Increased Aortic Intima-Media Thickness

A Marker of Preclinical Atherosclerosis in High-Risk Children

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Background—Autopsy studies in children have shown that atherosclerotic lesions begin to develop first in the intima of the aorta. Recent developments in ultrasound techniques have made it possible to visualize the intima-media thickness of the abdominal aorta directly (aIMT). Therefore, we examined the feasibility of measuring aIMT in children and studied its value in distinguishing high-risk children from healthy controls compared with a more established marker of subclinical atherosclerosis, the common carotid artery intima-media thickness (cIMT).

Methods and Results—IMTs were measured using high-resolution (13 MHz) ultrasound in 88 children (aged 11 ± 2 years); 16 had hypercholesterolemia (LDL cholesterol, 5.1 ± 1.2 mmol/L), 44 had type 1 diabetes (mean duration, 4.4 ± 3.1 years; LDL cholesterol, 2.3 ± 0.7 mmol/L), and 28 were healthy (controls; LDL cholesterol, 2.5 ± 0.8 mmol/L). High-risk children had significantly increased aIMTs and cIMTs (both \( P < 0.001 \)) compared with controls. In controls, aIMT was similar to cIMT (\( P = NS \)), but aIMT was higher than cIMT in the children with hypercholesterolemia and diabetes (both \( P < 0.01 \)). Both markers showed excellent and approximately equal between-observer (<4%) and between-subject variation (<5%).

Conclusions—Children with hypercholesterolemia and diabetes show increased IMTs compared with healthy controls, with a relatively greater increase in the aIMT than in the cIMT. Because atherosclerosis begins first in the intima of the aorta, these data suggest that the aIMT might provide the best currently available noninvasive marker of preclinical atherosclerosis in children. (Circulation. 2001;104:2943-2947.)

Key Words: aorta ■ atherosclerosis ■ pediatrics

The atherosclerotic process begins in childhood and develops inconspicuously for many decades before cardiovascular complications, such as myocardial infarction or stroke, occur in middle and late age.¹ The first signs of atherosclerosis include lipid deposits, resulting in fatty streaks in the intima of systemic arteries. Advanced atherosclerotic lesions arise from these fatty streaks, and their progression during childhood and adolescence is accelerated in the presence of risk factors for adult coronary artery disease, such as elevated LDL cholesterol, hypertension, and smoking.²–⁴ These observations, therefore, emphasize that risk factor control for the long-range prevention of atherosclerosis and its sequelae should begin in childhood.⁵ A lack of accurate diagnostic tests of preclinical atherosclerosis, however, has hampered the ability to detect and monitor such early atherosclerotic lesions. The development of such noninvasive tests could facilitate the early diagnosis and management of high risk individuals.

Recent improvements in imaging technology (increased resolution and accuracy) have identified early vascular changes that can be assessed noninvasively using ultrasound.⁶ These early changes include thickening of vessel walls and impairment of arterial vasodilatory function. Studies in adults have shown that the measurement of the thickness of carotid intima-media complex (cIMT) represents an excellent marker of subclinical atherosclerosis.⁷,⁸ Similarly, studies in children with hypercholesterolemia have demonstrated increased cIMT compared with control children.⁹,¹⁰ The carotid artery has been the target in these studies because it is located rather superficially on the neck and can be easily visualized by ultrasound. Autopsy studies, however, have shown that the first atherosclerotic lesions actually begin to develop in the abdominal aorta.¹¹ Because it is now possible to visualize the abdominal aorta and accurately assess its wall thickness (aortic intima-media thickness, aIMT), measuring aIMT might provide a better index of preclinical atherosclerosis in high-risk children than cIMT. In the present study, therefore, we examined the feasibility of accurately and reproducibly measuring aIMT in children and studied its value in...
distinguishing high-risk children with hypercholesterolemia and diabetes from healthy controls in comparison with cIMT.

Methods

Subjects
We studied 44 children with type 1 diabetes (DM group), 16 children with hypercholesterolemia (HC group; 11 children had familial hypercholesterolemia), and 28 healthy children with normal cholesterol levels. The groups were matched in terms of age, sex, and body size. The clinical characteristics of the study groups are shown in Table 1.

Children with diabetes and hypercholesterolemia were recruited from the outpatient clinic of the Department of Pediatrics, Turku University Central Hospital. The mean duration of diabetes was 4.4±3.1 years. No diabetic children were taking regular medications other than daily insulin. The daily insulin dose was 0.95±0.24 IU/kg (range, 0.62 to 1.53 IU/kg). None of the diabetic patients had evidence of microvascular complications, such as diabetic retinopathy, neuropathy, or microalbuminuria. In the diabetic group, the mean glycosylated hemoglobin level was 8.8±1.4% (range, 6.2% to 12.8%; reference range, 4.2% to 6.0%).

In the HC group, every child had a serum total cholesterol level >6.0 mmol/L or LDL cholesterol level >4.5 mmol/L, but none of the children had tendon xanthoma, arcus lipoides, or xanthalmas. Eleven children in the HC group were diagnosed with familial hypercholesterolemia, which was confirmed by lymphocyte testing. Nine of the children with familial hypercholesterolemia were on lipid-lowering medication: 6 were on statin treatment (simvastatin 40 mg, 1 child; lovastatin 40 mg, 2 children; lovastatin 20 mg, 3 children); 3 were on bile acid sequestrant treatment with cholestyramine 4 to 8 g/d; and 2 were on diet therapy only. None of the children in the HC group had any other chronic diseases or took medications other than lipid-lowering agents. The 5 other children in the HC group had total cholesterol and/or LDL cholesterol levels above the age- and sex-specific 90th percentile for Finnish children and were not on lipid-lowering medication.

The healthy control children included in the study were friends of the diabetic children studied and children of the staff members of the Turku University or Turku University Central Hospital. All the children included in the study were lifelong nonsmokers. Written, informed consent was acquired from the legal guardians of the children and, when possible, it was also obtained from the child. The study was conducted according to the declaration of Helsinki, and the study protocol was approved by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital.

Ultrasound Studies
Before beginning the ultrasound imaging, subjects lay quietly for 10 to 15 minutes in a dark, temperature-controlled room. The abdominal aorta and both carotid arteries were scanned according to a predetermined, standardized scanning protocol using an Acuson Sequoia 512 high-resolution ultrasound mainframe. All the ultrasound studies were performed by a single experienced vascular sonographer who was unaware of the clinical and laboratory characteristics of the study children. Blood pressure was measured using a pneumatic sphygmomanometer from the nondominant arm 3 times during the study, and the readings were averaged. Mean blood pressure was calculated as [(diastolic blood pressure+[(systolic blood pressure–diastolic blood pressure)/3]].

Measurement of aIMT
The abdominal aorta was first identified in the upper abdomen using a 7.5-MHz pediatric phased array transducer, and it was then followed distally until the aortic bifurcation was reached. The depth (anterior-posterior direction) and location (cranio-caudal direction) of the distal 15 mm of the abdominal aorta was measured from these images and used as an aid to locate the aortic intima-media complex using a 13-MHz linear array transducer. For the assessment of aIMT, the image was focused on the far wall (dorsal arterial wall of the most distal 15 mm of the abdominal aorta), and gain settings were used to optimize image quality. Images 15 mm in width were magnified using a resolution box function (Figure 1). The dorsal arterial wall of the most distal abdominal aorta was chosen for the area of interest because post mortem series have shown it is the most lesion-prone site. A scanning frequency of 13 MHz was preferred but, when necessary (to reach sufficient tissue penetration), scanning frequencies of 11.5 and 10 MHz were used. Several images of the most distal 15 mm aortic far wall were captured in every case, and the images were digitally stored for subsequent off-line analysis. All images were taken at end-diastole, incident with the R-wave on a continuously recorded ECG. Two images of the best quality were chosen for analysis in each study subject. IMT was measured using ultrasonic calipers in each case by 2 independent and blinded observers. At least 4 to 6 measurements covering the entire far wall segment of interest were taken for each image by both observers. The measurements of each observer were first averaged for each and then the average of both observers was used to calculate aIMT.

### Table 1. Characteristics of the Study Groups

<table>
<thead>
<tr>
<th></th>
<th>Control Children</th>
<th>DM Group</th>
<th>HC Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects (boys)</td>
<td>28 (17)</td>
<td>44 (31)</td>
<td>16 (7)</td>
</tr>
<tr>
<td>Age, y</td>
<td>11±1</td>
<td>11±2</td>
<td>11±3</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.50±0.08</td>
<td>1.50±0.12</td>
<td>1.52±0.16</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>41.7±8.5</td>
<td>43.0±10.1</td>
<td>48.6±14.8</td>
</tr>
<tr>
<td>Ponderal index, kg/m³</td>
<td>12.2±1.9</td>
<td>12.6±1.2</td>
<td>13.5±2.2</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>110±9</td>
<td>110±9</td>
<td>116±11</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>64±6</td>
<td>64±7</td>
<td>67±6</td>
</tr>
<tr>
<td>Mean blood pressure, mm Hg</td>
<td>80±6</td>
<td>80±7</td>
<td>84±7</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.3±0.8</td>
<td>4.3±0.8</td>
<td>6.9±1.2†</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.5±0.8</td>
<td>2.3±0.7</td>
<td>5.1±1.2†</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.50±0.34</td>
<td>1.61±0.37</td>
<td>1.3±0.34</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>0.75±0.45</td>
<td>0.72±0.51</td>
<td>1.1±0.41*</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.2±0.2</td>
<td>8.8±1.4†</td>
<td>5.4±0.4</td>
</tr>
</tbody>
</table>

Values are mean±SD unless otherwise indicated. HbA1c indicates glycosylated hemoglobin.

*P<0.01, †P<0.001 compared with the control group.
Measurement of cIMT
All studies were done following a predetermined, standardized scanning protocol for the right and left carotid arteries, as described previously. The proximal part of the carotid bulb was identified, and the segment of the common carotid artery 1 to 2 cm proximal to the bulb was scanned. The image was focused on the posterior (far) wall, and the resolution box function was used to magnify the arterial far wall. Two angles were used in each case: anterior oblique and lateral. All scans were digitally stored for subsequent off-line analysis. Two end-diastolic frames were selected and analyzed for mean IMT, and the average reading from these 2 frames was calculated for both right and left carotid arteries. The images were analyzed by 2 independent readers who were blinded to the subject’s clinical details, and the average values were used in the analysis. The far wall of the abdominal aorta, the common carotid arteries, and the carotid bulb region were also scanned for the presence of atherosclerotic plaques, which were defined as distinct areas of the vessel protruding >50% from the adjacent parts of the intima-media layer.

Serum Lipids, Lipoproteins, and Glycosylated Hemoglobin
Fasting blood was drawn for serum total, LDL, and HDL cholesterol and triglyceride determinations. Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured using standard enzymatic methods and Boehringer Mannheim reagents with a fully automated analyzer (Hitachi 704). LDL cholesterol concentration was calculated using Friedewald’s equation. Glycosylated hemoglobin was measured with high-performance liquid chromatography (Variant Analyser, Bio-Rad).

Statistical Methods
Results are expressed as mean±SD, unless stated otherwise. Data on serum triglycerides were skewed toward high values and were therefore included as their logarithms in the analyses. The comparison between the 3 groups was done using ANOVA with the post-hoc Bonferroni procedure to allow for multiple pair-wise comparisons. Univariate associations between the study variables were analyzed by calculating Pearson’s correlation coefficients. Multivariate analyses were done using the linear regression technique. The between-observer and between-subject repeatability of IMT measurements were analyzed by calculating the intraclass correlation coefficients (ρ) and coefficients of variation. The intraclass correlation coefficient is an estimate of the reliability of the measurement and varies from 0 (no reliability) to 1 (total reliability, when test=retest measure) and is expressed by the following equation: ρ=(MSBS−MSWS)/(MSBS+(n−1)×MSWS), where MSBS and MSWS are the sum of squares between and within subjects, respectively, and n is the number of within-subject measurements. All statistical analyses were performed using SAS.

Results
The characteristics of study groups are shown in Table 1. Groups were matched in terms of age, sex, and body size. Serum lipids, excluding HDL cholesterol, were significantly higher in the HC group compared with the other groups, and glycosylated hemoglobin was significantly higher in the DM group (Table 1). There were no differences in aortic luminal diameter between the study groups (controls, 9.5±1.7 mm; DM group, 9.7±1.8 mm; HC group, 10.3±1.5 mm; P=NS), but carotid diameter was significantly smaller in diabetic children (5.2±0.6, 4.8±0.4, and 5.2±0.5 mm for controls, HC group, and DM group, respectively; P<0.01).

Both aIMT and cIMT were significantly increased in high-risk groups compared with healthy control children, but the difference was relatively greater for aIMT, as shown in Figure 2. In control children, aIMT was similar and not statistically different from cIMT (0.44±0.05 versus 0.42±0.04 mm; P=NS), whereas in the HC and DM groups, aIMT was significantly higher than cIMT (DM group, 0.50±0.09 versus 0.47±0.04 mm, P<0.01; HC group, 0.53±0.10 versus 0.46±0.04 mm, P<0.01), thus indicating a more pronounced increase in aIMT in diabetes and hypercholesterolemia. This resulted in a significantly higher aIMT/cIMT ratio in risk
children compared with controls (1.10±0.20 versus 1.05±0.14, P<0.05).

To further compare aIMT and cIMT in detecting abnormal arterial wall thickening in high-risk children, we arbitrarily defined IMT values exceeding the 95th cut-point of control as abnormal (aIMT >0.491 mm and cIMT >0.475 mm). There were 14 high-risk children (23%) with an abnormal aIMT and normal cIMT, but only 8 high-risk children (13%) with a normal aIMT and abnormal cIMT. Nineteen high-risk children (32%) had both abnormal aIMTs and cIMTs.

To test the reproducibility of IMT measurements, the between-observer (2 observers) variations of aIMT and cIMT were calculated. For all measurements, the between-observer variations of aIMT and cIMT were similar, with mean between-observer errors of 0.027±0.035 mm (range, 0 to 0.21 mm) and 0.018±0.017 mm (range, 0 to 0.09 mm), respectively. The between-observer intraclass correlation coefficients and coefficients of variation were excellent and comparable between aIMT and cIMT (Table 2). To assess the between-subject repeatability of IMT measurements, 21 children attended the ultrasound study twice (several months apart). The mean between-subject error for aIMT was 0.032±0.029 mm (range, 0 to 0.11 mm). The between-subject repeatability of cIMT measurements was assessed by studying 22 subjects twice. The mean between-subject error for cIMT was 0.041±0.025 mm (range, 0.0 to 0.09 mm). The between-subject intraclass correlation coefficients and coefficients of variation for both aIMT and cIMT are shown in Table 2.

In healthy children, both aIMT and cIMT showed no univariate associations with measured risk factors. In the DM group, aIMT correlated with systolic (r=0.31, P=0.04) and diastolic blood pressures (r=0.29, P=0.05), and cIMT correlated with serum cholesterol (r=0.30, P=0.04), LDL cholesterol (r=0.40, P=0.008), and systolic (r=0.29, P=0.05) and diastolic blood pressures (r=0.29, P=0.05). In the HC group, cIMT correlated with age (r=0.63, P=0.009).

In multivariate regression models for aIMT, significant associations included the subject group (P=0.001), age (P=0.014), and diastolic blood pressure (P=0.075). The multivariate correlates for cIMT included subject group (P=0.001) and diastolic blood pressure (P=0.046; Table 3). The multivariate determinants for the IMT sum variable (aIMT+cIMT) were essentially similar to those of aIMT and cIMT (Table 3).

**Discussion**

This study demonstrates that IMT of the distal abdominal aorta can be reproducibly measured with ultrasound in children. Both aIMT and cIMT become higher in children with type 1 diabetes and hypercholesterolemia compared with healthy controls, but the increase in arterial wall thickness is relatively greater in the aorta than in the carotid artery. These findings are consistent with observations in autopsy studies, which have noted increased early atherosclerotic lesions in the diabetic state and in hypercholesterolemic children. The results are also consistent with the finding that atherosclerosis begins to develop first in the intima of abdominal aorta, and they show that aIMT may be used as a noninvasive ultrasound marker of preclinical atherosclerosis in children.

Fatty streaks develop in many areas of the abdominal aorta; however, those on the dorsal surface of the distal abdominal aorta develop and progress most rapidly to become raised lesions. Therefore, in the present study, this site of the far (dorsal) wall was scanned to measure aIMT to correspond to the most lesion-prone site seen in autopsy series. Fatty streaks are commonly found in the aortas of adolescents in their second decade of life, whereas the development of raised lesions mainly occurs after the age of 20 years. According to these postmortem findings, it is plausible that the increased aortic wall thicknesses seen in the high-risk children in the present study reflect increased fatty streak formation. Consistent with previous studies, blood pressure was an independent predictor of IMT in the present study. The relationship between increased IMTs and blood pressure may suggest that smooth muscle proliferation plays a role in the early diffuse thickening of the arterial wall. In the present study, however, differences in IMT were not explained by blood pressure, because both systolic and diastolic blood pressures were comparable between the study groups.

**TABLE 2. Between-Observer and Between-Subject Variation of aIMT and cIMT**

<table>
<thead>
<tr>
<th></th>
<th>Between Observer</th>
<th>Between Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ρ</td>
<td>CV, %</td>
</tr>
<tr>
<td>aIMT</td>
<td>0.86</td>
<td>4</td>
</tr>
<tr>
<td>cIMT</td>
<td>0.86</td>
<td>3</td>
</tr>
</tbody>
</table>

ρ indicates intraclass correlation coefficient; CV, coefficient of variation.

**TABLE 3. Multivariate Regression Analysis for the Determinants of aIMT, cIMT, and Their Sum**

<table>
<thead>
<tr>
<th>Explanatory Variable</th>
<th>aIMT Regression Coefficient β</th>
<th>P</th>
<th>cIMT Regression Coefficient β</th>
<th>P</th>
<th>aIMT + cIMT Regression Coefficient β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group variable</td>
<td>0.053</td>
<td>0.001</td>
<td>0.031</td>
<td>0.001</td>
<td>0.083</td>
<td>0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>0.012</td>
<td>0.014</td>
<td>0.000</td>
<td>0.975</td>
<td>0.012</td>
<td>0.042</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.002</td>
<td>0.075</td>
<td>0.001</td>
<td>0.046</td>
<td>0.004</td>
<td>0.020</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>−0.002</td>
<td>0.78</td>
<td>−0.004</td>
<td>0.234</td>
<td>−0.007</td>
<td>0.452</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>−0.004</td>
<td>0.643</td>
<td>0.008</td>
<td>0.544</td>
<td>0.004</td>
<td>0.891</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>−0.034</td>
<td>0.085</td>
<td>−0.005</td>
<td>0.655</td>
<td>−0.038</td>
<td>0.106</td>
</tr>
</tbody>
</table>
This study confirms the results of previous studies showing increased cIMTs in children with hypercholesterolemia. Several studies have demonstrated increased cIMT in adults with type 1 diabetes, and our results show that increased vascular thickness occurs in children with type 1 diabetes.

Aortic wall thickness has not been previously studied in children, and the reports on adult subjects are few. In adults, aIMT in the thoracic region has been measured using transesophageal ultrasound, which yields better image quality than transcutaneous imaging. These studies have shown increased aIMT in patients with coronary artery disease and familial hypercholesterolemia. Labropoulos et al. measured abdominal aIMT using transcutaneous ultrasound in a group of adults and observed increased aIMTs in subjects with atherosclerosis; however, with scanning frequencies of 4 to 7 MHz, aIMT resolution would be poorer compared with the higher frequency scanning used in the current study. It is likely that the high scanning frequencies (10 to 13 MHz) required for visualization of aIMT may provide inadequate tissue penetration in adults and thereby limit the accuracy of imaging the aorta in older subjects. Therefore, although these data indicate that the measurement of aIMT can be considered an accurate and reproducible means to assess early atherosclerosis in high-risk children, measurement of cIMT may still be the preferred technique in adult subjects.

Study Limitations
The present study examined the relationships between hypercholesterolemia, diabetes, and IMTs in a cross-sectional fashion. We included a relatively small number of subjects but, nevertheless, demonstrated that aIMT is significantly increased in children and adolescents with hypercholesterolemia and diabetes compared with healthy children. Although the imaging of the aIMT in light of our results is reproducible, it is technically more demanding than the visualization and measurement of cIMT. Therefore, imaging the aIMT requires an experienced sonographer and high-resolution ultrasound equipment with scanning frequencies of 10 to 13 MHz.

Clinical Implications
The primary prevention writing group III for the AHA Prevention Conference V stated that a carefully performed carotid ultrasound examination with cIMT measurement can add incremental information to traditional risk factor assessment in asymptomatic persons >45 years of age and can thereby be used as a clinical tool. Our results show that the measurement of aIMT is equally reproducible and more affected by early atherosclerosis than cIMT in childhood, when atherogenesis begins. Children with hypercholesterolemia and diabetes show increased IMTs compared with healthy controls, with relatively greater increases in the aorta than in the carotid artery. Because atherosclerosis begins to develop first in the intima of abdominal aorta, these data suggest that aIMT may be used as a noninvasive marker of preclinical atherosclerosis in children.

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References
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