

Original Article

Evaluation of Plaque Stabilization by Fluvastatin with Carotid Intima-Medial Elasticity Measured by a Transcutaneous Ultrasonic-Based Tissue Characterization System

Toshio Yamagishi¹, Makoto Kato², Yoshiro Koiwa³, Ken Omata⁴, Hideyuki Hasegawa^{5,6}, and Hiroshi Kanai^{6,5}

¹Department of Internal Medicine, Tohoku Kosai Hospital, Sendai, Japan

²Panasonic Shikoku Electronics Co., Ltd, Yokohama, Japan

³Oizumi Memorial Hospital, Shiroishi, Japan

⁴Health Administration Center, Miyagi University of Education, Sendai, Japan

⁵Department of Biomedical Engineering, Graduate School of Biomedical Engineering, Tohoku University, Sendai, Japan

⁶Department of Electronic Engineering, Graduate School of Engineering, Tohoku University, Sendai, Japan

Aim: As an approach to tissue characterization, we attempted to classify in vivo carotid plaque tissues in terms of arterial wall elasticity instead of echogenicity on B-mode scanning and investigated whether the effect of fluvastatin on carotid elasticity can be detected in hypercholesterolemic patients.

Methods: In 170 subjects, simultaneous measurements of intima-media thickness (IMT) and elastic modulus in the circumferential direction (E_{θ}) were performed using a new transcutaneous high-resolution Doppler technique.

Results: From the observation of various tissues, an elasticity library was obtained as follows: lipid core, 22 ± 15 kPa; calcification, 674 ± 384 kPa; lipid core- and calcification-free plaques, 173 ± 69 kPa; smooth muscle, 104 ± 32 kPa; blood clot, 85 ± 68 kPa; fibrosis, 273 ± 173 kPa.

The effect of fluvastatin (30 mg/day, $n=62$) was assessed over 12 months using the elasticity distribution and serum markers. The statin reduced low-density lipoprotein cholesterol, high-sensitivity CRP, mean IMT and mean E_{θ} , and increased nitrite/nitrate. In the max IMT ≥ 1.1 mm group, both E_{θ} and IMT decreased significantly. On the other hand, in the max IMT < 1.1 mm group, E_{θ} but not IMT decreased significantly. The histogram of the subgroups showing increased E_{θ} with max IMT ≥ 1.1 mm revealed a decrease in areas corresponding to E_{θ} of 20–200 kPa (lipid/smooth muscle-rich tissue) and an increase in relatively hardened areas of < 250 kPa (collagen fibers).

Conclusion: Non-invasive echographic carotid arterial elasticity measurement is useful for the classification of atherosclerotic plaques and evaluation of chronological and histopathological changes.

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Key words; Elasticity, Statin, Echo, Carotid artery

Introduction

Ultrasonography of the carotid arteries is a common imaging study performed for the diagnosis of atherosclerosis, not only in carotid but also in systemic arteries. Conventional evaluations are based on the

measurement of carotid arterial intima-media thickness (IMT) to detect morphological changes and remodeling, such as thickening and calcification¹⁻⁵ and on integrated backscatter (IBS) analysis using echogenicity on B-mode scanning for the soft/hard characterization of plaque composition⁶⁻⁸; however, these procedures provide no information on the mechanical stability of plaque against rupture and for tissue characterization. Recently, a new ultrasonic system that can measure regional elasticity in the arterial wall utilizing the phased tracking method has been developed⁹⁻¹⁵. Measurements of the elasticity distribu-

Address for correspondence: Toshio Yamagishi, Department of Internal Medicine, Tohoku Kosai Hospital, 2-3-11, Kokubuncho, Aoba-ku, Sendai 980-0803, Japan

E-mail: tyamagishi@tohokukosai.com

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Table 1. Characteristics of the study population

	Healthy volunteers in 20s-40s	Patients with hyperlipidemia	<i>p</i> -value
N	46	72	
age	35.4 ± 2.7	60.4 ± 7.6	< 0.0001
mean IMT (mm)	0.54 ± 0.08	0.88 ± 0.25	< 0.0001
max IMT (mm)	0.68 ± 0.08	1.15 ± 0.20	< 0.0001
mean <i>E</i> _θ (kPa)	118.8 ± 26.8	158.5 ± 62.0	0.0006
SBP (mmHg)	111.2 ± 7.8	135.2 ± 13.8	< 0.0001
DBP (mmHg)	70.1 ± 7.4	81.3 ± 8.5	< 0.0001
PP (mmHg)	41.1 ± 9.0	54.0 ± 11.7	0.0001
PR (bpm)	70.8 ± 8.9	71.3 ± 9.6	NS
TC (mg/dL)	177.6 ± 45.5	255.4 ± 32.8	< 0.0001
TG (mg/dL)	119.2 ± 70.7	141.9 ± 96.1	NS
HDL-C (mg/dL)	50.7 ± 10.3	59.8 ± 15.9	NS
LDL-C (mg/dL)	98.1 ± 29.8	154.7 ± 21.8	< 0.0001
FPG (mg/dL)	87.0 ± 9.3	98.8 ± 13.1	0.0128
HbA _{1c} (%)	4.7 ± 0.5	5.2 ± 0.7	0.0222
baPWV (cm/s)	1,106.4 ± 220.4	1,592.0 ± 306.9	< 0.0001

SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, PR: pulse rate, TC: total cholesterol, TG: triglyceride, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, FPG: fasting plasma glucose, baPWV: brachio-ankle pulse velocity wave.

tion of lipids, blood clots, fibrous tissue and calcified tissue *in vitro* have been reported previously, and an elasticity image can be classified into tissue components using the reference data obtained by *in vitro* experiments¹⁶. The usefulness of measuring the elastic modulus of the common carotid arteries (CCAs) by ultrasound imaging to detect early-stage atherosclerotic lesions caused by risk factors with regard to lifestyle-related disease has also been reported¹⁵.

The aim of the present study was to evaluate and classify *in vivo* carotid plaque tissues in terms of arterial wall elasticity using this novel real-time ultrasonic measurement system rather than conventional methods. Carotid plaque was evaluated to assess atherosclerotic lesions, such as plaques, that showed an increase of elasticity with progressive lipid deposition and fibrosis resulting from chronological and histopathological changes. In addition, we assessed whether the effects of fluvastatin on plaque stabilization can be detected by carotid elasticity distribution in patients with hypercholesterolemia.

Methods

Study Population

A total of 170 subjects, including healthy volun-

Table 2. Characteristics of patients with lipid core or calcification in carotid arteries

	Patients with lipid core	Patients with calcification
N	18	33
age	56.8 ± 9.1	61.8 ± 8.9
mean IMT (mm)	0.91 ± 0.16	0.93 ± 0.17
max IMT (mm)	1.21 ± 0.20	1.26 ± 0.27
mean <i>E</i> _θ (kPa)	145.7 ± 40.6	193.7 ± 55.4
SBP (mmHg)	129.8 ± 20.0	140.1 ± 17.7
DBP (mmHg)	79.8 ± 14.1	81.2 ± 9.9
PP (mmHg)	50.0 ± 13.5	58.9 ± 12.6
PR (bpm)	71.5 ± 12.8	72.3 ± 10.6
TC (mg/dL)	230.4 ± 36.8	228.6 ± 34.5
TG (mg/dL)	148.5 ± 82.3	117.5 ± 52.7
HDL-C (mg/dL)	52.6 ± 13.9	56.4 ± 13.4
LDL-C (mg/dL)	140.5 ± 37.5	137.0 ± 30.1
FPG (mg/dL)	116.9 ± 45.5	104.1 ± 24.6
HbA _{1c} (%)	5.5 ± 1.3	5.2 ± 0.6
baPWV (cm/s)	1,421.1 ± 144.4	1,644.4 ± 306.6

SBP: systolic blood pressure, DBP: diastolic blood pressure, PP: pulse pressure, PR: pulse rate, TC: total cholesterol, TG: triglyceride, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, FPG: fasting plasma glucose, baPWV: brachio-ankle pulse velocity wave.

teers, outpatients with untreated hypercholesterolemia, patients with lipid core or calcification, and a single case of acute-onset carotid artery dissection, were enrolled in this study from 2004 to 2006.

The characteristics of the study population, except the case of carotid artery dissection, are shown in **Table 1, 2**. Seventy-two subjects with hypercholesterolemia were defined in accordance with the guidelines of the Japanese Society of Atherosclerosis. Hypercholesterolemia was defined as total cholesterol ≥ 220 mg/dL or low-density lipoprotein cholesterol (LDL-C) ≥ 140 mg/dL and triglyceride (TG) ≥ 150 mg/dL. None of the subjects had hypertension, diabetes, a history of recent cardiovascular events, ischemic heart disease, atrial fibrillation, arteriosclerosis obliterans (ASO, defined as an ankle/brachial blood pressure index [ABI] < 0.9), renal insufficiency with serum creatinine greater than 3 mg/dL, or severe hepatic disorder. Patients with congestive heart failure were excluded from this study.

Some patients with lipid core and/or calcification on B-mode images included untreated patients with hypertension, diabetes mellitus, hypertension being defined as systolic blood pressure (BP) ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg. Diabetes mellitus was defined as a fasting plasma glucose (FPG) ≥ 126

mg/dL. They did not have any complications.

None of the patients were taking any drugs, such as inhibitors of the renin-angiotensin system, Ca channel blockers and anti-diabetic agents.

Similar examinations were conducted in 46 healthy subjects between 20 and 49 years of age, who served as the control group.

In addition, we assessed the effect of fluvastatin (30 mg/day, $n=62$) during a period of 12 months, evaluating IMT, elasticity distribution, brachial-ankle pulse wave velocity (baPWV) and serum and markers, including high-sensitivity CRP (hs-CRP) and nitrite/nitrate (NOx). In the control group ($n=10$), no statin treatment was performed.

The subjects provided informed consent, and the study was approved by the Institutional Ethics Committees of Tohoku Kosai Hospital and Matsushita Electrical Industrial Co., Ltd.

Carotid IMT and Elasticity Measurements

The subjects were studied after having abstained from caffeine, alcohol, and smoking during the previous 12 hours. They rested in a supine position for 15 min in a quiet room before the baseline hemodynamic measurements were obtained. Brachial blood pressure and heart rate (HR) (mean of three readings) were measured in the right arm with an automated digital oscillometric sphygmomanometer (EW-3110; Matsushita Electric Works, Osaka, Japan). An imaging study of the bilateral common carotid arteries was performed using a newly developed high-resolution ultrasonic measurement system (Panasonic, prototype) with a center frequency of 7.5 MHz. One well-trained sonographer who regularly participates in quality control measurement sessions performed all ultrasound scans^{13, 15}. The sonographer has been trained to obtain an elastic modulus measurement deviation of only 6% in the same subject (inter- and intra- variations were less than 10% at our institution). In order to obtain an accurate measurement of the same sites of carotid arteries in repeated measurements, the sonographer performed IMT and elastic modulus measurements by comparing current B-mode images with past B-mode images on the PC display and positioned the probe so as to measure a similar position in the carotid arteries.

IMT can only be determined accurately in the far wall position because only the far wall IMT is defined by leading edges, which enables correct ultrasonographic representation; therefore, the far walls of the following sites were measured: (1) the distal 3 cm of the straight part of both CCAs and (2) the distal 1 cm of both carotid bulbs. As in several previous studies, we defined a plaque as a focal raised lesion, using

an IMT cutoff value of 1.1 mm. Mean IMT and max IMT were determined to be the mean value of IMT and the maximum value of IMT, respectively, at six locations in the bilateral CCAs, including the carotid bulb.

This system measures the radial strain of the arterial wall during one cardiac cycle and calculates the elasticity from radial strain and pulse pressure. The distribution of elasticity is displayed as a 2D cross-sectional color-coded image in B-mode imaging, and the image is updated at every heartbeat. To measure minute changes in thickness during one cardiac cycle, this system uses the phased tracking method⁵⁻¹⁰. Elasticity was estimated at intervals of 80 μm along each ultrasonic beam in the depth direction and 400 μm in the arterial longitudinal direction. From the maximum change in thickness, ($h_{\text{max}} - h_{\text{min}}$), during one heartbeat, the radial strain ε_r of each assigned layer in the artery wall was calculated as follows:

$$\varepsilon_r = (h_{\text{max}} - h_{\text{min}}) / h_{\text{max}}, \quad (1)$$

where h_{max} and h_{min} are the maximum and minimum thickness of an assigned layer in the wall during one heartbeat, respectively.

The elastic modulus in the radial direction (E_r) and that in the circumferential direction (E_θ) were calculated as follows:

$$E_r = \text{Pulse Pressure} / \varepsilon_r \quad (2)$$

$$E_\theta = (1/2) \times (r_0/h_0 + 1) \times E_r \quad (3)$$

In these formulae, pulse pressure (PP) is the difference between systolic and diastolic brachial blood pressure (carotid blood pressure would be more accurate but is difficult to measure), and h_0 and r_0 are the initial wall thickness and radius of the vessel in end-diastole, respectively. The radial strain ε_r is caused by radial compression and circumferential stretching due to the increase of blood pressure Δp , with longitudinal strain being disregarded. As this circumferential stretching produces radial strain due to the incompressibility of the arterial wall, both radial and circumferential stresses should be considered when evaluating elasticity; however, as shown in Eq. (2), E_r is defined as the ratio of pulse pressure (which corresponds to radial stress) and radial strain. The circumferential stress acting on the cylindrical shell is not identical to pulse pressure, but also depends on r_0/h_0 . Thus, E_r is apparent elasticity in the radial direction and is dependent on r_0/h_0 as well as on the elasticity of the arterial wall. Alternatively, E_θ of Eq. (3) was introduced to evaluate the elasticity of the arterial wall by suppressing the influence of r_0/h_0 ⁷. The elastic modulus in each instrumentation locus was expressed in a histo-

gram, and the elasticity distribution of each tissue composition was determined.

The length and thickness of the ROI (region of interest), in which the elasticity distribution and average elasticity were calculated, can be changeable and depend on the effective size of the interested area, including IMT. When we obtained the elasticity library, by referring to B-mode echo images, we changed the sizes and positions of ROI within plaques of patients in order to confirm that areas with extreme differences in brightness and/or echo pattern are not included in the region of interest.

PWV Measurements

Brachial-ankle PWV (baPWV) was measured using a volume-plethysmographic apparatus (Form/ABI; Colin Co., Ltd., Komaki, Aichi, Japan) by simultaneous BP and waveform measurements on all four limbs, in addition to ECG and phonogram tracings. PWV was automatically calculated by time-phasic analysis using the following formula: distance between two sites divided by pulse wave transit time. Values for baPWV were measured from the ascending point shown by the right brachial pulse volume recorder to the ascending point indicated by each ankle pulse volume recorder. The distance between the right brachium and ankle was estimated as described previously¹⁶.

Blood Examination, NO_x and hs-CRP

The patients were requested to fast overnight without medication until blood samples were drawn. Venous blood samples were then drawn for analysis of FPG and serum concentrations of total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C) and HbA1c by standard laboratory methods. Serum LDL-C was calculated by Friedewald's equation. None of the patients had a TG >400 mg/dL, at which the Friedewald equation is known to yield unreliable results.

The serum level of NO_x (nitrite/nitrate; NO₂-/NO₃-), an indicator of NO, was measured by the Griess reaction after nitrate reductase treatment¹⁷ with a flow injection analysis system (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan). The serum NO_x level was 10–71 μmol/L and the coefficient of its variation was 3.0%.

The serum level of hs-CRP was measured by the BNII High Sensitivity CRP assay using anti-CRP monoclonal antibody coated with polystyrene particles and fixed-time kinetic nephelometric measurements (N High Sensitivity CRP; DadeBehring Inc., Marburg, Germany) with a lower detection level of 0.2

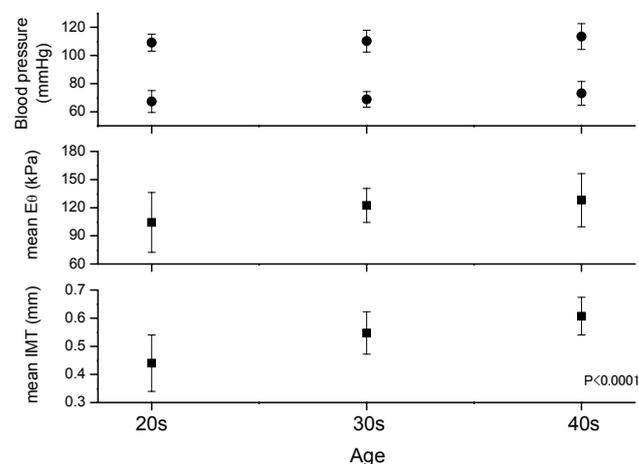


Fig. 1. Effects of aging on IMT and elastic modulus in healthy volunteers.

IMT increased with greater age, but not with higher blood pressure. Overall, E_{θ} in healthy volunteers was not affected by aging.

mg/L and a coefficient of variation of 3.0%¹⁸).

Statistical Analysis

Data were expressed as the mean \pm SD. Statistical analysis was performed using a JMP[®] software package (JMP 7.0.1; SAS Institute, USA), a value of $p < 0.05$ being considered significant. Differences between groups were analyzed by one-way analysis of variance (ANOVA). A post-hoc test for multiple comparisons was used when ANOVA showed significance. Linear regression analysis was performed to evaluate the association between IMT, elasticity or baPWV, and other clinical variables.

Results

Table 1 shows the characteristics of the study population of healthy volunteers ($n=46$), hypercholesterolemia patients ($n=72$), patients with a lipid core in carotid artery plaque ($n=18$), and patients with calcification ($n=33$). The average blood pressure (systolic/diastolic) was significantly lower in healthy volunteers than in other subjects (ANOVA, $p < 0.0001$) and other risk factors for cardiovascular disease were also lower in healthy volunteers.

Elasticity Library

The effects of aging on the mean IMT and mean E_{θ} in healthy volunteers in their twenties ($n=15$), thirties ($n=12$) and forties ($n=19$) are shown in **Fig. 1**. We were unable to obtain data for healthy volunteers over fifty. Although IMT increased signifi-

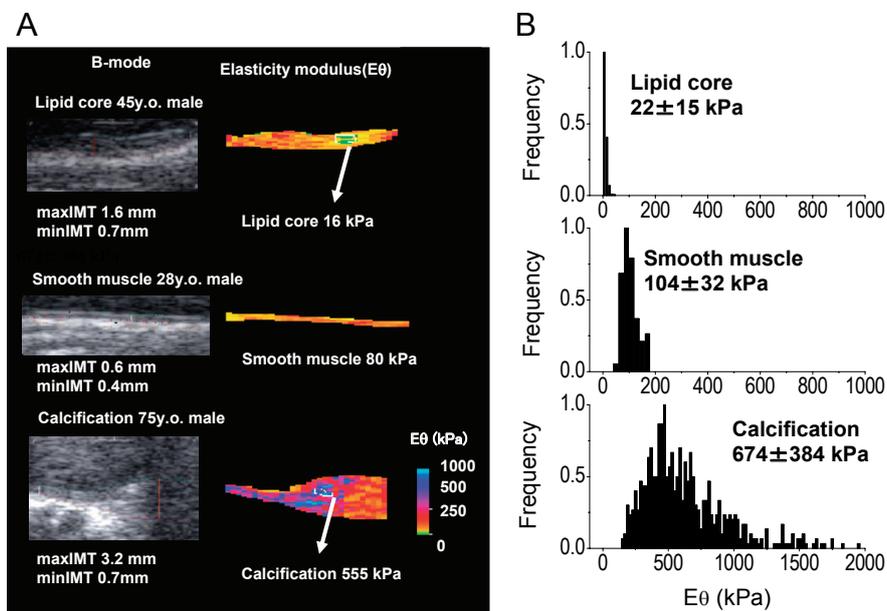


Fig. 2. Elastic modulus of lipid core, smooth muscle and calcification.

The right panel (A) shows the relation between B-mode findings and calculated E_{θ} . The value of E_{θ} was related to tissue composite elements. The left panel (B) shows a histogram for E_{θ} (lipid core, smooth muscle and calcification).

cantly by age, there was no significant increase in E_{θ} or blood pressure. In healthy volunteers in their thirties and forties, no significant increases in TC, HDL-C, LDL-C, TG or FPG were found in comparison with levels in healthy volunteers in their twenties (data not shown).

Fig. 2A shows the relationship between B-mode findings and E_{θ} in three representative cases. The dark central region of soft plaques was considered to be the lipid core (16 kPa). The normal carotid intima-media complex in a 28-year-old man was considered to be smooth muscle (80 kPa). The white regions, which were not accompanied by an acoustic shadow, were considered to be calcified regions (555 kPa). All data are summarized as histograms in **Fig. 2B** (lipid core, 22 ± 15 kPa (lesions: $n = 18$); calcification, 674 ± 384 kPa (lesions: $n = 36$)). In the 15 healthy subjects who were free from vascular risk factors, the normal carotid intima-media complex was considered to be smooth muscle (104 ± 32 kPa, sites: $n = 71$).

Next, in order to obtain the elasticity of blood clots and fibrosis, the following case was used. A 43-year-old woman came to our department because of a pulsatile mass 2.5 cm in size in the right submandibular area. Her blood pressure was 108/80 mmHg, and ECG showed sinus tachycardia (92/min). Imaging of enhanced CT and ultrasound showed acute-onset carotid artery dissection. The patient had no

cardiovascular risk and no history of stroke, and had experienced a spontaneous cure from intra-pseudocystic thrombosis. During B-mode scanning to observe the healing process at our clinic, with permission from the patient and approval from our ethics committee, elastic modulus was measured several times (**Fig. 3**). Elasticity E_{θ} , as determined from the time course of changes, was 85 ± 68 kPa in areas corresponding to thrombi and 273 ± 173 kPa in areas of fibrosis.

From the observation of various tissues, the elasticity library of E_{θ} by transcutaneous ultrasound was obtained, as shown in **Fig. 4**. The data indicate that the E_{θ} value rises depending on the tissue constituents. The mean E_{θ} value of lipid core- and calcification-free plaques was 173 ± 69 kPa (lesions: $n = 270$), falling between the E_{θ} values of fibrosis and blood clot.

Effect of Fluvastatin On Elastic Modulus and IMT

At baseline, patients with hypercholesterolemia showed significantly higher LDL-C, baPWV, mean IMT, max IMT and mean E_{θ} than seen in healthy volunteers (**Table 1**). As shown in **Fig. 5**, in the control group of hypercholesterolemia ($n = 10$), no change of LDL-C was seen. In the group treated with fluvastatin ($n = 62$), LDL-C was significantly reduced immediately. By fluvastatin treatment, hs-CRP and baPWV decreased gradually but NO increased over time ($p <$

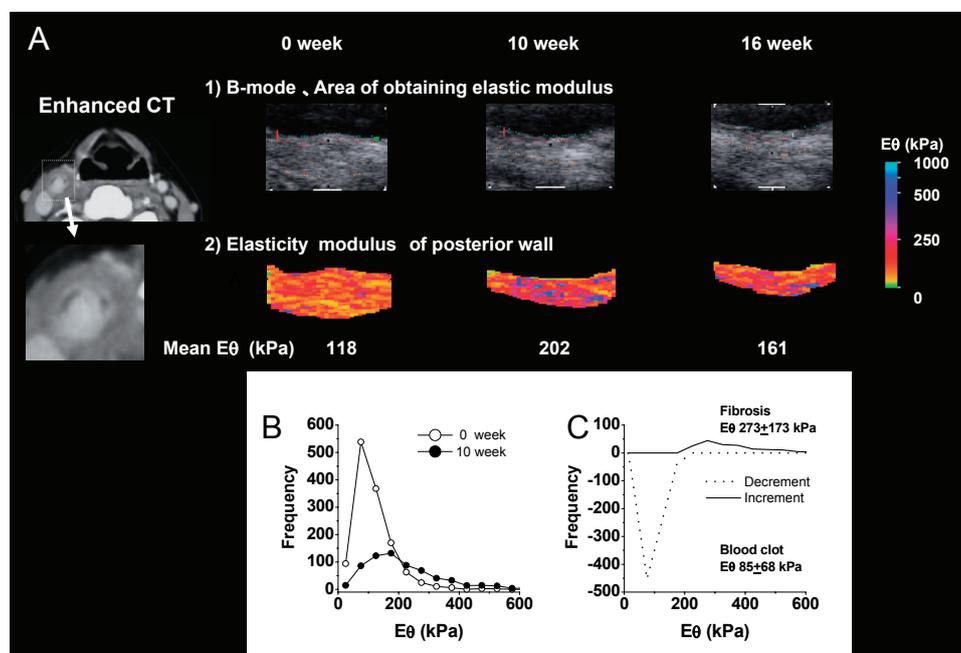


Fig. 3. Aneurysm of right cervical artery in a 43-year-old woman: time course of changes of thrombosis in the pseudolumen.

Computed tomography at 0 weeks showed dissection, and the B-mode and elasticity modulus of the lesion in the upper panel at 0 week showed relatively soft tissue elements; however, E_{θ} values rose over time, indicating the disappearance of the blood clot. In the lower panel (B), histograms of E_{θ} at 0 weeks and 10 weeks are shown.

0.05). No significant changes in blood pressure or pulse rate were seen (data not shown). Fluvastatin decreased the mean IMT and mean E_{θ} significantly in the analysis of all sites of all patients (Fig. 6A, ANOVA, $p < 0.05$). Mean E_{θ} decreased significantly from 3 to 12 months of administration, while mean IMT decreased only after 12 months of administration, compared with control values at 0 months. On the other hand, no change of mean IMT or mean E_{θ} between 0 and 12 months was seen in the control group of hypercholesterolemia (Fig. 6A, $n = 10$).

Since it has been reported that native Japanese reach an IMT of 1.1 mm at age 70 and subjects with max IMT < 1.1 mm are often classified as not having atherosclerosis⁵, we divided all analyzed sites ($n = 242$) into two groups with a max IMT cut off value of 1.1 mm. In the group with max IMT ≥ 1.1 mm (sites: $n = 144$), both the mean E_{θ} and mean IMT decreased significantly (Fig. 6B, ANOVA). In comparison with the values at 0 months, the values of mean E_{θ} at 6 and 12 months and mean IMT at 12 months were significantly decreased. In the group with max IMT < 1.1 mm (sites: $n = 98$), the mean E_{θ} significantly decreased and IMT showed a tendency to decrease (Fig. 6C).

Next, we divided all sites into three groups by

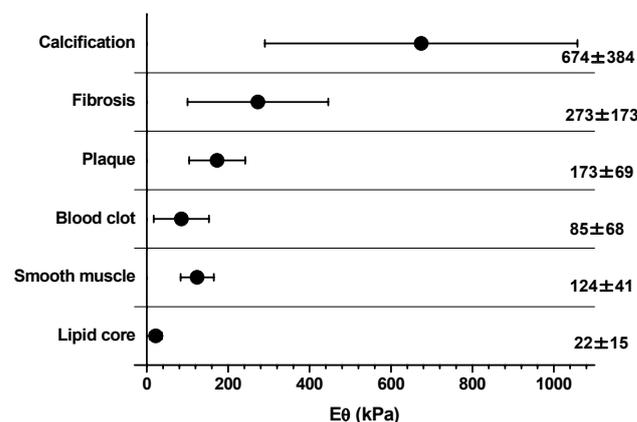


Fig. 4. Elasticity library for E_{θ} (kPa).

Data of the elasticity library of E_{θ} by transcutaneous ultrasound were obtained from the observation of various tissues: lipid core, 22 ± 15 kPa; calcification, 674 ± 384 kPa; lipid core- and calcification-free plaques, 173 ± 69 kPa; smooth muscle, 104 ± 32 kPa; blood clot, 85 ± 68 kPa; fibrosis, 273 ± 173 kPa. The dark central region of soft plaques was considered to be the lipid core. In healthy subjects free from vascular risk factors, we considered the normal carotid intima-media complex to be smooth muscle. The white regions, which are not accompanied by acoustic shadow, were considered to be calcification regions. Elastic moduli, equivalent to blood clot and fibrosis, were obtained from the observation of an uncomplicated healing process of carotid artery dissection, as shown in Fig. 3.

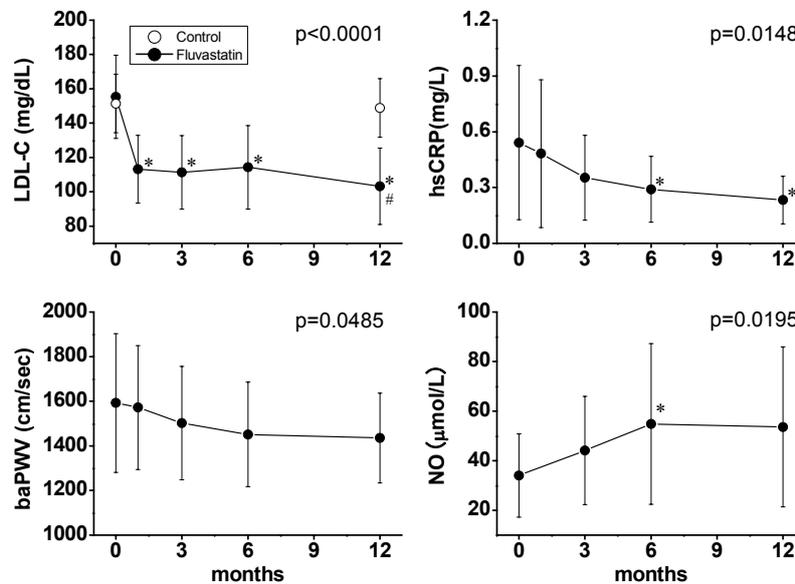


Fig. 5. Effects of fluvastatin on LDL-C, hs-CRP, baPWV and NOx.

A reduction in LDL-C and decreases in baPWV and hs-CRP were noted, along with a gradual increase in NOx. No difference of LDL-C was seen between the control ($n = 10$) and fluvastatin group ($n = 62$) at 0 months. Fluvastatin significantly reduced LDL-C in the comparison of the two groups at 12 months. P -values were obtained by ANOVA for the fluvastatin group. * $p < 0.05$ vs. 0 months of the fluvastatin group; # $p < 0.05$ vs. 12 months of the control.

the mean E_{θ} values at 0 months (before administration of fluvastatin), $E_{\theta} \leq 100$ kPa, $100 \text{ kPa} < E_{\theta} < 200$ kPa, and $200 \text{ kPa} \leq E_{\theta}$. In the three groups, IMT did not change and showed a tendency to decrease (Fig. 7). On the other hand, in the group with $E_{\theta} \leq 100$ kPa, the mean E_{θ} significantly increased from 1 month after administration of the drug (Fig. 7A, sites: $n = 42$). In the group of $200 \leq E_{\theta}$, the mean E_{θ} significantly decreased from 1 month after administration of the drug (Fig. 7C, sites: $n = 46$). In the group of $100 \text{ kPa} < E_{\theta} < 200$ kPa, the mean E_{θ} significantly decreased (Fig. 7B, sites: $n = 154$).

Two typical examples are shown in Fig. 8, 9. In the advanced thick plaque (max IMT ≥ 1.1 mm and $E_{\theta} \leq 100$ kPa) shown in Fig. 8, fluvastatin decreased IMT and increased E_{θ} at month 12, particularly in the hardened areas in the superficial layer. Histogram analysis of the data revealed a decrease in areas corresponding to E_{θ} of 20–200 kPa and an increase in the relatively hardened areas of $E_{\theta} > 250$ kPa for subgroups showing increased E_{θ} , thought to represent a decrease of lipid/smooth muscle-rich tissue and/or an increase of collagen fibers (based on the elasticity library and Fig. 8).

In the early lesion (max IMT < 1.1 mm) that showed heterogeneous distribution of E_{θ} ($E_{\theta} \geq 200$

kPa) in the control group, the administration of fluvastatin produced no changes in IMT but resulted in decreased E_{θ} (Fig. 9). Histogram analysis of the data revealed an increase in areas corresponding to E_{θ} of 20–200 kPa, as well as a decrease in relatively hardened areas.

Discussion

We first obtained an *in vivo* elasticity library with transcutaneous ultrasound from observation of various tissues of the carotid arterial wall by the phased tracking method (Fig. 4). In *in vitro* experiments, as reported previously, the main components of tissue have their own elasticities¹⁶. By referring to the elasticity library, we can speculate as to the tissue type whose elastic modulus is close to that of each pixel.

Second, this was a new challenge to show the detection of the effects of fluvastatin on the elasticity of the carotid artery (mean E_{θ}). The mean E_{θ} can be changed by statin treatment; that is, in the group with max IMT ≥ 1.1 mm, both the mean E_{θ} and mean IMT decreased significantly. On the other hand, in the max IMT < 1.1 mm group, the mean E_{θ} but not the mean IMT decreased significantly.

B-mode ultrasound is widely used to measure

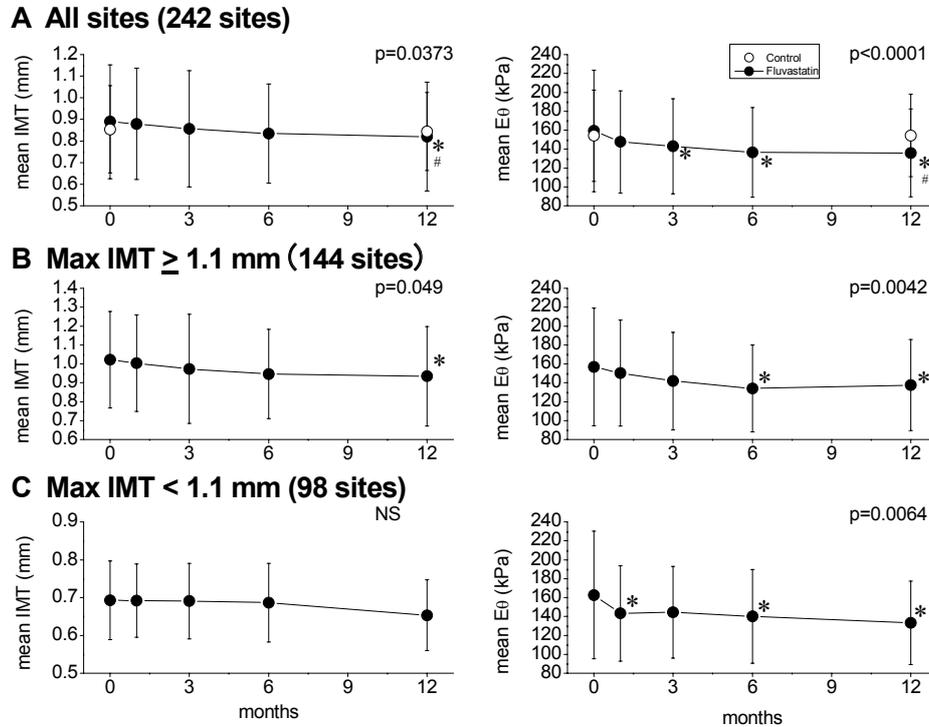


Fig. 6. Effects of fluvastatin on the mean IMT and mean E_{θ} .

(A) At all sites of all patients (242 sites), both the mean IMT and mean E_{θ} were significantly decreased (ANOVA). Compared with the values at 0 months, the values of mean E_{θ} at 3, 6 and 12 months and mean IMT at 12 month were significantly decreased. No difference of the mean IMT or mean E_{θ} was seen between the control (40 sites in 10) and the fluvastatin group at 0 months. Fluvastatin significantly reduced both the mean IMT and mean E_{θ} in the comparison of the two groups at 12 months. (B) In the group with max IMT ≥ 1.1 mm (144 sites), both mean IMT and mean E_{θ} were significantly decreased. (C) In the group with max IMT < 1.1 mm (98 sites), mean E_{θ} but not mean IMT was significantly decreased. P -values were obtained by ANOVA for the fluvastatin group. * $p < 0.05$ vs. 0 months of the fluvastatin group; # $p < 0.05$ vs. 12 months of the control.

carotid IMT, an early change that precedes the development of macroscopic plaques. Carotid IMT is increasingly being used for cardiovascular risk stratification and as an intermediate end point in clinical trials¹⁹), but it has limited ability to determine the extent of plaque burden and the nature of plaque composition. IBS analysis has shown associations between echo-lucent carotid plaques and lower HDL-C cholesterol, elevated triglycerides, elevated blood pressure and elevated blood sugar levels^{7, 8}); however, the IBS index simply reflects differences in echogenicity, but does not show the true elasticity of the arterial wall. Carotid stiffness, considered an index of sclerosis, can be determined by the following equation: $\beta = \ln(\text{SBP}/\text{DBP}) / [(D_s - D_d)/D_d]$, where D_s and D_d are the end-systolic and end-diastolic diameters of the common carotid artery, respectively^{20, 21}); however, this value does not indicate the two-dimensional distribution of carotid arterial wall elasticity. Under the assumption

of a blood vessel is a uniform cylinder pipe, PWV and stiffness β can be used to evaluate average elastic properties of the whole transmission passage and the entire circumference by measuring pulse wave velocity between dozens of centimeters and alteration of the diameter by beat, respectively. These methods are not necessarily suitable for comparing plaques or individuals, or for assessing qualitative changes. In contrast, our method enables the measurement of elasticity distribution in the intima-medial region by ultrasound and the evaluation of local (lesion) elastic properties by measuring mural thickness change. In a previous study, age, blood pressure and pulse rate were shown to be independent determinants of the average values E_{θ} of elasticity distribution¹⁵).

In the present study, an elasticity library with transcutaneous ultrasound was obtained by observing various tissues of the carotid arterial wall (Fig. 4). We were fortunate to be able to observe an uncomplicated

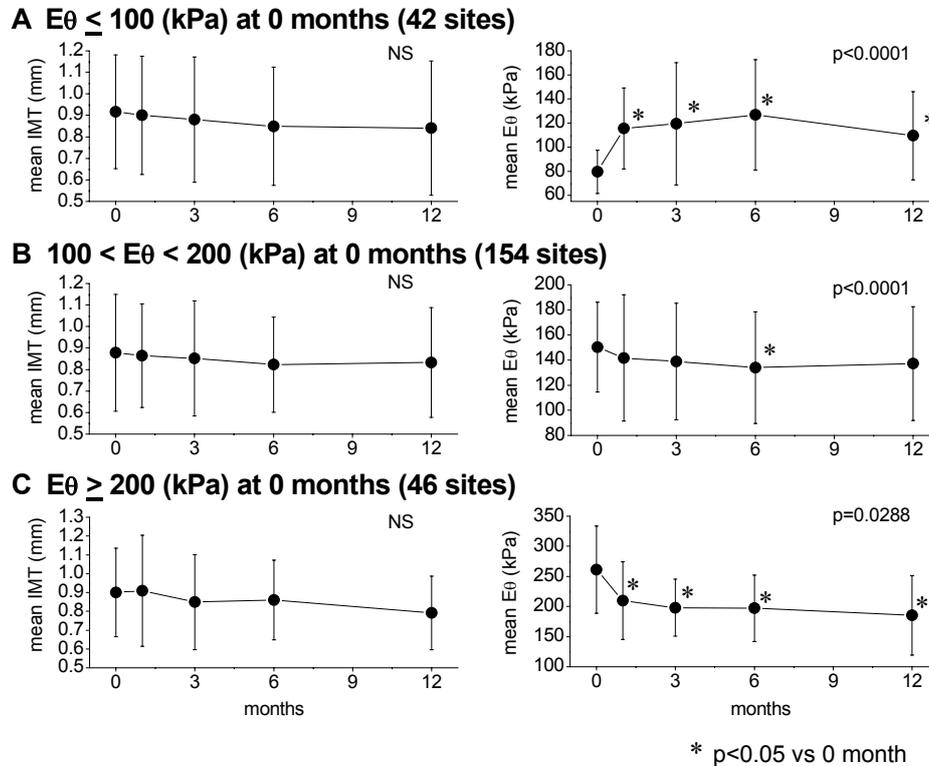


Fig. 7. Effects of fluvastatin on the mean IMT and mean E_{θ} in the three groups according to the mean E_{θ} values at 0 months.

(A) In the group with $E_{\theta} \leq 100$ (42 sites), the mean E_{θ} was significantly increased after 1-month administration of the drug. (B) In the group with $100 \text{ kPa} < E_{\theta} < 200 \text{ kPa}$ (154 sites), mean E_{θ} was significantly decreased. (C) In the group with $200 \leq E_{\theta}$ (46 sites), the mean E_{θ} was significantly decreased after 1-month administration of the drug.

healing process following carotid artery dissection, since such cases usually heal spontaneously²², and to obtain the elastic modulus, equivalent to blood clot and fibrosis information by histogram. When the relative interrelationships for elasticity in the corresponding vascular tissues were considered in this study, a remarkable similarity was noted to the elasticity findings for lipids, thrombus, smooth muscle, fibrotic tissue and calcification in isolated atherosclerotic vascular specimens as documented by Inagaki *et al.*²³.

Many risk factors are associated with endothelial dysfunction, including hypercholesterolemia, hypertension, cigarette smoking and diabetes mellitus. Increasing evidence indicates that oxidized low-density lipoprotein cholesterol (ox-LDL) plays an important role in endothelial dysfunction. Ox-LDL induces endothelial injury, inhibits apoptosis, monocyte adhesion and platelet aggregation, and also inhibits the expression/activity of endothelial nitric oxide synthase (eNOS)²⁴. Previous research has shown that 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors

(statins) improve endothelial function through mechanisms that go beyond their primary therapeutic effects²⁵⁻²⁸. Alteration of the endothelial function might result from increases in eNOS activity, reduced production of free radicals, inhibition of ox-LDL action, or other undefined mechanisms. In addition, coronary spasm is associated with endothelial dysfunction, and fluvastatin has been reported to reduce the number of patients with ACh-induced coronary spasm, which is associated with endothelial dysfunction, in comparison with conventional calcium channel blocker therapy²⁶. In our study, fluvastatin increased NO and decreased hs-CRP. In the group with max IMT < 1.1 mm, considered to be in the range of subclinical atherosclerotic findings, the elastic modulus was significantly reduced. This may be related to fluvastatin-induced improvement in endothelial dysfunction. Additionally, statin treatment is reported to decrease carotid arterial stiffness but not IMT (initial max IMT 0.95 ± 0.4 mm) in patients with hypercholesterolemia²⁰. Ultrasonic strain imaging has the

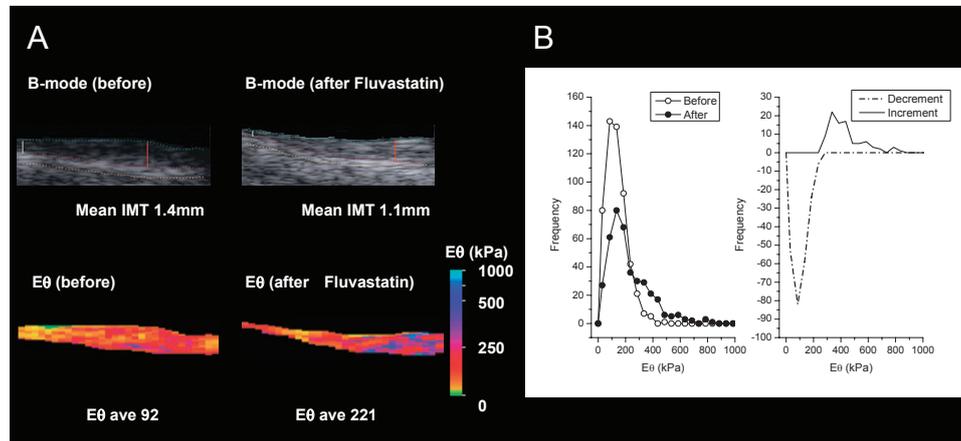


Fig. 8. A case of increased mean E_{θ} after fluvastatin administration (50-year-old man).

Images before and after administration of the drug for 12 months are shown. In the histograms, the increase in E_{θ} showed a decrease of lipid/smooth muscle-rich tissue and/or an increase of collagen fibers, deterring B-mode findings.

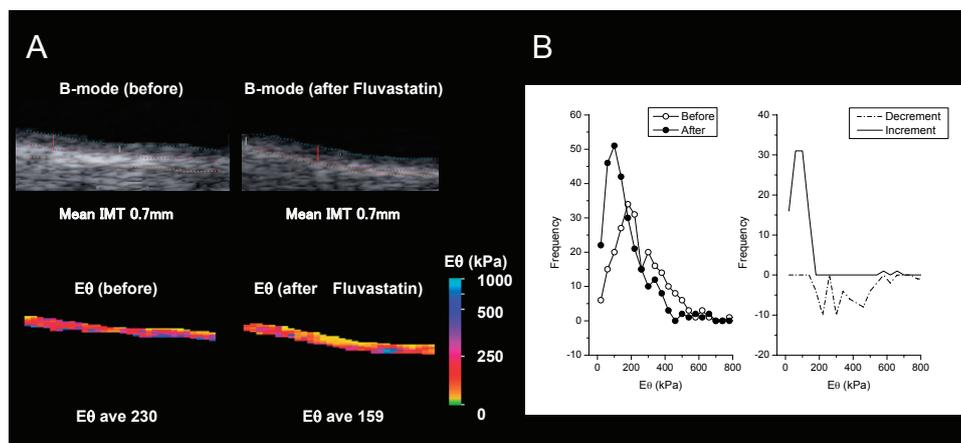


Fig. 9. A case of decrease in mean E_{θ} after fluvastatin administration (50-year-old man).

Images before and after administration of the drug for 12 months are shown. In the histograms, the decrease in E_{θ} shows an increase in areas corresponding to E_{θ} of 20–200 kPa, as well as a decrease in relatively hardened areas, indicating improvement of endothelial dysfunction.

potential to become a sensitive tool for detecting the effects of early statin intervention.

Recent studies, including those on advanced plaque formation, have also shown benefits from intensive statin therapy in slowing and even reversing the progression of atherosclerosis, as assessed by surrogate imaging measures such as IVUS and carotid IMT. In a study of the effectiveness of rosuvastatin against increases of intima media thickness, a statistically significant slowing in the progression rate of maximum carotid IMT (initial max IMT 1.15 ± 0.19 mm) was observed in the rosuvastatin group relative to the placebo group for 12 carotid sites, as well as for each

individual segment²⁷). Subanalysis of this METEOR study found that in the rosuvastatin group, a greater trend toward regression was seen in patients with more risk factors and greater baseline thickness²⁸). Similarly, in our study, a greater trend toward regression was seen in the advanced plaque group (max IMT ≥ 1.1 mm, **Fig. 6**).

As shown in **Fig. 7**, changes in mean E_{θ} may be related to an incremental beneficial effect on plaque stabilization with statins; for example, in the group with $E_{\theta} \leq 100$ kPa, the softer areas, mean E_{θ} significantly increased from 1 month after administration of the drug. This may be the result of a diminution of

the lipid-rich core, a reduction of inflammatory cells within the plaques, decreased macrophage activation as well as foam cell formation, and events related to thickening of the fibrous cap as reported by several investigators²⁹⁻³¹). On the other hand, in the group with $200 \text{ kPa} \leq E_{\theta}$, the harder areas, mean E_{θ} was significantly decreased. This may be related to improvement of endothelial dysfunction, such as paradoxical vasoconstriction of cardiac vessels. In the group with $100 \text{ kPa} < E_{\theta} < 200 \text{ kPa}$, the middle range of the elastic modulus, mean E_{θ} significantly decreased. This may be the result of variable changes of plaque components mentioned above.

On the basis of comparing histograms for tissue characterization (Fig. 4) and fluvastatin-induced changes in the intima-media complex (Fig. 6, 7, 8, 9), it may be possible to perform tissue characterization by comparing elasticity distribution and chronological changes in the distribution as measured by ultrasound. Collagen and smooth-muscle content have been reported to be important factors in determining the stability of the fibrous cap on atherosclerotic plaques; therefore, correlations between elasticity and tissue elements within the arterial wall may provide useful information for the noninvasive diagnosis of plaque vulnerability²³).

The present study has the following limitations: Although plaque can be evaluated three-dimensionally and relatively more easily with certain methods, such as computed tomography and magnetic resonance imaging, conventional IMT measurement is performed in one cross-section, making it difficult to construct three-dimensional images, which would be a future hurdle to overcome. In addition, measurement of one cross-section influences not only elasticity, but also the IMT. In the present study, to measure the same sites of carotid arteries, one well-trained sonographer performed the IMT and elastic modulus measurements by comparing current B-mode images with past B-mode images on a PC and positioned the probe so as to measure a similar position. Finally, the spatial averages of the elastic moduli were compared so that the mean value would be the same when the same area was included.

Plaques consist of various tissue components, and we previously reported the comparison of the elastic modulus with histologically corresponding tissues, such as lipids, blood clots and calcification¹⁶). In that report, the distributions of lipids and blood clots were relatively narrow and pure; however, those of collagen, smooth muscle and calcification were wide and rather scattered. The rank of the center of elasticity distributions obtained in the present study was similar

to that of *in vitro* experiments, indicating that it is impossible to obtain completely pure plaque tissue components, although the major components of the plaque can be detected and a classification can be made based on the elastic modulus.

E_{θ} may be an ideal marker to show the elasticity of the arterial wall itself because E_r (apparent elasticity) is influenced not only by an increase in elasticity but also by the increasing thickness of the arterial wall at the same location, especially in areas of relatively great thickness; however, the influence of blood pressure on the library cannot be neglected. The effects of aging on the mean IMT and mean E_{θ} are shown in Fig. 1. It seems that E_{θ} does not increase greatly in normotensives, even with advancing age. In hypertensives, however, E_{θ} was found to increase not only with decrements of strain (the denominator of Eq. (3) for elastic modulus) via tissue characteristics such as fibrosis, calcification and local contraction of smooth muscle, but also with increments of pulse pressure (the numerator of Eq. (3) for elastic modulus). A comparison with pathological findings for the elastic modulus is needed; however, in humans it is ethically unacceptable to compare ultrasonic images with pathological findings in early-stage carotid arterial atherosclerosis. In addition, an elastic modulus obtained by means of transcutaneous ultrasound differs from that obtained from isolated atherosclerotic vascular specimens, since the former is also affected by subcutaneous tissue, such as muscle and fat.

In summary, the present non-invasive echographic carotid arterial elasticity data suggest that this method is useful for classification and evaluation of atherosclerotic plaques that increase with progressive lipid deposition and that the measurement data may reflect chronological and histopathological changes.

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