To assess the clinical relevance of carotid artery elasticity, we compared our elasticity values to those obtained with currently established methods for evaluating atherosclerosis: max IMT, plaque score, baPWV and ABI. Carotid artery elasticity showed significant positive correlations with max IMT ($r=0.291$, $p<0.01$) (Fig. 2A), the plaque score ($r=0.220$, $p<0.01$, $n=160$) (Fig. 2B) and baPWV ($r=0.345$, $p<0.01$) (Fig. 2C) in subjects with type 2 diabetes. It should be kept in mind that the plaque score can be obtained only in subjects with IMT ≥ 1.1 mm ($n=160$), such that the correlation was studied only in those having definite atherosclerosis based on IMT criteria [22,23]. Arterial elasticity showed no correlation with the ABI value ($r=-0.087$, $p=0.176$) (Fig. 2D). However, when we performed multiple linear regression analysis adjusted with independent parameters, age, systolic blood pressure and hyperlipidemia (Table 4), the correlations between elasticity and atherosclerosis markers (max IMT, plaque score and baPWV) were no longer present.

In a subject with more than one risk factor, the atherosclerotic process would be accelerated and thus affect the values of atherosclerosis markers. Four modifiable risk factors, diabetes, hypertension, hyperlipidemia and current smoking,

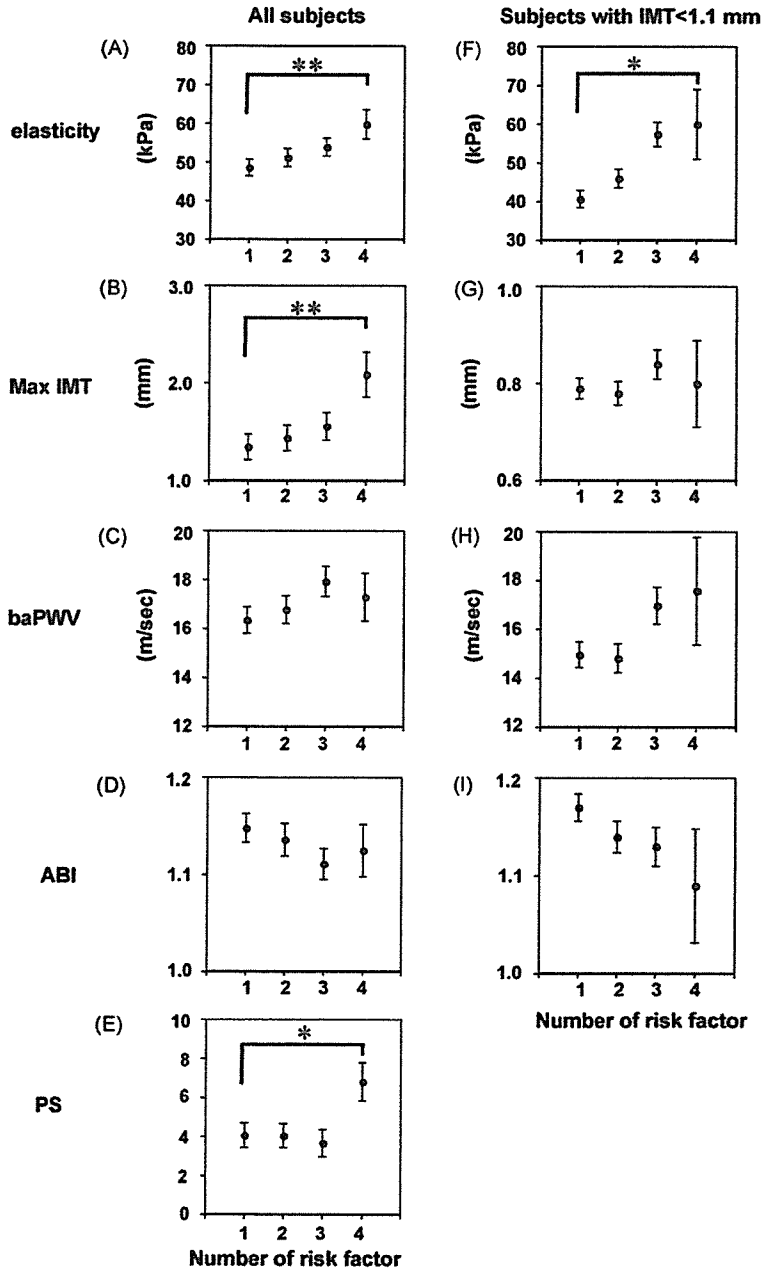

Fig. 3. Correlations of values reflecting atherosclerosis with the number of risk factors in all study subjects (A–E, $n=242$) and subjects with max IMT <1.1 mm (F–I, $n=82$). Data are presented as means ± S.E. $^{*}p<0.05$, $^{**}p<0.01$.

Please cite this article in press as: Okimoto H, et al., A novel method for evaluating human carotid artery elasticity: Possible detection of early stage atherosclerosis in subjects with type 2 diabetes, Atherosclerosis (2006), doi:10.1016/j.atherosclerosis.2006.11.020

were taken into account in this study. All subjects had at least one risk factor, diabetes. When all the subjects were analyzed by ANCOVA, adjusted with age as a covariate, the higher the number of risk factors, the greater the attenuation of arterial elasticity values, max IMT and the plaque score (Fig. 3A, B, E). However, very interestingly, when subjects with max IMT <1.1 mm, who are regarded as not having atherosclerosis based on IMT criteria, were analyzed ($n = 82$), only age-adjusted carotid artery elasticity correlated with an increasing number of risk factors (Fig. 3F). Other age-adjusted parameters for evaluating atherosclerosis, max IMT, baPWV and ABI, showed no significant correlations with a greater number of risk factors in subjects with max IMT <1.1 mm (Fig. 3G–I).

4. Discussions

Our most important finding is that in subjects with max IMT <1.1 mm, who are regarded as being free of atherosclerosis based on IMT criteria [22,23], only carotid artery elasticity as measured with our novel non-invasive method correlated with an increasing number of risk factors. No other values obtained with the currently available methods showed correlations with the number of risk factors in these "non-atherosclerotic" subjects. Thus, our novel method of measuring arterial wall elasticity raises the possibility of detecting atherosclerosis in its early stage.

Carotid artery elasticity correlates well with results obtained with currently established methods for evaluating atherosclerosis in subjects with type 2 diabetes. These results strongly suggest that elasticity as measured with our current method reflects the severity of atherosclerosis. The measurement procedures are relatively simple, essentially the same as those of B-mode ultrasonography. In addition, arterial wall elasticity is shown as a color coded cross-sectional image with a side by side B-mode ultrasonogram, which is very practical in the clinical setting.

This novel ultrasonic method accurately tracks the movement of the arterial wall based on both the phase and the magnitude of demodulated signals, allowing instantaneous determination of the position of an object. With this method, it is possible to accurately detect small-amplitude velocity signals, less than a few micrometers, that are superimposed on arterial wall motion due to the heartbeat. This method thus allows the elasticity, a qualitative feature, of the arterial wall to be evaluated. In addition to detecting the early stage atherosclerosis, this method may enable us to evaluate progression or regression of atherosclerosis in a much shorter time than currently available methods. This possibility is extremely interesting because a means of evaluating whether or not a treatment is effective for preventing atherosclerosis is urgently needed. It usually takes years to detect the progression or regression of atherosclerosis, while it may take only months with our present method of qualitative arterial wall measurement. For example, it may be possible to detect an improvement in response to statin treatment within a few months. Similarly, we will be able to assess the effects on atherosclerosis of altering risk factors within months. These possibilities clearly merits further study.

A variety of methods are widely used for evaluating atherosclerosis. Measuring carotid IMT with ultrasound is one of the most well-established methods because it is safe, non-invasive, reproducible and easy to perform. IMT provides quantitative information, i.e. vessel-wall thickness. Depicting changes in IMT is thus generally thought to take a long time. baPWV is also a non-invasive method, which assesses atherosclerosis, as a reflection of arterial stiffness, and the usefulness of baPWV has been reported in clinical studies [26–28]. However, the pulse wave velocity depends on the ratio of the inner radius of the artery to wall thickness, which is not related to regional elasticity. It also reportedly depends on heart rate [29].

While the elasticity average of the intima and media of the carotid artery wall was calculated and used for evaluation of atherosclerosis in this study, another interesting aspect of elasticity is its distribution. The elasticity distribution, which is depicted in a histogram, might provide additional information regarding qualitative changes in atherosclerosis, and should be comprehensively studied in the future. In conclusion, our novel method for evaluating carotid artery wall elasticity holds promise for early detection of atherosclerosis.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research (17790599) to Y. Ishigaki and the 21st Century COE Programs "Innovative Therapeutic Development towards the Conquest of Signal Transduction Diseases" to Y. Oka from the Ministry of Education, Science, Sports and Culture of Japan. This work was also supported by a Grant-in-Aid for Research on Human Genome, Tissue Engineering (H17-genome-003) to Y. Oka. We thank Healthcare Business Company, Matsushita Electric Industrial Co., Ltd. (Panasonic), Yokohama, Japan for supplying the prototype elasticity measurement system for this study.

References

[1] Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. JAMA 1997;241:2035–8.
[2] O'Leary DH, Polak JF, Kronmal RA, et al. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. Cardiovascular Health Study Collaborative Research Group. N Engl J Med 1999;340:14–22.
[3] Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of the arterial wall: a direct measurement with ultrasound imaging. Circulation 1986;74:1399–406.
[4] Salonen JT, Korpela H, Salonen R, Nyyssonen K. Precision and reproducibility of ultrasonographic measurement of progression of common carotid artery atherosclerosis. Lancet 1993;341:1158–9.

Please cite this article in press as: Okimoto H, et al., A novel method for evaluating human carotid artery elasticity: Possible detection of early stage atherosclerosis in subjects with type 2 diabetes, Atherosclerosis (2006), doi:10.1016/j.atherosclerosis.2006.11.020

[5] Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96:1432–7.
[6] Touboul PJ, Elbaz A, Koller C, et al. Common carotid artery intima-media thickness and brain infarction: the Etude du Profil Genetique de l'Infarctus Cerebral (GENIC) case-control study. The GENIC Investigators. Circulation 2000;102:313–8.
[7] Yamasaki Y, Kawamori R, Matsushima H, et al. Atherosclerosis in carotid artery of young IDDM patients monitored by ultrasound high-resolution B-mode imaging. Diabetes 1994;43:634–9.
[8] Folsom AR, Eckfeldt JH, Weitzman S, et al. Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Atherosclerosis Risk in Communities (ARIC) Study Investigators. Stroke 1994;25:66–73.
[9] Kawamori R, Yamasaki Y, Matsushima H, et al. Prevalence of carotid atherosclerosis in diabetic patients. Ultrasound high-resolution B-mode imaging on carotid arteries. Diabetes Care 1992;15:1290–4.
[10] Minamikawa J, Tanaka S, Yamauchi M, Inoue D, Koshiyama H. Potent inhibitory effect of troglitazone on carotid arterial wall thickness in type 2 diabetes. J Clin Endocrinol Metab 1998;83:1818–20.
[11] Furberg CD, Adams Jr HP, Applegate WB, et al. Effect of lovastatin on early carotid atherosclerosis and cardiovascular events. Asymptomatic Carotid Artery Progression Study (ACAPS) Research Group. Circulation 1994;90:1679–87.
[12] Lonn E, Yusuf S, Dzavik V, et al. Effects of ramipril and Vitamin E on atherosclerosis: the study to evaluate carotid ultrasound changes in patients treated with ramipril and Vitamin E (SECURE). Circulation 2001;103:919–25.
[13] Kodama M, Yamasaki Y, Sakamoto K, et al. Antiplatelet drugs attenuate progression of carotid intima-media thickness in subjects with type 2 diabetes. Thromb Res 2000;97:239–45.
[14] Beishuizen ED, van de Ree MA, Jukema JW, et al. Two-year statin therapy does not alter the progression of intima-media thickness in patients with type 2 diabetes without manifest cardiovascular disease. Diabetes Care 2004;27:2887–92.
[15] Rantala AO, Paivansalo M, Kauma H, et al. Hyperinsulinemia and carotid atherosclerosis in hypertensive and control subjects. Diabetes Care 1998;21:1188–93.
[16] Kanai H, Hasegawa H, Ichiki M, Tezuka F, Koiwa Y. Elasticity imaging of atheroma with transcutaneous ultrasound: preliminary study. Circulation 2003;107:3018–21.
[17] Hasegawa H, Kanai H, Hoshimiya N, Koiwa Y. Evaluating the regional elastic modules of a cylindrical shell with nonuniform wall thickness. J Med Ultrason 2004;31:81–90.
[18] Kanai H, Sato M, Koiwa Y, Chubachi N. Transcutaneous measurement and spectrum analysis of heart wall vibrations. IEEE Trans Ultrason Ferroelectr Freq Control 1996;43:791–810.
[19] Kanai H, Koiwa Y, Zhang J. Real-time measurement of local myocardium motion and arterial wall thickening. IEEE Trans Ultrason Ferroelectr Freq Control 1999;46:1229–41.
[20] Hasegawa H, Kanai H, Hoshimiya N, Chubachi N, Koiwa Y. Accuracy evaluation in the measurement of a small change in the thickness of arterial walls and the measurement of elasticity of the human carotid artery. Jpn J Appl Phys 1998;37:3101–5.
[21] Kanai H, Hasegawa H, Chubachi N, Koiwa Y, Tanaka M. Noninvasive evaluation of local myocardinal thickening and its color-coded imaging. IEEE Trans Ultrason Ferroelectr Freq Control 1997;44:752–68.
[22] Salonen R, Seppanen K, Rauramaa R, Salonen JT. Prevalence of carotid atherosclerosis and serum cholesterol levels in eastern Finland. Arteriosclerosis 1988;8:788–92.
[23] Poli A, Tremoli E, Colombo A, et al. Ultrasonographic measurement of the common carotid artery wall thickness in hypercholesterolemic patients. A new model for the quantitation and follow-up of preclinical atherosclerosis in living human subjects. Atherosclerosis 1988;70:253–61.
[24] O'Leary DH, Polak JF, Kronmal RA, et al. Distribution and correlates of sonographically detected carotid artery disease in the Cardiovascular Health Study. The CHS Collaborative Research Group. Stroke 1992;23:1752–60.
[25] Handa N, Matsumoto M, Maeda H, et al. Ultrasonic evaluation of early carotid atherosclerosis. Stroke 1990;21:1567–72.
[26] Lehmann ED, Hopkins KD, Gosling RG. Increased aortic stiffness in women with NIDDM. Diabetologia 1996;39:870–1.
[27] Farrar DJ, Green HD, Wagner WD, Bond MG. Reduction in pulse wave velocity and improvement of aortic distensibility accompanying regression of atherosclerosis in the rhesus monkey. Circ Res 1980;47:425–32.
[28] Lehmann ED, Riley WA, Clarkson P, Gosling RG. Non-invasive assessment of cardiovascular disease in diabetes mellitus. Lancet 1997;350(Suppl. 1):SI14–9.
[29] Lantelme P, Mestre C, Lievre M, Gressard A, Milon H. Heart rate: an important confounder of pulse wave velocity assessment. Hypertension 2002;39:1083–7.

Please cite this article in press as: Okimoto H, et al., A novel method for evaluating human carotid artery elasticity: Possible detection of early stage atherosclerosis in subjects with type 2 diabetes, Atherosclerosis (2006), doi:10.1016/j.atherosclerosis.2006.11.020