Elevated Skeletal Muscle Blood Flow in Noncomplicated Type 1 Diabetes Mellitus
Role of Nitric Oxide and Sympathetic Tone

Gerald Vervoort, Jack F. Wetzels, Jos A. Lutterman, Laurus G. van Doorn, Jo H. Berden, Paul Smits

Abstract—Capillary hyperperfusion precedes and contributes to the occurrence of diabetic microangiopathy. Vascular tone is regulated by the balance of vasodilating and vasoconstricting factors, of which nitric oxide (NO; an endothelium dependent vasodilator) and norepinephrine (NE; a potent vasoconstrictor), respectively, are of primary importance. To investigate the role of these factors in hyperperfusion, we measured forearm blood flow (FFB) in 50 patients with uncomplicated type 1 diabetes (DP) and 50 healthy control subjects (CS) under baseline conditions and during intrabrachial infusion of Nω-monomethyl-L-arginine (L-NMMA), an endothelium-dependent vasoconstrictor, and acetylcholine (ACH), an endothelium-dependent vasodilator. Furthermore, we determined arterial plasma NE concentration at baseline and then determined α-adrenergic receptor sensitivity by measuring FBF response to intra-arterially infused NE. We found that basal FBF was increased in DP (2.9±0.1 versus 2.0±0.1 mL · min⁻¹ · Dl⁻¹ in CS; P<0.01). L-NMMA caused a similar vasoconstriction in both groups (28.5±1.7% in DP versus 31.2±2.2% in CS; P=NS). Maximum blood flow during infusion of ACH was not different (23.3±1.9 mL · min⁻¹ · Dl⁻¹ in DP versus 20.1±1.6 in CS). Arterial plasma NE concentrations were significantly decreased in DP (0.57±0.03 versus 0.81±0.05 nmol/L in CS; P<0.01). The vasoconstrictive effect of NE was increased in DP (slope log dose-response curve, 31.3±1.5 versus 24.3±1.8 in CS; P<0.01). We conclude that basal FBF is increased in uncomplicated type 1 diabetes. We found no evidence of a disturbance of basal or stimulated NO production. Arterial plasma NE concentrations are decreased in uncomplicated type 1 diabetes. This may explain the vasodilatation at baseline and the increased vascular response to intra-arterially NE. (Hypertension. 1999;34:1080-1085.)

Key Words: endothelium ■ L-NMMA ■ norepinephrine ■ acetylcholine ■ diabetes ■ blood flow

Considerable evidence shows that the onset of diabetic microangiopathy is preceded by a state of generalized capillary hyperperfusion. These observations have led to the hypothesis that an increase in capillary flow or pressure contributes to the development of endothelial dysfunction and microvascular complications. Vascular tone is determined by a balance between vasoconstricting and vasodilating factors. Nitric oxide (NO) is an important endothelial vasodilator and a candidate for mediation of increases in blood flow observed in early diabetes. Vasoconstriction is largely dependent on sympathetic adrenergic activity. In experimental and human diabetes, changes in sympathetic activity have been found. Thus, in diabetic patients (DP), the decrease in vascular tone and the ensuing increase in skeletal muscle blood flow may result either from an increased release of NO from the vascular endothelium or from a decrease in sympathetic nervous system activity.

This hypothesis was addressed by use of the perfused forearm technique to quantify baseline and stimulated NO release and α-adrenergic responsiveness. Furthermore, as a measure of sympathetic nervous system activity, we have quantified arterial norepinephrine (NE) levels (α-adrenergic tone).

Methods

Study Population

After the study was approved by the local ethics committee, 50 patients with type 1 diabetes (DP) and 50 age- and sex-matched healthy controls (CS) gave informed consent. All procedures followed were in accordance with institutional guidelines. Type 1 diabetes was defined as an acute onset before the age of 40 years and insulin treatment <1 year after diagnosis. DP fulfilled the following criteria: diabetes duration, 5 to 12 years; age, 18 to 40 years; blood pressure (BP), <140/90 mm Hg; no antihypertensive medication; no clinical evidence of macrovascular disease; and no signs of microvascular disease, such as microalbuminuria or retinopathy (except for simple background retinopathy). Persistent normoalbuminuria was defined as urinary albumin excretion <20 μg/min in 2 timed, overnight urine samples. DP were recruited from the outpatient diabetic clinics of the University Hospital Nijmegen and TweeSteden Hospital Tilburg. CS were recruited from the local population and were screened for the absence of hypertension and cardiovascular and renal disease. Medication was not allowed except for oral contraceptives, which do not influence the parameters measured.
Study Protocols

Participants attended the clinic after an overnight fast. DP received their neutral protamine Hagedorn insulin dose the evening before the study but did not receive their usual fast-acting morning insulin. Glucose was measured before and during the study but was not corrected by insulin, to avoid confounding by the vasodilator effect of insulin.7–9 If hypoglycemia occurred (glucose <3.0 mmol/L), the test was canceled. In addition to glucose, free fatty acids (FFA) were measured. All participants were instructed to abstain from alcohol and caffeine for 24 hours and to refrain from smoking for 12 hours before the study.

The study was performed between 8 AM and 12 AM in a temperature-controlled room (23°C). Dosages of all drugs were calculated per deciliter of forearm volume, which was measured by water displacement. Forearm blood flow (FBF), intra-arterial BP, and heart rate were measured after cannulation of the left brachial artery as described before.10 After an equilibration period of 45 minutes with subjects supine, arterial blood samples were collected for determination of plasma NE and epinephrine, and intra-arterial BP and heart rate were registered. Thereafter, the experiment started with the measurement of baseline FBF during placebo infusion (NaCl, 0.9%). After placebo infusion, FBF response to intra-arterial infusion of N\(^{-}\)monomethyl-L-arginine (L-NMMA) was recorded. Three dosages of L-NMMA were given (0.05, 0.10, and 0.20 mg • min\(^{-1}\) • dL\(^{-1}\); 5 minutes per dose) to produce a dose-response curve. Baseline FBF recordings were performed again ≥60 minutes after infusion of L-NMMA, after FBF had returned to baseline values. Then, FBF response to graded intra-arterial infusion of NE (10, 20, and 40 • ng • min\(^{-1}\) • dL\(^{-1}\); 5 minutes per dose) was registered. Thereafter, FBF returned to baseline values within 30 minutes. After baseline FBF measurements were taken, FBF response to increasing dosages of ACh (0.5, 2.0, and 8.0 mg • min\(^{-1}\) • dL\(^{-1}\); 5 minutes per dose) was recorded. By ≥15 minutes after the infusion of ACh was stopped, forearm ischemia was achieved by inflating a cuff around the upper arm up to 200 mm Hg for 13 minutes; dynamic exercise (20 to 30 hand contractions) was performed during the last minute of ischemia. Flow was measured at 20-second intervals for the first 2 minutes after ischemic release to determine maximal FBF. This postocclusive reactive hyperemia test (PORH) was used to exclude structural abnormalities and to assess maximal vasodilator capacity.11

After the final dose of L-NMMA, 2 to 4 µCi 125I-albumin (code IM 17 P, Amershams Intnl) was given as an intravenous bolus injection. During 60 minutes after injection, 7 blood samples were collected at regular intervals. Plasma radioactivity was measured in each sample with a scintillation detector (automatic γ-counter, 1480 Wizard 3; Wallac). Plasma volume was determined from retropilation of the disappearance curve to time zero and from the injected volume of the tracer. Calculations were made only when correlation coefficient between time points for blood sampling and the corresponding values of ln (plasma radioactivity) were >0.85.12 In a pilot study, we found no influence of local arterial ischemia of L-NMMA on the clearance of 125I-albumin.

After completion of the study, we selected 24 DP with arterial plasma NE concentrations in the highest (n=12) or lowest (n=12) quartile. In these DP, cardiovascular autonomic function was assessed from BP response to sustained handgrip and from heart rate variation during controlled forced breathing, standing up, andValsalva maneuver.13 These tests were performed to find whether the observed reduction in plasma NE in DP resulted from subclinical autonomic neuropathy. Findings were compared with reference values from healthy subjects matched for age and gender.13

In all participants, 24-hour ambulatory BP was measured with an automatic BP device (Profilomat, Disetronic Medical Systems AG) on a normal working day. Raw data were manually checked and inappropriate readings removed.14 BP readings between 10 AM and 11 PM were averaged as “daytime” BP and between 1 and 7 AM as “nighttime” BP.

Drugs

Drugs were dissolved in saline (NaCl, 0.9%), at the start of the study. L-NMMA was purchased from Clinalfa A.G.; NE from Centrafarm Services B.V.; and ACh from Dispersa A.G.

Biochemical Measurements

Plasma arterial NE and epinephrine were determined by an accurate assay.15 Plasma glucose was measured using a standard glucose oxidation method. During the study, glucose was measured hourly in arterial blood using Glucocard (Menarini Diagnostics). Insulin was measured with an insulin-specific double-antibody radioimmunoassay (interassay coefficient of variation, 6.2%). Hemoglobin A\(_{1c}\) (HbA\(_{1c}\)) was measured using a high-performance liquid chromatographic technique (Bio-Rad Diamat), with reference values of 4.8% to 6.2%. FFA were measured using an enzymatic colorimetric method (ACS-ACOD method, Wako Chemicals).

Statistical Analysis and Data Report

FBF is expressed as milliliters per minute per deciliter of forearm tissue. Because BP was not significantly affected by infusion of either drug (see Results), changes in FBF were assumed to represent changes in vascular tone. Therefore, forearm vascular resistance was not calculated.16 FBF measurements obtained during the last 3 minutes of each 5-minute period of placebo or drug infusion were averaged. During these 3 minutes, FBF showed a steady state for all infusion rates. For the vasodilator response to forearm ischemia, the maximal FBF after deflation of the cuff was used. To quantify the overall response to L-NMMA and NE, we calculated absolute and percentage changes by using the preceding baseline recordings. To overcome differences in baseline flow and to correct for systemic influences, responses are also described as FBF (left/right) ratio (FBBF \(_{\text{left}}\)/FBBF \(_{\text{right}}\)).16

Because we used fixed dosages based on forearm volume, (fictive) concentration of the drug in the forearm will depend on FBF. Because FBF varies considerably between individuals, dose-response curves corrected for flow were calculated; the standard dose of the infused drug was divided by the resulting FBF in steady state. The responses to ACh were expressed as the ratio of FBF during infusion of ACh and individual maximal FBF after ischemia.11 We used this ratio to overcome individual differences in baseline and maximal flow. Differences in baseline levels between groups were analyzed by the Student t test or Mann-Whitney U test when appropriate. We evaluated differences in vascular responses to the various drugs and dosages between groups by use of repeated-measures ANOVA.

To analyze differences in relative change in FBF between groups and related to concentration, a generalized mixed linear model was postulated with group, concentration, and their interaction as fixed factors and DP or CS as random factor. If residuals appeared to be nongaussian, the same analysis was done on ranks of observations. Computations were done using Proc Mixed (SAS) and the SPSS software package. Correlations were calculated using the Pearson r test for normally distributed data and Spearman test for nonparametric data.

Results

Baseline Data

Baseline characteristics of DP and CS are summarized in Table 1. Mean HbA\(_{1c}\) was 8.4+±0.2% in DP. Insulin concentration was significantly increased in DP. Baseline glucose in DP averaged 11.4±±0.6 mmol/L (range, 4 to 22 mmol/L) and remained stable throughout the experiment. FFA were slightly but not significantly increased in DP. Baseline FBF was significantly higher in DP (2.9±±0.1 versus 2.0±±0.1 mL • min\(^{-1}\) • dL\(^{-1}\) in CS, Table 2, P<0.01).

Baseline and Stimulated NO Release

L-NMMA produced a dose-dependent vasoconstriction (P<0.001 in both groups, Table 2). Maximal percentage change in absolute FBF was 28.5+±1.5% in DP and 31.2+±2.2% in CS (P=NS). L-NMMA (0.05, 0.10, and 0.20 mg • min\(^{-1}\) • dL\(^{-1}\)) produced a dose-dependent vasoconstriction. Baseline FBF during placebo infusion (NaCl, 0.9%) was significantly reduced in DP compared with CS (P<0.05). Maximal percentage change in FBF after infusion of L-NMMA was significantly greater in DP than in CS, although FBBF \(_{\text{max}}\) was similar between groups. Maximal percentage change in FBF after infusion of NE was also greater in DP than in CS, although FBBF \(_{\text{max}}\) was similar between groups. Maximal percentage change in FBF after infusion of ACh was not different between groups.

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TABLE 1. Characteristics of DP and CS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F), n</td>
<td>25/25</td>
<td>25/25</td>
</tr>
<tr>
<td>Age, y</td>
<td>28.4±1.0</td>
<td>28.3±0.9</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.2±0.4</td>
<td>22.7±0.5</td>
</tr>
<tr>
<td>Nonsmoker/smoker, n</td>
<td>31/19</td>
<td>35/15</td>
</tr>
<tr>
<td>Arterial systolic/diastolic BP, mm Hg</td>
<td>116±1/62±1</td>
<td>116±1/63±1</td>
</tr>
<tr>
<td>Daytime systolic/diastolic BP, mm Hg</td>
<td>121.4±1.9/64.3±1.8</td>
<td>121.4±2.1/64.0±1.7</td>
</tr>
<tr>
<td>Nighttime systolic/diastolic BP, mm Hg</td>
<td>102.8±1.7/69.4±1.5</td>
<td>104.0±1.9/69.8±1.5</td>
</tr>
<tr>
<td>Daytime/nighttime heart rate, bpm</td>
<td>80.9±2.5/62.5±1.7</td>
<td>77.7±2.5/63.8±1.8</td>
</tr>
<tr>
<td>Plasma volume, mL/L 1.73 m²</td>
<td>2808±59</td>
<td>2829±62</td>
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<tr>
<td>Ankle/brachial index</td>
<td>1.09±0.01</td>
<td>1.09±0.01</td>
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<tr>
<td>Forearm volume, mL</td>
<td>947±21</td>
<td>934±25</td>
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<tr>
<td>Waist-to-hip ratio</td>
<td>0.89±0.01</td>
<td>0.89±0.01</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>8.3±0.8</td>
<td>...</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>11.4±0.6</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>HbA₁c, %</td>
<td>8.4±0.2*</td>
<td>5.0±0.1</td>
</tr>
<tr>
<td>FFA, mmol/L</td>
<td>0.46±0.03</td>
<td>0.39±0.02</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.1±0.1</td>
<td>4.3±0.2</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>0.9±0.1</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>HDL-cholesterol, mmol/L</td>
<td>1.3±0.0</td>
<td>1.2±0.0</td>
</tr>
<tr>
<td>LDL-cholesterol, mmol/L</td>
<td>2.4±0.1</td>
<td>2.6±0.2</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>121±12*</td>
<td>46±3</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SE.
*P<0.01 for diabetic patients vs control subjects.

mg · min⁻¹ · dL⁻¹) reduced the FBF ratio in DP by 12.5±1.2%, 21.9±1.4%, and 29.8±1.4%, respectively, and in CS by 14.0±2.0%, 22.0±2.1%, and 29.9±2.1% (P=NS between groups). A dose-response curve corrected for flow (concentration-response curve) is shown in Figure 1. The concentration-response curves were exactly the same in both groups. The slopes of the linear relationship of the logarithm of concentration and vasoconstricting response to L-NMMA were not different between groups (27.8±1.5 in DP and 26.2±1.7 in CS; P=NS).

ACh (0.5, 2.0, and 8.0 mg · min⁻¹ · dL⁻¹) produced a dose-dependent increase in FBF (P<0.001, Table 2). The maximal FBF during the highest ACh dose was not different between the groups (23.3±1.8 in DP versus 20.1±1.6 mL · min⁻¹ · dL⁻¹ in CS). After 13 minutes of forearm ischemia, mean maximal FBF averaged 43.5±1.5 mL · min⁻¹ · dL⁻¹ in DP and was similar to the corresponding maximal FBF of 41.0±1.7 mL · min⁻¹ · dL⁻¹ in CS (P=NS). In DP, the ratio of FBF during ACh infusion and the maximal FBF (PORH) increased by 21.1±1.7%, 33.7±2.8%, and 52.8±3.7% for dosages of 0.5, 2.0, and 8.0 mg · min⁻¹ · dL⁻¹, respectively. ACh increased this ratio in CS by 13.9±1.2%, 26.8±2.4%, and 50.4±3.4%, respectively (Figure 2; P=NS between groups).

TABLE 2. Baseline FBF and Its Response to 3 Different Dosages of Vasoactive Drugs in Healthy CS and Type 1 DP

<table>
<thead>
<tr>
<th>Vasoactive Drug</th>
<th>Placebo</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-NMMA</td>
<td></td>
<td>2.0±0.1</td>
<td>1.7±0.1</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>Type 1 DP</td>
<td></td>
<td>2.9±0.1</td>
<td>2.5±0.1</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>Acetylcholine</td>
<td></td>
<td>2.0±0.1</td>
<td>5.5±0.5</td>
<td>10.7±1.0</td>
</tr>
<tr>
<td>Type 1 DP</td>
<td></td>
<td>3.0±0.2</td>
<td>9.0±0.7</td>
<td>14.6±1.2</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td></td>
<td>1.9±0.1</td>
<td>1.4±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>Type 1 DP</td>
<td></td>
<td>2.7±0.1</td>
<td>1.9±0.1</td>
<td>1.7±0.1</td>
</tr>
</tbody>
</table>

FBF is measured in mL · min⁻¹ · dL⁻¹. For discussion of the data, see Results.

Figure 1. Concentration-response curve of intra-arterial infusion of L-NMMA in type 1 DP (■) and healthy CS (□). Percentage changes in absolute FBF are shown on the y axis, whereas the dosage of L-NMMA (0.05, 0.10, and 0.20 mg·min⁻¹·dL⁻¹) divided by FBF (mL·min⁻¹·dL⁻¹) in steady state is shown on the x axis (P=NS).
**α-Adrenergic Receptor–Mediated Vascular Tone**

Mean arterial plasma NE concentration was 0.57 ± 0.03 nmol/L in DP and 0.81 ± 0.05 in CS (P < 0.01). Plasma epinephrine concentrations were not significantly different between the groups (0.19 ± 0.02 nmol/L in DP and 0.21 ± 0.02 in CS). Intra-arterially infused NE produced dose-dependent vasoconstriction (Table 2; P < 0.001 in both groups). Maximal percentage change of absolute FBF was 45.8 ± 2.4% in DP and 39.8 ± 3.0% in CS (P = 0.09). NE (10, 20, and 40 ng·min⁻¹·dL⁻¹) reduced FBF ratio in DP by 27.9 ± 5.1%, 36.8 ± 2.0%, and 46.9 ± 2.2%, respectively, and in CS by 21.8 ± 2.9%, 30.5 ± 3.1%, and 40.1 ± 3.2%, respectively (P = NS between groups).

A concentration-response curve for NE is shown in Figure 3. Analysis of the generalized linear model showed a dose-response relationship in both groups and showed that the slope of the linear logarithmic relationship is steeper in DP (31.3 ± 1.5 versus 24.3 ± 1.8 in CS, P < 0.01).

During the various procedures, no significant changes to contralateral FBF occurred. No significant changes were noted in BP after infusion of L-NMMA, ACh, or NE in either group (data not shown). Standardized cardiovascular autonomic tests disclosed no differences between DP in the higher or lower quartile of NE concentration or with values of age- and sex-matched controls (data not shown).

In DP, no significant correlation existed between the response to L-NMMA, ACh, or NE and the duration of diabetes; HbA₁c; levels of total cholesterol, triglycerides, and FFA; concentrations of insulin, glucose, and plasma NE; and baseline flow (data not shown). Weak correlation existed between basal FBF and blood glucose level (Spearman, r = 0.40 [95% CI, 0.14 to 0.62]; P = 0.01; Figure 4) and between basal FBF and FFA levels (Spearman, r = 0.29 [95% CI, 0.01 to 0.53]; P = 0.03). No correlations were observed between basal FBF and HbA₁c, plasma NE concentration, total cholesterol, insulin concentration, or diabetes duration (data not shown). In view of the observed correlations between basal FBF, blood glucose level, and FFA and the possible confounding effect of elevated glucose levels, we performed a subanalysis in 11 DP with glucose values during the study of 4 to 7 mmol/L (normoglycemic; mean glucose, 5.9 ± 0.7 mmol/L) and 11 DP with glucose values of 14 to 22 mmol/L (hyperglycemic; mean glucose, 17.3 ± 2.6 mmol/L) (Table 3). FFA and basal FBF were significantly increased in the hyperglycemic DP compared with the normoglycemic group. However, FBF was still increased in the normoglycemic DP compared with CS. The responses to L-NMMA, ACh, and NE were comparable between both diabetic groups. In the normoglycemic DP, plasma NE was significantly reduced compared with CS (0.51 ± 0.16 versus 0.81 ± 0.37 nmol/L, P < 0.01).

**Discussion**

In patients with type 1 diabetes, a generalized increase in blood flow is found.¹²,¹⁷–¹⁹ Such an increase in blood flow has been suggested to be instrumental in the development of diabetic microangiopathy. We have studied several parameters of vascular control in type 1 DP without evidence of complications. Specifically, all DP had normoalbuminuria, normal BP, and normal vascular response to ischemia and ACh, which are indicators of vascular injury.²²

Our study confirms that skeletal muscle blood flow is increased in the early phase of diabetes. Some investigators have found no significant difference in baseline flow in DP; however, the number of patients in these studies was small.²³
TABLE 3. Subanalysis of Normoglycemic and Hyperglycemic DP

<table>
<thead>
<tr>
<th>Variables</th>
<th>Controls</th>
<th>Normoglycemic</th>
<th>Hyperglycemic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mmol/L</td>
<td>5.1±0.4</td>
<td>5.9±0.7</td>
<td>17.3±2.6</td>
</tr>
<tr>
<td>FFA, mmol/L</td>
<td>0.39±0.14</td>
<td>0.34±0.20</td>
<td>0.67±0.35*</td>
</tr>
<tr>
<td>Insulin, pmol/L</td>
<td>45.6±19.2</td>
<td>169.8±117.6</td>
<td>116.4±65.4</td>
</tr>
<tr>
<td>FBF, mL·min⁻¹·dl⁻¹</td>
<td>2.0±0.6</td>
<td>2.4±0.7†</td>
<td>3.4±1.3‡</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>4.9±0.4</td>
<td>7.9±1.2</td>
<td>9.3±1.7</td>
</tr>
<tr>
<td>NE, nmol/L</td>
<td>0.81±0.37</td>
<td>0.51±0.16</td>
<td>0.62±0.25</td>
</tr>
<tr>
<td>NE % vasoconstriction</td>
<td>25.9/30/39.8</td>
<td>27.1/34/41/43.5</td>
<td>26.8/36.2/45.1</td>
</tr>
<tr>
<td>L-NMMA % vasoconstriction</td>
<td>17.4/25.4/31.2</td>
<td>11.8/23.2/29.3</td>
<td>12.2/21.6/27.6</td>
</tr>
<tr>
<td>ACh/PORH</td>
<td>50.4±24.6</td>
<td>41.9±20.5</td>
<td>66.5±23.8</td>
</tr>
</tbody>
</table>

Data are expressed as mean±SD.

*P<0.05 vs normoglycemic DP and CS; †P<0.05 vs CS; ‡P<0.01 vs normoglycemic DP and CS.

Normoglycemic DP had glucose levels of 4 to 7 mmol/L; hyperglycemic, 14 to 22 mmol/L. NE % vasoconstriction indicates percentage reduction in FBF during intra-arterial infusion of norepinephrine in a dosage of 10, 20, and 40 ng·min⁻¹·dl⁻¹, respectively; L-NMMA % vasoconstriction, percentage reduction in FBF during intra-arterial infusion of L-NMMA in a dosage of 0.05, 0.1, and 0.2 mg·min⁻¹·dl⁻¹, respectively; ACh/PORH, ratio of FBF during intra-arterial infusion of acetylcholine (ACh, 8.0 mg·min⁻¹·dl⁻¹) and maximal FBF during the PORH.

Furthermore, glucose was controlled by euglycemic clamping (see discussion below).

We found no evidence for a role of the endothelium-derived vasodilator NO in DP, because the vasoconstrictive response to L-NMMA was similar in DP and CS. In experimental diabetes, basal endogenous NO accounts for the increase in renal perfusion, because increases in renal blood flow can be blocked by inhibitors of constitutive NO synthesis. From in vivo animal experiments, no data are available on the role of NO in hyperperfusion of other vascular beds. To our knowledge, studies on the role of NO in glomerular hyperfiltration in humans have not been published. Therefore, our conclusions may be applicable only to the skeletal muscle vascular bed. Several investigators have determined vascular response to L-NMMA in the human forearm. In these studies, either no difference or a decreased vasoconstrictive response was observed for L-NMMA in the skeletal muscle vascular bed. Several investigators have speculated on the role of NO in hyperperfusion of other vascular beds. To our knowledge, studies on the role of NO in DP have been equivocal. The increase in vascular responsiveness of NE seen in our DP is compatible with receptor upregulation expected to occur in situations of sympathetic nerve denervation and low NE levels. On the basis of our findings, we suggest that reduced sympathetic drive may, at least in part, be responsible for the reduced peripheral vascular resistance seen in noncomplicated type 1 diabetes.

A decrease in other endogenous vasoconstrictive mediators could also be involved. Some investigators have found a decrease in plasma endothelin and angiotensin levels in early diabetes. An altered sensitivity to both mediators, however, has not unequivocally been proven yet in humans in vivo.

One could speculate as to potential mechanisms that underlie the decrease in sympathetic drive. Hyperglycemia and hyperinsulinemia increase tubular sodium reabsorption, which causes extracellular volume expansion. An increase in BP or cardiac filling pressure will lead to decreased sympathetic tone and reduced peripheral resistance. However, plasma volume and BP were similar in noncomplicated DP and CS. Furthermore, we found no relation between glucose values or HbA₁c and plasma NE concentration and vascular response to NE. Finally, one could speculate that autonomic diabetic neuropathy is responsible for the decrease in sympathetic drive. However, cardiovascular autonomic responses were not different between DP with plasma NE concentrations in the lowest and highest quartiles, nor did they differ from those of CS. The night/day ratios of BP and heart rate were the same in DP and CS. Nevertheless, we cannot rule out that more advanced techniques (ie, microneurography) will reveal early autonomic dysfunction in these patients.

One important aspect concerns the role of hyperglycemia per se. A main issue when studying DP is the selection of the most appropriate experimental conditions. The study of DP in conditions of normoglycemia and normoinsulinemia is difficult. The only way to achieve this is to infuse low doses of insulin into the portal vein, a method clearly not available for routine use. Therefore, investigators are left with 2 options, either to study DP under euglycemic (hyperinsulinemic) conditions or to study DP in a fasting state with insulin levels as low as possible. In the latter case, glucose levels will be moderately increased, as is often the case in normal daily life of these patients. We think that either condition is relevant for the study of patients with diabetes.

For the present study, we chose to withhold the morning insulin dose to obtain low insulin levels to overcome the well-documented effects of insulin on vascular tone (vasodilation) and sympathetic activity (stimulation). As a result, glucose levels in our DP were slightly to moderately elevated. Prolonged local hyperglycemia has not been shown to affect FBF in healthy humans. In contrast, hyperglycemia has been
reported to cause increases in retinal and renal blood flow.9,19
Furthermore, basal blood flow in DP has been claimed to have been normalized during euglycemic clamp.23 However, in the latter study, CS were not subjected to euglycemic clamp.23
Besides, infusion of insulin has been demonstrated to increase sympathetic activity and NE levels.9 In view of the increased vasoconstrictive response to NE in DP, a euglycemic clamp is likely to lead to “pseudonormalization” of FBF. In this way, the procedure itself could cause a difference in NE vasoreactivity between DP and CS to be missed.
In the present study, we found a weak correlation between fasting glucose and FFA on one hand and basal FBF on the other. An increase in blood glucose or metabolic factors related to elevated glucose levels (eg, an increase in FFA) may be, in part, responsible for the higher FBF in the subgroup of hyperglycemic DP. However, subanalysis of DP with normal fasting glucose levels (between 4 and 7 mmol/L) showed a significant increase in FBF compared with CS. In this subgroup, glucose and FFA were normal. The combination of observations in this selected group proves that hyperglycemia did not confound our key conclusions.
From the present study, we therefore conclude that resting basal FBF is increased in noncomplicated type 1 diabetes. There is no evidence that basal NO synthesis is increased, whereas the vasoconstricting response to NE is increased, which suggests a chronic decrease in sympathetic drive. The decrease in sympathetic tone may, in part, be responsible for the decrease in vascular tone.

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Elevated Skeletal Muscle Blood Flow in Noncomplicated Type 1 Diabetes Mellitus: Role of Nitric Oxide and Sympathetic Tone
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