Microalbuminuria in Type 2 diabetes indicates impaired microvascular vasomotion and perfusion

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1Diabetic Centre and Academic Unit, Maelor Hospital, University of Wales College of Medicine, Wrexham, United Kingdom; and 2Max-Plank-Institute for Human Cognitive and Brain Sciences, University of Leipzig, Leipzig, Germany

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Schmiedel O, Schroeter ML, Harvey JN. Microalbuminuria in Type 2 diabetes indicates impaired microvascular vasomotion and perfusion. Am J Physiol Heart Circ Physiol 293: H3424–H3431, 2007. First published October 12, 2007; doi:10.1152/ajpheart.00558.2007.—Vascular oscillation (vasomotion) occurs in the microcirculation and is thought to be a significant contributor to tissue perfusion. Our aims were to assess the relationship of vasomotion to perfusion in the cutaneous microcirculation of diabetic patients, to determine the influence on it of endothelium-dependent and nonendothelium-dependent vasodilatory stimuli, and to assess the relationship to perfusion and vasomotion of various biochemical markers of vascular function (HbA1c, LDL- and HDL-cholesterol, triglycerides, insulin resistance, high sensitive C-reactive protein, L- and E-selectin, soluble ICAM, von Willebrand factor) and microalbuminuria. Perfusion and vasomotion (spectral density at low and very low frequencies) were measured by laser-Doppler flowmetry after local heat and iontophoresis of ACh and sodium nitroprusside. Perfusion responses to all stimuli were impaired in patients with Type 2 diabetes (heat: \( F = 28.0, P < 0.001 \); ACh: \( F = 7.11, P = 0.003 \); sodium nitroprusside: \( F = 4.0, P = 0.028 \)). Responses to endothelium-dependent stimuli were further impaired in microalbuminuric patients (heat: \( P = 0.035 \); ACh: \( P = 0.034 \)). Vasomotion responses at low frequencies after endothelium-dependent stimuli were impaired in diabetic patients compared with that shown in controls (heat: \( F = 5.62, P = 0.002 \); ACh: \( F = 4.32, P = 0.015 \)). Multivariate modeling showed microalbuminuria to be the only consistent predictor of perfusion and vasomotion responses. The results suggest that microalbuminuria in Type 2 diabetes reflects a generalized disturbance of microvascular function related to endothelium-dependent mechanisms.

Many aspects of vasomotion, including its origin, regulation, physiological role, and the pathophysiological consequences of altered vasomotion, remain the subject of debate.

Rhythmic oscillations in the microcirculation can be recorded in single vessels by video microscopy, capillaroscopy, and capillary anemometry, in the resistance vessels by plethysmography or near-infrared spectroscopy, and in skin nutritive and thermoregulatory circuits by laser-Doppler flowmetry (LDF). LDF measurements in the skin reflect flow and vasomotion in terminal arterioles, capillary loops, and postcapillary venules of the superior horizontal plexus (6). Results obtained with LDF are dependent on skin anatomy and are different in hairy and glabrous skin (8, 31). It has been suggested that alterations in the neural regulation of vascular beds underlie the loss of microvascular reactivity in diabetes (31).

Analysis of vasomotion in the microcirculation identifies oscillation at different frequencies. These are very-low-frequency (VLF) oscillations in the range from 0.009 to 0.025 Hz, which are thought to reflect microvascular oscillation of endothelial origin, and low-frequency (LF) oscillations at 0.025–0.07 Hz, which are thought to be of sympathetic-neurogenic origin, and at 0.07–0.15 Hz, which are thought to be of parasympathetic-neurogenic or myogenic origin (17, 28, 29). Assessment of the oscillatory dynamics of the blood perfusion signal “vasomotion” may give additional information to the measurement of perfusion in microvascular disease. Vasomotion is likely to be an important parameter of small vessel function. The aims of this study were as follows. 1) We aimed to measure vasomotion by LDF in the small vessels of diabetic patients, assess its relation to microvascular perfusion, and determine the influence on it of endothelium-dependent and nonendothelium-dependent vasodilatory stimuli. 2) We also aimed to assess the relationship of microalbuminuria and other biochemical markers of vascular function with vasomotion measured in this way.

PATIENTS AND METHODS

Subjects

We recruited 39 subjects, 29 with Type 2 diabetes (mean age 65.3 ± 5.8 yr, range 52–77 yr) who attended the diabetic outpatient clinic. The 10 healthy controls were of similar age and body-mass index. Each participant gave written, informed consent after the nature of the procedure was explained. The protocol was approved by the Local Medical Research Ethics Committee and written in accordance with the Declaration of Helsinki. None of the patients used insulin.

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THE SKIN MICROCIRCULATION is characterized by continuous oscillations of vessel diameter, termed vasomotion, with corresponding variations in blood flow. These oscillations contribute to tissue oxygenation (13), cell adhesion, and migration (23), reduce peripheral vascular resistance (35), and maintain capillary pressure gradients (5). Microvascular oscillation may be absent under resting conditions. In in vivo experiments, animal and human studies have shown increased vasomotion under conditions of tissue hypoxia, acidosis, hypotension or vasodilatation (16), and reduced flow (26) due to cold (24) or vasoconstriction (27). It is thought that endothelial cells can initiate and modulate vasomotion via endothelium-derived factors such as endothelium-derived hyperpolarizing factor and nitric oxide (NO), the former predominant in small blood vessels and the latter predominant in larger vessels (3, 19).
and all had moderate glycemic (mean HbA1c of 7.7%) and lipid control (mean total cholesterol 4.5 mmol/l) (Table 1). Participants were Caucasians from the United Kingdom. Patients were stable on antihypertensive, hypoglycemic, and lipid-lowering medications. There was no treatment change during the study. Patients with known allergic skin conditions were excluded.

Experimental Procedures

Patient studies were undertaken in the morning after an overnight fast, in a room with stable ambient temperature of 22 ± 1°C. The patients reclined on a couch with their left arm supported at heart level. All LDLF assessments were made in duplicate. Blood pressure at the brachial artery was measured with a Dinamap Pro 400 monitor (Critikon, Tampa, FL) after 5 min of rest on two occasions. Fasting blood was analyzed for total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride levels. Glucose, HbA1c, insulin, C-peptide, and von Willebrand factor (vWF) were measured by an in-house ELISA using a monoclonal antibody that recognizes the functional epitope on the vWF molecule. Soluble L-selectin, soluble E-selectin, and soluble ICAM were measured as specific activity using kits (R&D Systems, Abingdon, UK). High sensitive C-reactive protein (hsCRP) was measured by a high-sensitivity, two-site immunochemical chemiluminescent assay (Diagnostic Products, Llanberis, North Wales). To assess glycemic control, as it might have influenced vascular function, we used the mean of all available HbA1c measurements (liquid chromatography) taken at clinic visits over the preceding three years. Insulin resistance was calculated with the homeostatic model assessment method (HOMA-IR) (35) and the quantitative insulin resistance index (QUICKI) (10, 15). Microalbuminuria was assessed from the mean of two to four morning urine samples using the albumin-creatinine ratio (double-antibody sandwich ELISA) with assay done in duplicate. History of cardiovascular events, microvascular complications (presence of retinopathy), smoking status, and medications that patients were taking were recorded. Retinopathy data were obtained from the All Wales Diabetes Retinopathy Screening Register. Data on family history of heart disease, diabetes, and ischemic stroke were obtained. The family history was considered positive when the condition was present in at least one first-degree relative of the probate.

Iontophoresis

Iontophoresis allows noninvasive drug delivery to the skin without systemic effects and perturbation of the skin. The technique of iontophoresis has been described in detail elsewhere (18, 22). We measured endothelial-dependent vasodilatation and vasomotion to 1% ACh, endothelium-independent vasodilatation and vasomotion to 0.1% sodium nitroprusside (SNP), and the maximal hyperemic vasodilatation and oscillation response to local heating to 44°C. ACh (Miochol-E, Novartis) diluted in 3% mannitol to a final concentration of 1% was delivered by an anodal current of 2 × 0.1 mA for 30 s in accordance with protocols by Morris et al. (22). SNP (Mayne Pharma) diluted in 0.45% NaCl to a concentration of 0.01% was iontophoresed with a cathodal current of 2 × 0.1 mA for 30 s and 1 × 0.2 mA for 30 s. Flow and oscillations were recorded immediately before the iontophoresis and 60 s after the last application for at least 4 min continuously. During iontophoresis, the skin temperature under the probe was maintained at 32°C.

LDF and Spectral Analysis

LDF continuously monitors microvascular perfusion by measuring red blood cell flow using the Doppler principle. The photo detector electrode detects a light signal reflected from moving red blood cells and generates an electrical signal in response. Perfusion is calculated from the area under the curve of this signal according to the formula

\[ \text{Perfusion} = \frac{k}{\text{DC} \int_{t_{\text{begin}}}^{t_{\text{end}}} f P(f) df} \]

where k is an instrumentation constant, DC is the current applied to the detector, f is the frequency (high- and low-frequency cutoffs are used to reduce signal reflection from other structures), and \( P(f) \) is the power spectral density (PSD) of the signal generated by the detector.

Table 1. Characteristics of the 3 groups of subjects studied, biochemical parameters of glucose and lipid metabolism, and markers of vascular function

<table>
<thead>
<tr>
<th></th>
<th>Control Group Without MAU (n = 10)</th>
<th>Diabetic Patients With MAU (n = 17)</th>
<th>Diabetic Patients Without MAU (n = 12)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>66.6 ± 1.7</td>
<td>66.8 ± 1.9</td>
<td>63.5 ± 2.1</td>
<td>0.484</td>
</tr>
<tr>
<td>Duration of diabetes, yr</td>
<td>14.4 ± 1.6*</td>
<td>10.45 ± 1.05</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>Body-mass index, kg/m^2</td>
<td>27.8 ± 0.8</td>
<td>28.1 ± 0.9</td>
<td>29.3 ± 1.1</td>
<td>0.763</td>
</tr>
<tr>
<td>Smokers (n)</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>0.041</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>89.4±3.7*</td>
<td>96.7±1.8</td>
<td>103.9±2.9‡</td>
<td>0.008</td>
</tr>
<tr>
<td>HbA1c, % (mean over 3 yr)</td>
<td>(5.0±0.07)</td>
<td>7.8±0.25</td>
<td>7.5±0.24</td>
<td>0.82</td>
</tr>
<tr>
<td>Albumin-creatinine ratio, mg/mmol</td>
<td>0.84±0.17†</td>
<td>1.33±0.14*</td>
<td>9.95±3.7*</td>
<td>0.005</td>
</tr>
<tr>
<td>Triglycerides, mmol/l</td>
<td>1.4±0.29</td>
<td>2.1±0.32</td>
<td>1.8±0.3</td>
<td>0.29</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/l</td>
<td>1.46±0.15*</td>
<td>1.01±0.07*</td>
<td>0.97±0.06†</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/l</td>
<td>3.2±0.3</td>
<td>2.7±0.23</td>
<td>2.6±0.18</td>
<td>0.24</td>
</tr>
<tr>
<td>Insulin resistance (HOMA)</td>
<td>1.5±0.3*</td>
<td>6.7±1.9*</td>
<td>3.8±0.8*</td>
<td>0.015</td>
</tr>
<tr>
<td>Insulin resistance (QUICKI)</td>
<td>0.161±0.005†</td>
<td>0.138±0.005</td>
<td>0.140±0.004*</td>
<td>0.01</td>
</tr>
<tr>
<td>Insulin, mU/l</td>
<td>6.8±1.5</td>
<td>14.8±2.3</td>
<td>11.2±1.9</td>
<td>0.19</td>
</tr>
<tr>
<td>C-peptide, pmol/l</td>
<td>742±109</td>
<td>1,167±174</td>
<td>1,138±183</td>
<td>0.21</td>
</tr>
<tr>
<td>hsCRP, mg/dl</td>
<td>0.25±0.08*</td>
<td>0.27±0.04*</td>
<td>2.4±1.09*</td>
<td>0.014</td>
</tr>
<tr>
<td>L-selectin, ng/ml</td>
<td>1.06±0.17*</td>
<td>72±6.64*</td>
<td>957±65*</td>
<td>0.023</td>
</tr>
<tr>
<td>E-selectin, ng/ml</td>
<td>46±5</td>
<td>55±7</td>
<td>61±10</td>
<td>0.49</td>
</tr>
<tr>
<td>ICAM, mg/dl</td>
<td>280±36</td>
<td>252±14</td>
<td>250±23</td>
<td>0.64</td>
</tr>
<tr>
<td>von Willebrand factor, U/ml</td>
<td>0.87±0.09</td>
<td>0.86±0.14</td>
<td>1.06±0.16</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAU, microalbuminuria; hsCRP, high sensitive C-reactive protein. One-way ANOVA results for each parameter are shown. For post hoc analyses, asterisks against the control group values indicate significant difference from normoalbuminuric diabetes values, asterisks against the normoalbuminuric diabetic group values indicate significant difference from MAU diabetes values, and asterisks against the MAU diabetic group indicate significant differences from the normal control subjects. P < 0.05; † P < 0.01; ‡ P < 0.001.
Calibration was undertaken before each study by the use of small polystyrene spheres dissolved in water; the Brownian motion of these spheres generates a reproducible signal that is used to calibrate the instrument. Perfusion was recorded in arbitrary perfusion units (PU) (20) and expressed as maximal flow minus baseline flow. Measurements were made with a Periflux 5000 laser-Doppler system (Perimed, Stockholm, Sweden), which delivers light generated by a near-infra-red laser diode at a wavelength of 780 nm with a power of 1.0 mW.

Microvascular oscillations were calculated in the frequency domain using the Fast-Fourier analysis algorithm. A bandpass filter with cutoff frequencies at 20 Hz and 20 kHz and a time constant of 0.2 s was used. The sampling frequency was at 32 Hz. A data window (Parzen window) of 0.009–0.8 Hz was selected to detect LF oscillations with the highest possible resolution and to avoid loss of information, which is inevitable when using larger windows. Periodic oscillations with three characteristic frequency peaks can be observed within this window as demonstrated in Fig. 1. These are 1) VLF oscillations in the range of 0.009–0.025 Hz and 2) LF oscillations at 0.025–0.07 Hz and at 0.07–0.15 Hz. Results are measured as area under the curve in the specified frequency interval of interest (mean of 2 separate measurements) and expressed in PSD units.

Vasomotion measurements are influenced by changes in perfusion, as vasodilatation results in an increased volume of moving red blood cells affecting both perfusion and vasomotion measurements. To assess the change in oscillation independently of change in perfusion, we also expressed measured oscillation values relative to perfusion measured from the same time window as suggested by Benbow et al. (2). This relative amplitude (RA) was calculated as follows: RA (%) = (oscillation power in PSD/perfusion in PU) × 100.

Statistics

Because most of the data describing microvascular perfusion and oscillation responses were not normally distributed, all of these values and the biochemical measures were log transformed before analysis. Perfusion responses to heat, ACh, and SNP were analyzed by one-way ANOVA with post hoc analyses by Tukey’s least significant difference test. Pearson’s χ²-test was used to compare dichotomous data between groups. Oscillation responses to these stimuli were assessed by two-way ANOVA with control, diabetic without microalbuminuria, and diabetic with microalbuminuria as between-subject factors and frequency domain (endothelial, neurogenic-sympathetic, and myogenic) as within-subject factors. Multilinear regression analysis was used to identify predictors of impaired perfusion and oscillation responses. The following clinical and metabolic parameters were tested in the model: age, smoking history, albumin-creatinine ratio, HOMA-IR, C-peptide, HbA1c, mean arterial blood pressure, LDL-

![Fig. 1. Spectral analysis of microvascular oscillations [mean power spectral density (PSD)] for control patients, diabetic patients without microalbuminuria (DM without MAU), and diabetic patients with microalbuminuria (DM with MAU). Peaks at very low frequency (0.009–0.025 Hz), low frequency-neurogenic (0.025–0.07 Hz), and low frequency-myogenic (0.07–0.15 Hz) are present.](http://ajpheart.physiology.org/)

![Fig. 2. Skin microvascular perfusion response to 44°C heat, ACh, and sodium nitroprusside (SNP) for control and diabetic patients with and without microalbuminuria.](http://ajpheart.physiology.org/)
cholesterol, HDL-cholesterol, triglycerides, hsCRP, vWF, E-selectin, L-selectin, and ICAM. Family history and data on microvascular and macrovascular complications were analyzed in further linear regression models. All analyses were performed with statistical software SPSS version 14. Data are given as means ± SE.

RESULTS

Table 1 gives clinical and biochemical details of the three groups of subjects.

Perfusion Responses

Baseline skin perfusion. Basal forearm skin perfusion was not different between diabetic patients and controls (10.14 ± 0.97 vs. 9.63 ± 1.45 PU; \( P = 0.49 \)) or between diabetic patients with and without microalbuminuria (10.22 ± 0.94 vs. 10.03 ± 0.74 PU; \( P = 0.91 \)).

Stimulated perfusion responses. The perfusion responses of skin microvascular flow to local heat, ACh, and SNP in normal control subjects and patients with Type 2 diabetes with and without microalbuminuria are shown in Fig. 2. ANOVA indicated clear differences between the three patient groups in their responses to temperature (\( F = 28.0, P < 0.001 \)), ACh (\( F = 7.11, P = 0.003 \)), and SNP (\( F = 4.0, P = 0.028 \)). Post hoc analysis showed that, with all three stimuli, there were significant differences in perfusion responses between the normal control and the diabetic groups. For endothelium-dependent stimuli (heat, ACh), the microalbuminuric patients showed significantly poorer perfusion responses than the diabetic patients without microalbuminuria. For responses to SNP, there was no significant difference between diabetic patients with and without microalbuminuria (\( P = 0.55 \)).

There was a significant linear correlation between log albumin-creatinine ratio and the perfusion responses to heat (\( r = -0.49, P = 0.002 \)) and ACh (\( r = -0.46, P = 0.003 \)) (Fig. 3) but not the perfusion response to SNP (\( r = -0.26, P = 0.125 \)).

Vasomotion Response

In the two-way ANOVA, the between-factor analysis showed differences between control patients and diabetic patients with and without microalbuminuria in absolute oscillation responses to local heat (\( F = 7.46, P = 0.002 \)) and ACh (\( F = 4.54, P = 0.017 \)). There was no difference between the groups in response to SNP (\( F = 1.81, P = 0.18 \)). With the stimuli that were significant, further analysis was undertaken with one-way ANOVA. The results of this are given in Table 2. Post hoc testing of the responses after endothelium-dependent stimuli (local heat, ACh) showed that the responses in the microalbuminuric diabetic subjects were significantly impaired compared with those in normal controls. After local heat, the microalbuminuric diabetic subjects also showed significantly impaired responses compared with diabetic patients without microalbuminuria.

Assessment of the Relative Vasomotion Amplitude

The results of calculations of vasomotion RA are shown in Table 3. With two-way ANOVA, there were significant differences in the RA response to local heat (\( F = 8.55, P = 0.001 \)) but not after ACh or SNP. One-way ANOVA showed differences at each of the three frequency windows. In post hoc analysis, controls were different from the diabetic groups at all three frequencies in so far that they had the lowest relative vasomotion amplitude. The normoalbuminuric and microalbuminuric groups were not significantly different. The perfusion and oscillation responses used in these calculations are shown in Fig. 4.

Biochemical Parameters of Vascular Function

In addition to biochemical measures of glucose and lipid metabolism, indicators of endothelial activation and inflammation were analyzed before their inclusion into a multivariate model to assess their relationship to abnormal microvascular perfusion and oscillation responses. The results are given in Table 1. Differences between the groups were seen in insulin resistance (via HOMA-IR and QUICKI), HDL-cholesterol levels, hsCRP, and plasma L-selectin. vWF levels showed a trend to higher levels in microalbuminuric patients, although it was not significant.

Multivariate Analysis

Considering the diabetic subjects only, there was no relationship between duration of diabetes and any of the microvascular function parameters. Multivariate linear regression
modeling identified independent predictors of perfusion and oscillation responses, as given in Table 4.

**Family History and Vascular Complications**

When we compared the three groups for presence of microvascular complications (diabetic retinopathy), a significant difference was found ($\chi^2 = 15.38, P < 0.001$), with the numbers being highest for the diabetic patients with microalbuminuria. Nevertheless, there was no difference in cardiovascular complications ($\chi^2 = 4.12, P = 0.13$).

With regard to family history, we found a significant difference between groups in family history of cardiovascular events ($\chi^2 = 6.06, P = 0.048$); family history was most frequent among diabetic patients with microalbuminuria. There was no difference between groups in family history of ischemic stroke. For family history of diabetes, there was a difference between patients with diabetes and the control group but not between the two diabetic groups. Regression analysis demonstrated family history of cardiovascular events to be a predictor of reduced endothelial-dependent vasomotion (ACh: $F = 3.73, P = 0.034$) and oscillation responses (ACh: $F = 6.13, P = 0.005$, heat: $F = 3.46, P = 0.042$).

**DISCUSSION**

Vasomotion can be measured as a spontaneous phenomenon or after vasodilatory or vasoconstrictor stimuli. Because the assessment of spontaneous oscillations is greatly influenced by the cutaneous microarchitecture, the spatial distribution of capillaries, arterioles, and venules (6), skin type (glabrous or hairy), and skin temperature, we performed a series of stimulation tests. Endothelium-dependent and -independent vasodilators were used, and perfusion and oscillation responses were measured simultaneously. The diabetic patients had a significantly reduced perfusion response to local heat, ACh, and SNP as shown previously (22). The microalbuminuric patients had a further reduction in their microvascular perfusion response.
when compared with diabetic subjects without microalbuminuria in response to heat and ACh but not in response to SNP. Thus diabetes impairs microvascular perfusion generally, but microalbuminuria confers additional impairment as a result of impaired endothelium-dependent mechanisms. This is consistent with studies measuring flow in conduit vessels that have shown endothelial dysfunction in diabetic patients with microalbuminuria (1, 11).

Altered microvascular vasomotion was also identified in the diabetic group. Vasomotion was measured in a frequency range from 0.0095 to 0.8 Hz. We found oscillation peaks at 0.0095–0.025 Hz, 0.025–0.07 Hz, and 0.07–0.15 Hz that were similar to previous studies in normal subjects (14, 16). We found these peaks to be similar in control subjects and diabetic patients (Fig. 1). Absolute vasomotion was measured after endothelium-dependent stimuli and after SNP exposure. After endothelium-dependent stimuli but not SNP, there was a clear impairment of the response in the microalbuminuric diabetic patients vs. results in diabetic patients without microalbuminuria and in normal controls. There was no difference between controls and diabetic patients without microalbuminuria. Thus microalbuminuria reflects an impairment of endothelium-dependent microvascular perfusion and vasomotion.

Vasomotion is thought to be an independent phenomenon that does not represent a downstream effect of blood pressure and heart rate variations (4, 28). The exact mechanism of causation of altered vasomotion in patients with diabetes remains uncertain, with evidence of both altered production of local mediators, particularly NO, and the influence of diabetic neuropathy (34). Oscillations of \(0.009–0.025\) Hz, termed VLF vasomotion, are thought to relate to NO production from endothelial cells. Infusions of \(N\textsuperscript{G}-\text{monomethyl-L-arginine}\) abolish and arginine exposure restores this phenomenon (17). Oscillations of \(0.025–0.06\) Hz and \(0.06–0.14\) Hz, described as LF vasomotion, are thought to be related to the autonomic nervous system. Meyer et al. (20) described reduced vasomotion at \(0.1\) Hz in diabetic patients with sympathetic dysfunction. Stansberry et al. (32) identified a relationship between vasomotion and altered thermal threshold. The skin response to local heating is a complex phenomenon, which includes suppression of \(\alpha\)-receptor affinity for norepinephrine, activation of small C fiber nociceptive neurons, and release of neuropeptides (substance P, CGRP, histamine, bradykinin, and VIP), referred to as the axon flare response (21, 34). Local NO-mediated endothelial mechanisms are also involved (12). In our measurements, microvascular oscillations at all three frequencies

![Diagram showing relative vasomotion amplitude (RA) for control and diabetic patients with and without microalbuminuria: perfusion response in perfusion units (PU) to heat (open bars) and oscillation response to heat (solid bars). As shown, predominant difference between the groups relates to perfusion and demonstrates that the relative increase of oscillations (RA) in diabetic patients is caused by less difference in oscillations.](image)

Table 4. Results of the multivariate analyses to identify biochemical predictors of the microvascular perfusion and oscillation responses to local heat, ACh, and SNP

<table>
<thead>
<tr>
<th>Outcome Parameter</th>
<th>Model Adjusted (R^2)</th>
<th>Independent Predictor</th>
<th>Regression Coefficient (B)</th>
<th>Standard Coefficient ((\beta))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perfusion to heat</td>
<td>0.24</td>
<td>ACR</td>
<td>-0.30</td>
<td>-0.51</td>
<td>0.001</td>
</tr>
<tr>
<td>Perfusion to ACh</td>
<td>0.20</td>
<td>ACR</td>
<td>-0.22</td>
<td>-0.47</td>
<td>0.003</td>
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<tr>
<td>Perfusion to SNP</td>
<td>0.09</td>
<td>HbA1c</td>
<td>-0.15</td>
<td>-0.33</td>
<td>0.044</td>
</tr>
<tr>
<td>VLF oscillation</td>
<td>0.34</td>
<td>ACR</td>
<td>-0.26</td>
<td>-0.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LF oscillation</td>
<td>0.33</td>
<td>Age</td>
<td>0.03</td>
<td>0.44</td>
<td>0.005</td>
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<tr>
<td></td>
<td></td>
<td>L-selectin</td>
<td>-0.49</td>
<td>-0.35</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACR</td>
<td>-0.15</td>
<td>-0.29</td>
<td>0.047</td>
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<tr>
<td>Heat</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>VLF oscillation</td>
<td>0.21</td>
<td>LDL-C</td>
<td>0.83</td>
<td>0.36</td>
<td>0.022</td>
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<tr>
<td>LF oscillation</td>
<td>0.13</td>
<td>ACR</td>
<td>-0.21</td>
<td>-0.32</td>
<td>0.042</td>
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<tr>
<td>ACh</td>
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<td></td>
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<tr>
<td>VLF oscillation</td>
<td>0.13</td>
<td>vWF</td>
<td>0.05</td>
<td>0.39</td>
<td>0.019</td>
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<tr>
<td>LF oscillation</td>
<td>0.13</td>
<td>vWF</td>
<td>0.05</td>
<td>0.39</td>
<td>0.019</td>
</tr>
<tr>
<td>SNP</td>
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Oscillation responses were measured at very low frequency (VLF) and low frequency (LF). ACR, albumin-creatinine ratio; vWF, von Willebrand factor.
were impaired in microalbuminuric patients in response to endothelium-dependent stimuli, suggesting a common mechanism of vasomotion generation probably involving NO synthesis.

In vitro techniques such as confocal fluorescence microscopy or luminal cross-sectional analysis measure vasomotion independent of blood flow. In vivo techniques such as LDF and plethysmography assess blood flow and vasomotion simultaneously. To assess whether the changes in vasomotion could be due to changes in perfusion, we calculated the relative vasomotion response that corrects in part for changes in perfusion (2). Corrected for perfusion in this way, the vasomotion responses to heat in diabetic patients remained different from responses in healthy controls, but there were no longer differences after ACh. The results suggest that the changes in vasomotion in diabetes are not fully explained by changes in perfusion. Among our patients, the proportionally greater reduction in perfusion than in oscillation responses to endothelium-dependent mediators demonstrates increased relative vasomotion amplitude (Fig. 4).

Increased vasomotion may be an important compensatory mechanism where tissue perfusion is compromised (23). However, like us, others have also found reduced LF and VLF oscillations in diabetes. Stansberry et al. (32) and Kvandal et al. (17) found reduced vasomotion in patients with long-duration Type 1 and Type 2 diabetes. Our data are consistent with this, although our data may be interpreted as suggesting that impaired perfusion responses occur first. Impaired perfusion responses may occur at the stage of fasting hyperglycemia and have been related to insulin resistance (14), although our study did not test this. An impaired vasomotion response was most clearly demonstrated in the microalbuminuric patients. If increased microvascular vasomotion is a part of the normal physiological response to compensate for impaired tissue perfusion, then microalbuminuric patients will be at greater risk of hypoxic tissue damage as a result of their inability to mount this response.

Multilinear regression modeling was undertaken to indicate the influence of different demographic and biochemical factors on microvascular perfusion and oscillation. Age had an influence on vascular responsiveness, although we showed no effect of duration of diabetes. Unsurprisingly, HDL-cholesterol was lower in patients with diabetes than in control patients, and patients with diabetes had greater insulin resistance as measured by the HOMA-IR and QUICKI indexes. hsCRP was higher in the microalbuminuric subjects, and vWF showed a similar trend (Table 1). Despite these differences between the three patient groups, microalbuminuria was the only consistent predictor of perfusion and oscillation responses when measured after endothelium-dependent stimuli (heat or ACh). In response to SNP, there was a perfusion rise in all three groups, but no effect on oscillation and no difference between diabetic patients with and without microalbuminuria. Mean HbA1c was a weak predictor of the perfusion response to SNP, and this probably reflects vascular change accumulated in relation to less diabetic control. Interestingly, mean arterial blood pressure was not a predictor of microvascular function in these experiments.

We demonstrated an association between microalbuminuria and endothelial-dependent perfusion and vasomotion; nevertheless, the mechanistic explanation for this relationship remains speculative. Endothelial function appears to have a major influence on vascular bed perfusion. Thus endothelial dysfunction may contribute to glomerular hemodynamic abnormalities. Increased albumin filtration may be the result of increased intraglomerular pressure or structural change due to abnormal renal microvascular perfusion and vasomotion. Alternatively, or in addition, endothelial dysfunction is closely associated with atherosclerotic plaque formation in large vessels. There is a low level of release of inflammatory mediators into the circulation; these inflammatory mediators may be taken up by mesangial cells or podocytes and adversely affect their function (33).

It is important to know whether family history of vascular disease or possession of susceptibility genes predisposes an individual to endothelial dysfunction and microalbuminuria. Because identification of genes that contribute to the development of vascular dysfunction and nephropathy in diabetes has not yet progressed to the point where we can link our findings to specific gene variants (9, 28), we evaluated family history of diabetes, cardiovascular events, and ischemic stroke. We also checked for the coexistence of other microvascular and macrovascular complications. Our findings demonstrate a significant association between endothelial function and family history of ischemic heart disease. The most interesting result was the association between family history of cardiovascular events and reduced endothelium-dependent microvascular oscillation. These findings warrant further study, as the predisposition to develop endothelial dysfunction may be inherited.

We conclude that microvascular endothelial dysfunction is related more strongly to microalbuminuria than to other biochemical markers of vascular disease in diabetes. The microvascular dysfunction associated with microalbuminuria seems to be a result of derangement of endothelium-dependent mechanisms. This suggests that hemodynamic or vascular structural or functional change rather than renal-specific abnormalities initiates microalbuminuria in Type 2 diabetes.

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GRANTS

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