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ORIGINAL ARTICLE

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Usefulness of measurement of carotid arterial wall elasticity distribution in detection of early-stage atherosclerotic lesions caused by cigarette smoking

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Abstract
Purpose. The aim of this study was to evaluate early-stage changes in the arterial wall caused by smoking.
Methods. A newly developed real-time ultrasonic measurement system was used to measure the elasticity distribution of the carotid arterial intima-media complex in 53 healthy male volunteers (mean age: 37.6 years), including 27 smokers. Simultaneous measurement of the elasticity distribution and intima-media thickness (IMT) was performed at six locations in the bilateral carotid arteries.
Results. The mean elastic modulus in the radial direction (Er) of the carotid arterial area where the IMT was less than 1.1 mm in smokers was larger than that in age-matched nonsmokers. There were no significant correlations between IMT and Er at the same location. However, a significant positive correlation was observed between the maximum IMT (maxIMT) and that of Er (maxEr) in six locations. In smokers, maxEr had a better correlation with the smoking index, and areas of IMT less than 1.1 mm containing harder lesions of Er ≥ 160 kPa were significantly more frequent than in nonsmokers.
Conclusion. Measurement of carotid arterial wall elasticity is useful for detecting distortion in the intramural elasticity distribution that occurs prior to IMT thickening caused by smoking as an early-stage atherosclerotic sign.

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Introduction

Smoking is known to be a major risk factor for atherosclerosis, which can induce stroke and/or myocardial infarction, and cause endothelial dysfunction and arterial plaque in the early stages of atherosclerosis. Carotid arterial intima-media thickness (IMT) is one of the established indices of chronic changes in atherosclerosis. For example, an IMT that is valued equal to or thicker than an absolute threshold (such as 1.1 mm) or a predicted IMT based on age and other potential covariates is considered to be atherosclerotic.[1–4] Measurement of increases or decreases in IMT provides information about the speed of atherosclerotic progression and the efficacy of drugs for improving atherosclerotic lesions. However, the IMT does not directly indicate the stiffness of the arterial wall.

Recently, a new ultrasonic system that can measure the regional elasticity of an arterial wall by using the phased-tracking method was developed.[5–11] The modulus of elasticity has also been successfully derived from measurement of the change in arterial wall thickness, as an index of "stiffness/softness." This technology has been successfully combined with a system for displaying the modulus of elasticity in color on two-dimensional ultrasound images of arteries, enabling evaluation of the characteristics of the arterial wall in real time.

The aim of the present study was to determine whether early-stage atherosclerotic changes can be detected by using an ultrasonic-based tissue characterization system for arterial walls. We found that measurement of the carotid arterial wall elasticity distribution was useful for detecting early-stage atherosclerotic lesions caused by cigarette smoking.

Table 1. Characteristics of the study population

	Nonsmokers	Smokers	*P*
n	26	27	
Smoking index (cigarettes × years)	–	343.5 ± 172.0	
Age (years)	37.9 ± 6.6	38.7 ± 7.2	0.67
BMI (kg/m^2)	23.4 ± 3.1	23.5 ± 3.4	0.87
Systolic BP (mmHg)	114.1 ± 9.3	117.9 ± 10.7	0.18
Diastolic BP (mmHg)	72.5 ± 7.5	73.7 ± 10.1	0.64
Pulse pressure (mmHg)	41.6 ± 10.4	44.2 ± 7.5	0.31
Heart rate (bpm)	68.2 ± 9.4	70.0 ± 7.2	0.46

Values are expressed as mean ± SD
BMI, body mass index; BP, blood pressure

Materials and methods

Subjects

The study group comprised 53 healthy male volunteers aged 20 to 49 years (mean age 37.6 ± 7.2 years). None of the participants had systemic hypertension (blood pressure <140/90 mmHg), diabetes mellitus (fasting glucose <110 mg/dl, HbA1c <5.8%), hypercholesterolemia (fasting total cholesterol <220 mg/dL), or cardiac disease, and none was taking any medication. Chronic smokers (n = 27) were matched for age, height, and weight with nonsmokers (n = 26). Table 1 shows the characteristics of the study population. There was no significant difference between smokers and nonsmokers with respect to any parameter. Smoking status was categorically evaluated on the basis of self-reports. The smokers smoked an average of 19.2 ± 5.3 cigarettes per day for 17.3 ± 6.6 years. The smoking index was defined as the product of the number of cigarettes smoked per day and smoking years. The average smoking index was 343.5 ± 172.0 (cigarettes × years). The nonsmoking group was composed of only men who had never smoked; it did not include past smokers or passive smokers in the office and/or at home.

Carotid IMT and elasticity measurements

The subjects were studied after having abstained from caffeine, alcohol, and smoking during the previous 12 h. The subjects 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 (mean of three readings) were measured in the right arm with an automated digital oscillometric sphygmomanometer (EW-3110; Matsushita Electric Works, Osaka, Japan). The subjects provided informed consent, and the study was approved by the institutional ethics committee of Tohoku Kosai Hospital and Matsushita Electrical Industrial Co., Ltd.

An imaging study of the bilateral common carotid arteries was performed using a 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.

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 common carotid arteries; (2) the distal 1 cm of both carotid bulbs; and (3) the right and left carotid bulbs (from 1 cm proximal to the level of the flow divider).

This system measures the radial strain of the arterial wall and calculates the elasticity from the radial strain and the blood pressure. The distribution of the elasticity is displayed as a 2D cross-sectional color image on a B-mode image, and the image is updated at every heartbeat. For measurement of minute changes in thickness, this system uses the phased tracking method.[5–10] Elasticity was estimated at intervals of 80 μm in the depth direction and 400 μm in the axial direction. From the maximum change in thickness during one heartbeat, the radial strain of each assigned layer in the artery wall (ε_r) was calculated as follows:

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

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

Elastic modulus in the radial direction (Er) and elastic modulus in the circumferential direction (Eθ) were calculated as follows:

$$Er = \text{pulse pressure}/\varepsilon_r \quad (2)$$

$$E\theta = 1/2 \times (r_0/h_0 + 1) \times Er \quad (3)$$

where pulse pressure is the difference between the systolic and diastolic brachial blood pressure (the carotid blood pressure would be more accurate but is not easy to measure), h_0 is the initial thickness of the wall, and r_0 is the initial radius of the vessel. The measurement reproducibility for this system has been reported previously.[11] The sonographer trained to obtain an Er measurement deviation of less than 10% in the same subject.

Pulse Wave Velocity (PWV) measurements

Brachial-ankle PWV (baPWV) was measured using a volume-plethysmographic apparatus (Form/ABI; Colin,

Aichi, Japan) from simultaneous blood pressure and waveform measurements from all four limbs along with electrocardiogram 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. baPWV was measured from the ascending point of the right brachial pulse volume recorder to the ascending point of each ankle pulse volume recorder. The distance between the right brachium and ankle was estimated as described previously.[12]

Statistical analysis

Data are expressed as the mean ± SD. Statistical analysis was performed using the SPSS software package (SPSS, Chicago, IL, USA), and a value of $P < 0.05$ was considered significant. The differences between smokers and nonsmokers were analyzed by one-way analysis of variance (ANOVA). Linear regression analysis was performed to evaluate the association between IMT, elasticity, or baPWV and other clinical variables. Multiple linear regression analysis was applied to evaluate whether the relationship between the two parameters was independent of other related factors.

Results

Figure 1 shows a typical image of the right carotid arterial wall of a 44-year-old male smoker. In this image, the maximum IMT was 0.8 mm, and the average values of Er and Eθ in the colored area were 82 kPa and 473 kPa, respectively. The pattern of distribution of the elasticity modulus was scattered and heterogeneous (Figs. 1B, 4A).

The relationships between the maximum IMT and the average Er at the right carotid artery in all cases are shown in Fig. 2A. In this area, the maximum IMT was less than 1.1 mm, and there was no significant relationship between the maximum IMT and Er. The average Er but not average Eθ in smokers was significantly higher than that in nonsmokers (Er, 39.2 ± 13.7 kPa vs 29.3 ± 7.3 kPa, $P = 0.0020$, Fig. 2C; Eθ, 147 ± 50.1 kPa vs 127.6 ± 37.4 kPa, $P = 0.10$), although there was no difference in the maximum IMT values between the two groups (0.63 ± 0.12 mm vs 0.61 ± 0.13 mm, $P = 0.52$, Fig. 2B). Calcified plaque on B-mode imaging was found in two smokers in their 40s (data not shown).

By examining six locations on the bilateral carotid arteries, we defined the maximum IMT value as maxIMT, the maximum value of average Er as maxEr, and the maximun

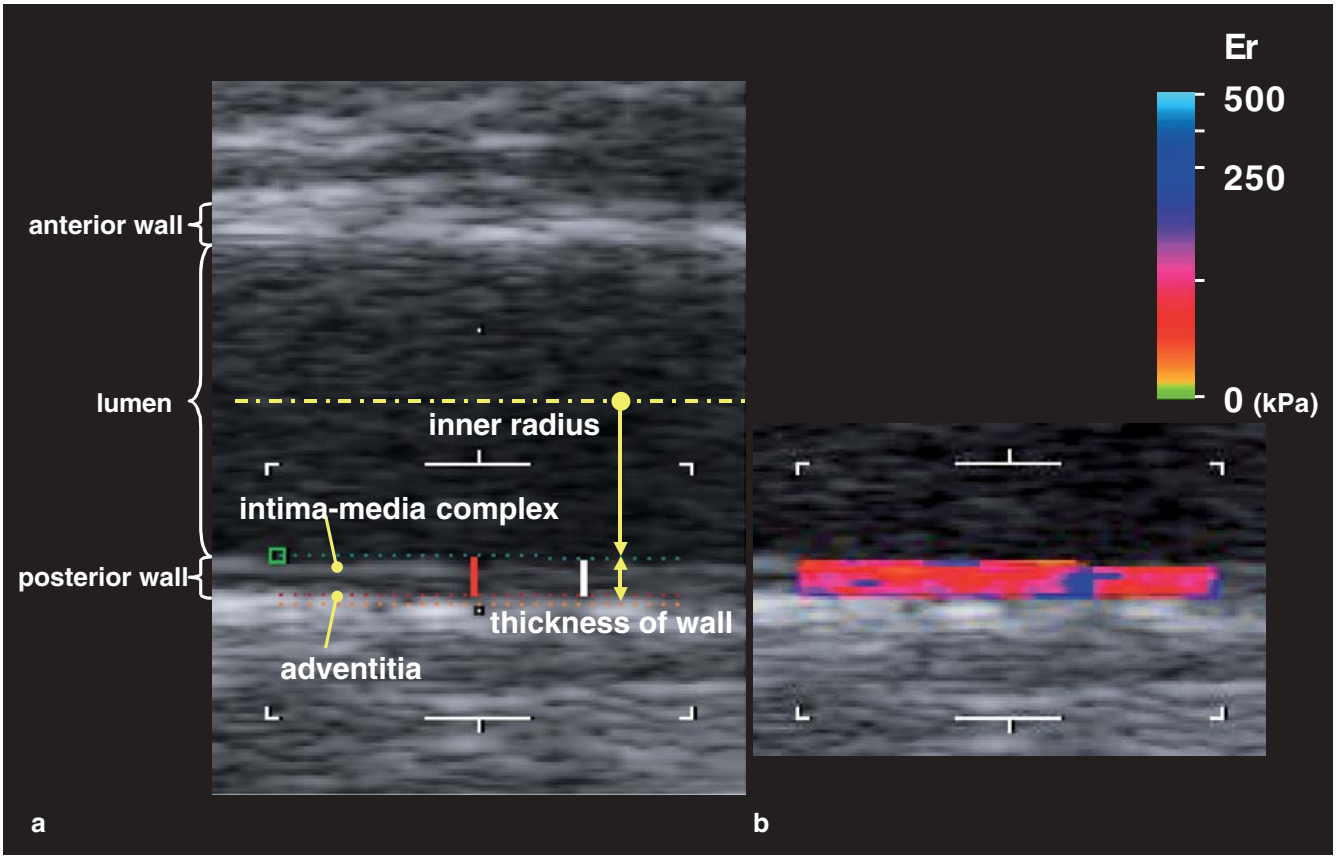

Fig. 1A,B. Typical imaging of the right carotid arterial wall with the IMT and elasticity recorded simultaneously, shown here for a 44-year-old male smoker. **a** B-mode image. In the intima-media complex area, the *solid red line* shows the position of the maximum IMT (0.8 mm) and the *solid white line* shows that of the minimum (0.6 mm). **b** Distribution of the elastic modulus in the radial direction (Er). The *colored area* in the *white box* shows the distribution of Er at intervals of 80 μm in the depth direction and 400 μm in the axial direction according to the color scale at the *upper right*. The averages of Er and Eθ are 82 kPa and 473 kPa, respectively

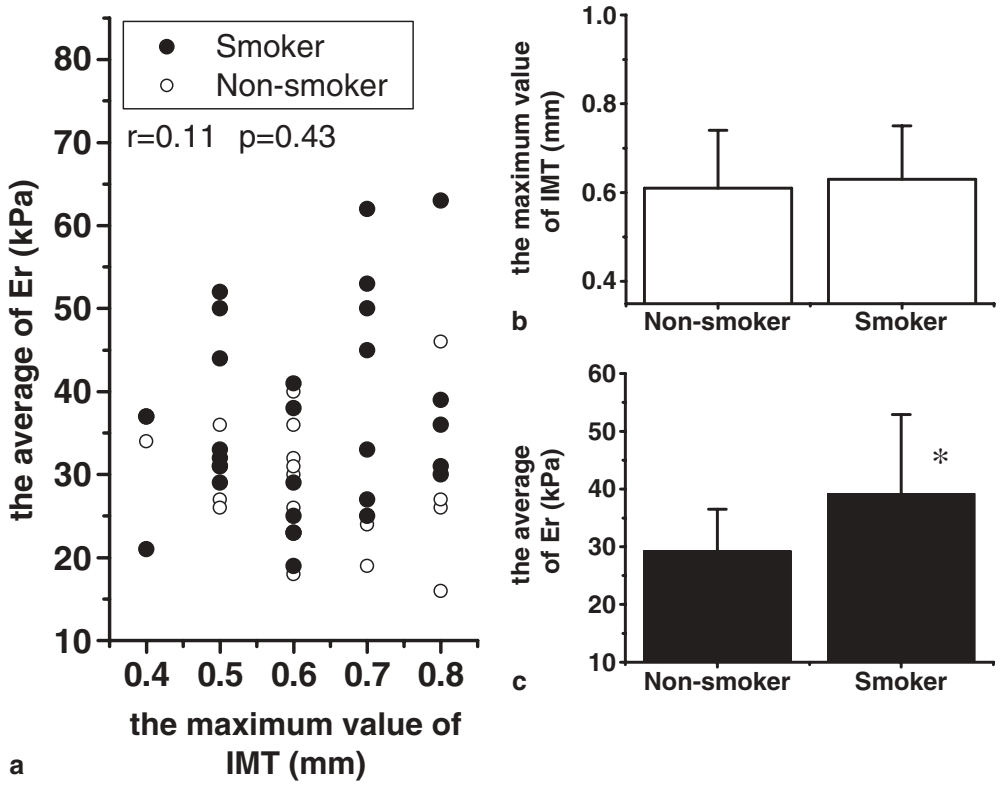

Fig. 2. **a** Plots of maximum IMT and average Er at the central side of the right carotid artery. No significant correlation between the two was seen. **b** Average maximum IMT values in smokers and nonsmokers were not significantly different (0.63 ± 0.12 mm vs 0.61 ± 0.13 mm, $P = 0.52$). **c** The average Er in smokers was significantly higher than that in nonsmokers (39.2 ± 13.7 kPa vs 29.3 ± 7.3 kPa, $P = 0.0020$)

Table 2. Comparison of atherosclerotic parameters

	Nonsmokers ($n = 26$)	Smokers ($n = 27$)	P
maxIMT (mm)	0.86 ± 0.14	0.99 ± 0.26	0.0284
maxEr (kPa)	37.3 ± 10.3	44.5 ± 14.2	0.0394
maxEθ (kPa)	171.0 ± 59.0	190.0 ± 52.0	0.23
baPWV (cm/s)	1211.2 ± 132.4	1220.8 ± 139.0	0.80
Number of cells with Er ≥ 160 kPa[a]	2.6 ± 4.5	10.2 ± 13.1	0.0078

Values are expressed as mean ± SD
IMT, intima-media thickness; baPWV, brachial-ankle pulse wave velocity; maxIMT, maxEr, and maxEθ, maximum values of IMT, Er, and Eθ for six locations, respectively
[a] Details are shown in Fig. 4

value of average Eθ as maxEθ. As shown in Table 2, there were significant increases in maxIMT and maxEr in smokers compared with nonsmokers. MaxEθ showed only a statistically nonsignificant tendency to increase in smokers. There was no difference in baPWV between the two groups (Table 2).

Figure 3 shows that in smokers, maxEr had a better correlation with the smoking index ($r = 0.59$, $P = 0.0013$, Fig. 3C) than with age ($r = 0.48$, $P = 0.0109$, Fig. 3A), and maxIMT had a better correlation with age ($r = 0.66$, $P = 0.0001$, Fig. 3B) than with the smoking index ($r = 0.46$, $P = 0.0161$, Fig. 3D). As shown in Table 3, maxEθ had similar correlations with age ($r = 0.35$, $P = 0.08$) and smoking index ($r = 0.50$, $P = 0.0080$). We compared the relationship of each of maxIMT, maxEr, maxEθ, and baPWV with age and smoking index, as shown in Table 3. In nonsmokers, baPWV had the highest correlation with age, and the rank of the correlation coefficients with age was baPWV > maxIMT > elastic modulus (maxEθ > maxEr). In smokers, maxIMT had the highest correlation with age, and the rank of the correlation coefficients with age was maxIMT > baPWV > elastic modulus (maxEr > maxEθ). In contrast, the rank of the correlation coefficients with smoking index was elastic modulus (maxEr > maxEθ) > maxIMT > baPWV. Therefore, maxEr reflected the effects of smoking better than maxIMT and baPWV.

In smokers, areas of intima-media complex less than 1.1 mm in thickness were observed to contain harder lesions of 160 kPa or higher (Er ≥ 160 kPa) more frequently than in nonsmokers, and the number of cells with Er ≥ 160 kPa counted from the histogram of Er was compared between the two groups. For example, in the case shown in Fig. 1, the number of cells with Er ≥ 160 kPa was 19 (8%, Fig. 4A). Figure 4B shows that the number of cells with Er ≥ 160 kPa

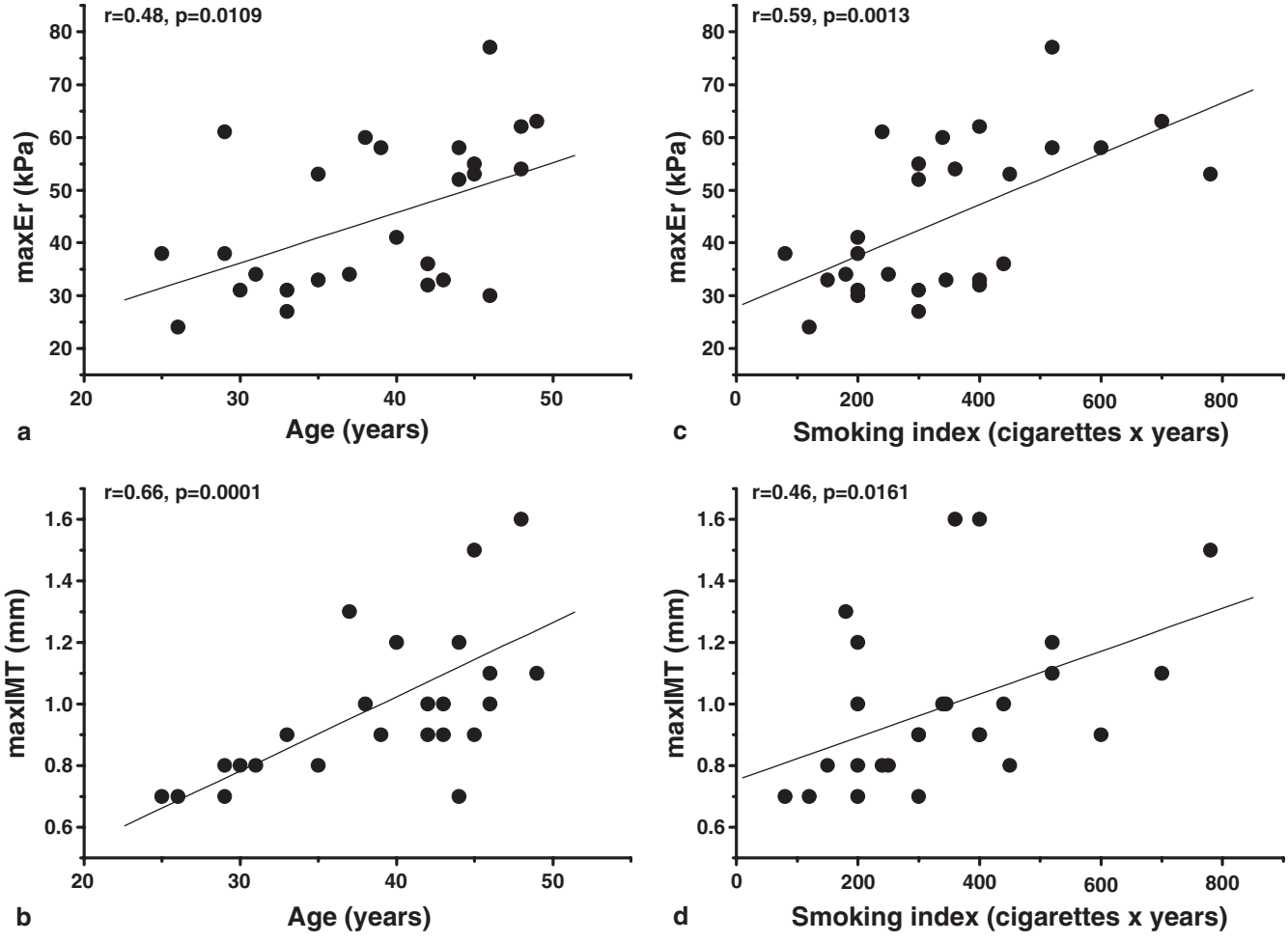

Fig. 3. Correlation of age with maxEr (**a**) and maxIMT (**b**), and correlation of smoking index with maxEr (**c**) and maxIMT (**d**) in smokers

Table 3. Correlation coefficients for parameters in nonsmokers and smokers

	r	*P*
Nonsmokers		
maxIMT vs maxEr	0.06	0.78
maxIMT vs maxEθ	0.10	0.63
Age vs maxIMT	0.42	0.0325
Age vs maxEr	0.24	0.24
Age vs maxEθ	0.36	0.08
Age vs baPWV	0.47	0.0183
Smokers		
maxIMT vs maxEr	0.39	0.0456
maxIMT vs maxEθ	0.33	0.09
Age vs maxIMT	0.66	0.0001
Age vs maxEr	0.48	0.0109
Age vs maxEθ	0.35	0.08
Age vs baPWV	0.57	0.0021
Smoking index vs maxIMT	0.46	0.0161
Smoking index vs maxEr	0.59	0.0013
Smoking index vs maxEθ	0.50	0.0080
Smoking index vs baPWV	0.26	0.20

IMT, intima-media thickness; baPWV, brachial-ankle pulse wave velocity; maxIMT, maxEr, and maxEθ, maximum values of IMT, Er, and Eθ for six locations, respectively

was significantly and positively associated with age for smokers but not for nonsmokers. Some younger smokers in their 20s had harder carotid lesions. As shown in Table 2, there were significant increases in the number of cells with Er ≥ 160 kPa in smokers compared with nonsmokers, which according to age were 1.3 in nonsmokers in their 20s (n = 3), 9.0 in smokers in their 20s (n = 4), 1.2 in nonsmokers in their 30s (n = 10), 6.9 in smokers in their 30s (n = 9), 4.0 in nonsmokers in their 40s (n = 13), and 12.6 in smokers in their 40s (n = 14, Fig. 4C). In multiple regression analysis, the number of cells with Er ≥ 160 kPa showed a significant correlation with age and smoking (r = 0.49, F = 7.7496, P = 0.0012).

Discussion

Cigarette smoking is a risk factor for atherosclerosis, and therefore myocardial infarction and stroke later in life. The aim of the present study was to show a direct relationship between chronic smoking and carotid arterial stiffness in a population without additional cardiovascular risk factors.

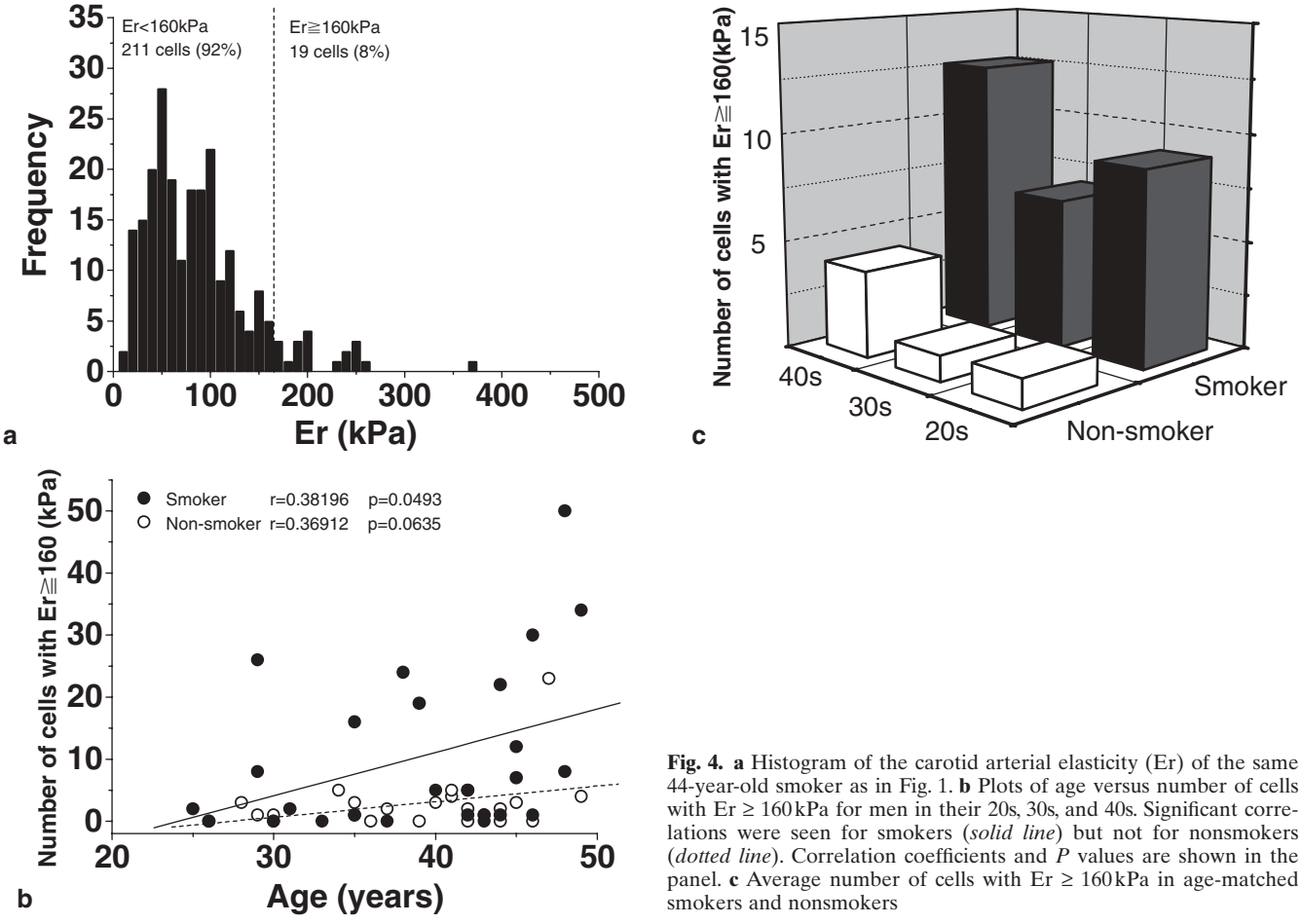

Fig. 4. **a** Histogram of the carotid arterial elasticity (Er) of the same 44-year-old smoker as in Fig. 1. **b** Plots of age versus number of cells with Er ≥ 160 kPa for men in their 20s, 30s, and 40s. Significant correlations were seen for smokers (*solid line*) but not for nonsmokers (*dotted line*). Correlation coefficients and *P* values are shown in the panel. **c** Average number of cells with Er ≥ 160 kPa in age-matched smokers and nonsmokers

We found that there was heterogeneity and increased elasticity of the carotid arterial wall in smokers, which could be a good marker of early-stage atherosclerosis caused by chronic smoking. We focused only on relatively young men because in women it is sometimes difficult to rule out the effects of female hormones, and subjects older than 50 years would be more affected by aging.

Measurement of IMT allows the detection of atherosclerotic lesions with morphological changes and remodeling such as thickening and calcification, which would happen perhaps later in the time course. IMT is also correlated with age, and it increases in older subjects. However, it cannot be used to assess acute or early changes in the arterial wall caused by cigarette smoking. In the present study, we demonstrated that IMT in smokers in their 40s was significantly increased and that some smokers showed calcified plaque, which was previously thought not to occur at such an early age, while some younger subjects in their 20s without thickening of the intima-media complex already showed harder carotid lesions, at least in terms of elasticity (Fig. 4B).

PWV and the augmentation index (AIx) are established markers of atherosclerosis,[13] but they cannot reveal regional atherosclerotic changes, even though they are related to ultrasonographically detected atherosclerotic changes in the carotid artery. Several studies[14,15] have indicated that acute cigarette smoking increases the arterial stiffness measured by PWV and/or AIx. However, the chronic effects of smoking are controversial. Wollersheim et al.[16] described increased arterial stiffness of the popliteal artery and a tendency toward stiffening of the common femoral and carotid arteries in 13 habitual smokers measured ultrasonographically by using the pressure–strain elastic modulus. Mahmud et al.[15] observed a much higher AIx in smokers compared with nonsmokers in a young homogenous group of healthy adults who were matched for age, height, weight, and hemodynamic status, although there was no statistically significant difference in PWV. However, others have found no difference in the regional arterial compliance between smokers and nonsmokers.[17,18] Liang et al.[19] reported that smoking does not influence the lumen-to-wall ratio but has a significant effect on wall stiffness. Munakata et al.[12] reported that age, systolic blood pressure, and the stage of hypertensive organ damage are major determinants of baPWV. Such discrepant results for chronic smoking effects on increases in arterial wall stiffness might be caused, at least partly, by differences in experimental methodologies.

Therefore, in order to focus on the chronic effects of smoking, elasticity was measured in the present study. All measurements were carried out after the subjects had not

smoked during the previous 12 h to rule out the acute effects of smoking. Although PWV was not different between smokers and nonsmokers, the carotid arterial elasticity in smokers was significantly higher than that in age-matched nonsmokers (Table 2). A positive significant correlation was observed between maxIMT and maxEr in the measurements at the six locations. Although maxEr_ and maxIMT were significantly correlated with age and the smoking index, maxEr reflected the effects of smoking better than maxIMT and baPWV (Table 3). In addition, the harder areas (Er ≥ 160 kPa) were found to be more highly reproducible and were seen more frequently in smokers than in nonsmokers. Thus, it seems that distortion in the intramural elasticity distribution occurs prior to IMT thickening caused by smoking.

However, there are limitations to our study that should be considered. First, in this noninvasive study, the actual structure cannot be determined, and we can only speculate as to what the underlying changes may be. Different cardiovascular risk factors, such as high blood pressure, changes in lipid and sugar profiles, endothelial factors, and changes in arterial wall structure and function, appeared to influence the relationships among arterial geometry, wall stress, and stiffness in different ways. Our results, however, are consistent with those showing that smoking initially causes increased stiffness. Endothelium-dependent functional changes are also likely to contribute to the increased stiffness, given the adverse effects of smoking on endothelial function. It has been previously demonstrated that there is a good relationship between elasticity obtained using the current method and the tissue characteristics obtained from isolated iliac arteries with plaques.[7] The cross-sectional elasticity distribution could be classified into three categories: lipids, a mixture of smooth muscle and collagen fibers, and others. Lee et al.[20] reported that the stiffness of fibrous caps from human atherosclerotic plaques is related to the underlying histological structure. It is possible that the harder lesions (Er ≥ 160 kPa) represent changes due to chronic spasms caused by endothelial dysfunction or a remodeled region that contains more collagen fibers or minor calcification. Inagaki et al.[21] reported that there is a relationship between collagen content and elasticity. Based on this report, we estimated that Er = 160 kPa, still a provisional figure, could be the elasticity associated with 10% collagen, and used this as a borderline value for early changes in atherosclerosis.

Second, Eθ may be an ideal marker to indicate the elasticity of the arterial wall itself because Er (an apparent elasticity) is influenced not only by an increase in elasticity but also by the thickness of the arterial wall at the same location, especially in the thicker areas. However, a limitation of the measurement of Eθ with the current technique is that echoic demonstration of the borders of the anterior wall and the adventitia of the posterior wall of the carotid artery is fuzzy and imprecise, especially on curved boundaries such as the bulb and severely atherosclerotic regions. Without an accurate measurement of h_0/r_0 as described in the materials and methods section, Eθ cannot represent the real elasticity. In fact, the statistical values for Eθ were not as good as those for Er and IMT in the present study. As shown in Fig. 2, within the range of IMT values of our subjects, the increase in Er was not related to the increase in IMT at the same location. Therefore, we believe that increases in Er in smokers are increases in stiffness associated mainly with smoking. However, we cannot distinguish chronic spasms caused by smoking from early atherosclerotic remodeling at this stage.

Further studies will be needed to determine the actual meaning of the changes in the elastic modulus in smokers and to refine the technique for measuring such changes.

Conclusion

Measurement of carotid arterial wall elasticity distribution to detect early-stage atherosclerotic lesions caused by smoking may be more useful than conventional IMT measurement.

References

1. Pignoli P, Tremoli E, Poli A, et al. Intimal plus medial thickness of the arterial wall; a direct measurement with ultrasound imaging. Circulation 1986;74:1399–406.
2. Howard G, Sharrett AR, Heiss G, et al. Carotid artery intimal-medial thickness distribution in general populations as evaluated by B-mode ultrasound. ARIC Investigators. Stroke 1993;24: 1297–304.
3. Simon A, Gariepy J, Chironi G, et al. Intima-media thickness: a new tool for diagnosis and treatment of cardiovascular risk. J Hypertens 2002;20:159–69.
4. Watanabe H, Yamane K, Fujikawa R, et al. Westernization of lifestyle markedly increases carotid intima-media wall thickness (IMT) in Japanese people. Atherosclerosis 2003;166:67–72.
5. Kanai H, Sato M, Koiwa Y, et al. Transcutaneous measurement and spectrum analysis of heart wall vibrations. IEEE Trans Ultrason Ferroelectr Freq Control 1996;43:791–810.
6. Kanai H, Hasegawa H, Chubachi N, et al. Noninvasive evaluation of local myocardial thickening and its color-coded imaging. IEEE Trans Ultrason Ferroelectr Freq Control 1997;44:752–68.
7. Kanai H, Hasegawa H, Ichiki M, et al. Elasticity imaging of atheroma with transcutaneous ultrasound: preliminary study. Circulation 2003;107:3018–21.
8. Hasegawa H, Kanai H, Hoshimiya N, et al. Evaluating the regional elastic modulus of a cylindrical shell with nonuniform wall thickness. J Med Ultrasonics 2004;31:81–90.
9. Hasegawa H, Kanai H, Koiwa Y. Detection of lumen-intima interface of posterior wall for measurement of elasticity of the human carotid artery IEEE Trans Ultrason Ferroelectr Freq Control 2004;51:93–108.
10. Kanai H, Koiwa Y, Zhang J. Real-time measurements of local myocardium motion and arterial wall thickening. IEEE Trans Ultrason Ferroelectr Freq Control 1999;46:1229–41.
11. Koiwa Y, Katoh G, Okimoto H, et al. The distortion of the intramural arterial wall elasticity distribution precedes the intima-media thickening in the arteriosclerosis. Circulation 2005;112: S428.
12. Munakata M, Ito N, Nunokawa T, et al. Utility of automated brachial ankle pulse wave velocity measurements in hypertensive patients. Am J Hypertens 2003;16:653–7.
13. Mitchell GF, Parise H Benjamin EJ, et al. Changes in arterial stiffness and wave reflection with advancing age in healthy men and women: the Framingham Heart Study. Hypertension 2004;43: 1239–45.

14. Kim JW, Park CG, Hong SJ, et al. Acute and chronic effects of cigarette smoking on arterial stiffness. Blood Press 2005;14:80–5.
15. Mahmud A, Feely J. Effect of smoking on arterial stiffness and pulse pressure amplification. Hypertension 2003;41:183–7.
16. Wollersheim H, Firestone G, Fronek A. Selective increase in popliteal artery wall stiffness in long-term smokers. J Hypertens 1993;11:S84–5.
17. Kool MJ, Hoeks AP, Struijker Boudier HA, et al. Short and long-term effects of smoking on arterial wall properties in habitual smokers. J Am Coll Cardiol 1993;22:1881–6.
18. Halimi MJ, Gireadeau B, Vol S, et al. The risk of hypertension in men: direct and indirect effects of chronic smoking. J Hypertens 2002;20:187–93.
19. Liang YL, Shiel LM, Teede H, et al. Effects of blood pressure, smoking, and their interaction on carotid artery structure and function. Hypertension 2001;37:6–11.
20. Lee RT, Grodzinsky AJ, Frank EH, et al. Structure-dependent dynamic mechanical behavior of fibrous caps from human atherosclerotic plaques. Circulation 1991;83:1764–70.
21. Inagaki J, Hasegawa H, Kanai H, et al. Construction of reference data for classification of elasticity images of arterial wall. In: IEEE International Ultrasonics Symposium Proceedings; 2004. p. 2161–4.