Vascular Function and Carotid Intimal-Medial Thickness in Children With Insulin-Dependent Diabetes Mellitus

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METHODS

We studied 31 diabetic teenagers (age 15.0 ± 2.4 years; duration of diabetes 6.8 ± 3.9 years) and 35 age-matched healthy children (age 15.7 ± 2.7 years). Using high-resolution vascular ultrasound, we compared carotid IMT and brachial artery responses to reactive hyperemia (endothelium-dependent vasodilation) and to sublingual nitroglycerin (endothelium-independent vasodilation).

RESULTS

There was no difference in baseline brachial artery diameter between the two groups. Endothelium-dependent vasodilation was significantly lower in diabetic children compared with healthy children (4.2 ± 3.8% vs. 8.2 ± 4.2%, p < 0.001). There was no difference in endothelium-independent vasodilation (17 ± 6% vs. 18 ± 6%, p = NS) or mean carotid IMT between the groups (0.33 ± 0.05 vs. 0.32 ± 0.08 mm, p = NS). Endothelium-dependent brachial vasodilation correlated with blood glucose levels (r = 0.58, p = 0.001) and was weakly and inversely related to the duration of diabetes (r = -0.4, p = 0.02), total cholesterol, and low-density lipoprotein cholesterol levels.

CONCLUSIONS

Endothelial function is impaired in children with diabetes mellitus within the first decade of its onset and precedes an increase in carotid IMT. The relative timing of these events is important in the evaluation of strategies to prevent progression of atherosclerosis and other vascular complications in this patient population. (J Am Coll Cardiol 2003;41:661–5)

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Subjects rested in a quiet, temperature-controlled room for 15 min in the supine position and then underwent vascular ultrasound imaging of the brachial and carotid arteries, as described. After completion of imaging, a blood sample was obtained by venipuncture, and blood levels of glycosylated hemoglobin (HbA1c), lipid profile, total plasma homocysteine, blood glucose, creatinine, and von Willebrand factor (vWF) antigen, a marker of endothelial activity, were measured.

**Imaging protocol.** A single experienced vascular sonographer, who had no knowledge of the clinical or laboratory profile of the study subjects, performed all imaging studies. The images were obtained using a standard 10/5-MHz linear array transducer and an ATL HDI 3000 system (Phillips, Bothell, Washington) with the subject in the supine position. A continuous three-lead electrocardiogram (ECG) was recorded for timing diastole. A sphygmomanometer cuff was placed on the proximal right forearm. The right brachial artery images were obtained above the antecubital fossa using B-mode imaging in the longitudinal plane of the artery (10). A baseline image was acquired using a resolution box function to magnify this part of the artery. Blood flow was estimated by time-averaging the pulsed Doppler velocity signal obtained from a mid-artery sample volume. The cuff was inflated to 100 mm Hg above the systolic pressure to occlude arterial flow for 5 min. The cuff was then released, and the longitudinal image of the brachial artery was recorded continuously from just before to 1 min after cuff deflation. A mid-artery pulse Doppler signal was obtained immediately after cuff release to assess hyperemic velocity. Endothelium-dependent, flow-mediated dilation (FMD) was assessed by measurement of the brachial artery diameter 60 s after release of the cuff. Endothelium-independent vasodilation was estimated as the percent increase in brachial artery diameter 3 min after the nitroglycerin dose. We related brachial vascular reactivity (endothelium-dependent and -independent vaso-dilation) to the subjects’ glucose level, risk factors (lipid profile, homocysteine), HbA1c, and, in diabetic subjects, duration of diabetes. A bi-variate correlation matrix was created to examine the strength of the relationship between the variables. We then performed multiple stepwise linear regression analysis using SPSS statistical software to identify the variables that best predicted the relationship. All measures are expressed as the mean value ± SD. The diabetic and control groups were compared using the unpaired Student t test. A p value <0.05 was used to define statistical significance.

**RESULTS**

Table 1 demonstrates a comparison of the metabolic profile between diabetic and control children. There was no difference in the lipid levels (total, low-density lipoprotein [LDL], and high-density lipoprotein cholesterol and triglycerides) between the two groups. As expected, however, plasma glucose and HbA1c values were higher in diabetics. Total plasma homocysteine levels were lower in diabetics than in control subjects, the mean values being within

### Table 1. Comparison of Metabolic Profile

<table>
<thead>
<tr>
<th></th>
<th>Diabetics</th>
<th>Control Subjects</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>159 ± 82</td>
<td>72 ± 13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.6 ± 1.5</td>
<td>4.8 ± 0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Homocysteine (μmol/l)</td>
<td>7.0 ± 2.2</td>
<td>10.0 ± 4.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>165 ± 38</td>
<td>159 ± 31</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>55 ± 13</td>
<td>51 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>86 ± 33</td>
<td>87 ± 29</td>
<td>NS</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>129 ± 104</td>
<td>107 ± 58</td>
<td>NS</td>
</tr>
<tr>
<td>Total HDL cholesterol</td>
<td>3.2 ± 1.0</td>
<td>3.3 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>vWF antigen (%)</td>
<td>109 ± 62</td>
<td>98 ± 38</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as the mean value ± SD. HbA1c = glycosylated hemoglobin; HDL and LDL = high- and low-density lipoprotein, respectively; NS = not significant.
normal range for both groups. There was no difference in vWF antigen levels between the two groups.

The brachial artery diameter at baseline was similar in diabetics and control subjects (3.13 ± 0.44 vs. 3.20 ± 0.60 mm, p = NS). The degree of reactive hyperemia was similar in the two groups. The brachial artery diameter increased in response to reactive hyperemia (FMD) in both groups. However, the increase in diameter was significantly lower in diabetics than in control subjects (0.13 ± 0.11 vs. 0.25 ± 0.12 mm, p < 0.001) (Fig. 1). As a result, the percent increase in diameter from baseline was lower in diabetics than in control subjects (4.2 ± 3.8% vs. 8.2 ± 4.2%, p < 0.001) (Fig. 2). In contrast to endothelium-dependent FMD, endothelium-independent vasodilation, determined as the increase in brachial artery diameter with nitroglycerin, was similar in diabetics and control subjects (16.7 ± 5.6% vs. 18.2 ± 5.5%, p = NS). There was no difference in carotid IMT between the two groups (0.33 ± 0.05 vs. 0.32 ± 0.08 mm, p = NS).

When analyzed for all subjects (diabetics and control subjects) as a single population, FMD (percent change in brachial artery diameter) was weakly related to homocysteine (r = 0.26, p = 0.04) and HbA1c levels (r = −0.3, p = 0.02). There was no relationship between FMD and blood glucose levels or the lipid profile. When analyzed only for diabetic subjects, FMD correlated best with blood glucose levels (r = 0.58, p = 0.001). Flow-mediated dilation was also inversely related to the duration of diabetes (r = −0.39, p = 0.02), total cholesterol (r = −0.36, p = 0.05), and LDL cholesterol levels (r = −0.37, p = 0.05) on univariate analysis, but was unrelated to homocysteine or HbA1c levels. On multiple stepwise regression analysis, the blood glucose level was the single best predictor of FMD ($r^2 = 0.31$, p = 0.002); LDL cholesterol levels added another 12% to the explained variance. Importantly, FMD remained significantly lower in diabetics, even after adjustment for glucose levels as a co-variate.

**DISCUSSION**

The results of this study demonstrate that children with insulin-dependent DM develop endothelial dysfunction within the first decade after the onset of diabetes. Importantly, these changes are manifest before an increase in carotid IMT can be identified. These findings are important because they describe, for the first time, the relative timing of these two precursors of atherosclerosis in this cohort of children. This timing should be taken into account for designing studies that address the effect of therapeutic interventions on these preclinical events.

Endothelium-dependent vasodilation during reactive hyperemia is predominantly modulated by local release of nitric oxide (11). Impaired local availability of nitric oxide and endothelium-dependent vasodilation may result from
either short- or long-term exposure to several factors. A transient abnormality in endothelial function lasting a few hours may occur shortly after a high-fat meal, under mental stress, and within hours of induced hyperglycemia (12–14). Persistent or recurrent exposure to risk factors of atherosclerosis such as active or passive smoking, hypercholesterolemia, and diabetes results in abnormal vascular endothelial function, even without additional exposure to the aforementioned acute factors (2,15). The increase in IMT in response to these factors is likely to occur only after long-term exposure and is the likely explanation for the findings in our study. A similar relationship between the timing of endothelial dysfunction and the increase in IMT may occur in the presence of other risk factors, as well. It may be speculated that exposure to a risk factor initially leads to episodic and transient endothelial dysfunction. The length and severity of these episodes are related to the intensity of exposure to the risk factor. With recurrent or persistent exposure, there is a state of persistent endothelial dysfunction and altered vascular wall milieu that promotes structural changes of atherosclerosis.

Some arteries, such as the coronary arteries, and the abdominal aorta may be at particularly high risk of developing such changes; autopsy studies in the young have detected fatty streaks and fibrous plaques in these arteries that appear to relate to ante-mortem risk-factor exposure (16). A recent study in children in Finland demonstrated that an increase in IMT of the abdominal aorta may occur before these changes appear in the carotid artery (17). We did not examine the aortic wall in our study. However, our results are in contrast to this study, which found a significant, albeit small, increase in carotid IMT in Finnish diabetic children compared with control subjects. A closer comparison of the results between these two studies reveals an interesting observation—the carotid IMT in a teenaged Finnish control population was 30% higher than that in the U.S. teenaged control population. These differences in results in the two populations may represent the potential influence of additional genetic and environmental cardiovascular risk factors on atherosclerosis burden and thereby carotid IMT.

The measurements of IMT are accurate and reproducible (3,17). We minimized the variability in measurements by reporting for each subject a single value of IMT that was an average of 10 measurements (5 on each side) along a segment of the common carotid artery. The sample size in each group was adequate to detect (77% power) a 10% increase in IMT in diabetics ($p < 0.02$); a smaller difference was not considered to have substantive significance.

Several studies have demonstrated that a better control of diabetes in young subjects results in a lower incidence of long-term vascular complications (18). The mechanism of endothelial dysfunction in insulin-dependent DM is modulated by a decreased local nitric oxide availability, presumably due to superoxide-mediated nitric oxide destruction (19). Several short-term interventions have been studied in diabetics for their potential beneficial effect on endothelial dysfunction (20–24). Children and young adults would be ideal subjects for studying the effect of long-term interventions in preventing the onset or progression of atheroscle-
Vascular Function in Diabetic Children

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Singh et al.

Vascular Function in Diabetic Children

Identification of such a benefit in the young will require a clear understanding of the relative timing of various identifiable preclinical precursors of atherosclerosis.

Conclusions. Functional changes of endothelial dysfunction appear in children with insulin-dependent DM within the first decade of its onset and precede an increase in carotid IMT. These findings should be considered when designing intervention studies to prevent atherosclerosis and other vascular complications in these patients.

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REFERENCES