Early Endothelial Dysfunction in Young Type 1 Diabetics

R. Hurks, M.J. Eisinger, I. Goovaerts, L. van Gaal, C. Vrints, J. Weyler, J. Hendriks, P. van Schil, P. Lauwers

Department of Vascular Surgery, Antwerp University Hospital, Wilrijkstraat 10, B 2650 Edegem, Belgium
Department of Cardiology, Antwerp University Hospital, Wilrijkstraat 10, B 2650 Edegem, Belgium
Department of Diabetology, Metabolic and Nutritional disorders, Antwerp University Hospital, Wilrijkstraat 10, B 2650 Edegem, Belgium
Department of Epidemiology and Social Medicine, University of Antwerp, Universiteitsplein 1, B 2610 Antwerp, Belgium

Submitted 28 November 2008; accepted 24 January 2009
Available online 17 March 2009

Abstract

Objectives: Endothelial dysfunction is a known precursor of atherosclerosis and can be assessed by measuring the brachial artery flow-mediated dilatation (FMD) via ultrasonography. This study investigated endothelial function in young type 1 diabetics without cardiovascular morbidity or diabetes-related pathology.

Methods: Young diabetics and healthy controls were recruited, both meeting strict inclusion and exclusion criteria. To prove absence of subclinical atherosclerosis, intima-media thickness (IMT) measurements at the carotid bifurcation were done in all of them. FMD was measured at the brachial artery. The results were compared using the t-test and the influences of different variables on FMD were assessed using multiple linear regression.

Results: Twenty-six diabetics (23.4 ± 5.8 years) and 36 healthy volunteers (23.1 ± 2.8 years) were recruited. The duration of diabetes was 9.2 ± 5.3 years; metabolic control was moderate (HbA1c 7.6 ± 1.0%) and IMT was normal in both groups. FMD was significantly impaired in type 1 diabetics (7.13 ± 0.43 vs. 8.77 ± 0.43%; p = 0.002). The FMD grade was associated with diabetes and age. Patients with a good metabolic control (HbA1c < 7.0%) had a better FMD.

Conclusions: In type 1 diabetics, even without preclinical or clinical atherosclerosis, endothelial function is already disturbed and can be detected using ultrasonography.

© 2009 European Society for Vascular Surgery. Published by Elsevier Ltd. All rights reserved.
Atherosclerosis is a chronic progressive vascular degeneration, which manifests itself mainly in coronary, carotid and peripheral arteries.\(^1\) An accurate and reproducible method to detect (sub)clinical atherosclerosis is to measure the intima-media thickness (IMT) at the common carotid artery, using ultrasound.\(^2,3\) The IMT has a predictive value for development of cardiovascular morbidity and mortality.\(^4\)

Endothelial dysfunction is considered to precede atherosclerosis and is mainly characterised by impaired vasomotor function, which results in a decreased vasodilatation.\(^5\) Nitric oxide (NO) is essential for the regulation of the vascular tonus. NO is formed from L-arginin by endothelial-derived NO synthase (eNOS) in reaction to mechanical, pharmacological and biochemical stimuli.\(^6\) Diminished NO release and eNOS expression form the fundament of endothelial dysfunction and are present in atherosclerosis.\(^5,6\) A noninvasive technique to determine endothelial function involves measuring flow-mediated dilatation (FMD) via ultrasound.\(^7\) The FMD is defined as the reactive vasodilatation of the brachial artery after hyperaemia and is considered to be a physiological reaction of the artery. Decreased FMD has been demonstrated in patients with risk factors for atherosclerosis, such as diabetic children and adolescents.\(^8\)–\(^10\) Endothelial dysfunction, defined as a decreased FMD, has a predictive value for future cardiovascular disease.\(^11\) Diabetes is an important risk factor for atherosclerosis, and both the incidence and mortality of cardiovascular disease are increased in diabetic patients.\(^12\)

Preclinical evaluation of atherosclerosis and early intervention are essential for reducing cardiovascular morbidity and mortality. Presence of endothelial dysfunction before development of preclinical atherosclerosis (defined as an increased IMT) has not been demonstrated before. This is relevant for future therapeutic intervention strategies.

In the present study, we investigated endothelial function in young adults with type 1 diabetes mellitus (T1DM) without preclinical or clinical atherosclerosis. T1DM had to be the only risk factor for atherosclerosis, and patients with diabetes-related co-morbidities were excluded.

Materials and Methods

Type 1 diabetics, aged 16–36 years, regularly attending the diabetes outpatient clinic of the Antwerp University Hospital were consecutively recruited. To minimise influences other than T1DM on endothelial function, strict exclusion criteria were met (Tables 1a and b). The FMD tests and IMT measurements were performed, together with the annual screening for diabetes-related co-morbidities. A control group of non-diabetics was recruited among medical students; they met exclusion criteria as well (Table 1a).

The conducted tests were standardised. All measurements were performed by the same technician with an extensive experience (>2300 measurements) in a neutral environment with a constant temperature to minimise the influence of stress. A strict preparation was conducted by each test subject to avoid factors that might influence vascular tone: no caffeine or alcohol use, no intensive physical activity 24 h before the testing and no food intake 6 h prior to the testing.\(^9,13,14\) All tests were preceded by measuring the blood pressure. Coefficients of variance (measured at six consecutive days at the same time) were 1% and 7% for the basal diameter and FMD, respectively.

Carotid IMT was evaluated in the supine position, with the head turned 45° away from the side being scanned. The reference point for measurement was the beginning of the dilatation of the carotid bulb. The two-dimensional B-mode image of the posterior wall of the right common carotid artery was gained 1–2 cm proximal to the carotid bifurcation. The radio-frequency signals originating from an M-line perpendicular to the longitudinal and transversal axes of the artery, R-wave triggered, were analysed thrice using three different interrogation angles: 0° from midline, 30–60° from midline (anterior oblique) and 90–100° from midline (lateral). The mean IMT of the three measurements was calculated. This method has been validated and is reproducible.\(^15\)

The FMD was evaluated by ultrasonography of the right brachial artery at rest (baseline arterial diameter) and during reactive hyperaemia after inflating and deflating a forearm blood pressure cuff (200 mmHg or at least 50 mmHg above peak systolic blood pressure for 4 min). Continuous electrocardiogram (ECG) registration was used to measure the correct end-diastolic diameter, coincident with the R-wave. Post-occlusion measurements were taken every 30 s over the following 4 min. To calculate the maximal vasodilatation compared with baseline (peak FMD percentage), the mean of the three measurements at baseline and the maximal post-occlusion value were used.

Ultrasound studies were performed using the AU5 Ultrasound system (Esaote, Biomedica, Genova, Italy), equipped with a 10-MHz linear-array transducer. Data analysis was performed using the Wall-Tracker System (WTS, P-Medical, Maastricht, the Netherlands).

<table>
<thead>
<tr>
<th>Table 1a</th>
<th>Exclusion criteria for all tested individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obesity (Body mass index &gt; 25 kg m(^{-2}))</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
</tr>
<tr>
<td>Arterial hypertension</td>
<td></td>
</tr>
<tr>
<td>Age &lt;18 years or &gt;35 years</td>
<td></td>
</tr>
<tr>
<td>Any underlying disease, other than diabetes</td>
<td></td>
</tr>
<tr>
<td>Use of any medication</td>
<td></td>
</tr>
<tr>
<td>Proven clinical atherosclerosis</td>
<td></td>
</tr>
<tr>
<td>Family history positive for cardiovascular disease</td>
<td></td>
</tr>
</tbody>
</table>

HDL and LDL are high- and low-density lipoproteins, respectively.

Table 1b | Additional exclusion criteria for diabetics |
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL - cholesterol &gt; 4 mmol l(^{-1})</td>
<td></td>
</tr>
<tr>
<td>HDL - cholesterol &lt; 0.9 mmol l(^{-1}) (male), &lt;1.1 mmol l(^{-1}) (female)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides &gt; 2.8 mmol l(^{-1})</td>
<td></td>
</tr>
<tr>
<td>HbA1c &gt; 10%</td>
<td></td>
</tr>
<tr>
<td>Micro-albuminuria &gt; 20 µg min(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Neuropathy</td>
<td></td>
</tr>
<tr>
<td>Retinopathy</td>
<td></td>
</tr>
<tr>
<td>Use of any medication, other than insulin</td>
<td></td>
</tr>
</tbody>
</table>

HbA1c is glycylated haemoglobin.
The population characteristics were described by their mean ± standard deviation (SD). The outcomes, IMT and FMD, are expressed as mean ± standard error (SE). The differences between groups were tested using a Student’s t-test; for non-Gaussian data, the Mann–Whitney U-test was used. Multiple linear regression was applied to identify the relationship among the various parameters. An interaction variable (T1DM*gender) was entered to analyse the interaction between those two variables. \( R^2 \) is used to identify the proportion of the variability of the dependent variable explained by the regression model. Values of the coefficient B and the p-values are provided. Two-tailed p-values ≤ 0.05 are considered statistically significant.

Data are analysed using the statistical package SPSS Version 15.0 (SPSS Inc, Chicago, IL, USA). For this cross-sectional study, we calculated that ≥23 patients per group would be required.

Results

With regards to clinical characteristics (Table 2), the healthy control (CO) and diabetic groups (T1DM) did not differ significantly in age, gender distribution and blood pressure at the moment of testing. Body mass index (BMI) was higher in T1DM but was within the normal range (22.1 ± 2.0 vs. 21.1 ± 1.7 kg m\(^{-2}\), \( p = 0.020 \)).

Carotid IMT (0.482 ± 0.011 in T1DM vs. 0.499 ± 0.011 mm in CO, \( p = 0.275 \)) was not significantly different in diabetics and was not affected by gender. The influence of different variables on the dependent variable IMT was analysed using multiple linear regression, but no significant model could be fitted.

Endothelial function as assessed by the brachial artery FMD was significantly impaired in T1DM compared to CO (7.13 ± 0.43 vs. 8.77 ± 0.43; \( p = 0.002 \) power = 85.2%). Split for gender, FMD remained significantly lower in male and female T1DM patients compared to CO (6.72 ± 0.76 vs. 8.95 ± 0.49% for male patients (\( p = 0.024 \)) and 7.48 ± 0.46 vs. 8.66 ± 0.50% for female patients (\( p = 0.036 \)), respectively. No statistically significant differences were found for gender within T1DM or CO. Multiple linear regression in the entire study group for the dependent variable FMD (\( R^2 = 0.355 \); \( p < 0.001 \)) resulted in a model with T1DM (\( B = 2.393; \ p < 0.001 \)), age (\( B = 0.197; \ p = 0.004 \)) and BMI (\( B = 0.256; \ p = 0.096 \)). An interaction term for gender and T1DM was entered in the model to further analyse the influence of both the variables on each other. This turned out to be negative and was therefore excluded from the final model.

T1DM split for good (HbA1c ≤ 7.0%) and moderate/bad (HbA1c > 7.0%) metabolic controls resulted in no differences in gender, age, duration of diabetes, BMI, IMT, blood pressure, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and triglycerides.

The FMD was significant better in the group with good metabolic control compared to poor metabolic control (8.71 ± 0.84 vs. 6.55 ± 0.44%, \( p = 0.047 \); power = 62.6%). Multiple linear regression (stepwise backward) in T1DM for the dependent variable HbA1c (\( R^2 = 0.500 \); \( p = 0.042 \)) resulted in a model with significant associations with age (\( B = -0.071; \ p = 0.042 \)), HDL (\( B = -0.245; \ p = 0.006 \)) and total cholesterol (\( B = 0.095; \ p = 0.006 \)).

Discussion

In this study, we describe endothelial dysfunction in young type 1 diabetes patients with good-to-moderate metabolic control (HbA1c ≤ 7.0%, without (pre)clinical atherosclerosis and without other risk factors hereon. We compare these patients with healthy controls matched by our strict inclusion criteria as mentioned in Tables 1a and b.

Although BMI differs between both the groups, both values are considerably under the set cut-off point of 25 kg m\(^{-2}\), and BMI did not turn out to be significant in the multiple linear regression analysis. We therefore conclude that this difference will not influence the outcome. To investigate the effect of gender on FMD, both the independent variable FMD and gender itself were entered as an interaction term (gender * T1DM) in the model. This showed no direct or indirect effect of gender on FMD.

The IMT measurements are accurate and reproducible, and we minimised variability by performing measurements in three angles 12 times in total. The IMT was not significantly different between T1DM and CO. None of the individuals had elevated IMT values, thus allowing us to conclude that the endothelial function can be properly assessed in this study without impacting the effects of atherosclerosis.

Previous studies of endothelial function to compare T1DM and CO showed significant differences in IMT in both high (42 years) and low (11 years) age groups.
be noted that inclusion criteria were less strict (smokers were not excluded) and the metabolic control was worse (HbA1c = 8.9 ± 1.4%) compared to our study, respectively. We expect our comparable IMT measurements to result from our strict inclusion criteria.

Age is a known risk factor for atherosclerosis; increasing IMT with age has been documented in several studies. In the present study, no association was found between age and IMT or FMD and IMT, probably because of the narrow age distribution and the cross-sectional study design. A study in patients with atherosclerosis (aged 61 ± 2 years) did show an association between IMT and FMD. Another group found an association between IMT and duration of disease, which could not be confirmed by either us or others. This might be due to a selection bias owing to their specific criteria, mainly including patients with prolonged duration of diabetes and without clinical atherosclerosis.

In our study, type 1 diabetics have a significantly lower FMD (p = 0.002; power = 85.2%). Split for gender, the difference remains lower for T1DM between males (p = 0.024) and females (p = 0.036). No differences in FMD were found when males were compared with females. Remarkable results are described in literature. In a study with older T1DM and CO (41.5 ± 13.2 and 42.2 ± 13.4 years, respectively), increased disease duration (21 ± 10 years), worse metabolic control (HbA1c = 8.0 ± 1.1%) and specific risk factors, but without clinical atherosclerosis, no difference in FMD was found.

This study was limited by the relatively small groups (17 vs. 17) and the lack of power calculations. Another group did not find a difference in FMD in a younger age group (13.5 ± 3.5 years), a short duration of disease (<5 years) and a bad metabolic control (HbA1c = 9.4 ± 2.1%). Other researchers found a significantly lower FMD in T1DM compared to CO. These studies differ from the present one by significantly different IMT between both the groups and elevated IMT values in two of the studies. In the present study, we found associations between FMD and both T1DM (p = 0.001) and age (p = 0.006) in all the studied subjects. The positive weak coefficient for age (β = 0.192) was unexpected, but our population was characterised by a narrow age distribution (23.2 ± 4.3 years) and the study design was cross-sectional; therefore, these findings cannot be extrapolated to other age categories. To further explore the reasons for the positive association for age in T1DM, we found a negative association between age and HbA1c (r = −0.071; p = 0.042), so older patients had a better metabolic control in our study.

The mean HbA1c (7.6 ± 1.0%) represents moderate metabolic control, but is considerably better compared to previously mentioned studies. The endothelial function (FMD) turned out to be better in patients with good metabolic control compared to moderate/bad control (p = 0.047); no other differences between both the groups were found, suggesting a positive influence of good metabolic control on endothelial function. FMD was not associated with HbA1c.

Brazilian researchers found that FMD and HbA1c were positively associated in a young group of patients (13.4 ± 3.3 years) with a short duration of diabetes (2.9 ± 1.2 years) and a bad metabolic control (HbA1c = 9.35 ± 2.1%). They hypothesised, based upon animal experiments, that chronic hyperglycaemia would modify eNOS as an early adaptive mechanism of the endothelium. Their results are not being confirmed by us or others. The same group found no association between FMD and duration of disease. Another study with a relatively small number of patients (n = 17) did not find this association in older patients (41.5 ± 13.2 years) with prolonged disease duration (21.5 ± 10.2 years) or in patients with atherosclerotic risk factors. A recent study found a negative association between FMD and disease duration, FMD being already impaired in the first 10 years of disease. Their study population was older (46.6 ± 9.7 years), had a prolonged duration of diabetes (16.2 ± 9.3 years), increased IMT (0.644 ± 0.033 mm) and atherosclerotic risk factors. Remarkably, these authors deny any influence of increasing BMI, percentage smokers and HbA1c on FMD with increasing duration of disease. Comparable to these results, others report decreased FMD during the first 10 years of disease in a population with increased IMT. In a study on young diabetics (15.0 ± 2.4 years) without IMT alterations, a decreasing FMD with disease duration was found. We have no explanation for the lack of this association in our patient group, but to draw conclusions from associations like this, a longitudinal study design is needed.

Lipids were associated with HbA1c, and not with IMT or FMD; since the obtained values were within the normal range, their influence is considered not substantial.

In conclusion, the results of this study demonstrate endothelial dysfunction in type 1 diabetics without atherosclerotic risk factors, without clinical or preclinical atherosclerosis (increased IMT). The present study differs from previously conducted studies by lack of a difference in IMT in patients vs. controls.

Endothelial function is better in T1DM with a good metabolic control (HbA1c ≤ 7.0%) compared to moderate/bad metabolic control (HbA1c > 7%), suggesting a positive influence on endothelial function of well-regulated diabetics.

A longitudinal study is needed to analyse the evolution of atherosclerotic risk factors, ageing and increasing duration of diabetes and their influence on endothelial function within both the T1DM and CO groups to further explore this entity and to seek for novel therapeutic targets.

Conflict of Interest

None.

Acknowledgements

We thank Marjolein Sikkema and Vera Eenkhoorn for their support in organising the current study. We also thank Christophe de Block and Dominique Ballaux for supporting the recruitment of our patients.

References

Early Endothelial Dysfunction in Young Diabetics


15 Barth JD. Which tools are in your cardiac workshop? Carotid ultrasound, endothelial function, and magnetic resonance imaging. Am J Cardiol 2001 Feb 13;87(4A):8A–14A.


