Impaired Hyperaemic and Rhythmic Vasomotor Response in Type 1 Diabetes Mellitus Patients: A Predictor of Early Peripheral Vascular Disease

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Objectives. The smooth muscle of distal vascular networks exhibits periodical contraction and relaxation known as rhythmic vasomotion. The nature of microvascular vasomotion has been shown to correlate with severity of peripheral vascular disease. We present basal and post-ischaemic hyperaemic laser doppler flowmetry vasomotion in control and type 1 adult diabetic patients.

Design. Prospective case control study.

Methods. Laser Doppler flowmetry was used to measure vasomotion and hyperaemic responses in age and body mass index matched male subjects (25 type 1 Diabetes Mellitus and 13 controls), all with ankle/brachial pressure index (ABPI) >1.0 but <1.2.

Results. The frequency of resting vasomotion was raised in diabetics compared to controls 8 (5–9) min⁻¹ vs. 5 (4–6) min⁻¹ (median (range); p < 0.0001). The post ischaemic hyperaemia response was significantly higher in the diabetic group compared to the controls 11 (7–12) min⁻¹ vs. 6 (5–7) min⁻¹ (median (range); p < 0.05). Post ischaemic hyperaemic flux (expressed as percent increase from resting) was significantly lower in the diabetic group compared to controls (234 ± 62 vs. 453 ± 155%, p < 0.01). The time to achieve peak post ischaemic response was also significantly increased in the diabetic group compared to control: 21.4 ± 0.4 vs. 12.8 ± 5.4 sec (mean ± SD, p < 0.05).

Conclusions. Vasomotion frequency and its change during hyperaemic insult is significantly different in Type 1 Diabetes Mellitus subjects compared to controls. The results are similar to patients with macrovascular atherosclerosis. Long term studies of these groups of patients will be required to determine the significance of these findings and whether these changes could be used as a non invasive screening test to predict peripheral early vascular disease in type 1 diabetic patients.

Keywords: Peripheral vascular disease; Type 1 diabetes mellitus; Microvascularity.

Introduction

The smooth muscle of distal vascular networks displays an inherent property of periodical contraction and relaxation. This is known as rhythmic vasomotion, a process thought to be part of active microvascular autoregulation (reviewed by Nilsson and Asakjaer).¹

Laser Doppler flowmetry (LDF) is a non-invasive measure of tissue perfusion and reliably assesses cutaneous blood flow.² LDF measurements have been shown to reliably differentiate patients with peripheral arterial occlusive disease from controls.³ The ankle brachial pressure index is known to be an unreliable indicator of peripheral vascular disease (PVD) in diabetic patients with medial artery calcification and supra-normal pressures.

We sought to define the relationship between LDF flux levels and rhythmic vasomotion pressures before, during and after a hyperaemic insult in type 1 diabetic patients with no clinical signs or symptoms of peripheral vascular disease or neuropathy.

Methods

Patients and controls with peripheral pulses, demonstrable ankle reflexes and normal vibration sense on testing at the medial malleolus were recruited. Systolic blood pressure >160 mmHg and diastolic >90 mmHg were excluded as were patients with microalbuminuria. None were on oral pharmacological agents.
Cardiovascular autonomic tests were carried out on all subjects: Heart rate variability in response to standing and deep breathing; Postural variation in systolic blood pressure was recorded.

The laser Doppler flowmeter (Moores Instruments Ltd., Axminster, Devon, UK), illuminates a small area of tissue with monochromatic light at a wavelength of 780 nm. Reflected, back scattered, light falls onto a photo multiplier. The light that is received onto the photo multiplier consists of Doppler shifted light from moving objects, mainly erythrocytes and non shifted light from fixed connective and stromal elements. Interaction of this shifted and non-shifted light at the photo multiplier produces the laser Doppler flowmetry signal, which is directly recorded into a computer with a DRTSOFT (copyright Moores Instruments Ltd.) program.

The instrument was calibrated in a standard manner according to manufacturer’s instruction with probes in a probe holder against a stable white surface. Subjects were positioned supine and the leg was held stable with the aid of a vacuum cushion. The site of the laser Doppler probe was the pulp surface of the great toe. Laser Doppler recordings were made with a holder heated to 30 °C. Room temperature was maintained at 25 °C.

Ultrasonic Doppler ankle pressures were recorded followed by a 20-minute rest period during which continuous measurements of resting flux were taken with the LDF until a steady state level had been achieved. This was taken to be the resting flux (Fr).

A tourniquet was then applied five centimetres below the knee and the cuff inflated rapidly to a pressure 70 mmHg above the systolic pressure at the ankle for three minutes after Morales et al. At this point biological zero was taken. At three minutes the cuff was rapidly deflated and the post ischaemic peak hyperaemic response was recorded. Local ethics committee approval was obtained.

**Tracing and Statistical Analysis**

Changes in blood flux measurements of microvascular vasomotion are measured in arbitrary perfusion units (PU). Three patterns of fluctuation in LDF measured vasomotion are recognised: low frequency (2 to 10 min⁻¹); high frequency (15–25 min⁻¹); and fluctuations corresponding to heart rate. Low frequency undulations (3–5 cycles/min) were taken to represent autonomic nervous system affected microvascular vasomotion. Flow motion was analysed using fast Fourier transform analysis and displayed on a power spectral density graph. Peak flux (Fp) and time taken to reach peak flux (Tp; see Fig. 1).

The two tailed Mann-Whitney U test was used to compare parameters between groups, and P < 0.05 was considered significant. Data was analysed using Microsoft Excel and SPSS version 14.

**Results**

25 male type 1 Diabetes Mellitus subjects and 13 males were studied (Table 1). There were no significant differences between the groups regarding age or body mass index (BMI). All subjects had ankle brachial pressure index >1.0 but below 1.2. All diabetic subjects had normal autonomic tests. Mean duration

![Fig. 1. Typical laser doppler flowmetry recording showing baseline flow, occlusion and hyperaemic response (AU. arbitrary units).](image-url)
of diabetes was 19, ranging from 5 to 38 years. HbA1c was 7.9 ± 0.9 (%; mean ± SD).

The frequency of resting vasomotion was significantly raised in patients with diabetes compared to controls 8 (5–9) min⁻¹ vs. 5 (4–6) min⁻¹ (median (range); p < 0.0001; Fig. 2).

The post ischaemic hyperaemia response was significantly higher in the diabetic group compared to the controls 11 (7–12) min⁻¹ vs. 6 (5–7) min⁻¹ (median (range); p < 0.05).

Post ischaemic hyperaemic flux (expressed as percent increase from resting) was significantly lower in the diabetic group compared to controls (234 ± 62 vs. 453 ± 155%, p < 0.01). The time to achieve peak post ischaemic response was also significantly increased in the diabetic group compared to control: 21.4 ± 0.4 vs. 12.8 ± 5.4 sec (mean ± SD, p < 0.05).

Discussion

LDF has been shown to measure the flux of erythrocytes within a tissue volume and as such has been used to assess microvascular vasomotion, a process intrinsic to active microvascular autoregulation. Flow motion or vasomotion is the cyclical variation in blood flow owing to the rhythmic opening and closing of arterioles. First reported by Jones in 1852 after his observations on a bat’s wing, this and the related cyclical variation in blood flow has received little attention until recent years.

Amplitude and frequency of vasomotion has been shown to characterise degree of peripheral arterial occlusive disease. Previous studies have suggested that cutaneous flow motion may be altered in type 1 diabetics with neuropathy but not in those without neuropathy.

In this study we sought to define the relationship between laser Doppler measured vasomotion before, during and after a hyperaemic insult in a series of type 1 diabetic patients without clinically apparent peripheral vascular disease and age and BMI matched control subjects.

Regional nerve blockade has previously been reported not to influence LDF measured vasomotion implying that vasomotion and the factors affecting it are local in nature. Other studies have demonstrated attenuation of the low frequency vasomotion fluctuations in diabetic patients with somatic and autonomic neuropathy, however these studies do not report on frequency of vasomotion response or the post ischaemic hyperaemic response.

The time taken to reach the peak of the post ischaemic hyperaemic response was significantly prolonged and the flux significantly reduced in the diabetic group. Fagrell et al. report a similar delay in the post-occlusion hyperaemic responses in diabetics and controls utilising capillary microscopy. Delay and reduction in the post ischaemic hyperaemic response has been reported to be a good indicator of degree of ischaemia in patients with peripheral vascular disease.

Our study demonstrates that both rhythmic vasomotion frequency prior to post ischaemic peak hyperaemic response and maximum frequency post ischaemia are significantly increased in type 1 diabetes mellitus patients which may result in increasingly turbulent microvascular flow in these patients compared to controls. Dardik et al. have shown in cultured cells that endothelial cells exposed to turbulent shear stress as opposed to laminar shear stress results in increased proliferation and apoptosis. Similarly, Hutcheson and Griffith have demonstrated a frequency related pulsatile flow dependant release of NO in isolated rat aortic rings supporting turbulent flow as central in development of micro-angiopathy in these patients.

Nitric oxide (NO) is an endogenous vasodilator substance which regulates basal blood flow in an endothelium dependant manner. Forearm venous occlusion plethysmography results from type 1 diabetic and controls has demonstrated that agonist induced NO mediated vasodilation is impaired in

### Table 1. Demographic of the diabetic and control subjects

<table>
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<tr>
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<th>IDDM</th>
<th>Control</th>
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<tbody>
<tr>
<td>Number</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Age (years; mean ± SD)</td>
<td>32.7 ± 8.1</td>
<td>34.1 ± 9.9</td>
</tr>
<tr>
<td>BMI (kg/m²; mean ± SD)</td>
<td>24.8 ± 3.1</td>
<td>24.9 ± 3.2</td>
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<tr>
<td>Duration of diabetes (years; mean (range))</td>
<td>19 (5–38)</td>
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<tr>
<td>HbA1c (%; mean ± SD)</td>
<td>7.9 ± 0.9</td>
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![Fig. 2. Boxplot showing resting (Fr) and peak (Fp) vasomotion frequency in diabetic patients and controls.](image-url)
type 1 diabetic patients, an effect independent of direct smooth muscle reactivity. These findings suggest a role for NO in the aberrations of vascular reactivity seen in these diabetic patients.

Our results show that there are functional microvascular disturbances in the toes of asymptomatic type 1 diabetic patients without neuropathy, adding to our knowledge regarding microvascular disturbances in patients with uncomplicated diabetes. Long term studies of these groups of patients will be required to determine the significance of these findings and whether these changes could be used as a non invasive screening test to predict peripheral early vascular disease in type 1 diabetic patients.

References

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