Endothelial dysfunction of conduit arteries in insulin-dependent diabetes mellitus without microalbuminuria

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Abstract

Objective: Previous studies have shown that endothelial dysfunction, an early sign of atherosclerosis, occurs in animal models of diabetes mellitus and in resistance vessels of patients with insulin-dependent diabetes. In the present study we examined whether young patients with insulin-dependent diabetes without microalbuminuria present abnormal endothelial function of large peripheral arteries.

Methods: Twenty-six patients with insulin-dependent diabetes without microalbuminuria were compared with 26 normal controls and 5 patients with insulin-dependent diabetes with microalbuminuria. Brachial artery diameter was measured at rest, during reactive hyperaemic flow (endothelium-dependent dilatation) and after sublingual isosorbide dinitrate (endothelium-independent dilatation).

Results: Baseline artery diameter and flow as well as the degree of reactive hyperaemia were similar in all groups compared to controls. Flow-mediated dilatation was lower in patients with diabetes without microalbuminuria \(5.8 \pm 7\%\) vs \(11 \pm 7\%, \ P \leq 0.01\) as well as in patients with diabetes without microalbuminuria \(0.75 \pm 2.5\%\) vs \(11 \pm 7\%, \ P = 0.003\); nitrate-induced dilatation was normal in patients without microalbuminuria and attenuated in patients with microalbuminuria. In the group of diabetes patients without microalbuminuria, those with disease duration > 10 years and HbA1c > 6% had the worse endothelial function. Conclusions: Our results demonstrate that endothelial dysfunction of conduit arteries can be detected in patients with insulin-dependent diabetes mellitus without microalbuminuria, probably contributing to the high prevalence of atherosclerosis in these patients.

Keywords: Diabetes; Endothelium; Human

1. Introduction

Cardiovascular disease is a major cause of excess mortality and morbidity in patients with insulin-dependent diabetes mellitus; atherosclerosis in these patients is usually diffused with early onset. Normal vascular endothelium contributes to the control of vessel wall homeostasis; there is accumulating evidence that impairment of endothelial function is an important feature of vascular disease not only in subjects with established atherosclerosis but also in subjects with risk factors for vascular disease before anatomic evidence of atherosclerosis [1–3]. There is now substantial evidence that endothelial dysfunction occurs in animal models of diabetes mellitus [4,5]; endothelial dysfunction also appears to be present in forearm resistance vessels of patients with insulin-dependent diabetes [6]. Some investigators included in their studies patients with microalbuminuria which appears to be a marker of widespread vascular damage [6,7].

The objective of the present study was to determine whether young patients with insulin-dependent diabetes mellitus without microalbuminuria present abnormal endothelial function of large peripheral arteries. Furthermore, we sought to determine whether a possible vascular dysfunction is confined to endothelium only, or whether it reflects an intrinsic impairment of the capacity of the smooth muscle cell to relax.
2. Methods

2.1. Patient population

Twenty-six patients with insulin-dependent diabetes mellitus participated in the study; 9 were men and 17 women with a mean age of 32 ± 8 years (range 16–46 years); duration of diabetes was 12.9 ± 8 years (range 1–24 years). No patient had evidence of atherosclerosis as judged by absence of angina, claudication and cerebrovascular ischaemia; all patients had a normal electrocardiogram. No patient had microalbuminuria (albumin excretion rate < 20 μg/min), systemic hypertension or cholesterol > 240 mg/dl; 8 patients were chronic smokers. No patient had evidence of hepatic or renal dysfunction. Mean cholesterol level was 194 ± 25 mg/dl (range 140–240 mg/dl) and mean triglycerides level was 89 ± 34 mg/dl (range 49–174 mg/dl); body mass index was 25.5 ± 3.6 kg/m² (range 20–33 kg/m²). Mean HbA1c during the last 6 months before the study (average of at least 2 measurements) was 6.5 ± 1.5% (range 5–11%); 11 patients had HbA1c < 6% and 15 patients HbA1c > 6%. Mean daily dose of insulin was 46 ± 16 IU (range 20–80 IU). Patients were taking no medications other than insulin. Results were compared with 26 normal subjects matched for sex; age was similar in the two groups (32 ± 8 vs 34 ± 9 years, n.s.); 10 normal subjects were smokers and no control subject had hypertension or cholesterol > 240 mg/dl. A third group comprised of 5 patients with insulin-dependent diabetes mellitus and microalbuminuria. Age, body mass index, blood pressure, smoking habits, daily insulin dose and HbA1c were comparable in the two diabetic groups, while disease duration tended to be longer in patients with microalbuminuria, but the difference did not reach statistical significance (20 ± 8.5 vs 12.9 ± 8.4 years, P = 0.09). Characteristics of the patients and control subjects are shown in Table 1. Informed consent was obtained from all participants in the study. The investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Measurements

Each subject was studied in the morning having abstained from alcohol, caffeine and tobacco as well as food for 8 h before the study. High-resolution echo-Doppler ultrasound (Acuson 128 XP, Mountain View, CA) with a 7.0 MHz transducer was used to measure flow velocity and diameter of the right brachial artery. In all studies scans were taken at rest, during reactive hyperaemia (an endothelium-dependent stimulus to vasodilation), again at rest and after sublingual isosorbide dinitrate (an endothelium-independent vasodilator). The subject rested quietly for 10 min before the scan; when a satisfactory position was found, the skin was marked; a resting scan was recorded and arterial flow velocity was measured using a pulsed Doppler signal at 60° angle in the centre of the artery. Blood flow through the brachial artery was altered with an occluding cuff placed on the forearm approximately 8 cm distal to the site of brachial artery measurement [1]. By inflating the cuff to 250–300 mmHg distal circulatory arrest was obtained and flow was reduced through the brachial artery measured proximal to the cuff. By deflating the cuff after 5 min of inflation, flow through the brachial artery was increased (reactive hyperaemia). The brachial artery was scanned continuously 30 s before and 90 s after cuff deflation. Ten minutes later a second rest scan was recorded. Isosorbide dinitrate (5 mg) was then administered sublingually and the artery was scanned 5 min later.

Artery diameter was measured by consensus of two observers (J.L., C.P.) unaware of the stage of experiment. Measurements were made at end-diastole (peak of R-wave of ECG); 5 cardiac cycles were analyzed and measurements were averaged; volume flow was calculated by multiplying the time-averaged velocity of the Doppler flow signal by the heart rate and the vessel cross-sectional area (\( \pi r^2 \)) [1]. Flow-mediated dilatation was calculated as the percent increase in arterial diameter during hyperaemia compared to the corresponding resting value. Nitrate-induced dilatation was also calculated similarly. Reactive

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Table 1

<table>
<thead>
<tr>
<th>Characteristics of patients with insulin-dependent diabetes and control subjects</th>
<th>Diabetics with microalbuminuria</th>
<th>Diabetics without microalbuminuria</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>5</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>33 ± 6</td>
<td>32 ± 8</td>
<td>34±19</td>
</tr>
<tr>
<td>Male/Female</td>
<td>1/4</td>
<td>9/17</td>
<td>9/17</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24 ± 2.2</td>
<td>25.5 ± 3.6</td>
<td>26 ± 5.1</td>
</tr>
<tr>
<td>Smokers (no.)</td>
<td>2</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>209 ± 22</td>
<td>194 ± 25</td>
<td>198 ± 27</td>
</tr>
<tr>
<td>Total triglycerides (mg/dl)</td>
<td>104 ± 23</td>
<td>89 ± 34</td>
<td>92 ± 32</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>122 ± 15</td>
<td>117 ± 10</td>
<td>119 ± 11</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 ± 14</td>
<td>75 ± 6</td>
<td>77 ± 6</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>20 ± 8.5</td>
<td>12.9 ± 8.4</td>
<td>–</td>
</tr>
<tr>
<td>Daily insulin dose (IU)</td>
<td>44 ± 17</td>
<td>46 ± 16</td>
<td>–</td>
</tr>
<tr>
<td>Glycosylated haemoglobin (%)</td>
<td>7.1 ± 1</td>
<td>6.5 ± 1.5</td>
<td>–</td>
</tr>
</tbody>
</table>
Table 2
Brachial artery diameter, flow and response to hyperaemia or nitrates in diabetes and normal subjects

<table>
<thead>
<tr>
<th></th>
<th>Diabetics with microalbuminuria</th>
<th>Diabetics without microalbuminuria</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessel size (mm)</td>
<td>3.3 ± 0.3</td>
<td>3.4 ± 0.5</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Flow at rest (ml/min)</td>
<td>135 ± 32</td>
<td>125 ± 38</td>
<td>132 ± 31</td>
</tr>
<tr>
<td>Hyperaemia (%)</td>
<td>492 ± 190</td>
<td>478 ± 215</td>
<td>522 ± 230</td>
</tr>
<tr>
<td>Flow-mediated dilatation (%)</td>
<td>0.75 ± 2.5 *</td>
<td>5.8 ± 7 b</td>
<td>11 ± 7</td>
</tr>
<tr>
<td>Nitrate-induced dilatation (%)</td>
<td>15 ± 2.9 c</td>
<td>19 ± 6.9</td>
<td>24 ± 9</td>
</tr>
</tbody>
</table>

* P = 0.003 vs controls. b P = 0.01 vs controls. c P = 0.03 vs controls.

hyperaemia was calculated as the maximum flow during the first 15 s after cuff deflation divided by the corresponding rest flow [1].

2.3. Statistical analysis

Descriptive data are expressed as a mean ± s.d. Student’s t-test was used to analyze the difference between the means in each group. Linear regression analysis was used to assess the relationship between selected variables. Statistical significance was taken as P < 0.05.

3. Results

Baseline brachial artery diameter was comparable in patients with microalbuminuria (3.3 ± 0.3 mm), patients without microalbuminuria (3.4 ± 0.5 mm), and control subjects (3.5 ± 0.5 mm, n.s.). Flow at rest and the degree of reactive hyperaemia were similar in patients and control subjects (Table 2).

Flow-mediated dilatation was significantly lower in patients with microalbuminuria (0.75 ± 2.5%, P = 0.003) and without microalbuminuria (5.8 ± 7%, P = 0.01) compared to normal control subjects (11 ± 7%). Nitrate-induced dilatation was normal in patients without microalbuminuria and attenuated in patients with microalbuminuria (15 ± 2.9 vs 24 ± 9%, P = 0.03). These data suggest abnormal endothelial function in diabetic patients with or without microalbuminuria and smooth muscle dysfunction in patients with microalbuminuria (Table 2). Linear regression analysis did not reveal any statistically significant correlation between the vasodilatory response to hyperaemic flow and HbA1c, disease duration, age and daily dose of insulin.

To better characterize the possible influence of metabolic control on endothelial function, we analyzed the data in the group of patients with a mean HbA1c during the last 6 months higher or lower than 6%. Patients with good metabolic control (HbA1c > 6%) tended to have a higher flow-mediated dilatation (7.5 ± 6.8 vs 4.6 ± 7.2%), but the difference did not achieve statistical significance (ns). By comparing the two groups of patients separately to the control subjects, only patients with HbA1c > 6% presented a significantly lower endothelium-dependent vasodilatation (P = 0.008) (Fig. 1). We also analyzed data on the basis of a disease duration higher or lower than 10 years. Patients with long disease duration tended to have a worse flow-mediated dilatation (5.0 ± 8.3 vs 7.9 ± 6.7%) without statistically significant differences (n.s.); when

![Fig. 1. Flow-mediated dilatation in control subjects, patients with diabetes without microalbuminuria and HbA1c < 6% or > 6%](image-url)
comparing the data of each group with the control subjects, only patients with long disease duration showed a significant difference ($P = 0.03$) (Fig. 2).

4. Discussion

In the present study we examined vascular endothelial and smooth muscle function in the large vessels of insulin-dependent diabetic patients using a simple non-invasive method which enabled accurate and reproducible assessment of the vascular responses to flow increase and nitrates [8]. In animals, flow-dependent dilatation is endothelium-dependent and mediated by nitric oxide [9,10]; the same mechanism is essential for flow-mediated dilatation of large human arteries [11]. Hence, this test can be used as an estimate of the capacity of human endothelial cells to release nitric oxide in response to a physiological stimulus as well as an estimate of endothelial dysfunction in diseased states. To distinguish endothelial from intrinsic smooth muscle dysfunction, we used isosorbide dinitrate, an endothelium-independent vasodilator; nitrates cause smooth muscle to relax, acting directly by increasing cyclic GMP levels of smooth muscle cells [12]. We preferred to use this non-invasive and accurate method to test endothelial function of conduit arteries, instead of using intrarterial infusion of acetylcholine which is an invasive method and in the forearm is usually used in conjunction with venous occlusion plethysmography to assess endothelial function of small resistance vessels.

The results of the present study in brachial artery clearly indicate the presence of endothelial dysfunction in insulin-dependent diabetes mellitus even in the absence of microalbuminuria. The most striking endothelial dysfunction was observed in patients with microalbuminuria, and in patients without microalbuminuria but with long disease duration and poor metabolic control. Zenere et al. [13] has also reported a reduced vasodilatation capacity using the same method in femoral artery; however, we should note that femoral artery diameter in adults is greater than 7.0 mm; for arteries of more than 6.0 mm in diameter flow-mediated dilatation is small and data from studies in adult femoral arteries should be interpreted with caution [8]. Previous studies in diabetic subjects have focused on endothelial function of small resistance vessels; most of these studies reported that endothelium-dependent vasodilatation is abnormal in forearm resistance vessels [6,7,14], although in two reports [15,16] a deficit in endothelium-dependent vasodilatation of resistance vessels was not observed.

The response to nitrates was normal in patients without microalbuminuria and attenuated in diabetics with microalbuminuria although not to the same degree as the impairment of endothelium-dependent vasodilatation. This response indicates an abnormality of smooth muscle cells in patients with diabetes and microalbuminuria. It is known that microalbuminuria in diabetic patients reflects widespread vascular damage [17] and our observation of an impairment of the capacity of smooth muscle cells to relax in addition to endothelial dysfunction is in accord with this previous knowledge.

Various mechanisms have been proposed to explain endothelial dysfunction in diabetes. Reduced synthesis or accelerated inactivation of nitric oxide and excessive release of vasoconstrictor substances like thromboxane A2 and endothelin [18,19] are among the proposed mechanisms. High levels of oxygen-derived free radicals or the accumulation of advanced glycosylation end-products may participate in the pathogenesis of endothelial dysfunction in these patients [20].

There is ample evidence that impairment of endothelium-dependent vasodilatation is an early phenomenon of
atherogenesis and it is present in humans before the anatomic evidence of atherosclerosis [1–3]. Abnormal endothelial function results in abnormal reactions between the vessel wall and platelets, facilitates the adhesion of monocytes to the endothelial surface and enhances the proliferation of vascular smooth muscle cells [21–23], contributing to the genesis of atherosclerosis. Apparently the observed endothelial dysfunction in our patients reflects an early atherosclerotic process of large vessels.

In conclusion, the results of this study indicate that endothelium-dependent dilatation is impaired in the systemic arteries of young adults with insulin-dependent diabetes, even in the absence of microalbuminuria. Endothelial dysfunction is striking in patients with poor metabolic control, longer duration of diabetes and microalbuminuria and may represent early large vessel disease. Strategies to reduce or retard endothelial dysfunction in these patients may lead to decreased cardiovascular morbidity and mortality.

References


