Long-term hyperglycaemia impairs vascular smooth muscle cell function in women with type 1 diabetes mellitus

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Abstract

Observations of increased stiffness in the elastic aorta in women with diabetes, but not men, emphasise the need for further analysis regarding early abnormalities in arterial wall properties of women with type 1 diabetes mellitus (DM).

Ultrasound was used to study the wall properties of the distal brachial artery (BA) in 37 type 1 diabetic women (aged 22–45 years) without evident complications and in 53 controls (C). Blood samples were drawn for later analysis.

Flow-mediated dilatation (FMD) was slightly lower in DM than C, 8.1±4.3% vs. 10.3±4.9% (p<0.05), and nitrate-mediated dilatation (NMD) was markedly lower, 21.7±6.6% vs. 31.4±5.7% (p<0.001). Lumen diameter, intima-media thickness and distensibility were similar in DM and C. Insulin-like growth factor (IGF-1) was lower in DM than C, 231±65 vs. 349±68 ng/ml (p<0.001). Glycosylated haemoglobin (HbA1C) and matrix metalloproteinase (MMP-9) were independent predictors of the reduced NMD in the DM.

Brachial artery responsiveness to an exogenous donor of nitric oxide (NO) was markedly reduced in type 1 diabetic women despite only limited reduction in endothelium-dependent dilatation. The negative association between NMD and HbA1C suggests that long-term hyperglycaemia impairs vascular smooth muscle cell function in DM.


Key words: brachial artery, hyperglycaemia, type 1 diabetes, ultrasound, vasodilatation, women.

Introduction

An increased cardiovascular risk has been shown in type 1 diabetes (DM) patients, especially in women. The reason why women have a greater relative risk is not clear at present, but arterial stiffness, an independent predictor of cardiovascular morbidity and mortality, could be of importance since it has been reported to be raised in central arteries of female diabetic patients. Poor glycaemic control increases the risk of diabetic vascular complications, possibly through greater oxidative stress, generating an inflammatory response and the formation of advanced glycation end-products, which affect both arterial stiffness and endothelial function negatively. Matrix metalloproteinases (MMPs) are enzymes that degrade components of the extracellular matrix, an important determinant of properties of the arterial wall. Serum MMP-9 levels correlate with arterial stiffness, and higher levels of circulating MMP-9 have been reported in patients with hypertension and DM. Moreover, recent data suggest that insulin-like growth factor 1 (IGF-I) is of importance for vascular function and associated with atherosclerotic events. Low levels of circulating IGF-I have been found in type 1 diabetes.

The aims of this study were: 1) to evaluate the properties of the brachial artery (BA) wall in women with type 1 diabetes and to compare them with healthy control subjects; 2) to test the association between these physiological variables and biomarkers which are of importance in diabetes and arterial disease.

Methods

Subjects

Thirty-seven women with type 1 diabetes mellitus (DM) who fulfilled the following criteria were studied: 1) diabetes duration of at least eight years; 2) age 21–45 years; 3) no history of cardiovascular disease or hypertension; 4) normoalbuminuria (albumin excretion ratio < 20 µg min⁻¹); 5) normal retinal photographs or only slight background retinopathy; and 6) no symptoms or signs of peripheral neuropathy. Of these women, 24 individuals had background retinopathy. The control group (C) consisted of 53 healthy female volunteers (mean age 34±7 years, range 22–45). All C had glycosylated haemoglobin (HbA1C) < 5.0% and fasting plasma

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Wall tracking of brachial artery diameter change (strain)

Measurements were performed using an ultrasound scanner (Esaote AU5, Esaote Biomedica, Florence, Italy) equipped with a 7.5 MHz linear transducer. The scanner is connected to a personal computer which has a Wall Track System (WTS2, Pie Medical, Maastricht, The Netherlands) installed. Details of the study technique have been described previously. In short, ECG leads are connected to the subject. After visualisation of the artery in a longitudinal section, the scanner is switched to M-mode and the line is positioned perpendicular to the anterior and posterior wall. A window of sufficient width to include the envelope from both anterior and posterior wall is chosen, and the radiofrequency (RF) signal is transferred to the PC, where it is stored. A sample volume is positioned automatically on both the anterior and posterior wall. Manual adjustment of the sample volume is possible before arterial distension waveforms are finally calculated and data on average diastolic vessel diameter, absolute and relative diameter change are given.

Sampling of distension waveforms for four seconds was repeated three times, and the mean was used for statistical analysis. Non-invasive upper arm blood pressure (BP) and heart rate (HR) were recorded using the oscillometric technique (Dinamap PRO 200 Monitor, Critikon, Tampa, FL, US).

B-mode ultrasound imaging

A digital ultrasound system (HDi 5000, Philips Medical Systems, ATL Ultrasound, Bothell, WA, US) equipped with a broadband linear transducer (L12-5) was used for scanning the brachial artery in longitudinal section. ECG leads were connected. For determination of lumen diameter (LD) and intima-media thickness (IMT), three consecutive frozen images with special focus on lumen-intima echo and media-adventitia echo of the far arterial wall were saved for later analysis.

In order to measure flow-mediated dilatation (FMD), the transducer was held in position with a stereotactic clamp without compressing soft tissue. Baseline B-mode images and flow velocity were recorded, followed by five minutes’ 200 mmHg inflation of the cuff placed on the proximal left forearm. In addition, the subject squeezed a rubber ball in the left hand 20 times between the third and fourth minute of ischaemia in order to augment further post-occlusive hyperaemia. After cuff deflation, velocity data were recorded at 10 and 120 seconds and frozen B-mode images in end-diastole at 45, 75, 105, 150 and 240 seconds. Following 10 minutes’ rest, B-mode images were recorded before and five minutes after sublingual administration of 0.4 mg glyceryl trinitrate (GTN).

The digital B-mode images were transferred later to a personal computer with installed software for offline measurement of LD and IMT (Artery Measurement System II, Image and Data Analysis, Gothenburg, Sweden). Calibration and subsequent measurement was performed by manually tracing a cursor along the leading edge of the intima-lumen echo of the near wall, the leading edge of the lumen-intima echo and the media-adventitia echo of the far wall. A 5 mm section of the distal brachial artery was selected to obtain mean LD and far wall IMT. During analysis, the measurement window was hidden from the reader and values were saved in a text file.

**Table 1. Clinical data and demographics in the study subjects**

<table>
<thead>
<tr>
<th>Variable</th>
<th>C (n=53)</th>
<th>DM (n=37)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>34 (7)</td>
<td>33 (8)</td>
<td>NS</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.68 (0.06)</td>
<td>1.67 (0.07)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (m/kg)</td>
<td>24.1 (3.9)</td>
<td>24.8 (3.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Former smokers (n)</td>
<td>11</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.7 (0.8)</td>
<td>4.5 (0.8)*</td>
<td>NS</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.8 (0.8)</td>
<td>2.5 (0.6)*</td>
<td>NS</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.6 (0.4)</td>
<td>1.6 (0.2)*</td>
<td>NS</td>
</tr>
<tr>
<td>Apo A1 (g/L)</td>
<td>1.6 (0.4)</td>
<td>1.6 (0.5)*</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>0.8 (0.2)</td>
<td>0.7 (0.2)*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>0.9 (0.5)</td>
<td>1.0 (0.6)*</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>66 (9)</td>
<td>71 (9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hb (g/L)</td>
<td>135 (9)</td>
<td>133 (7)</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1C (%)</td>
<td>4.0 (0.3)</td>
<td>7.1 (1.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>18 (8-39)*</td>
<td>18 (8-39)*</td>
<td></td>
</tr>
<tr>
<td>Insulin dose (units-1 kg-1day)</td>
<td>0.62 (0.15)</td>
<td>0.62 (0.15)</td>
<td></td>
</tr>
</tbody>
</table>

Data are means (SD) unless otherwise stated. *Three DM are excluded from (lipid-lowering therapy). †Median(range)

Key: LD = low-density lipoprotein; HDL = high-density lipoprotein; HDL = low-density lipoprotein; C = controls; DM = diabetes mellitus; BMI = body mass index; Hb = haemoglobin; HbA1C = glycosylated haemoglobin.

end-diastole at 45, 75, 105, 150 and 240 seconds. Following 10 minutes' rest, B-mode images were recorded before and five minutes after sublingual administration of 0.4 mg glyceryl trinitrate (GTN).

The digital B-mode images were transferred later to a personal computer with installed software for offline measurement of LD and IMT (Artery Measurement System II, Image and Data Analysis, Gothenburg, Sweden). Calibration and subsequent measurement was performed by manually tracing a cursor along the leading edge of the intima-lumen echo of the near wall, the leading edge of the lumen-intima echo and the media-adventitia echo of the far wall. A 5 mm section of the distal brachial artery was selected to obtain mean LD and far wall IMT. During analysis, the measurement window was hidden from the reader and values were saved in a text file.

**Study protocol**

All subjects refrained from drinking beverages containing caffeine or alcohol for 12 hours before the examination. First, with the subject in a fasting state, venous blood was drawn from the antecubital vein. After a light breakfast, the subject entered the examination room (temperature 22–24°C), and rested in supine position for 10 minutes before systolic blood pressure (SBP) in the upper arm and ankle was registered bilaterally, using cuff and pen-Doppler. Capillary plasma glucose concentration was checked, and values in the range 4–12 mmol/L were considered acceptable to continue with the two-hour examination. Using
ultrasound, the distal part of the left brachial artery (BA) was scanned in a longitudinal section 0–5 cm proximal to the antecubital crease to assess: 1) arterial diameter change; 2) end-diastolic IMT and LD; 3) FMD; and 4) nitrate-mediated dilatation (NMD). Ipsilateral upper arm BP and HR were recorded before and after the set of distension waveforms, and after FMD and NMD data collection. Finally, plasma glucose concentration was measured again in DM patients.

Calculations and data analysis
The distensibility coefficient (DC) is the relative increase of arterial cross-section area for a given increase in pressure.\(^{16}\)

\[
DC = \frac{2\Delta d\Delta \theta + \Delta \theta^2}{\Delta P \theta^2}
\]

The unit for DC is 10\(^{-3}\)/kPa, where \(\Delta P\) is pulse pressure in kPa, \(\theta\) is the minimum diastolic diameter in mm, \(\Delta \theta\) is pulsatile diameter change and \(\Delta \theta^2\) is the square of the pulsatile diameter change in mm.

In addition, the compliance coefficient (CC) was calculated. The CC is the absolute increase in cross-section area for a given increase in arterial pressure, with the assumption that the length of the vessel is unaffected by the pulse wave. Consequently, the measured change in cross-section area is supposed to correspond to the volume change per unit of length (buffering capacity).

\[
CC = \frac{\pi(2\delta\Delta \theta + \Delta \theta^2)}{4\delta P}
\]

The unit for CC is mm\(^2\)/kPa.

The relative change in end-diastolic diameter of the BA in response to increased blood flow (FMD) or administration of 0.4 mg glyceryl trinitrate (NMD) was defined as:

\[
\text{FMD} \% = 100 \left( \frac{\delta_{45s} + \delta_{75s}}{\delta_{baseline}} \right)^2 - 1
\]

where \(\delta_{45s}\) and \(\delta_{75s}\) is BA diameter (mm) 45 and 75 seconds after release of forearm arterial occlusion pressure.

\[
\text{NMD} \% = 100 \left( \frac{\delta_{5min}}{\delta_{baseline}} \right)^2 - 1
\]

where \(\delta_{5 \text{ min}}\) is BA diameter (mm) measured five minutes after the exogenous NO donor is given.

Post-velocity was defined as the sum of peak systolic and end-diastolic velocity (\(\text{VELOCITY}\)) in the BA during hyperaemia.

Velocity response (%) was defined as post-velocity divided by peak systolic velocity at baseline \(\times 100\).

Laboratory measurements
Standard analysis included serum and plasma levels of cholesterol, triglycerides, haemoglobin (Hb), creatinine, glucose and HbA\(_1C\). Additionally, serum and plasma were separated and stored frozen at -70°C. A turbidimetric method was used for analysis of C-reactive protein (CRP) with antibodies (Bayer, Solna, Sweden). Serum MMP-9, MMP-3 and tissue inhibitor of metalloproteinase 1 (TIMP-1) were determined using commercially available ELISA (Quantikine, R & D Systems, Abingdon, UK). The intra-assay coefficient of variation was < 8%. C-peptide was measured with ELISA from DakoCytomation (DakoCytomation Ltd., Ely, Cambridgeshire, UK), based on two monoclonal antibodies against C-peptide. Plasma free total insulin was measured by Mercodia Iso-Insulin ELISA (Mercodia AB, Uppsala, Sweden) using a two-site enzyme immunoassay containing two monoclonal antibodies cross-reacting equally with human insulin and insulin aspart.

Total serum IGF-1 was measured by ELISA after acid-ethanol-extraction from its binding protein with a commercial kit from Diagnostic System Laboratories (Webster, TX, US). Plasma insulin-like growth factor binding protein 1 (IGFBP-1) was determined by a two-step ELISA using a kit from Diagnostic System Laboratories (Webster, TX, US). HbA\(_1C\) was determined with the Mono S standardisation (a high resolution ion-exchange method with reference range 3.2–5.0%).

Statistical analysis
Version 15 of the SPSS statistical package was used. The difference in continuous variables between controls and diabetics was tested using unpaired student’s t-testing or analysis of covariance (ANCOVA). Correlation or multiple stepwise regressions were used to evaluate the association between continuous data, whereas chi-squared testing was used for the evaluation of categorical data. For unevenly distributed variables, the data were logarithmically transformed (ln) before analysis. Values were presented as mean ± SD except as noted. P values < 0.05 were considered significant.

Results
Figure 1 shows the distribution of FMD and NMD in DM and C. FMD was slightly lower in DM than C, 8.1±4.3% vs. 10.3±4.9% (p<0.05), and NMD was markedly reduced, 21.7±6.6% vs. 31.4±5.7% (p<0.001) in DM compared to C. FMD was still significantly lower in DM after adjustment for BA velocity response, but not after adjustment for absolute flow velocity during peak hyperaemia.

Table 2 shows heart rate, blood pressure and brachial
artery data in the studied women. Resting heart rate, systolic and diastolic blood pressure were higher in DM than C (p<0.05). The size and mechanical properties of the BA, assessed as DC, CC, IMT and LD, were similar in both groups.

CRP levels were higher in DM than C, 2.30±2.7 (median 1.10) vs. 1.45±2.3 mg/ml (median 0.35), respectively (p<0.05).

Figure 2 shows serum TIMP-1 concentrations in DM and C. DM had higher TIMP-1 (151±25 vs. 140±21 ng/ml, p<0.05). MMP-3 and MM-9 were similar in DM and C, 10.8±6.1 vs. 9.0±2.8 ng/ml (NS) and 225±95 vs. 213±117 ng/ml (NS).

Levels of IGF-1 were lower in DM than C (231±65 vs. 319±68 ng/ml, p<0.001), whereas IGFBP-1 levels were higher (66±34 and 26±12 ng/ml, p<0.001).

C-peptide was 641±215 pmol/L in the C group, and in the DM group only three subjects had detectable C-peptide. Iso-insulin was below the detectable limit in three DM and eight C subjects, median value 38 vs. 30 pmol/L (NS) in DM and C, respectively.

Table 3 shows selected parameters and their associations with FMD, NMD and HR. HbA1C was positively associated with HR in both groups, r=0.34 (p<0.05). In controls only, CRP was associated with BMI (r=0.36, p<0.01), MMP-9 (r=0.42, p<0.01) and TIMP-1 (r=0.37, p<0.01). FMD was lower in DM than C, 151±25 vs. 140±21 ng/ml (NS). HR was higher in DM than C, 67±8 vs. 63±8 (p<0.05).}

### Table 2. Heart rate, blood pressure and brachial artery data in the study subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>C (n=53)</th>
<th>DM (n=37)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>63 (8)</td>
<td>67 (8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>106 (11)</td>
<td>111 (11)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>65 (8)</td>
<td>69 (8)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>79 (9)</td>
<td>83 (9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>41 (7)</td>
<td>42 (5)</td>
<td>NS</td>
</tr>
<tr>
<td>Lumen diameter (mm)</td>
<td>2.96 (0.32)</td>
<td>2.96 (0.33)</td>
<td>NS</td>
</tr>
<tr>
<td>Intima-media thickness (mm)</td>
<td>0.27 (0.02)</td>
<td>0.28 (0.03)</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline velocity (m/s)</td>
<td>0.70 (0.15)</td>
<td>0.56 (0.10)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Post-velocity (m/s)</td>
<td>1.98 (0.36)</td>
<td>1.71 (0.33)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Velocity response (%)</td>
<td>291 (53)</td>
<td>311 (65)</td>
<td>NS</td>
</tr>
<tr>
<td>Distensibility coefficient (10⁻³/kPa)</td>
<td>8.2 (4.4)</td>
<td>7.2 (4.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Compliance coefficient (mm²/kPa)</td>
<td>0.57 (0.30)</td>
<td>0.58 (0.26)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Key:** bpm = beats per minute; C = controls; DM = diabetes mellitus; BP = blood pressure

### Table 3. Univariate correlation coefficient (r) between brachial artery vasodilatation response, HR and selected parameters presented in C (n=53), DM (n=37) and all subjects (n=90)

<table>
<thead>
<tr>
<th>Variable</th>
<th>FMD</th>
<th>NMD</th>
<th>HR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>DM</td>
<td>All</td>
</tr>
<tr>
<td>FMD</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LD</td>
<td>-0.38**</td>
<td>NS</td>
<td>0.34**</td>
</tr>
<tr>
<td>Post-velocity</td>
<td>0.36**</td>
<td>0.37**</td>
<td>0.41***</td>
</tr>
<tr>
<td>InDC</td>
<td>-0.27*</td>
<td>-0.36*</td>
<td>-0.27*</td>
</tr>
<tr>
<td>MAP</td>
<td>0.29*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>InHbA1C</td>
<td>NS</td>
<td>-0.35**</td>
<td>-0.28*</td>
</tr>
<tr>
<td>InCRP</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>InMMP-9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Key:** HR = heart rate; C = controls; DM = diabetes mellitus; FMD = flow-mediated dilatation; NMD = nitrate-mediated dilatation; LD = lumen diameter; In = log-transformed data; DC = distensibility coefficient; MAP = mean arterial pressure; HbA1C = glycosylated haemoglobin; CRP = C-reactive protein; MMP = matrix metalloproteinase; *p<0.05, **p<0.01, ***p<0.001
negatively related to DC, r = -0.36 (p<0.05), r = -0.27 (p<0.05) in DM and C, respectively.

Figure 3 shows the association between NMD and HbA1C. Since there was a similar negative association between HbA1C and NMD both in DM (r = -0.44, p < 0.01) and C (r = -0.30, p < 0.05), both groups were combined in a single graph (r = -0.69, p < 0.001). Using a multiple stepwise regression model, HbA1C (R^2 48%, p < 0.001) and LD (R^2 9%, p < 0.001) were found to be negative independent predictors of NMD, in distinction to mean arterial pressure (MAP), MMP-9 and FMD. When the DM patients' data were analysed separately, HbA1C (R^2 17%, p < 0.01), LD (R^2 14%, p < 0.01) and MMP (R^2 8%, p < 0.05) were found to be negative independent predictors of NMD, whereas MAP and FMD were excluded from the model.

Discussion
In addition to microvascular complications, accelerated loss of central artery distensibility, which is an independent predictor of cardiovascular morbidity and mortality, has been reported in diabetes patients in general and interestingly, in diabetes patients of female, but not male, gender. Peripheral muscular arteries have been less well studied: results are variable, showing either unchanged brachial artery or increased radial artery stiffness in type 1 diabetes patients of mixed gender or unchanged femoral artery stiffness in both males and females. The behaviour of the brachial artery wall is of specific interest since it is often used as a model artery in the study of the endothelium, where dysfunction is suggested to precede atherogenesis. In contrast to earlier findings of increased stiffness in central arteries, the women with DM in our study had unchanged distensibility in the brachial artery (BA). This may be due to the fact that we studied young subjects (mean age 34 years) without vascular complications (table 1 and 2). Furthermore, the distensibility of the distal BA is little affected by ageing, in contrast to central arteries; this may make the BA less sensitive to accelerated vascular ageing, which is proposed to occur in DM. Thus, the mechanical properties of muscular arteries seem to be well preserved in DM.

Flow-mediated dilatation was reduced in DM, implying impaired endothelial function (figure 1). The FMD impairment was, however, rather subtle, which might be due to the absence of microalbuminuria in the DM cohort since impairment of FMD has often been more convincingly shown in patients with microalbuminuria, even in those without diabetes. Experimental studies indicate that acute hyperglycaemia may impair endothelium-dependent vasodilatation, also in non-diabetic subjects. Whereas normalisation of glycaemia in combination with antioxidant treatment improves FMD, hyperglycaemia may induce oxidative stress that negatively affects endothelial function. Despite this, earlier studies have failed to find a significant negative association between FMD and HbA1C level, as in the present study. Confounding factors that might influence FMD response are brachial artery diameter and flow velocity response during hyperaemia; after adjustment for the absolute flow velocity, the difference in FMD response failed to reach significance. Increased arterial stiffness has been suggested to impair FMD falsely. This was not confirmed in the present study, where a negative correlation between DC and FMD was seen. There was a striking decrease in responsiveness to the exogenous nitric oxide (NO) donor in DM (figure 1). Earlier studies have shown dominant endothelial dysfunction in DM, but only a minor, or no, reduction in vascular smooth muscle cell responsiveness. Whether this is due to the fact that earlier studies have included diabetic men and women, or whether it is a finding independent of gender is present unknown. Since IMT and DC were unchanged, the main impairment seems to be related to functional factors at the level of the smooth muscle cell rather than to structural factors or reduced production of endogenous NO. The biotransformation of GTN to NO metabolites is not completely understood but is believed to be initiated by mitochondrial enzymes within vascular smooth muscle cells. Since GTN reacts readily with superoxide radicals, one plausible factor for the impaired action of exogenous NO might be oxidative stress, induced by long-term poor glycaemic control. HbA1C, but not plasma glucose, was independently correlated with NMD in a negative manner. A negative correlation has previously been reported between NMD and short-term glycaemic control in patients with type 1 diabetes, but to our knowledge this is the first study to find a negative independent correlation between long-term glycaemic control and NMD in DM. Furthermore, a similar relation was also found in controls, indicating a generalised effect of glycaemic control on vascular smooth muscle behaviour (figure 3). A reduced NMD response is associated with cardiovascular risk factors, as well as a higher incidence of future cardiovascular events in patients with coronary artery disease. In controls, HbA1C also correlated positively with C-peptide, negatively with IGFBP-1 and positively with BMI. This last finding is
in line with earlier observations, and suggests that the correlation between HbA1c and NMD is due to overweight and insulin resistance. The decreased NMD response seen in the DM patients should be interpreted with some caution, since GTN was administered sublingually. The response to sublingual GTN seems, however, to be consistent between three to nine minutes after administration in DM subjects.

A higher resting HR has convincingly been shown to predict cardiovascular risk in men, but seems to be a risk factor also in women. The higher HR in DM is in accordance with some earlier studies, the reason being unclear. An increased sympathetic tone often accompanies an increase in resting HR. This was not evaluated in the present study, but baseline sympathetic tone has not previously been demonstrated to be higher in uncomplicated type 1 diabetes. Autonomic neuropathy with a reduced parasympathetic drive might also play a role. In both DM and C, HR was positively associated with HbA1c, but the expected positive relation between HR and triglyceride, CRP and MMP-9 was only found in controls (table 3), suggesting that autonomic neuropathy possibly interacts and depresses the HR response to these mediators in DM. Cardiovascular fitness influences parasympathetic activity, and we cannot rule out the possibility that the increase in HR in DM may reflect a difference in fitness between groups rather than incipient parasympathetic dysfunction, but the difference in resting heart rate between groups was still present after correction for aerobic capacity on a bicycle (data not shown).

A reduced concentration of IGF-1 has been suggested to be involved in the pathogenesis of atherosclerotic events. The DM had decreased serum levels of IGF-1, in agreement with previous reports. It is conceivable that low total IGF-I and high IGFBP-1 negatively affect vascular smooth muscle cell function, even if we failed to find any association between NMD and IGF-1 in the present study. The higher CRP in DM is consistent with earlier reports, but not associated with the altered wall properties. Previous studies have shown that MMP-3 in serum correlates with arterial stiffness and moreover MMP-3 and MMP-9 genotype have been shown to influence large artery stiffness. MMP-9 has also emerged as an indirect marker of diabetes-related abnormality of vascular reactivity. In the present study, similar MMP-3 and MMP-9 levels were seen in DM and C although MMP-9 was found to be an independent predictor of NMD. However, the significantly higher TIMP-1 levels in the DM may suggest that TIMP-1 constitute an early marker of vascular remodelling. Interestingly, higher serum levels of TIMP-1 have been shown to be independently associated with the presence of carotid atherosclerosis.

In conclusion, brachial artery responsiveness to an exogenous NO donor was markedly reduced in women with type 1 diabetes without evident complications, despite only limited reduction in endothelium-dependent dilatation, unaltered arterial size, stiffness and wall thickness. A negative association between the GTN response and HbA1c suggests that poor long-term hyperglycaemia impairs vascular smooth muscle cell function in DM. In future studies, the prognostic importance of NMD impairment and response to intervention should be evaluated in DM.

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Conflicts of interest statement

None declared.

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