased vascular resistance is proposed as a major mechanism by which local atherosclerosis progresses in paretic side by increasing vascular injury [42]. In fact, we have previously found that hemiparetic patients exhibited greater fa PWV in the paretic lower limbs than in non-paretic counterpart [14]. Conversely, an increase of basal vascular resistance in the hindlimb in the sedentary spontaneously hypertensive rat (SHR) is significantly reduced after training [43]. Its mechanism is explained by training-induced increase of venule density in skeletal muscle. Since physical functioning score assesses physical activities such as walking and climbing stairs, it may improve muscle condition preferentially in the peripheral components rather than central components. These backgrounds may rationalize the preferential association of physical functioning score with PWV in peripheral segments.

Another interesting finding in the present study is that calcaneus OSI emerged as an independent factor signifi-

cantly associated with FA IMT but not CA IMT (Table 4). Previous studies have reported that symmetrical training of the upper limbs is accompanied by a greater distensibility of the middle-sized arteries of the more trained side [44] and that improved vascular endothelial function in the upper limb after hand-grip exercise training in the patients with congestive heart failure [45,46], suggesting that the beneficial effect of training on vascular endothelial function may be localized to the side where muscle mass increased. Since calcaneus OSI is a reliable marker of ADL particularly that of lower limb [32], it is reasonable that calcaneus OSI was associated preferentially with FA IMT but not CA IMT. Calcaneus OSI was independently associated with FA IMT but not with CA IMT and all parts of PWV. On the other hand, lumbar spine BMD was not independently associated with either IMT or any parts of PWV in multiple regression analysis in the present study. As IMT indicates arterial thickening and PWV indicates arterial stiffening, respectively, the results of this study indicated that bone loss at calcaneus, but not at lumbar spine, enhanced arterial thickening selectively at femoral artery but not at carotid artery, suggesting the local mechanism of increased bone loss to increase arterial thickening [1].

The limitation of the present study is that the multiple regression model explained only 27.4% of the variance of FA IMT. This may indicate the presence of other factors affecting FA IMT that were not included in the models. Furthermore, due to small numbers of subjects, analyses were performed in subjects including both pre- and post-menopausal women, although calcaneus OSI emerged as a factor independently associated with FA IMT even after adjustment for existence of menopause in the multiple regression analysis. Another limitation of the present study is that the reduced blood flow to the lower extremities resulting from atherosclerotic vascular disease might reduce total hip and calcaneus BMD, as previously described [47]. Furthermore, we have no direct histopathological demonstration that increased IMT and PWV is due to atherosclerosis. The arterial thickening and stiffening measured by ultrasonography and waveform analyzer, respectively, might have been due to another, non-atherosclerotic arteriopathy. However, IMT and PWV measurement is still useful in that these parameters strongly correlated with the presence of coronary artery diseases [8–11].

In summary, it was suggested that physical activity and bone status might be intimately associated with arterial wall condition even in healthy Japanese women. Especially, physical activity may be preferentially associated with arterial stiffening in peripheral arteries and bone status of calcaneus locally with that of femoral artery.

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Paraarticular Trabecular Bone Loss at the Ultradistal Radius and Increased Arterial Stiffening in Postmenopausal Patients with Rheumatoid Arthritis

KAZUMASA TANAKA, MASAAKI INABA, HITOSHI GOTO, MAYUMI NAGATA-SAKURAI, SHINREI SAKAI, SHINSUKE YAMADA, MISAKO UEDA, EIJI ISHIMURA, and YOSHIKI NISHIZAWA

ABSTRACT. ***Objective.*** We recently reported enhanced arterial thickening in patients with rheumatoid arthritis (RA) and the importance of increased bone resorption in this process. Our aim was to examine whether arterial stiffening, another aspect of atherosclerosis, is also increased in patients with RA, and to determine if it is an important risk factor.

Methods. The subjects were 47 patients with RA and 49 healthy controls, all postmenopausal women. Subjects having risk factors for atherosclerosis were excluded. Femoral-ankle (fa) pulse wave velocity (PWV) and brachial-ankle (ba) PWV were measured in all patients using a waveform analyzer. Bone mineral density (BMD) at the ultradistal radius was assessed by peripheral quantitative computed tomography. Inflammation markers (C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor, platelet count) and bone resorption markers (urinary excretion of deoxypyridinoline and N-terminal telopeptide) were also measured.

Results. The median values of faPWV and baPWV in RA patients were 1124 cm/s [interquartile range (IQR) 1040–1175] and 1539 cm/s (IQR 1297–1738), respectively, which were significantly greater than the respective values of 982 cm/s (IQR 819–1054; $p < 0.001$) and 1322 cm/s (IQR 1112–1398; $p = 0.004$) in controls. In multiple regression analysis, the presence of RA emerged as an independent factor associated with the greater faPWV and baPWV when adjusted for age, blood pressure, and smoking. In RA patients alone, BMD in the trabecular bone component, but not for the total bone (cortical plus trabecular), at the ultradistal radius correlated significantly with both faPWV and baPWV. Multiple regression analysis showed that trabecular BMD at the distal radius was a significant factor independently associated with greater faPWV and baPWV when adjusted for age, blood pressure, and smoking. None of the measured inflammation markers or bone resorption markers correlated with either faPWV or baPWV in patients with RA.

Conclusion. Patients with RA show increased arterial stiffening, in addition to the arterial thickening we have previously reported, supporting the notion of enhanced atherosclerosis in RA patients. Paraarticular bone loss in the trabecular bone component at the ultradistal radius is a factor significantly associated with increased arterial stiffening in RA patients. (J Rheumatol 2006;33:652–8)

Key Indexing Terms:
ATHEROSCLEROSIS RHEUMATOID ARTHRITIS ARTERIAL STIFFENING
PULSE WAVE VELOCITY PARAARTICULAR OSTEOPOROSIS

An accumulation of evidence indicates accelerated atherosclerosis in patients with rheumatoid arthritis (RA)[1-4], and we recently conducted a cross-sectional study that showed patients with RA exhibit greater intima-media thickness (IMT) in the common carotid artery, compared to healthy controls, and that the increase is independently associated with the presence, duration, and severity of RA[5]. Further, we reported in a subsequent longitudinal study that RA patients have a higher rate of increased arterial wall thickening[6], to which increased bone resorption was a contributory factor. Atherosclerosis has 2 key components, arterial thickening (atherosis) and stiffening (sclerosis)[7], which can now be quantified by measuring far-wall IMT by ultrasonography and pulse wave velocity (PWV), respectively[8].

Atherosclerosis and osteoporosis progress simultaneously with advancing age[9] and share common risk factors, such as smoking[10] and menopause[11]. There is an association between aortic calcification and bone mineral density (BMD) in the hip or lumbar spine in postmenopausal women, suggesting that

From the Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, and Senboku Second Hospital, Osaka, Japan.

Supported in part by a grant-in-aid from Health Science Research, Ministry of Health and Welfare of Japan.

K. Tanaka, MD, Research Associate; M. Inaba, MD, PhD, Associate Professor; H. Goto, MD, PhD, Lecturer; M. Nagata-Sakurai, MD, PhD, Research Associate; S. Sakai, MD; S. Yamada, MD, Graduate Student; M. Ueda, MD, PhD; E. Ishimura, MD, PhD, Associate Professor; Y. Nishizawa, MD, PhD, Professor.

Address reprint requests to Dr. M. Inaba, Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. E-mail: inaba-m@med.osaka-cu.ac.jp

Accepted for publication November 23, 2005.

development of osteoporosis may be a risk factor for advanced atherosclerosis after menopause[9,12]. We recently reported that even in healthy people a significant association exists between IMT in the femoral artery and the calcaneus osteo-sono assessment index (OSI)[13], and that paraarticular bone in the trabecular bone component at the ultradistal radius and calcaneus is preferentially lost at an early stage of RA, probably due to RA joint inflammation and impairment of physical activity, respectively[14].

This background prompted us to examine (1) whether patients with RA exhibit increased arterial stiffening in addition to arterial thickening; and (2) whether bone loss, and particularly paraarticular trabecular bone loss at the ultradistal radius, might be involved in increased arterial stiffening in patients with RA.

MATERIALS AND METHODS

Subjects. The subjects enrolled in our study were all postmenopausal women. They comprised 47 RA patients and 49 healthy controls; all provided written informed consent to the study protocol. The RA patients were recruited from the Outpatient Clinic of Rheumatology at Osaka City University Hospital, and were diagnosed according to the 1987 revised criteria of the American College of Rheumatology (formerly, the American Rheumatism Association)[15]. Healthy controls were selected from participants in a local health-check program at Osaka City University Hospital. To avoid complication by other known risk factors for atherosclerosis, both groups of subjects were selected on the basis of the following exclusion criteria: (1) hypertension, as defined by blood pressure > 150/90 mm Hg, or use of antihypertensive medication; (2) hyperlipidemia, as diagnosed by the National Cholesterol Education Program ATP III criteria[16] [total cholesterol > 240 mg/dl, low density lipoprotein (LDL) cholesterol > 160 mg/dl, or triglyceride > 150 mg/dl], or use of lipid-lowering medication; (3) diabetes mellitus, based on a history of diabetes or consistency with the criteria of the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus[17], or use of antidiabetic medication; (4) a history of ischemic heart disease or cerebrovascular events; and (5) receiving hormone replacement therapy.

The clinical characteristics of the RA patients and controls are shown in Table 1. No significant difference existed between the 2 groups in age, body mass index (BMI), smoker to nonsmoker ratio, serum level of total cholesterol and LDL cholesterol, or systolic blood pressure. The mean duration of RA was 8.7 years (range 1 to 36 yrs). All 47 RA patients were receiving multiple medications, with 21 patients taking nonsteroidal antiinflammatory drugs (NSAID), 19 receiving methotrexate (MTX), 11 receiving actarit, 8 receiving salazosulfapyridine, 8 receiving bucillamine, 7 receiving MTX supplemented with folate, and 28 receiving low-dose prednisolone (one patient taking 1.0 mg/day, 5 patients 2.0 mg/day, 3 patients 2.5 mg/day, 5 patients 4.0 mg/day, 6 patients 5.0 mg/day, 6 patients 7.5 mg/day, and 2 patients 10.0 mg/day).

Measurement of clinical variables. Blood was collected after an overnight fast at the time of PWV measurement. A morning void sample, which subjects were asked to bring to the hospital with them, was used for measurement of urinary parameters. Blood samples were immediately centrifuged and the serum samples were stored at –70°C until analysis. Laboratory variables relevant to RA activity [erythrocyte sedimentation rate (ESR), platelet count (Plt), serum C-reactive protein (CRP), and rheumatoid factor (RF)] were measured by routine methods in RA patients. Serum levels of total cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol were determined using an autoanalyzer. LDL cholesterol was calculated by the formula of Friedewald, *et al*[18]. The urinary excretion of deoxypyridinoline (DPD) and N-terminal telopeptide (NTX) was measured as bone resorption markers, as described[19].

Information on smoking habits was obtained using a self-administered questionnaire.

PWV measurement. PWV was measured as an index of arterial stiffening as we reported[20,21]. Resting blood pressure was determined in the right arm with a sphygmomanometer after at least 15 min of supine rest at the time of PWV measurement. The systolic blood pressure was taken upon appearance of Korotkoff sounds, and the diastolic blood pressure upon disappearance of such sounds. Results are reported as the average of 3 measurements. PWV was measured in the supine position after 5 min of bed rest, using an automatic waveform analyzer (model BP-203RPE; Colin, Komaki, Japan). Pressure waveforms of the brachial and tibial arteries were recorded by an oscillometric method, using occlusion/sensing cuffs adapted to both arms and both ankles. Pressure waveforms of the femoral arteries were recorded using multi-element tonometry sensors placed at the femoral artery. The electrocardiogram was monitored with electrodes placed on both wrists. Heart sounds S1 and S2 were detected by a microphone positioned at the left edge of the sternum at the third intercostal space. The waveform analyzer measures time intervals between S2 and the notch of the brachial pulse wave (Thb), between

Table 1. Clinical characteristics of patients with RA and healthy controls. Values are shown as mean ± SD.

	Controls	RA Patients	p
No. of subjects	49	47	
Age, yrs	56.7 ± 7.4	59.6 ± 14.1	0.198
Body mass index, kg/m^2	20.4 ± 2.2	21.1 ± 2.4	0.240
Smoker/nonsmoker	4/45	2/45	0.240
Total cholesterol, mg/dl	209.5 ± 19.0	198.8 ± 34.2	0.058
LDL cholesterol, mg/dl	116.5 ± 31.5	110.6 ± 24.9	0.346
Systolic BP, mm/Hg	129.3 ± 17.3	131.9 ± 21.4	0.505
CRP, mg/dl	ND	1.0 (0.1–8.0)	—
RF, IU/ml	ND	151.1 (9–1270)	—
ESR, mm/h	ND	49 (8–110)	—
Platelet count, × 10^4/μl	ND	26.3 (17.2–56.1)	—
DPD/Cre, nmol BCE/mmol Cr	ND	7.9 (4.8–21.2)	—
NTX/Cre, nmol BCE/mmol Cr	ND	69.5 (29.3–153.9)	—

ND: not determined. LDL: low density lipoprotein; BP: blood pressure; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DPD: deoxypyridinoline; NTX/Cre: N-terminal telopeptide/creatinine ratio.

pulse waves of the femoral arteries (Tcf), and between pulse waves of the femoral and tibial (ankle) arteries (Tfa). Estimates of the path lengths of the heart-carotid (Dhc), heart-brachial (Dhb), heart-femoral (Dhf), and femoral-ankle (Dfa) segments were obtained based on the height (HT, in centimeters), using the following formulas; Dhc = 0.2437 × HT – 18.999; Dhb = 0.2195 × HT – 2.0734; Dhf = 0.5643 × HT – 18.381; and Dfa = 0.2486 × HT + 30.709. PWV was calculated for each arterial segment as the path length divided by the corresponding time interval. Reproducibility of the PWV measurement was evaluated by repeated measurements in 17 healthy subjects on 2 different occasions. The coefficients of variation were 1.9% and 3.3% for baPWV and faPWV, respectively[20,21], which were significantly lower than the respective value of flow-mediated dilatation (4.3%)[22] or IMT (3.4%)[5].

Peripheral quantitative computed tomography (pQCT) measurement. PQCT measurements were performed at 4% to the ulnar length proximal to the end of the radius (ultradistal site) with a single 2.5-mm thick CT slice on the nondominant side, using an XCT-960 scanner (Stratec Inc., Pforzheim, Germany) as described[23-25]. Briefly, the bone mineral content (mg/mm), the cross-sectional bone area, and the BMD (mg/cm^3) were determined at the ultradistal site for the entire cross-section, as well as for the trabecular compartment. After determination of the entire bone contour, the outer 65% of voxels were concentrically peeled off. The remaining 35% of voxels were defined as the trabecular region, while the peeled-off area was defined as the cortical plus subcortical area[23,24]. Image processing and calculation of numerical values were performed using the manufacturer's software. The precision of the pQCT procedure ranged from 1% to 2%, depending upon the parameter being assessed[26].

Quantitative ultrasound assessment of the calcaneus. Quantitative ultrasound assessment of the calcaneus was performed using an ultrasound system (Acoustic Osteo-Screener, AOS-100; Aloka, Tokyo, Japan) as described[5]. Briefly, the instrument measures both speed of sound (SOS) and an attenuation-related parameter, the transmission index (TI). These measurements yield a derived parameter, the OSI, which has been proposed to be an estimate of the elastic modulus of the calcaneus. The precision of the OSI parameter was 2.2%[13,14,27].

Statistical analysis. For categorical data, the difference in prevalence was evaluated by a chi-square test. Variables with a normal distribution were expressed as the mean ± SD, and differences between the mean values were examined by Student t test. Variables showing a non-normal distribution were summarized as the median and the range, and a nonparametric Mann-Whitney U test was used to evaluate differences between the median values. Linear regression analysis was performed to examine univariate correlation, and multiple linear regression analysis was performed to assess independent associations between variables. Variables with skewed distributions were subjected to univariate and multivariate regression models after log-transformation of the data. P values less than 0.05 were considered significant. Statistical analysis was performed with StatView 5.0 for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Effects of RA on PWV. The median faPWV was 1124 cm/s [interquartile range (IQR) 1040–1175] in RA patients, which was significantly greater than the value of 982 cm/s (IQR 819–1054) in healthy controls ($p < 0.001$). The median baPWV was also significantly greater in RA patients (1539 cm/s; IQR 1297–1738) than in controls (1322 cm/s; IQR 1112–1398; $p = 0.004$).

Since PWV values exhibited skewed distribution, the values were log-transformed to fit linear models thereafter. To examine whether the presence of RA was an independent factor associated with the greater faPWV in RA patients, multiple regression analysis was performed. Results of multiple regression analysis of factors associated with log faPWV in the entire group of 96 subjects are shown in Table 2. In model 1, which included age, systolic blood pressure (BP), RA prevalence, and smoking habit as independent variables, RA prevalence and systolic BP were found to be significantly associated with log faPWV. In models 2 and 3, which included total cholesterol and BMI, respectively, in place of smoking habit, RA prevalence still emerged as an independent factor associated with log faPWV. Examination of the association with log baPWV, using the same models, showed that RA prevalence was again a significant and independent factor associated with log baPWV (Table 2). These data indicated that RA prevalence was a significant factor independently associated with increased log faPWV and log baPWV, when classical cardiovascular factors were adjusted.

Univariate analysis of factors correlated with PWV in RA patients. Because of the non-normal distribution, logarithmic transformations of various clinical variables were performed (Table 3). Log trabecular bone density at the ultradistal radius in RA patients correlated significantly in a negative manner with log faPWV and log baPWV ($r = -0.425$, $p = 0.007$, and $r = -0.553$, $p < 0.001$, respectively; Table 3 and Figure 1), although log total bone density at the ultradistal radius failed to correlate significantly with both log faPWV and log baPWV (Table 3). Log calcaneus OSI correlated significantly with log baPWV ($r = -0.357$, $p = 0.017$), but not with log faPWV ($r = -0.021$, $p = 0.893$). Neither inflammation markers including serum log CRP, log ESR, and log RF, nor bone resorption markers including urinary log NTX/Cre and log DPD/Cre were significantly correlated with either log faPWV or log baPWV (Table 3).

Multiple regression analysis of factors associated with the level of PWV. Finally, we evaluated factors independently associated with the level of faPWV and baPWV in the RA patients, using multiple regression models (Table 4). Three variables (age, smoking habit, and systolic BP) in these models were included as classical risk factors for atherosclerosis. Factors that had shown a significant correlation or tendency to correlate with log PWV were included as the fourth variable, in order to determine whether the variable was independently associated with log PWV. These variables included the log trabecular density (model 1) and the log total bone density (model 2) at the log ultradistal radius and the log calcaneus OSI (model 3), and the models were tested for association with log faPWV and log baPWV. Of all the variables examined, systolic BP and log trabecular density at the ultradistal radius were found to be significant factors independently associated with log faPWV and log baPWV.

Association of NSAID, corticosteroid, and MTX treatment with arterial stiffening in RA patients. As shown in Table 5, the differences in faPWV and baPWV did not reach statistical significance between the RA patients who were taking NSAID, corticosteroids, or MTX and those who were not. This lack of association between PWV values and the treatment for RA was also found by multiple regression analysis (data not shown).

Table 2. Multiple regression analysis to evaluate the association of RA and other risk factors with log femoral-ankle pulse wave velocity (faPWV) and log brachial ankle (ba)PWV in controls and patients with RA. Standard regression coefficients (ß) are given in the table.

Independent Variables	Log faPWV			Log baPWV		
	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3
Age	0.153	0.190	0.186†	0.398*	0.435*	0.439*
Systolic BP	0.339*	0.328**	0.378*	0.418*	0.423*	0.427*
RA	0.316**	0.294**	0.256**	0.264*	0.188*	0.188†
Smoking habit (–/+)	–0.049			0.026		
Total cholesterol		–0.023			–0.023	
BMI			–0.131			–0.102
R^2	0.318*	0.301*	0.328*	0.541*	0.539*	0.552*

BP: blood pressure; BMI: body mass index; R^2: multiple coefficient of determination. * $p < 0.001$, ** $p < 0.01$, † $p < 0.05$.

Table 3. Univariate analysis of factors correlated with femoral-ankle pulse wave velocity (faPWV) and brachial ankle (ba) PWV in patients with RA.

	Log faPWV		Log baPWV	
	r	p	r	p
Age	0.192	0.213	0.520	0.001*
Duration of RA	0.108	0.537	0.110	0.530
Log total density at the ultradistal radius	–0.078	0.631	–0.294	0.060
Log trabecular density at the ultradistal radius	–0.425	0.007**	–0.553	< 0.001*
Log calcaneous OSI	–0.021	0.893	–0.357	0.017†
Log CRP	–0.059	0.711	0.002	0.988
Log RF	–0.001	0.997	0.017	0.630
Log ESR	0.096	0.588	0.115	0.519
Log DPD/Cre	–0.011	0.965	0.065	0.799
Log NTX/Cre	–0.076	0.642	0.046	0.779

BMD: bone mineral density; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DPD: deoxypyridinoline; NTX: N-terminal telopeptide; Cre: creatinine; OSI: osteo-sono assessment index. * $p < 0.001$, ** $p < 0.01$, † $p < 0.05$.

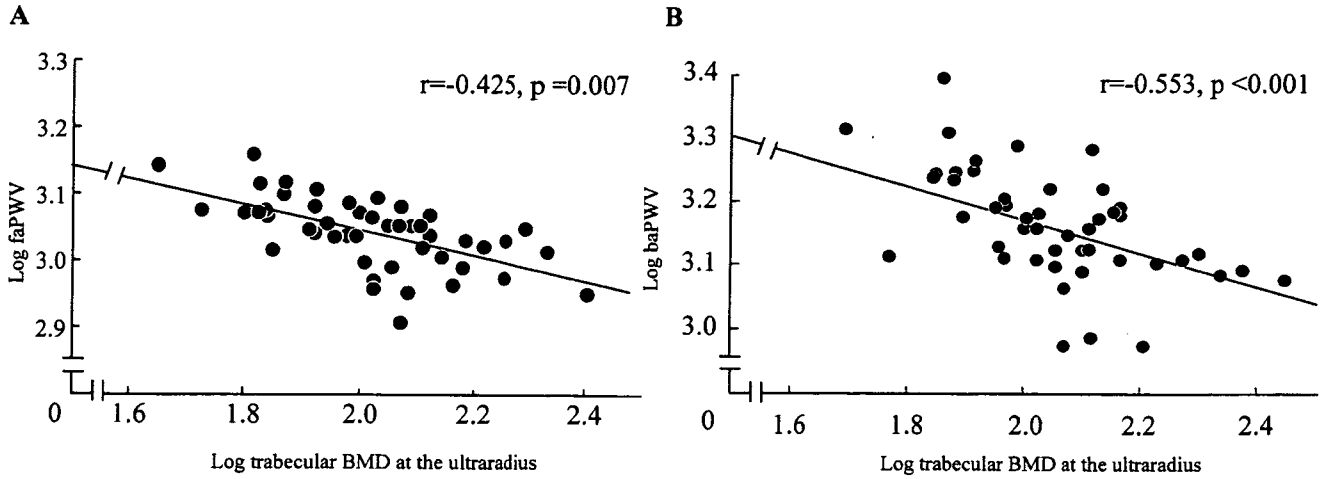

Figure 1. Correlation of trabecular bone density at the ultradistal radius with faPWV (A) and baPWV (B) in 47 RA patients. A significant positive correlation was found between trabecular bone density at the ultradistal radius and faPWV ($r = -0.425$, $p = 0.007$) and baPWV ($r = -0.553$, $p < 0.001$).

Table 4. Multiple regression analysis to evaluate the association of bone status and other risk factors with log femoral-ankle pulse wave velocity (faPWV) and log brachial ankle (ba)PWV in patients with RA. Standard regression coefficients (ß) are given in the table.

Independent Variables	Log faPWV Model 1	Log faPWV Model 2	Log faPWV Model 3	Log baPWV Model 1	Log baPWV Model 2	Log baPWV Model 3
Age	0.193	0.325	0.220	0.337**	0.421**	0.421**
Smoking/nonsmoking (–/+)	0.100	0.025	–0.050	0.105	0.076	0.121
Systolic BP	0.378†	0.386†	0.375†	0.452*	0.482*	0.420**
Log trabecular density at the ultradistal radius	–0.325†			–0.360**		
Log total density at the ultradistal radius		-0.006			–0.201	
Log calcaneous OSI			0.157			–0.081
R^2	0.426*	0.358**	0.230†	0.711*	0.655*	0.562*

BP: blood pressure; OSI: osteo-sono assessment index; R^2: multiple coefficient of determination. * $p < 0.001$, ** $p < 0.01$, † $p < 0.05$.

Table 5. Association between arterial stiffening and treatment with NSAID, corticosteroid, and methotrexate in 47 patients with RA. Values are shown as mean ± SD.

	Treated	Not Treated	p
NSAID			
No.	21	26	
Age, yrs	59.8 ± 9.8	61.0 ± 2.1	0.702
faPWV, cm/s	1130.5 ± 216.4	1102.7 ± 116.8	0.999
baPWV, cm/s	1614.1 ± 669.5	1549.9 ± 319.6	0.526
Corticosteroids			
No.	28	19	
Age, yrs	60.6 ± 9.5	60.3 ± 10.0	0.918
faPWV, cm/s	1077.1 ± 120.4	1149.5 ± 202.8	0.212
baPWV, cm/s	1489.3 ± 300.0	1659.2 ± 645.4	0.358
Methotrexate			
No.	19	28	
Age, yrs	59.0 ± 9.2	61.7 ± 10.2	0.372
faPWV, cm/s	1073.9 ± 122.0	1155.5 ± 203.1	0.169
baPWV, cm/s	1465.1 ± 277.9	1688.7 ± 657.5	0.129

faPWV: femoral-ankle pulse wave velocity; baPWV: brachial-ankle pulse wave velocity.

DISCUSSION

We observed that patients with RA exhibit increased arterial stiffening specifically associated with the prevalence of RA, as reflected by significant increases of baPWV and faPWV in these patients and an independent association of RA prevalence with increases in baPWV and faPWV when adjusted for age, systolic blood pressure, smoking habit, total cholesterol, and BMI. In a recent cross-sectional study, we showed that RA patients exhibit increased IMT of the common carotid artery, compared to healthy controls[5,6]. In a subsequent longitudinal study, we found that the annual increase in IMT of the common carotid artery was significantly greater in RA patients than in healthy controls, and that inflammation markers and increased bone resorption were significantly and independently associated with the increased rate of IMT[6]. Taken collectively, these data strongly suggest that RA patients might exhibit increased arterial stiffening in addition to arterial thickening through either RA-associated inflammation or increased bone resorption. Since RA patients might preferentially lose bone from the trabecular bone component at the ultradistal radius and calcaneus[14], we examined which site of bone loss might be important for increased arterial stiffening in RA patients. Multiple regression analysis revealed the trabecular BMD of the ultradistal radius, but not the calcaneus OSI, as an independent factor negatively associated with the greater PWV, even after adjustment for major risk factors for atherosclerosis, such as age, smoking habit, and systolic blood pressure (Table 4), indicating the importance of paraarticular bone loss in increased arterial stiffening in patients with RA. Trabecular bone density at the ultradistal radius, where bone loss occurs specifically for RA inflammation[14], may provide further support for the theory of independent association of RA prevalence with increased PWV values. The major mechanisms through which bone loss occurs in the trabecular bone component at the ultradistal radius and calcaneus are RA inflammation and impairment of activities of daily living (ADL), respectively[14]. Further, we reported that bone resorption around RA-inflamed joints contributes to an increase of the serum bone resorption markers pyridinoline and deoxypyridinoline[28]. These observations may explain the sig-

nificant association of ultradistal trabecular BMD with PWV values. In contrast, calcaneus OSI was not found to be associated with either baPWV or faPWV. These data may suggest the lesser importance of impairment of ADL in the increased arterial stiffening of patients with RA[14].

We previously described a positive association between the Larsen score for metacarpophalangeal joints and IMT of the common carotid artery in a cross-sectional study, suggesting the simultaneous progression of arterial wall thickening with bone destruction in RA patients[5]; and showed that in patients with early-stage RA, reductions in the trabecular BMD, but not the cortical BMD, at the ultradistal radius occurred when there was no decrease in BMD of the lumbar spine, suggesting the ultradistal radius as a major site of bone loss in patients with RA. In accord with the recent hypothesis on an intimate association of bone loss and atherosclerosis in non-RA patients[9,11,29], our study illustrates the relationship between bone loss at the ultradistal radius and increased arterial stiffening in patients with RA.

Administration of bisphosphonate, a bone antiresorptive drug, prevents an increase in IMT in diabetic patients, along with promoting an increase in BMD of the lumbar spine[30]. Further, in postmenopausal women, estrogen derivatives are known to protect against the development of atherosclerosis, while increasing the BMD of the lumbar spine and femur[12]. Although these patients exhibit generalized bone loss, while RA patients exhibit focal bone loss at the paraarticular trabecular bone in RA-involved joints[28], these data suggest that bone loss, either systemically or locally, might play an important role in the stiffening of arterial walls.

The possible mechanism underlying the association between bone loss and atherosclerosis may be explained by the similarity of several aspects of these processes. Recent studies have shown that atherogenic stimulation can induce the expression of molecules originally found in skeletal tissue, such as type I collagen, proteoglycans, osteonectin, osteopontin[31], osteocalcin[32], and also hydroxyapatite[31]. It was recently reported that smooth muscle cells, which derive from bone marrow, exist in atherosclerotic lesions. These results suggest that preferential differentiation of bone marrow cells into smooth muscle cells, rather than osteoblasts, may be one of the mechanisms linking bone loss and atherosclerosis.

Inflammatory cytokines such as tumor necrosis factor-α (TNF-α) play an important role in osteoporosis and atherosclerosis in patients with RA[33,34]. Increased production of TNF-α causes paraarticular bone loss and enhances arterial stiffening simultaneously in patients with RA. However, we found that bone resorption markers (DPD, NTX) and inflammation markers (CRP, ESR) were not significantly correlated with the PWV values. Since it is assumed that a long time period is required for RA inflammation and bone resorption to enhance atherosclerosis, the lack of a significant association between a single measurement of inflammation or bone resorption markers and PWV values in a cross-sectional study may be possible. In contrast, since paraarticular bone loss is assumed to reflect the sum of longterm effects of RA inflammation and bone loss, a significant association between paraarticular trabecular BMD and PWV values might be anticipated.

As a series of epidemiological studies showed that corticosteroid treatment is not associated with increased cardiovascular disease in RA patients[35,36], the use of corticosteroid, in addition to NSAID and methotrexate, did not affect the PWV value in the RA subjects in our study, as we previously described[5,6].

The limitation of our study is the small number of subjects and the restriction of subjects to postmenopausal women. However, since a significant association was observed between paraarticular bone loss at ultradistal radius and arterial stiffening in postmenopausal patients with RA, our study clearly demonstrates the relationship between metabolisms of bone and vessel in postmenopausal patients with RA, as well as in patients with other disease status[37], although a large-scale study is needed to draw a final conclusion.

We demonstrate that patients with RA have increased arterial stiffening, and we suggest that such stiffening may be explained in part by paraarticular trabecular bone loss at the ultradistal radius in these patients.

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Independent Association of Increased Trunk Fat with Increased Arterial Stiffening in Postmenopausal Patients with Rheumatoid Arthritis

MASAAKI INABA, KAZUMASA TANAKA, HITOSHI GOTO, SHINREI SAKAI, SHINSUKE YAMADA, HIROSHI NAKA, YASUO IMANISHI, and YOSHIKI NISHIZAWA

ABSTRACT. ***Objective.*** We recently reported increased arterial thickening and stiffening in patients with rheumatoid arthritis (RA) to which inflammation and increased bone resorption contributed. The current study examined the possible involvement of trunk fat in increased arterial stiffening in postmenopausal patients with RA.

Methods. RA patients (n = 30) and healthy controls (n = 30), all postmenopausal women, were examined for body adiposity and brachial-ankle pulse wave velocity (baPWV) by dual-energy x-ray absorptiometry and waveform analyzer, respectively. Subjects having other diseases and predisposed to atherosclerosis were excluded. Trunk:peripheral fat ratio was calculated as the fat mass of the trunk divided by the sum of the fat mass of arms and legs. Bone mineral density (BMD) at ultradistal radius was measured by peripheral quantitative computed tomography. Inflammation markers and bone resorption markers were also measured.

Results. Age, body mass index, and systolic blood pressure (BP) of RA patients were 60.8 ± 9.8 years, 22.5 ± 3.3, and 129.6 ± 20.8 mm Hg, respectively, which did not differ from data from healthy controls. Duration of RA was 10.4 years with mean daily dose of prednisolone 3.02 ± 3.85 mg. RA patients exhibited a significantly greater trunk:peripheral fat ratio (1.041 ± 0.253 vs 0.839 ± 0.223; $p < 0.001$) and baPWV value (1544.7 ± 304.9 vs 1373.8 ± 256.1; $p < 0.005$) than healthy controls. In RA patients, age ($r = 0.588$, $p < 0.001$), systolic BP ($r = 0.553$, $p < 0.005$), trabecular BMD at ultradistal radius ($r = -0.346$, $p = 0.061$), and trunk:peripheral fat ratio ($r = 0.366$, $p = 0.046$) were correlated with baPWV. Trunk:peripheral fat ratio did not differ significantly between RA patients with and those without prednisolone treatment. In multiple regression analysis that included age, systolic BP, and trunk:peripheral fat ratio as independent variables, the trunk:peripheral fat ratio emerged as an independent factor significantly associated with baPWV in RA patients. When trabecular BMD at ultradistal radius was inserted in place of trunk:peripheral fat ratio, it emerged as a factor that was significantly associated with baPWV.

Conclusion. We showed that increased trunk fat was significantly and independently associated with increased arterial stiffening in postmenopausal patients with RA. (J Rheumatol 2007;34:290–5)

Key Indexing Terms:

RHEUMATOID ARTHRITIS | ATHEROSCLEROSIS | ARTERIAL STIFFENING
PULSE WAVE VELOCITY | METABOLIC SYNDROME | CENTRAL OBESITY

Large epidemiological studies showed that one of the most important causes of death in patients with rheumatoid arthritis (RA) is cardiovascular disease[1-3]. We recently reported that RA prevalence by itself is a risk factor for accelerated atherosclerosis by cross-sectional[4] or longitudinal study[5], and that RA inflammation and enhanced bone loss, particularly at the paraarticular trabecular component, is responsible for the increased arterial wall thickening and stiffening[5,6]. Recently, evidence has accumulated that excess body fat, particularly visceral fat, is associated with the prevalence of the metabolic syndrome that increases cardiac risk[7,8]. Obesity might also promote preclinical atherosclerotic changes by a direct effect on vascular physiology[9,10]. We have reported that bone loss at weight-bearing bones, such as calcaneus, was significantly associated with impairment of physical activity in patients with RA, where it occurs preferentially[4,11], as well as in healthy subjects[12]. Therefore, the reduction of calcaneus osteo-sono index might result from the impairment of physical activity in patients with RA. It has been increasingly recognized that visceral obesity is intimately involved in the pro-

From the Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, Osaka, Japan.

M. Inaba, MD, PhD, Associate Professor; K. Tanaka, MD, Research Associate; H. Goto, MD, PhD, Lecturer; S. Sakai, MD, Research Associate; S. Yamada, MD, PhD, Research Associate; H. Naka, MD, Research Associate; Y. Imanishi, MD, PhD, Lecturer; Y. Nishizawa, MD, PhD, Full Professor, Department of Metabolism, Endocrinology and Molecular Medicine.

Address reprint requests to Dr. M. Inaba, Department of Metabolism, Endocrinology and Molecular Medicine, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan. E-mail: inaba-m@med.osaka-cu.ac.jp

Accepted for publication October 10, 2006.

gression of atherosclerosis due partly to the lack of physical activity[13]. RA patients' physical activity decreases as joint destruction progresses[14], and thus the loss of muscle mass is assumed to occur preferentially in the limb region as a result of impaired joint function. Although computed tomography is the gold standard to measure visceral fat mass, the amount of radiation exposure is too great for use in the general population. Alternatively, dual-energy x-ray absorptiometry (DEXA) allows us to determine the fat mass separately in trunk and peripheral regions; the resultant trunk:peripheral fat ratio provides a clinically relevant measure of central obesity[15].

These assumptions prompted us to examine whether patients with RA exhibited increased trunk:peripheral fat ratio, and to determine the involvement of central obesity in development of increased arterial wall stiffening in RA.

MATERIALS AND METHODS

Subjects. The subjects were all postmenopausal women, comprising 30 patients with RA and 30 healthy controls; all provided written informed consent to the study protocol. RA patients were recruited from the Outpatient Rheumatology Clinic at Osaka City University Hospital, and were diagnosed according to the 1987 revised criteria of the American College of Rheumatology (formerly the American Rheumatism Association)[16]. Healthy controls were selected from participants in a Local Health Check Program at Osaka City University Hospital. The study was approved by the Institutional Review Board of Osaka City University Graduate School of Medicine.

To avoid confounding by other known risk factors for atherosclerosis, both groups were selected on the basis of the following exclusion criteria: (1) hypertension, defined as blood pressure > 150/90 mm Hg, or use of antihypertensive medication; (2) hyperlipidemia, as diagnosed by the NCEP ATP III criteria[17] [total cholesterol > 240 mg/dl, low-density lipoprotein (LDL) cholesterol > 160 mg/dl, high-density (HDL) cholesterol < 35 mg/dl, or triglyceride > 150 mg/dl], or use of lipid-lowering medication; (3) diabetes mellitus, based on a history of diabetes or the expert committee criteria[18], or use of antidiabetic medication; (4) history of ischemic heart disease or cerebrovascular events; and (5) receiving hormone replacement therapy. Further, to avoid the effect of joint destruction on ultradistal radius bone mineral density (BMD) by peripheral quantitative computerized tomography (pQCT), RA patients whose bone structure of distal radius was destroyed as shown by radiographic examination were also excluded. All 30 RA patients were receiving multiple medications — 19 taking nonsteroidal antiinflammatory drugs, 13 methotrexate (MTX), 4 actarit, 1 salazosulfapyridine, 3 bucillamine, 5 MTX supplemented with folate, and 15 low-dose prednisolone (2 patients taking 1.0 mg/day, 2 patients 3.0 mg/day, 1 patient 4.0 mg/day, 6 patients 5.0 mg/day, 1 patient 6.0 mg/day, 1 patient 7.5 mg/day, and 2 patients 10.0 mg/day).

Measurement of clinical variables. Blood was collected after an overnight fast at the time of PWV measurement. A morning void urine sample, which subjects were asked to bring to the hospital, was also used. Blood samples were immediately centrifuged and the resultant serum samples were stored at −70°C until analyzed. Laboratory variables relevant to RA activity [erythrocyte sedimentation rate (ESR), platelet count, serum C-reactive protein (CRP), and rheumatoid factor (RF)] were measured by routine methods in RA patients. Serum levels of total cholesterol, triglyceride, and HDL cholesterol were determined using an autoanalyzer. LDL cholesterol was calculated by the formula of Friedewald, *et al*[19]. Urinary excretion of deoxypyridinoline and N-terminal telopeptide was measured as bone resorption marker, as described[20].

Measurement of body fat by DEXA. The percentage of body fat of the total body, trunk, arms, and legs was measured by DEXA (QDR-4500A, Hologic, Waltham, MA, USA)[21,22] (Figure 1). Fat from arms and legs was first summed to estimate peripheral fat, and a trunk:peripheral fat ratio variable was created by dividing trunk regional fat (g) by peripheral regional fat (g), as described[15]. The precision of measurements of fat mass are 1.5%, 0.8%, and 1.1% for the arms, legs, and trunk, respectively, according to the report of the manufacturer.

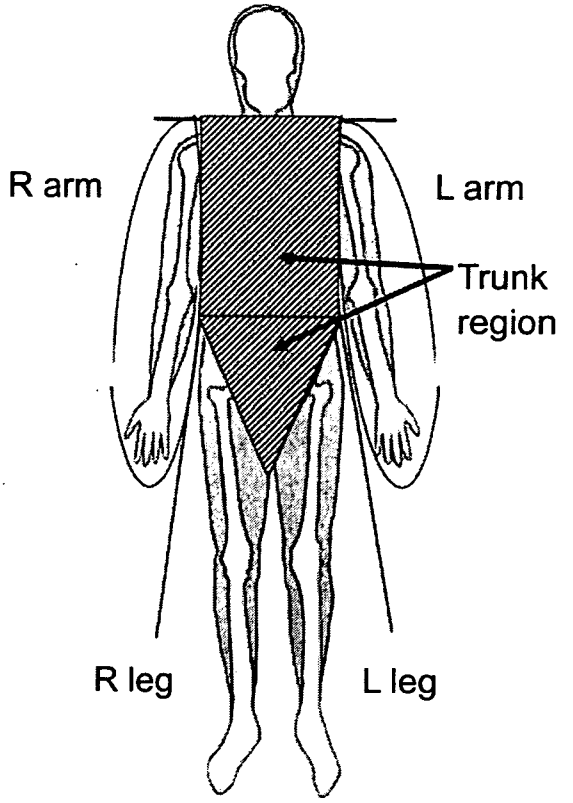

Figure 1. Estimation of trunk:peripheral fat ratio. Fat from both arms and legs was first summed to estimate peripheral fat. A trunk:peripheral fat ratio variable was created by dividing trunk regional fat (g) by peripheral regional fat (g).

Measurement of arterial stiffening by PWV. PWV was measured as an index of arterial stiffening as reported[6,23]. Resting blood pressure was determined in the right arm with a sphygmomanometer after at least 15 min of supine rest at the time of PWV measurement. The systolic blood pressure was taken upon appearance of Korotkoff sounds, and the diastolic blood pressure upon disappearance of such sounds. Results are reported as the average of 3 measurements. PWV was measured in the supine position after 5 min of bed rest, using an automatic waveform analyzer (model BP-203RPE; Colin, Komaki, Japan). Pressure waveforms of the brachial and tibial arteries were recorded by an oscillometric method, using occlusion/sensing cuffs adapted to both arms and both ankles. Pressure waveforms of the femoral arteries were recorded using multi-element tonometry sensors placed at the femoral artery. The electrocardiogram was monitored with electrodes placed on both wrists. Heart sounds S1 and S2 were detected by a microphone positioned at the left edge of the sternum at the third intercostal space. The waveform analyzer measures time intervals between S2 and the notch of the brachial pulse wave (Thb), between pulse waves of the femoral arteries (Tcf), and between pulse waves of the femoral and tibial (ankle) arteries (Tfa). Estimates of the path lengths of the heart-carotid (Dhc), heart-brachial (Dhb), heart-femoral (Dhf), and femoral-ankle (Dfa) segments were obtained based on the subject's height (HT, in cm), using the following formulas: Dhc = 0.2437 × HT − 18.999; Dhb = 0.2195 × HT − 2.0734; Dhf = 0.5643 × HT − 18.381; Dfa = 0.2486 × HT + 30.709. PWV was calculated for each arterial segment as the

path length divided by the corresponding time interval. Reproducibility of the PWV measurement was evaluated by repeated measurements in 17 healthy subjects on 2 different occasions. The coefficients of variation were 1.9% for baPWV and 3.3% for faPWV[21,24].

Measurement of BMD by pQCT. pQCT measurements were performed at 4% to the ulnar length proximal to the end of the radius (ultradistal site) with a single CT slice 2.5 mm thick on the nondominant side, using an XCT-960 scanner (Stratec Inc., Pforzheim, Germany) as described[11]. Briefly, the bone mineral content (mg/mm), cross-sectional bone area, and BMD (mg/cm^3) were determined at the ultradistal site for the entire cross-section, as well as for the trabecular compartment. After determination of the entire bone contour, the outer 65% of voxels were concentrically peeled off. The remaining 35% of voxels were defined as the trabecular region, while the peeled-off area was defined as the cortical plus the subcortical area[25]. Image processing and calculation of numerical values were performed using the manufacturer's software. The precision of the pQCT procedure ranged from 1% to 2%, depending upon the variable being assessed[26].

Statistical analysis. Variables with a normal distribution were expressed as mean ± SD, and differences between mean values were examined by Student's t test. Variables showing a non-normal distribution were summarized as median and range, and a nonparametric Mann-Whitney U-test was used to evaluate the differences between the median values. Linear regression analysis was performed to examine univariate correlation, and multiple linear regression analysis to assess independent associations between variables. Variables with skewed distributions were subjected to univariate and multivariate regression models after log-transformation of the data. P values < 0.05 were considered statistically significant. Statistical analysis was performed with StatView 5.0 for Windows (SAS Institute Inc., Cary, NC, USA).

RESULTS

Clinical characteristics of patients and controls. Clinical characteristics of the RA patients and healthy controls are shown in Table 1. The mean duration of RA was 10.4 ± 14.3 months (range 0.33–50 mo), with daily dose of prednisolone 3.02 ± 3.85 mg (range 0–15 mg). There was no significant difference between the 2 groups in age, body mass index, or systolic blood pressure, although trunk:peripheral fat ratio, faPWV, and baPWV were all significantly greater in RA patients than in controls. Trunk:peripheral fat ratio did not differ significantly between RA patients using and those not using steroid therapy (data not shown).

Factors correlated with PWV in RA patients. In RA patients, age ($r = 0.588$, $p < 0.001$) and systolic blood pressure ($r = 0.553$, $p = 0.0015$) showed a significant and positive correlation with baPWV, as reported[6] (Table 2). Trabecular, but not total, BMD at ultradistal radius showed a tendency to a negative correlation with baPWV ($r = -0.346$, $p = 0.061$), essentially as previously reported[11]. Of interest, the trunk:peripheral fat ratio showed a significant and positive correlation with baPWV ($r = 0.366$, $p = 0.046$).

Multiple regression analysis of factors associated with the level of PWV. Next, we tried to elucidate the factor independently associated with increased PWV value in RA patients. In multiple regression analysis that included age, systolic blood pressure and trunk:peripheral fat ratio as independent variables, the trunk:peripheral fat ratio and systolic blood pressure emerged as independent factors significantly associated with baPWV in RA patients. When trabecular or total BMD at ultradistal radius was inserted in place of the trunk:peripheral fat ratio, trabecular, but not total BMD, emerged as a significant factor (Table 3).

Table 1. Clinical characteristics of healthy controls and patients with RA.

Clinical Variables	Controls	RA Patients
No. of subjects	30	30
Age, yrs	65.6 ± 10.8	60.8 ± 9.8
Body mass index, kg/m^2	22.8 ± 4.1	22.5 ± 3.3
Systolic BP, mm Hg	139.3 ± 20.8	129.6 ± 20.8
Total cholesterol	210.3 ± 35.8	203.9 ± 35.9
HDL cholesterol	56.6 ± 16.3	63.5 ± 14.8
Triglyceride	113.0 ± 48.5	104.7 ± 36.1
Trunk:peripheral fat ratio	0.839 ± 0.223	1.041 ± 0.253
baPWV, cm/s	1373.8 ± 256.1	1544.7 ± 304.9
pQCT		
Total BMD at ultradistal radius (Z score, %)	101.2 ± 13.6	93.3 ± 26.0
Trabecular BMD at ultradistal radius (Z score, %)	121.2 ± 33.4	83.3 ± 45.0
RA duration, mo	–	10.4 ± 14.3
Prednisolone, mg/day	–	3.02 ± 3.85
CRP, mg/dl	ND	1.0 (0.1–8.0)
RF, IU/ml	ND	151.1 (9–1270)
ESR, mm/h	ND	49 (8–110)
Platelets (× 10^4/μl)	ND	26.3 (17.2–56.1)
DPD/Cre, nmol/mmol Cr	ND	7.9 (4.8–21.2)
NTX/Cre, nmol BCE/mmol Cr	ND	69.5 (29.3–153.9)

Values are shown as mean ± SD. Values of CRP, RF, ESR, platelets, DPD, and NTX are median (range). ND: not determined. HDL: high density lipoprotein; BP: blood pressure; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; DPD: deoxypyridinoline; NTX:/Cre, N-terminal telopeptide/creatinine ratio; baPWV: brachial-ankle pulse wave velocity; pQCT: peripheral quantitative computerized tomography.

Table 2. Univariate analysis of factors correlated with baPWV in patients with RA.

	baPWV	
	r	p
Age	0.588	0.0006*
Systolic blood pressure	0.553	0.0015*
RA duration	0.156	0.564
Total BMD at the ultradistal radius	–0.237	0.208
Trabecular BMD at the ultradistal radius	–0.346	0.061
Trunk:peripheral fat ratio	0.366	0.046**

* $p < 0.01$, ** $p < 0.05$.

DISCUSSION

Our study showed that trunk:peripheral fat ratio was significantly greater in patients with RA than in healthy controls, and that increased trunk:peripheral fat ratio in RA patients was independently associated with increased arterial stiffening observed in those patients. These data showed that an increase of abdominal fat might contribute to the increased arterial stiffening in RA patients, and also probably to increased cardiovascular mortality.

Inflammatory disease activity in RA is frequently accompanied by loss of body cell mass, known as rheumatoid cachexia[27]. This condition may manifest as low BMI and can also potentially contribute to excess cardiovascular burden. Recently, an epidemiological study confirmed that low BMI is associated with a significantly increased cardiovascular death rate[28], although no data were shown on RA disease activity. When the treatment of RA is not sufficient to suppress disease activity, patients with RA should lose body weight due to persistence of RA-associated inflammation. Since sustained inflammation is known to accelerate atherosclerosis in RA patients, those patients should have higher cardiovascular risk. We examined RA patients with their RA activity almost controlled, as reflected by their serum median CRP levels around 1.0 mg/dl, thanks to the introduction of MTX into the RA therapeutic regimen. Therefore, the effect of RA inflammation on atherosclerosis should have been attenuated in the patients enrolled in this study. As RA activity has been controlled, patients might easily gain body weight to develop central obesity, leading to increased arterial stiffening. These data raise the possibility that recent improvements of RA disease control by new therapeutic regimens may induce patients to gain body weight preferentially in the trunk region and to develop metabolic syndrome, which might contribute to the increased arterial stiffening, and possibly to the increased cardiovascular risk in patients. Although the metabolic syndrome is defined as those having abdominal circumference > 90 cm, RA patients in our study did not show such a great circumference on the basis of their normal BMI of 22.5 ± 3.3. However, the older age of patients with RA might play a role in development of central obesity. Further, the smaller amounts of muscle due to ethnic variation may be partly responsible.

Trunk fat is known as a risk factor for chronic diseases including metabolic syndrome, such as diabetes mellitus and cardiovascular diseases[29]. Although overall obesity increases the cardiovascular risk, central adiposity contributes to it to a greater extent by several specific mechanisms[30]. Trunk adipose tissue is reported to secrete various adipocytokines[31] that may induce endothelial dysfunction, impaired metabolic state[32], and atherogenic serum lipid profile[33], to increase cardiovascular risk[34]. Excess subcutaneous trunk fat and abdominal adiposity have also been shown to induce insulin resistance to enhance atherosclerosis[35,36].

An exercise intervention study reported a greater reduction in total abdominal and subcutaneous abdominal fat in the exercise weight-loss group than in the diet weight-loss group[37]. Another study clearly showed that mean BMI, the percentage of body fat, and the waist:hip ratio were significantly lower for each increasing physical activity level[38], suggesting that impaired physical activity often observed in patients with RA might enhance deposition of adipose tissue in the trunk region. Supporting this notion is our previous finding that bone mass at calcaneus was preferentially reduced even in the early stage of RA[11], and that this had a significant negative correlation with the score on the modified Health Assessment Questionnaire[4], suggesting that impairment of patients' physical activity occurs even in the early stage of RA.

Our study confirmed our previous finding[6] that trabecular

Table 3. Multiple regression analysis to evaluate the association of bone status and other risk factors with baPWV in patients with RA. Standard regression coefficients (ß) are given.

Independent Variables	baPWV Model 1	Model 2	Model 3
Age	0.270	0.386**	0.270**
Systolic blood pressure	0.325*	0.336	0.294
Trabecular bone density at the ultradistal radius	–0.328*		
Total bone density at the ultradistal radius		–0.083	
Trunk:peripheral fat ratio			0.321**
R^2	0.514*	0.315*	0.423**

* $p < 0.01$, ** $p < 0.05$. R^2, multiple coefficient of determination.

bone loss, but not cortical bone loss, at the paraarticular ultradistal radius was associated with increased arterial wall stiffening. The reduction of physical activity in patients with RA might cause loss of muscle mass in the limb region, as described[39]. Glucocorticoid is known to increase visceral fat, as observed in Cushing syndrome, since fat cells in the abdominal region have been thought to be more sensitive to hormonal factors than fat cells in other regions[40]. However, the effect of glucocorticoid in increasing abdominal fat could be negated in patients with RA receiving low-dose prednisolone, since no essential difference was observed in the trunk:peripheral fat ratio between RA patients with and those without prednisolone therapy. The limitation of our study is that it is not possible to conclude that the increased fat was involved in the development of increased arterial stiffening in postmenopausal patients with RA, since the study design was cross-sectional. Thus, a longitudinal study is needed to draw conclusions about the significance of central obesity in arterial stiffening in RA.

In summary, postmenopausal patients with RA who had success with drug treatment had significantly higher trunk:peripheral fat ratios that were independently associated with increased baPWV values; these findings suggest that increased trunk fat, as well as paraarticular bone loss, might be involved in the increased arterial stiffening in postmenopausal patients with RA.

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連通多孔体ハイドロキシアパタイトと骨髄間葉系細胞を用いた骨再生*

西川昌孝
名井　陽
大串　始
池内正子
玉井宣行
吉川秀樹**

[別冊整形外科 47：7～11，2005]

はじめに

整形外科分野において骨腫瘍，外傷，リウマチ性疾患，人工関節置換術後の弛みなどに対し，骨盤や腓骨などからの自家骨移植が行われてきた．しかし，採骨に伴う手術侵襲，採骨部の術後骨折などの合併症や採骨量の限界などの問題がある．今日までこれらの問題を解決するためアルミナ，バイオグラス，ハイドロキシアパタイト（HA）などさまざまな素材が使用されてきた．HA は生体適合性と骨伝導能を有した骨補填材料であるが，近年まで用いられてきた合成多孔体 HA には十分な連通多孔体構造が存在していない．連通多孔体 HA［NEOBONE®：東芝セラミックス社，東京］は平均気孔径 150 μm，平均連通孔径 40 μm，気孔率 75%であるが，その連通構造のため深部気孔内にまで細胞が進入することができ，従来の多孔体 HA に比べ優れた骨伝導能を示す[1]．しかし，このように優れた骨伝導能をもつ NEOBONE® でさえ骨誘導能はもたず，巨大な欠損や感染後などの劣悪な骨再生環境では人工骨単体では十分な骨再生を得られない．

近年，再生組織工学の手法を用いた新鮮骨髄細胞や培養増殖させた骨髄間葉系細胞（marrow mesenchymal cells：MMCs）を導入した多孔体 HA の骨形成能が報告されており[2～9]，連通多孔体構造をもつ NEOBONE® は骨形成細胞を容易に気孔内に導入することができる．今回骨再生組織工学の担体としての NEOBONE® の有用性を検討した．

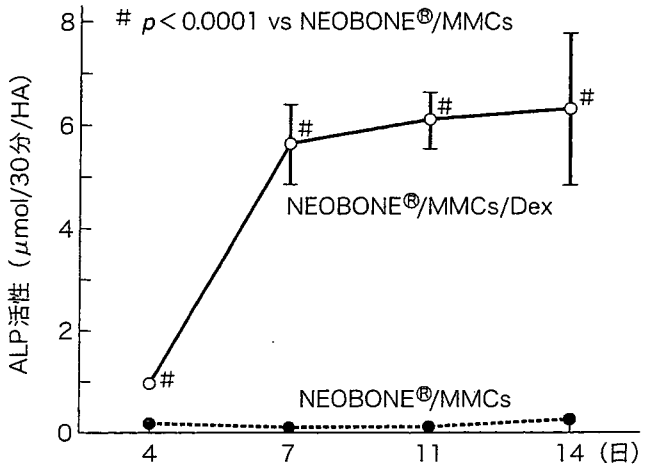


図 1．NEOBONE® 培養人工骨の *in vitro* でのアルカリホスファターゼ（ALP）活性． デキサメタゾン（Dex）の添加培養により骨芽細胞への初期分化マーカーである ALP 活性は 7 日目から高値を示した．

I．培養人工骨の作成

Fischer 344 ラットの大腿骨から採取した骨髄細胞を培養し，MMCs を回収した[10]．これを Yoshikawa ら[11]の方法に準じて 10^6細胞/ml の細胞浮遊液に調整し，直径 5 mm，厚さ 2 mm の円盤状に形成した NEOBONE® ならびにほかの 3 つの日本の合成多孔体 HA（HA-A，HA-B，HA-C）を一晩浸し，細胞を接着させた．次にデキサメタゾン（Dex），β-グリセロリン酸，ビタミン C 存在下で培養分化させたのち，同系ラットの皮下に移植した．

Key words
bone tissue engineering，hydroxyapatite，marrow mesenchymal cell

*Bone tissue engineering using interconnected porous calcium hydroxyapatite loaded marrow mesenchymal cells
要旨は第 25 回日本バイオマテリアル学会において発表した．

**M. Nishikawa, A. Myoui：大阪大学大学院医学系研究科器官制御外科学整形外科（Dept. of Orthop, Osaka University Graduate School of Medicine, Suita）；H. Ohgushi（グループ長），M. Ikeuchi：産業技術総合研究所セルエンジニアリング研究部門組織・再生工学研究グループ；N. Tamai, H. Yoshikawa（教授）：大阪大学大学院医学系研究科器官制御外科学整形外科．

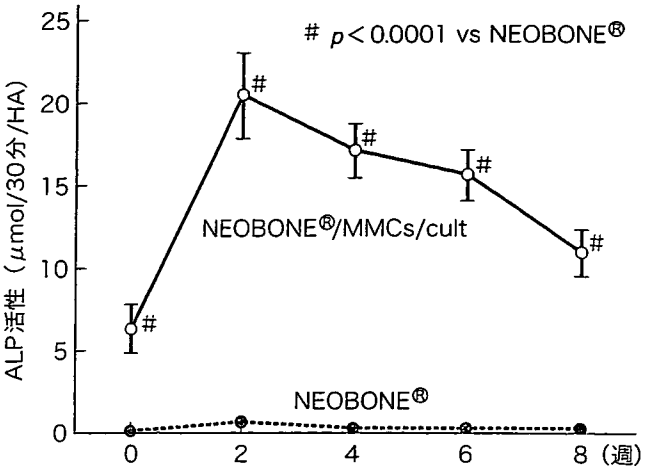


図2. NEOBONE®培養人工骨の *in vivo* でのアルカリホスファターゼ（ALP）活性. NEOBONE®培養人工骨（NEOBONE®/MMCs/cult）の骨芽細胞への初期分化マーカーであるALP活性は移植後2週目にピークとなり徐々に減少したが，移植後8週目でも高値を維持している.

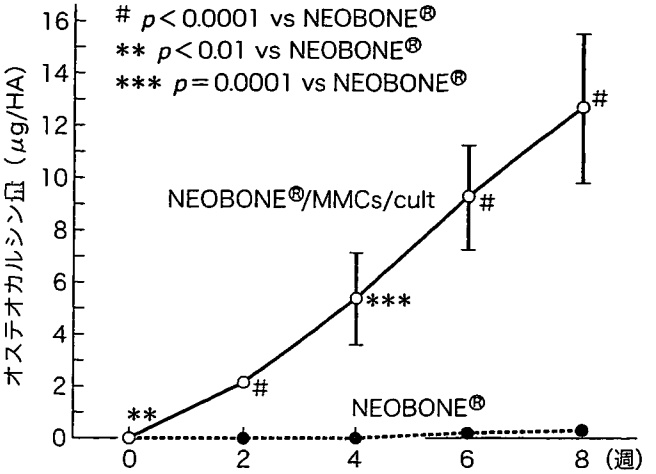


図3. NEOBONE®培養人工骨の *in vivo* でのオステオカルシン量. NEOBONE®培養人工骨（NEOBONE®/MMCs/cult）の骨芽細胞への後期分化マーカーであるオステオカルシン量は移植後経時的に増加している.

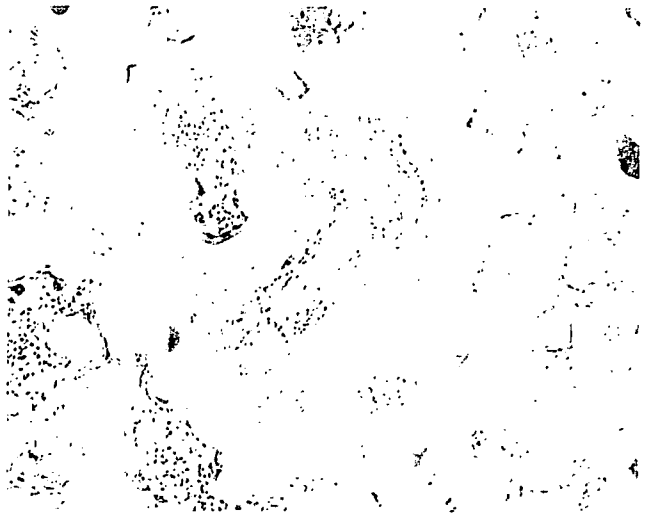


図4. NEOBONE®培養人工骨の移植後2週目の組織像（HE染色，100倍）. 気孔内に骨芽細胞による活発な新生骨の形成が多数観察される.

Ⅱ. 培養人工骨の生化学的分析

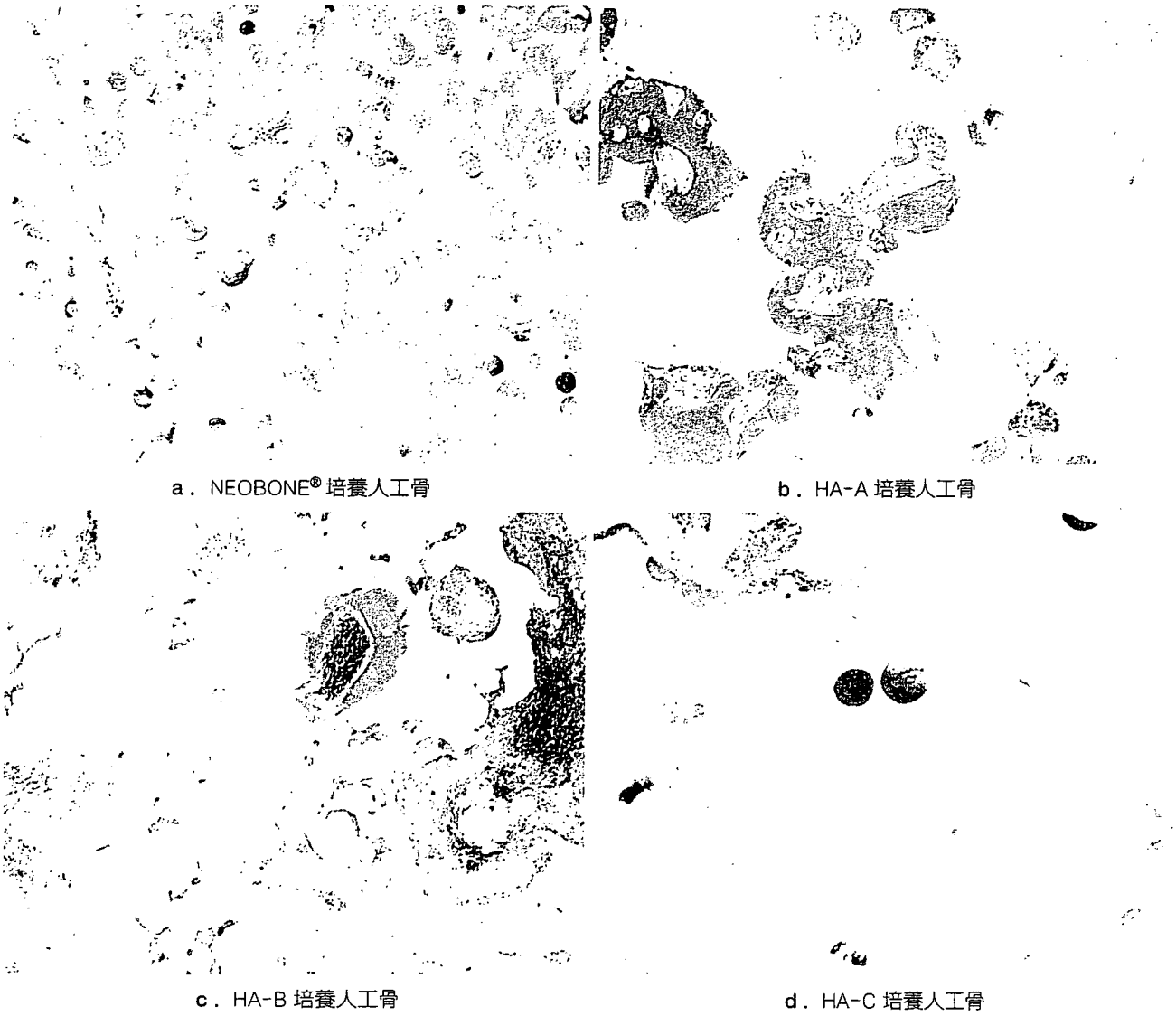
骨芽細胞への初期分化マーカーであるアルカリホスファターゼ（ALP）活性をNEOBONE®を担体とした培養人工骨（NEOBONE®培養人工骨）で測定したところ，培養7日目から高値を示し14日目の培養終了時まで維持された（図1）. その後ALP活性はラットの皮下移植後2週目にピークとなり徐々に減少したが，移植後8週目でも高値を維持した（図2）. また骨芽細胞への後期分化マーカーであるオステオカルシンの含有量は移植後経時的に増加した（図3）. 以上より，NEOBONE®上にて培養分化誘導されたMMCsは骨芽細胞へ分化したことが確認された.

Ⅲ. 培養人工骨による生体内での骨形成

図4は本来HA単体では骨形成が生じない皮下への移植後2週目のNEOBONE®培養人工骨の組織像である. NEOBONE®の気孔内に骨芽細胞による活発な新生骨形成が多数観察され，培養人工骨の骨形成能が証明された. また図5aは移植後8週目の組織像であるが，新生骨や骨髄細胞がほぼすべての気孔内で観察されたのに対し，ほかの日本の合成多孔体HAを担体とした培養人工骨は不十分な連通多孔体構造のためわずかな骨形成しか示さず，まったく組織侵入のない気孔も存在した（図5b～d）. 以上の結果は骨再生組織工学の担体としての多孔性HAにおいて，NEOBONE®の有する連通多孔体構造の重要性を示している.

Ⅳ. 培養人工骨内新生骨の三次元評価

培養人工骨中の新生骨量の定量測定のためにマイクロCT分析を行った. 前述の組織学的分析はサンプルの切断面レベルにより結果が異なり，サンプル全体の骨量を正確に反映しない. この点マイクロCTは三次元構造を再構築することができ，新生骨の三次元分布やサンプル全体の骨量を測定することができる[12]. 図6aは移植後8週目の培



a．NEOBONE® 培養人工骨　　b．HA-A 培養人工骨

c．HA-B 培養人工骨　　d．HA-C 培養人工骨

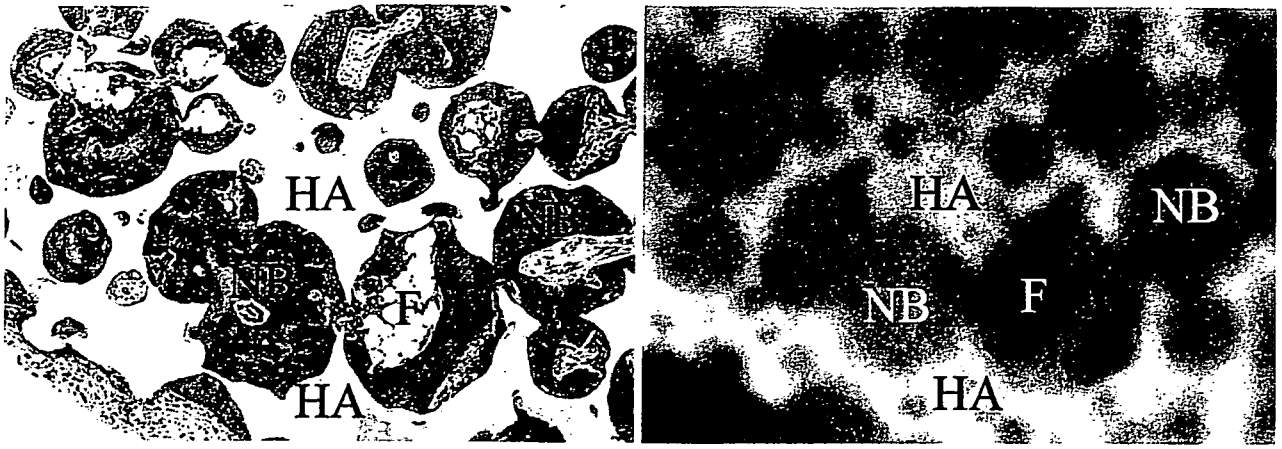
図 5．NEOBONE® 培養人工骨およびほかの市販合成多孔体 HA 培養人工骨の移植後 8 週目の組織像（HE 染色，40 倍）．NEOBONE® 培養人工骨ではほぼすべての気孔内で新生骨が確認されるが，ほかの市販合成多孔体 HA 培養人工骨はわずかな骨形成しか示さない．

養人工骨の組織像，図 6b はほぼ同部位のマイクロ CT である．マイクロ CT はグレースケールで表現されるが，これを組織像と比較して閾値を決定することにより HA，新生骨（NB），軟部組織（F）を区別することができる．コンピュータソフトにより中間色の新生骨領域を図 6c のようにオレンジ色に変換した．

移植後 8 週目の NEOBONE® 培養人工骨中央での新生骨の分布を評価した（図 7）．白い部分はアパタイトで，オレンジ色は図 6 で示した新生骨である．表面だけでなく中央の気孔内にまで新生骨が確認された．次にこのオレンジ色部分の体積を新生骨量として定量評価した．図 8 に示すように，新生骨は 2 週目から検出され，期間とともに増加した．また，移植後 8 週目の HA-A，B，C の各人工骨を担体とした培養人工骨での新生骨量も測定したところ，NEOBONE® を担体とした培養人工骨よりも有意に低値であった（表 1）．

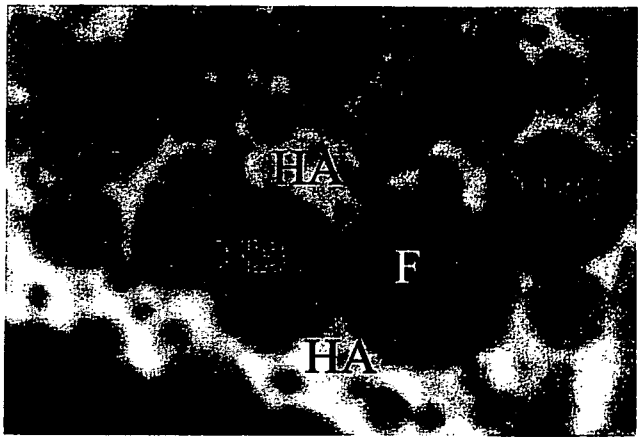
V．考　察

骨形成条件が不良な骨欠損の修復において，再生組織工学の手法を利用した再生医療には大きな期待が寄せられている．この手法は多孔体担体を必要とし，その担体は生体適合性と荷重下での骨格構造を支持するため十分な初期強度を要する．さらに細胞を導入するため三次元連通構造を必要とする．連通多孔体構造をもつ NEOBONE® の連通性および高気孔率は骨髄間葉系細胞などの骨形成細胞の導入



a．NEOBONE® 培養人工骨移植後 8 週目の組織像（HE 染色，100 倍）

b．マイクロ CT



c．コンピュータによる新生骨領域抽出像

図 6．NEOBONE® 培養人工骨内新生骨のマイクロ CT 評価．マイクロ CT（b）はグレースケールで表現されるが，これを組織像と比較して閾値を決定することにより HA，新生骨（NB），軟部組織（F）を区別することができる．コンピュータソフトにより中間色の新生骨領域を c のようにオレンジ色に変換した．

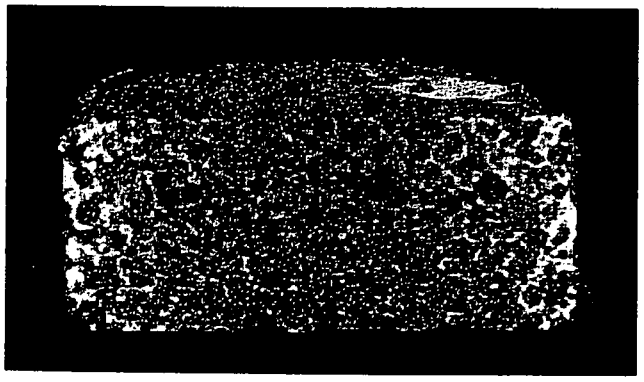


図 7．移植後 8 週目の NEOBONE® 培養人工骨中央での新生骨分布（白色：HA，オレンジ色：図 6 で示した新生骨）．表面だけでなく中央の気孔内にまで新生骨が確認される．

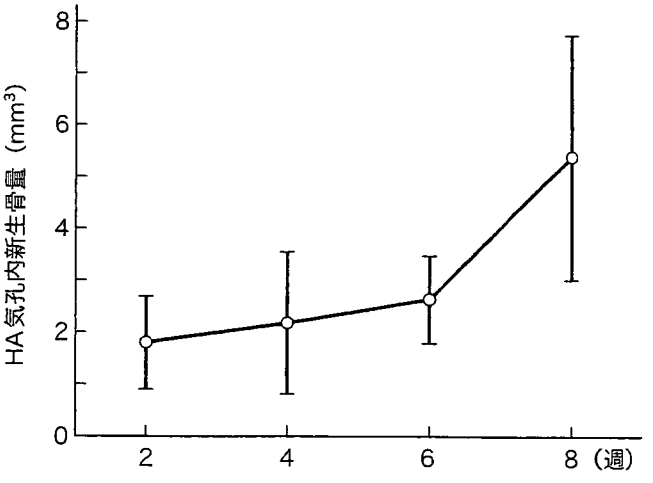


図 8．NEOBONE® 培養人工骨内新生骨量の経時変化．新生骨量は移植後経時的に増加している．