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ORIGINAL ARTICLE

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Effect of age on carotid arterial intima-media thickness in childhood

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Abstract To investigate relationships between carotid arterial intima-media thickness (IMT) and age in childhood, we performed high-resolution carotid arterial ultrasonography in 60 healthy children (27 boys, 33 girls; age range, 5–14 years) determined by screening to have no dyslipidemia or hypertension. No plaque formation was found, and irregularity of IMT (root mean square roughness of IMT) did not correlate with age. Mean IMT increased in a linear manner with age [IMT in millimeters = (0.009 × age in years) + 0.35] (r = 0.39, P = 0.002). This correlation remained significant after adjustment for gender, parental smoking, systolic and diastolic blood pressure, body mass index, and serum concentrations of low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides. None of these known cardiovascular disease risk factors in adults had a significant relationship with age-adjusted IMT in children. While circumferential wall stress and diastolic blood pressure were not correlated with age, mean IMT and lumen diameter showed significant positive relationships with circulating blood volume, which was calculated as the function of height and weight. These data suggested that age-dependent physiologic thickening of arterial walls begins in childhood.

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Introduction

The atherosclerotic process begins and accelerates in childhood,[1] when risk of cardiovascular disease already exists.[2,3] Initiation of measures to prevent coronary artery disease in childhood also has been advocated.[4,5] Accurate noninvasive evaluation of early subclinical atherosclerosis in children would be essential for identification and clinical management of individuals at risk.

Recent technological advances in ultrasonographic imaging permit noninvasive assessment of early carotid arterial atherosclerosis. In adults, carotid arterial intima-media thickness (IMT), measured ultrasonographically, has been reported to be a reliable marker of systemic subclinical atherosclerosis[6,7] and is associated with cardiovascular events.[8] Recently, similar ultrasonographic assessment of carotid IMT has been carried out in children;[9] high-risk children[10–14] showed greater carotid IMT than control children.

Cross-sectional studies in apparently healthy adults[15–17] demonstrated age-associated changes in carotid arteries, i.e., intima-media thickening and increases in vascular luminal diameter after the third decade of life. In adults, age is the one of the most important determinants of carotid IMT. Notably, the difference between older and younger persons without evidence of cardiovascular disease far exceeds the difference between older persons free of coronary disease and those with disease.[15,18] In assessing whether a given IMT is normal or pathologic, the effect of aging must be considered. However, to our knowledge, age-associated changes of IMT in children have not yet been fully explored. In the present study, we obtained carotid ultrasonographic measurements in children without apparent cardiovascular risk factors and determined age-specific reference ranges for these measurements in children.

Subjects and methods

Study subjects

This study is based on a school survey conducted in December 2002 in a rural town located approximately 50 km northeast of Tokyo. We studied 60 school-age subjects and their siblings who were free from abnormal serum lipid concentrations or hypertension, having low-density lipoprotein cholesterol (LDL-C) below 140 mg/dl, high-density lipoprotein cholesterol (HDL-C) of at least 40 mg/dl, fasting triglyceride (TG) below 150 mg/dl, and blood pressure below 140/90 mmHg. None took medication. All were nonsmokers with no history of cardiac disease. All subjects and their parents gave informed consent to the examinations after explanation of the study design.

Biochemical analyses

Blood was sampled for chemical analysis in the fasting state, with measurement of serum total cholesterol (TC), TG, and HDL-C. The concentration of LDL-C was calculated using Friedewald's formula.[19]

Demographic variables

Body weight and height were measured, and body mass index (BMI) was calculated as weight (kg)/height^2 (m^2). Radial artery blood pressure was measured in the right forearm. Pulse pressure was calculated as the difference between the systolic and diastolic blood pressure. Circulating blood volume was calculated as previously described[20] by the following formulae: $\log BV + 0.6459 \times Wt + 0.002743 \times Ht + 2.0324$ in boys aged 2–14 years and girls aged 2–6 years; $\log BV + 0.6412 \times Wt + 0.001270 \times Ht + 2.2169$ in girls aged 7–14 years, where BV is blood volume (ml), Wt is weight (kg), and Ht is height (cm).

Each participant and their parents completed a standard questionnaire concerning family history of sudden death, cardiovascular disease, and smoking habits. A family history of premature myocardial infarction was defined as a history of myocardial infarction before 55 years of age in first-degree relatives or grandparents.

Ultrasonography

A single experienced physician, kept unaware of the clinical and laboratory background data for the study children, performed all ultrasonographic studies and image analyses. Ultrasonography was performed with a Logiq 7 ultrasound system (GE Medical Systems, Hino, Tokyo, Japan) equipped with an M12L probe (active matrix array probe, 5–13 MHz, linear). The subjects were examined in the supine position with the head tilted slightly away from the side being scanned. The depth of scanning was set at 3 or 4 cm, being equivalent to 0.074 or 0.098 mm per pixel. The image was focused on the far wall of the artery. Several longitudinal images of the far wall of the right and left distal common carotid arteries, 1–2 cm proximal to the bulb, were obtained in every case. In each examination two scanning angles (anterior and lateral) were used to record IMT. Images were stored digitally for subsequent off-line analysis. All images were obtained at end-diastole. The two images of best quality were chosen for analysis in each study subject. The common carotid artery and the carotid bulb were also scanned for the presence of atherosclerotic plaques, defined as distinct areas of the vessel wall protruding inward more than 50% beyond adjacent parts of the intima-media layer.[11]

The IMT was manually traced using a computer program (US Distance Measure, ver.1.04, Hitachi Denshi Technosystem, Tokyo, Japan). Intima-media thickness was defined as the distance from the leading edge of the lumen-intima interface of the far wall to the leading edge of the media-adventitia interface of this wall (Fig. 1). The computer program automatically measured the distance between the interfaces, and calculated maximum and mean IMT and root mean square (RMS) differences between each IMT and the average IMT, to quantitate irregularity.[21] Root mean square roughness of the IMT was defined as follows:

$$\mathrm{RMS}\left(X_i, i = 1, \ldots, E\right) = \left\{\frac{1}{E}\sum_{i=0}^{E}\left(\overline{X} - X_i\right)^2\right\}^{\frac{1}{2}}$$

in which $\overline{X}$ represents the average IMT and X_i represents each IMT value. The common carotid luminal diameter was measured as the distance between the leading edge of the echo produced by the intima-media interface of the near wall and the leading edge of the echo produced by the lumen-intima interface of the far wall. The mean reading from these four frames for the right and left carotid arteries was calculated and used for analysis. Circumferential wall stress (dyne/cm^2) was calculated according to Lamé's equation:[22] Wall STRESS = DP $\times$ (LD/2)/IMT, where DP is diastolic blood pressure (dyne/cm^2) (used because IMT measurements were performed in diastole;[23,24] 1 mmHg equals 1333 dyne/cm^2), LD is lumen diameter (cm), and IMT is intima-media thickness (cm).

To assess the reproducibility of the method, images from 10 subjects were selected randomly and analyzed twice by two evaluators. The intraobserver mean absolute difference in measuring the common carotid arterial mean and maximum IMT were 0.010 $\pm$ 0.025 and 0.032 $\pm$ 0.053 mm (mean $\pm$ 2SD), respectively (intraclass correlation coefficient 0.91, 0.81). The interobserver mean absolute difference in measuring the common carotid arterial mean and maximum IMT were 0.043 $\pm$ 0.063 and 0.030 $\pm$ 0.076 mm (mean $\pm$ 2SD), respectively (intraclass correlation coefficient 0.63, 0.74).

Statistical analyses

Intima-media thickness data are expressed as the mean $\pm$ SD. Distributions were examined to determine whether

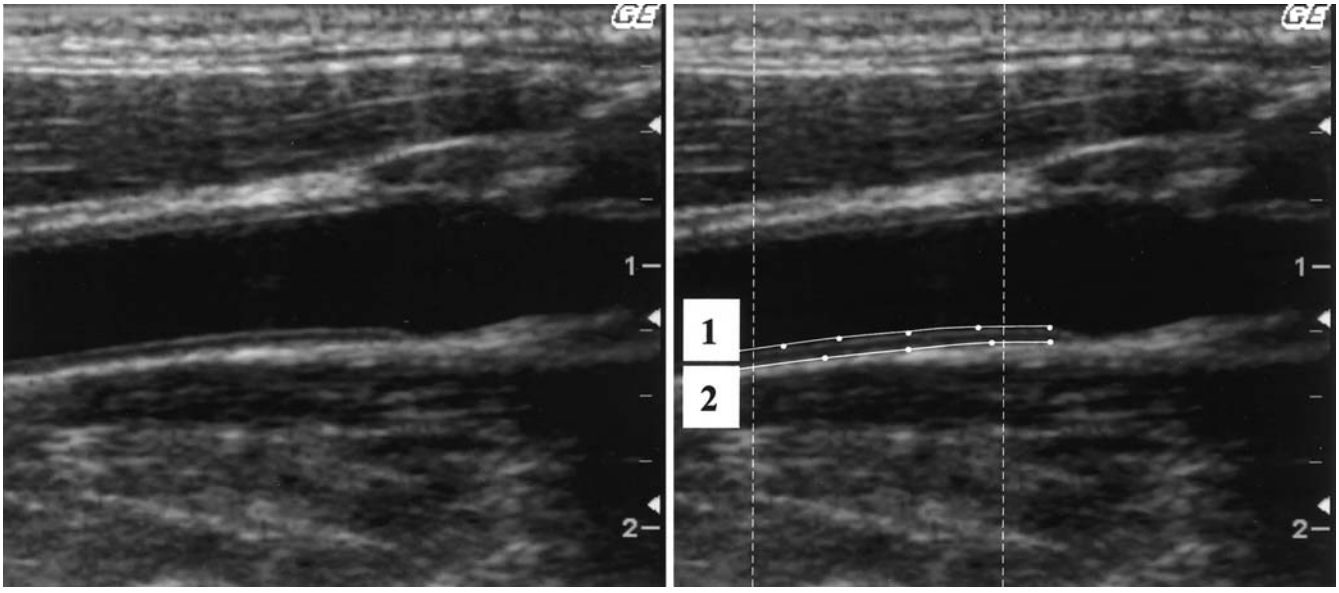

Fig. 1. Two-dimensional ultrasonogram of the common carotid artery. The far wall of the distal common carotid artery near the carotid bulb was scanned (*left*), and the intima-media thickness (IMT) was measured from the far wall image (*right*). This scan was obtained in healthy 10-year-old boy with a mean IMT of 0.46 mm, maximum IMT of 0.59 mm, root mean square roughness of IMT of 0.05 mm, and carotid diameter of 4.26 mm

Table 1. Subject profile

Variable		SD	Range
No. of subjects	60		
Age (years)	11	2	5–14
Body/girls (*n*)	27/33		
Familial history of premature CVD (*n*)	2		
Current parental smoking [*n* (%)]	16 (28%)		
Body mass index (kg/m^2)	22.1	4.9	15–34
Systolic blood pressure (mmHg)	108	13	73–134
Diastolic blood pressure (mmHg)	64	11	33–88
Pulse pressure (mmHg)	45	15	17–80
LDL cholesterol (mg/dl)	104	20	57–139
Triglycerides (mg/dl)	71	31	25–146
HDL cholesterol (mg/dl)	71	19	43–143

Data are presented as mean for continuous variables. Pulse pressure was calculated as the difference between systolic blood pressure and diastolic blood pressure

CVD, cardiovascular disease; premature CVD, first relatives or grandparents who suffered cardiovascular disease before 55 years of age; LDL, low-density lipoprotein; HDL, high-density lipoprotein

Table 2. Intima-media thickness and vessel diameter of the study subjects

Variable	Mean ± SD	Range
Mean IMT (mm)	0.44 ± 0.05	0.35–0.55
Maximum IMT (mm)	0.60 ± 0.08	0.44–0.88
RMS difference of IMT (mm)	0.07 ± 0.02	0.05–0.13
Lumen diameter (mm)	5.33 ± 0.45	4.26–6.26
Wall stress (10^5 dynes/cm^2)	5.20 ± 1.35	2.57–9.24

IMT, intima-media thickness; RMS, root mean square

they were normal. Variables showing non-normal distribution were logarithmically transformed. Statistical software (State, College Station, TX, USA) was used for analyses. A P value of less than 0.05 was considered to indicate statistical significance. To examine age-adjusted correlations between ultrasonic measurements and clinical variables, age was entered in a multivariate analysis as an independent variable. Associations between ultrasonographic measurements and age were examined with Pearson correlation coefficients, and 95% prediction intervals were calculated as predicted value ± 2.01 × standard errors of predictions. Multiple linear regression analysis was used to examine the effects of aging on carotid ultrasonographic measurements after controlling for other clinically important risk factors [gender, parental smoking status, systolic and diastolic blood pressure, body mass index (log), serum concentrations of LDL-C, HDL-C (log), and TG]. Stepwise multivariate analysis was performed upon several variables determined to be significant ($F \geqslant 4.0$) by univariate analysis.

Results

Subject characteristics

The characteristics of the children are shown in Table 1. Common carotid arterial IMT and luminal diameter were evaluated in all 60 children (age range, 5–14 years). No plaque was detected in any subject. The mean length of IMT for the tracing was 30 ± 9 mm, and 4015 ± 1255 data points were used for calculation of mean IMT and RMS roughness in each case. Table 2 shows the ultrasonographic measurements of the carotid arteries.

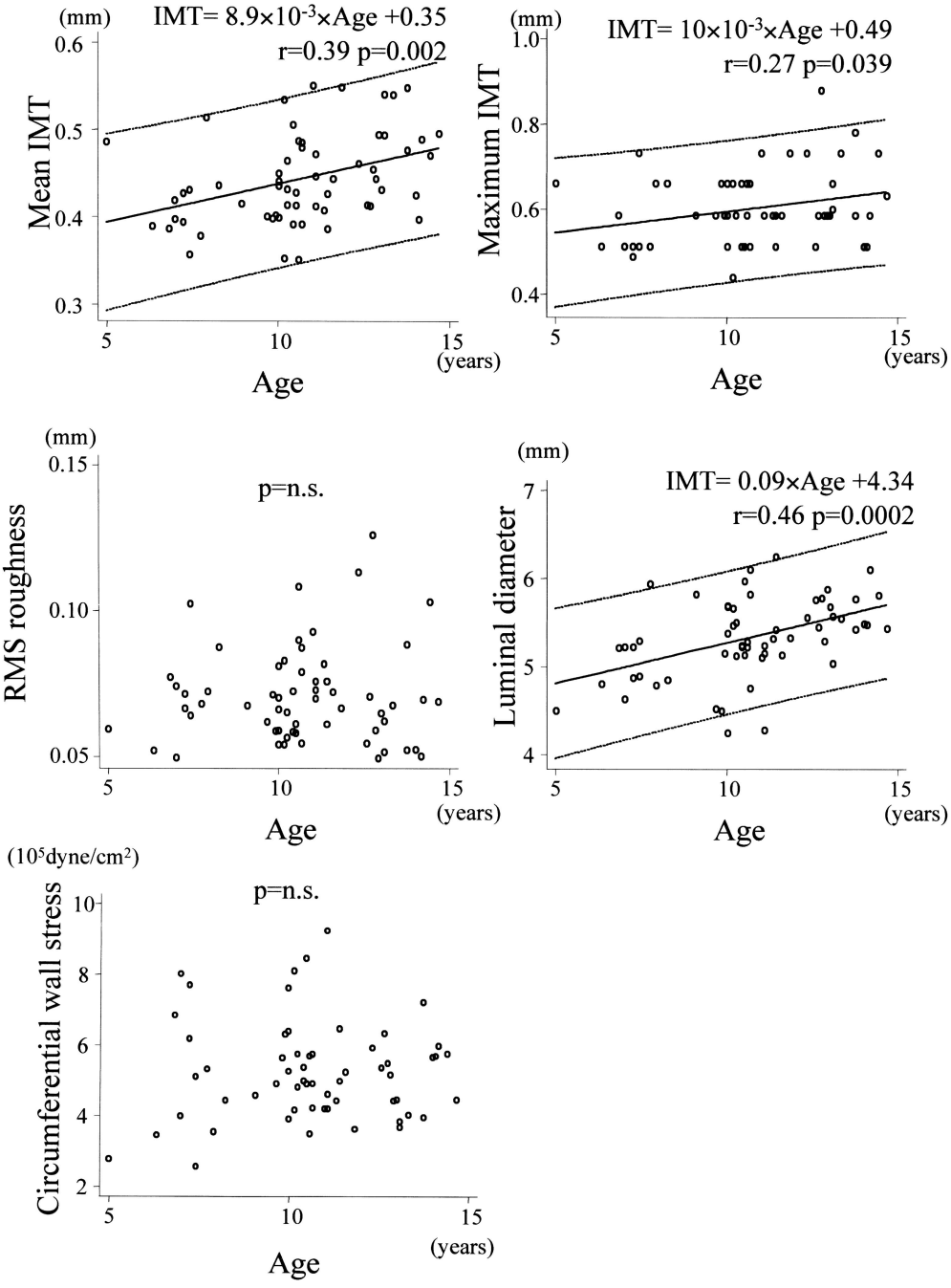

Fig. 2. Age-related change in mean intima-media thickness (*IMT*), maximum IMT, root mean square (*RMS*) roughness of IMT, luminal diameter, and circumferential wall stress in healthy children. The linear regression line (*solid line*) and 95% prediction intervals (*dotted lines*) are shown

Change of intima-media, thickness luminal diameter, and wall stress with age

On univariate analysis, mean and maximum IMT as well as luminal diameter increased in a linear manner with age in our subjects (Fig. 2). Root mean square roughness of IMT, wall stress, and diastolic blood pressure, however, showed no significant relationship with age. Multivariate regression models (Table 3) demonstrated that age remained significantly associated with mean IMT, maximum IMT, and luminal diameter, after adjustment for the clinically important risk factors as listed in the description of the statistical analysis.

Carotid ultrasonographic measurements and risk factors

To investigate the correlation of carotid ultrasonographic measurements and risk factors, adjustment by age was performed (Table 4). Current parental smoking correlated significantly with greater RMS roughness of IMT. Otherwise, no significant correlation was found for gender, BMI, blood pressure, or serum lipid concentrations.

Height was selected to be a significant determinant of mean IMT and luminal diameter ($r = 0.48$, $P = 0.001$ and $r = 0.48$, $P = 0.0002$, respectively) by stepwise multiple regression analysis among age, gender, height, weight, BMI, blood pressure, LDL-C, HDL-C, and TG. Height corre-

Table 3. Multiple regression of carotid ultrasonographic measurements with age adjusted for risk factors[a]

	r	Coefficient (10^{-3} mm/year)	95% CI (10^{-3} mm/year)	*P* value
Mean IMT	0.55	10.7	4.3, 17.1	0.002
Maximum IMT	0.52	12.0	1.2, 22.9	0.030
RMS difference of IMT	0.38	0.1	−2.2, 2.4	0.930
CCA diameter	0.65	88.2	41, 135	<0.001
Wall stress[b]	0.50	−0.3	−0.7, 0.2	0.266

CI, confidence interval; CCA, common carotid artery
[a] Adjusted by gender, parental current smoking, systolic blood pressure, diastolic blood pressure, body mass index (log), and serum concentrations of LDL cholesterol, HDL cholesterol (log), and triglycerides
[b] Diastolic blood pressure was excluded from the adjustment variables because of confounding

Table 4. Association between age-adjusted[a] carotid ultrasonographic variables and clinical measurements

Clinical variable	Mean IMT		Maximum IMT		RMS difference		Diameter		Wall stress	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Gender	0.40	>0.20	0.30	>0.20	0.10	>0.20	0.47	>0.20	0.12	>0.20
Parental current smoking	0.41	>0.20	0.33	0.13	0.28	0.043	0.53	0.10	0.09	>0.20
Body mass index (kg/m^2) (log)	0.39	>0.20	0.28	>0.20	0.08	>0.20	0.48	>0.20	0.20	0.15
Systolic blood pressure (mmHg)	0.40	>0.20	0.28	>0.20	0.06	>0.20	0.47	>0.20	0.25	0.068
Diastolic blood pressure (mmHg)[b]	0.46	0.049	0.31	>0.20	0.08	>0.20	0.50	0.18	–	–
Pulse pressure (mmHg)[b]	0.40	>0.20	0.28	>0.20	0.10	>0.20	0.49	>0.20	–	–
LDL cholesterol (mg/dl)	0.42	0.19	0.34	0.09	0.05	>0.20	0.49	0.16	0.06	>0.20
Triglycerides (mg/dl)	0.40	>0.20	0.28	>0.20	0.19	0.15	0.47	>0.20	0.06	>0.20
HDL cholesterol (mg/dl) (log)	0.41	>0.20	0.27	>0.20	0.08	>0.20	0.47	>0.20	0.19	0.18

[a] Adjustment by age was achieved by inclusion of age as an independent variable in multiple regression analyses
[b] Relationship between diastolic blood pressure or pulse pressure and wall stress were not analyzed because of collinearity

lated highly with age ($r = 0.88$, $P < 0.0001$; data not shown). Both mean IMT and lumen diameter correlated significantly with circulating blood volume ($r = 0.39$, $P = 0.0023$ and $r = 0.40$, $P = 0.0018$, respectively).

Discussion

Our data showed an increase with age of the carotid mean and maximum IMT as well as luminal diameter between ages 5 and 14 years in healthy children. For 10-year-old children, mean IMT in the distal far wall of the common carotid artery was estimated to be 0.44 mm (95% prediction range, 0.34–0.53 mm) based on the linear regression equation linking age and IMT. Mean IMT (mean ± SD) in the distal common carotid artery has been reported as 0.46 ± 0.06 mm for 30 healthy children of 14 ± 2 years,[11] 0.42 ± 0.04 mm for 28 control children of 11 ± 1 years with normal serum cholesterol,[25] and 0.39 ± 0.03 mm for 48 control children of 6 ± 3 years with normal cholesterol and triglyceride,[10] within the estimated range in our study. In addition, the reported mean IMT for subjects with familial hypercholesterolemia (aged 14 ± 2 years),[11] diabetes (11 ± 2 years),[25] and hypercholesterolemia (7 ± 3 years)[10] was higher than in controls (0.48 ± 0.07, 0.47 ± 0.04, and 0.40 ± 0.03 mm). Notably, differences between normal and abnormal groups were small even though statistically significant with the mean values distributed within the reference range estimated in the present study. This indicates that in assessing IMT in childhood, age as well as variability at a given age must be taken into account in children as in adults.[18]

Autopsy studies would suggest caution in extrapolation of the equation of the present study to ages below 5 years. Early atheromatous change such as diffuse intimal thickening or fatty streaks has been reported to develop soon after birth and increase until 8 months of age, with lesions then decreasing for the next 7 years.[1] Early lesions became more frequent again with the approach of puberty, slowly increasing subsequently until the end of third decade.[1] In the present study the youngest girl, 5 years of age, showed a mean IMT of 0.49 mm, exceeding the upper 75th percentile of study subjects. When her data was excluded from the analysis, the correlation coefficient for linear regression of age and mean IMT improved from 0.39 to 0.45. This implies that a nonlinear correlation should be assumed in assessment of carotid IMT of children less than 6 years of age.

Age-related increases in carotid IMT in healthy children do not appear to be strongly associated with pathologic atherosclerotic progression. First, our study excluded subjects with dyslipidemia and hypertension, which have been reported to be independent risk factors for IM thickening in childhood.[25] Also, no present subject showed pathologic plaque formation in the carotid arterial tree. Second, risk factors, including serum lipid concentration, obesity, blood pressure, and gender, did not show a significant relationship with IMT. Third, height, which can be regarded as a marker of the physiologic developmental status of children, corre-

lated closely with both IMT and luminal diameter. Finally, the IMT irregularity did not change with age. Taken together, these results suggested that the age-related increase in carotid IMT in healthy children may, at least in part, reflect the physiologic growing process rather than pathologic atherosclerosis.

While an annual increase of mean IMT was estimated to be 0.009 mm and the rate was similar to that previously reported in healthy adults,[17,26] the underlying mechanism resulting in arterial wall thickening may not be similar in childhood and adulthood. Although no detailed information is presently available concerning factors involved in age-dependent arterial wall thickening, this has been proposed to be attributed to the reaction to increased blood pressure in healthy adults.[27,28] Based on the law of LaPlace, increased arterial diameter, combined with increased arterial pressure, has been proposed to promote arterial medial thickening to maintain tensile stress upon the arterial wall at near-baseline levels as age increases.[27,28] In contrast, during the period of growth, the greater circulating blood volume associated with increasing body mass would be expected to generate an increased preload on the cardiovascular system.[29] Increased arterial diameter, combined with increased circulating blood volume, may promote arterial medial thickening to keep wall shear stress constant. In the present study, although diastolic blood pressure and shear stress did not change with age, lumen diameter and IMT showed a significant positive relationship with circulating blood volume. These results suggested that the physical growth accompanied with increased circulating blood volume may play an important role in wall thickening during the period of growth, while the pressure overload has been proposed to be the main factor attributed to the wall thickening in adults with aging.

The present study included obese children. In 16 out of 60 children, the BMI exceeded 25 kg/m^2, yet our data showed no significant correlation of BMI with carotid arterial IMT. Although obesity in adults has been reported to be an independent risk factor for mortality from coronary artery disease, an independent relationship between obesity and coronary artery disease risk is less clear in childhood.[4] The results of the present study suggest that obesity without dyslipidemia or hypertension might not accelerate atherosclerosis during childhood.

Parental smoking correlated with greater irregularity of carotid arterial IMT (larger RMS roughness of IMT). This finding suggested the possibility that parental smoking might contribute to the onset of atherosclerosis in childhood. This possibility deserves investigation in detail. Whether increased IMT in children contributes to cardiovascular events in adulthood is not clear; further studies are needed concerning this important issue as well.

Limitation of the study

The reproducibility of ultrasonographic measurements of the carotid artery should be strictly monitored to retrieve a subtle change of these parameters with age. Although variability based on repeat examinations in a person at different visits is potentially sizable, it was not presented in the present study because our study was based on the setting of an annual health check for schoolchildren. It could be estimated as about 1.6 times greater than the variability found in the intraobserver measurement according to previous reports.[30]

References

1. Stary HC (1989) Evolution and progression of atherosclerotic lesions in coronary arteries of children and young adults. Arteriosclerosis Suppl I9: I-19–I-32
2. Wissler RW, Strong JP (1998) Risk factors and progression of atherosclerosis in youth. PDAY Research Group. Pathological determinants of atherosclerosis in youth. Am J Pathol 153:1023–1033
3. Berenson GS, Srinivasan SR, Bao W, Newman WP 3rd, Tracy RE, Wattigney WA (1998) Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med 338:1650–1656
4. Van Horn L, Greenland P (1997) Prevention of coronary artery disease is a pediatric problem. JAMA 278:1779–1780
5. Kavey RE, Daniels SR, Lauer RM, Atkins DL, Hayman LL, Taubert K (2003) American Heart Association guidelines for primary prevention of atherosclerotic cardiovascular disease beginning in childhood. Circulation 107:1562–1566
6. Salonen JT, Salonen R (1991) Ultrasonographically assessed carotid morphology and the risk of coronary heart disease. Arterioscler Thromb 11:1245–1249
7. Cerne A, Kranjec I (2002) Atherosclerotic burden in coronary and peripheral arteries in patients with first clinical manifestation of coronary artery disease. Heart Vessels 16:217–226
8. Chambless LE, Heiss G, Folsom AR, Rosamond W, Szklo M, Sharrett AR, Clegg LX (1997) Association of coronary heart disease incidence with carotid arterial wall thickness and major cardiovascular risk factors: the atherosclerosis risk in communities (ARIC) study, 1987–1993. Am J Epidemiol 146:483–494
9. Raitakari OT (1999) Imaging of subclinical atherosclerosis in children and young adults. Ann Med 31 Suppl 1:33–40
10. Pauciullo P, Iannuzzi A, Sartorio R, Irace C, Covetti G, Di Costanzo A, Rubba P (1994) Increased intima-media thickness of the common carotid artery in hypercholesterolemic children. Arterioscler Thromb 14:1075–1079
11. Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D, Bonaa KH (1996) Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. Arterioscler Thromb Vasc Biol 16:984–991
12. Jarvisalo MJ, Harmoinen A, Hakanen M, Paakkunainen U, Viikari J, Hartiala J, Lehtimaki T, Simell O, Raitakari OT (2002) Elevated serum C-reactive protein levels and early arterial changes in healthy children. Arterioscler Thromb Vasc Biol 22:1323–1328
13. Jarvisalo MJ, Putto-Laurila A, Jartti L, Lehtimaki T, Solakivi T, Ronnemaa T, Raitakari OT (2002) Carotid artery intima-media thickness in children with type 1 diabetes. Diabetes 51:493–498
14. Cuomo S, Guarini P, Gaeta G, De Michele M, Boeri F, Dorn J, Bond M, Trevisan M (2002) Increased carotid intima-media thickness in children-adolescents, and young adults with a parental history of premature myocardial infarction. Eur Heart J 23:1345–1350
15. Nagai Y, Metter EJ, Earley CJ, Kemper MK, Becker LC, Lakatta EG, Fleg JL (1998) Increased carotid artery intimal-medial thickness in asymptomatic older subjects with exercise-induced myocardial ischemia. Circulation 98:1504–1509
16. Schmidt-Trucksass A, Grathwohl D, Schmid A, Boragk R, Upmeier C, Keul J, Huonker M (1999) Structural, functional, and hemodynamic changes of the common carotid artery with age in male subjects. Arterioscler Thromb Vasc Biol 19:1091–1097
17. Homma S, Hirose N, Ishida H, Ishii T, Araki G (2001) Carotid plaque and intima-media thickness assessed by B-mode ultra-

sonography in subjects ranging from young adults to centenarians. Stroke 32:830–835
18. Lakatta EG, Levy D (2003) Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a "set up" for vascular disease. Circulation 107:139–146
19. Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499–502
20. Linderkamp O, Versmold HT, Riegel KP, Betke K (1977) Estimation and prediction of blood volume in infants and children. Eur J Pediatr 125:227–234
21. Ishizu T, Ishimitsu T, Kamiya H, Seo Y, Moriyama N, Obara K, Watanabe S, Yamaguchi I (2002) The correlation of irregularities in carotid arterial intima-media thickness with coronary artery disease. Heart Vessels 17:1–6
22. Nichols WW, O'Rourke MF (1998) Properties of the arterial wall: theory. In: Nichols WW, O'Rourke MF (eds) McDonald's blood flow in arteries: theoretical, experimental and clinical principles, 4th edn. Edward Arnold, London, pp 54–72
23. Astrand H, Sandgren T, Ahlgren AR, Lanne T (2003) Noninvasive ultrasound measurements of aortic intima-media thickness: implications for in vivo study of aortic wall stress. J Vasc Surg 37:1270–1276
24. Girerd X, Mourad JJ, Copie X, Moulin C, Acar C, Safar M, Laurent S (1994) Noninvasive detection of an increased vascular mass in untreated hypertensive patients. Am J Hypertens 7:1076–1084
25. Jarvisalo MJ, Jartti L, Nanto-Salonen K, Irjala K, Ronnemaa T, Hartiala JJ, Celermajer DS, Raitakari OT (2001) Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. Circulation 104:2943–2947
26. Crouse JR 3rd, Tang R, Espeland MA, Terry JG, Morgan T, Mercuri M (2002) Associations of extracranial carotid atherosclerosis progression with coronary status and risk factors in patients with and without coronary artery disease. Circulation 106:2061–2066
27. Tanaka H, Dinenno FA, Monahan KD, DeSouza CA, Seals DR (2001) Carotid artery wall hypertrophy with age is related to local systolic blood pressure in healthy men. Arterioscler Thromb Vasc Biol 21:82–87
28. Glagov S, Zarins C, Giddens DP, Ku DN (1988) Hemodynamics and atherosclerosis. Insights and perspectives gained from studies of human arteries. Arch Pathol Lab Med 112:1018–1031
29. Anversa P, Capasso JM, Olivetti G, Sonnenblick EH (1992) Myocyte cellular hypertrophy and hyperplasia in the heart during postnatal maturation and ageing. Acta Paediatr Suppl 383:29–31
30. Chambless LE, Zhong MM, Arnett D, Folsom AR, Riley WA, Heiss G (1996) Variability in B-mode ultrasound measurements in the atherosclerosis risk in communities (ARIC) study. Ultrasound Med Biol 22:545–554