Effects of Autonomic Neuropathy on Coronary Blood Flow in Patients With Diabetes Mellitus

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Background—Cardiac sympathetic signals play an important role in the regulation of myocardial perfusion. We hypothesized that sympathetically mediated myocardial blood flow would be impaired in diabetics with autonomic neuropathy.

Methods and Results—We studied 28 diabetics (43±7 years old) and 11 age-matched healthy volunteers. PET was used to delineate cardiac sympathetic innervation with [11 C]hydroxyephedrine ([11 C]HED) and to measure myocardial blood flow at rest, during hyperemia, and in response to sympathetic stimulation by cold pressor testing. The response to cardiac autonomic reflex tests was also evaluated. Using ultrasonography, we also measured brachial artery reactivity during reactive hyperemia (endothelium-dependent dilation) and after sublingual nitroglycerin (endothelium-independent dilation). Based on [11 C]HED PET, 13 of 28 diabetics had sympathetic-nerve dysfunction (SND). Basal flow was regionally homogeneous and similar in the diabetic and normal subjects. During hyperemia, the increase in flow was greater in the normal subjects (284±88%) than in the diabetics with SND (187±680%, P=0.084) and without SND (177±72%, P=0.028). However, the increase in flow in response to cold was lower in the diabetics with SND (14±10%) than in those without SND (31±12%) (P=0.015) and the normal subjects (48±24%) (P<0.001). The flow response to cold was related to the myocardial uptake of [11 C]HED (P<0.001). Flow-mediated brachial artery dilation was impaired in the diabetics compared with the normal subjects, but it was similar in the diabetics with and without SND.

Conclusions—Diabetic autonomic neuropathy is associated with an impaired vasodilator response of coronary resistance vessels to increased sympathetic stimulation, which is related to the degree of SND. (Circulation. 1999;100:813-819.)

Key Words: nervous system, autonomic diabetes mellitus blood flow tomography endothelium

Autonomic neuropathy is a common and serious consequence of diabetes mellitus. Between 20% and 40% of randomly selected diabetics show abnormal autonomic function on clinical testing, which suggests significant subclinical autonomic nerve damage even when diabetes is first diagnosed. Recent evidence indicates that alterations of cardiac sympathetic pathways, as determined by myocardial scintigraphy, may be present in a substantial number of diabetics in the absence of apparent autonomic neuropathy as determined by autonomic reflex tests.

Symptomatic autonomic neuropathy is a marker of poor prognosis, particularly when it affects the sympathetic nervous system. Silent myocardial ischemia and infarction and sudden death are common causes of death and disability among diabetics with overt autonomic neuropathy. However, the mechanisms underlying these associations are not well understood. We have recently shown that cardiac efferent sympathetic signals play an important role in regulating myocardial perfusion. The present study was designed to test the hypothesis that sympathetically mediated myocardial blood flow would be impaired in diabetics with autonomic neuropathy.

Methods

Study Population
We studied 28 persons 24 to 50 years old with type 1 or type 2 diabetes mellitus (Table 1). The duration of diabetes ranged from 1 to 45 years, averaging 16±12 years. The control population consisted of 11 age-matched healthy volunteers. All subjects in the study had a low probability of coronary artery disease based on the absence of cardiovascular symptoms, a normal resting ECG, and a normal maximal exercise ECG and echocardiogram. All subjects had normal left ventricular function, and none had evidence of left ventricular hypertrophy.

Study Design
The Human Investigation Committee of Wayne State University approved the study protocol, and all participants gave written informed consent. Each subject made 2 visits to the study hospital, during which time cardiac sympathetic-nerve function, myocardial blood flow, and brachial artery reactivity were assessed. Cardiac...
sympathetic innervation and myocardial blood flow were evaluated with a whole-body PET scanner (Siemens/CTI EXACT HR).

All subjects refrained from caffeine-containing beverages and theophylline-containing medications for 24 hours before each hospital visit. Two patients with diabetes were receiving calcium channel blockers for mild hypertension, and 8 patients were receiving low-dose ACE inhibitors, all of which were withheld for 24 hours before the study. None of the patients received medications known to interfere with catecholamine uptake in presynaptic nerve terminals. All subjects were studied in the fasting state.

**PET Imaging**

**Assessment of Cardiac Sympathetic Nerve Terminals**
Cardiac sympathetic innervation was evaluated by use of the norepinephrine analogue [13 N]ammonia. A 15-minute transmission scan was acquired for correction of photon attenuation. Beginning with the bolus administration of [13 N]ammonia 0.286 mCi/kg IV, serial images were acquired for 40 minutes.

**Assessment of Myocardial Blood Flow**
By use of [11 C]hydroxyephedrine, myocardial blood flow was measured at rest, during hyperemia, and in response to cold pressor testing (CPT). A 15-minute transmission scan was acquired for correction of photon attenuation. Beginning with a bolus administration of [11 C]hydroxyephedrine 0.286 mCi/kg IV, serial images were acquired for 40 minutes. Thirty minutes later, adenosine 0.14 mg/kg was infused, and images were recorded in the same acquisition sequence. Three minutes into the adenosine infusion, a second dose of [11 C]hydroxyephedrine was injected, and images were recorded in the same acquisition sequence. The heart rate, systemic blood pressure, and 12-lead ECG were recorded at baseline and throughout the infusion of adenosine and the CPT.

**Data Analysis**
To quantify the regional myocardial catecholamine storage and coronary blood flow, identical regions of interest (ROIs) encompassing the left anterior descending, circumflex, and right coronary artery territories were automatically assigned to each of 4 midventricular short-axis slices of the [11 C]hydroxyephedrine and [13 N]ammonia images, as previously described. An additional small circular ROI was manually placed in the center of the left ventricular blood pool of each image set to obtain the arterial input function. The corresponding ROIs were then copied to the entire [11 C]hydroxyephedrine and [15 N]ammonia image sequences, and regional myocardial blood flow and blood pool time-activity curves were obtained. In each coronary territory, the retention fraction of [15 N]ammonia was calculated by dividing the [15 N]ammonia concentration in myocardial tissue at 12 minutes after injection by the integral of the [15 N]ammonia concentration in arterial blood. Regional myocardial blood flow was calculated by fitting the [15 N]ammonia time-activity curves with a 3-compartment tracer kinetic model. An index of coronary vascular resistance was calculated by dividing the mean aortic blood pressure by myocardial blood flow. The coronary vasodilator reserve was defined as the ratio between hyperemic and basal myocardial blood flow.

**Vascular Ultrasound**
Endothelial function was characterized by measuring the brachial artery response to reactive hyperemia (causing endothelium-dependent dilatation) and sublingual nitroglycerin (causing endothelium-independent dilatation), using high-resolution vascular ultrasound. Reactive hyperemia was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 300 mm Hg for 5 minutes, followed by release.

The brachial artery diameter was measured on B-mode ultrasound images with a standard 7- to 4-MHz linear-array transducer and an ATL Ultramark 9 HDI system. The intraobserver variability for repeated measurements of brachial arterial diameter in our laboratory is 0.021 ± 0.019 mm. The study subjects lay quietly for 15 minutes before the first scan and remained supine throughout the study.

The brachial artery was scanned in longitudinal sections 2 to 15 cm above the elbow. A baseline scan was obtained, and the arterial flow velocity was measured with a pulsed-Doppler signal at a 60° angle to the vessel, with the sample volume placed in the center of the artery. During reactive hyperemia, a series of scans were obtained for 90 seconds after cuff deflation, including a repeated recording of flow velocity at 30 seconds. Then, 20 to 30 minutes was allowed for recovery of the vessel, after which a second baseline scan was performed. Sublingual nitroglycerin spray (400 μg) was then administered, and 3 minutes later the last scan was performed.

Measurements of arterial diameter were taken at end diastole from the anterior to posterior adventitial-medial interface. Measurements

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**TABLE 1. Baseline Characteristics of Study Population**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Volunteers (n=11)</th>
<th>Diabetics SND− (n=15)</th>
<th>Diabetics SND+ (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>40±8</td>
<td>42±8</td>
<td>43±6</td>
</tr>
<tr>
<td>Male sex, n</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Type 1:type 2 diabetes, n</td>
<td>...</td>
<td>9.6</td>
<td>7.6</td>
</tr>
<tr>
<td>Duration of diabetes, y</td>
<td>...</td>
<td>18±15</td>
<td>14±10</td>
</tr>
<tr>
<td>Retinopathy, n</td>
<td>...</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Fasting blood glucose, mg/dL*</td>
<td>81±10</td>
<td>190±49†</td>
<td>197±62†</td>
</tr>
<tr>
<td>Glycohemoglobin, %*</td>
<td>...</td>
<td>10.1±2.7</td>
<td>10.6±1.8</td>
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<tr>
<td>Total cholesterol, mg/dL</td>
<td>163±25</td>
<td>199±42</td>
<td>189±32</td>
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<tr>
<td>HDL cholesterol, mg/dL</td>
<td>48±16</td>
<td>48±13</td>
<td>44±16</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>94±32</td>
<td>119±32</td>
<td>113±31</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>106±45</td>
<td>144±92</td>
<td>154±96</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.78±0.2</td>
<td>0.85±0.31</td>
<td>1.05±0.48</td>
</tr>
<tr>
<td>BUN, mg/dL</td>
<td>16±4</td>
<td>14±4</td>
<td>14±3</td>
</tr>
<tr>
<td>Urine albumin, μg/mg</td>
<td>8±7</td>
<td>40±45†</td>
<td>48±72†</td>
</tr>
<tr>
<td>vWF antigen, %</td>
<td>106±35</td>
<td>148±68†</td>
<td>135±29</td>
</tr>
</tbody>
</table>

*Fasting blood glucose and glycohemoglobin in the diabetics are average of 4 years.
†P<0.05 vs healthy volunteers.
of vessel diameter during reactive hyperemia were taken 60 seconds after deflation of the cuff. The vessel diameter in the scans obtained after reactive hyperemia and the administration of nitroglycerin was expressed as a percentage of the corresponding baseline scan. Volume flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by the cross-sectional area of the vessel. Reactive hyperemia was calculated as the maximal flow recorded in the first 30 seconds after cuff deflation divided by the flow during the first baseline scan.

**Autonomic Nerve Function Tests**

All subjects in the study were also evaluated for cardiac autonomic neuropathy by conventional cardiovascular reflex tests. Autonomic nerve function tests measuring mainly the parasympathetic limb included the heart rate variation during deep breathing, Valsalva ratio, and heart rate change with standing. In addition, we determined the blood pressure response to sustained handgrip and to standing, both of which are thought to reflect the integrity of the sympathetic nervous system.

**Laboratory Analyses**

Venous plasma and serum samples were taken after an overnight fast. Plasma glucose was measured by the glucose oxidase method. Serum cholesterol and triglyceride concentrations were measured by standard enzymatic methods. HDL cholesterol was measured with the Equal HDL Direct method and the Technicon DAX System (Bayer). LDL cholesterol was calculated by use of the Friedewald formula. Urine albumin excretion was measured by rate nephelometry (reference, 0 to 20 μg/min). von Willebrand factor (vWF) antigen was measured by immunoelectrohoresis. Glycated hemoglobin level was measured by high-performance liquid chromatography (reference, 4% to 8%). Serum creatinine and blood urea nitrogen were also obtained.

**Statistical Analysis**

Data are presented as mean±SD. Differences between groups were assessed with a paired or unpaired Student’s t test for continuous variables as appropriate and with a χ² test for discrete variables. Differences among multiple groups were investigated with repeated-measures ANOVA, followed by a Tukey test to allow pairwise testing for differences between groups. The determinants of myocardial blood flow in response to CPT were assessed by multiple linear regression analysis. Linear regression analysis was performed by least-squares fitting. A value of P<0.05 was used to define statistical significance.

**Results**

**Regional Myocardial Uptake and Storage of Catecholamines**

In the healthy volunteers, no regional differences in the myocardial uptake of [11C]hydroxyephedrine were noted by coronary artery territory (P=0.55) or by anatomic location within each territory (proximal versus distal) (P=0.49). In contrast, only the diabetics with cardiac sympathetic-nervous dysfunction (SND) (as defined below) showed a modest increase in [11C]hydroxyephedrine uptake in the left anterior descending compared with the right coronary artery territory (0.147±0.012 versus 0.140±0.013 minutes⁻¹, P=0.006). Within each vascular territory, however, diabetic subjects with (0.147±0.012 versus 0.139±0.017 minutes⁻¹, P<0.001) and without (0.204±0.023 versus 0.192±0.024 minutes⁻¹, P<0.001) SND showed increased [11C]hydroxyephedrine uptake in the proximal compared with the distal myocardial segments.

The results of [11C]hydroxyephedrine imaging were then used to determine the presence or absence of cardiac SND in the diabetic subjects. Accordingly, regional [11C]hydroxyephedrine retention values in each coronary artery territory were averaged in each subject. A mean myocardial uptake of [11C]hydroxyephedrine >2 SD below the normal mean value (0.19±0.02 minutes⁻¹, obtained by averaging the uptake values in the 11 healthy volunteers) was considered abnormal. On the basis of this definition, 13 diabetic patients had evidence of cardiac SND (0.14±0.02 minutes⁻¹), whereas 15 patients had no evidence of SND (0.20±0.02 minutes⁻¹) and thus served as diabetic control subjects. The 2 groups of diabetics were similar with respect to age, duration of diabetes, glycemic control, lipid profile, renal function, urine albumin, retinopathy, and vWF antigen levels (Table 1).

**Autonomic Reflex Tests**

The results of [11C]hydroxyephedrine imaging were also compared with those of conventional autonomic reflex tests. Clinical assessment of autonomic function in the diabetics showed abnormal responses to none of the 5 standard maneuvers in 21% of subjects, to 1 maneuver in 39%, to 2 maneuvers in 29%, and to 3 maneuvers in 11%. None had a positive response to maneuvers to assess the integrity of the sympathetic nervous system. Only 6 of 11 diabetics (55%) with clinical autonomic dysfunction based on classic diagnostic criteria (≥2 abnormal tests) had significant abnormalities in cardiac sympathetic innervation, as defined by PET. Conversely, 7 of 17 patients (41%) without clinical autonomic dysfunction had significant abnormalities in cardiac sympathetic innervation, consistent with previous data.

**Systemic Hemodynamics**

The heart rate and rate-pressure product increased similarly with CPT and the infusion of adenosine in both groups of diabetics and in the healthy volunteers (Table 2). Likewise, systolic and mean aortic blood pressure increased similarly in all 3 groups during CPT but remained unchanged during the infusion of adenosine.

**Regional Myocardial Blood Flow and Coronary Vascular Resistance**

**Baseline**

The baseline blood flow was regionally homogeneous and was similar in the diabetics and the healthy volunteers despite the differences in cardiac sympathetic-nerve function (Table 3).

**Blood Flow Response to the CPT**

During the CPT, blood flow increased significantly in the 3 groups studied (Table 3). However, the magnitude of flow increase was significantly lower in the diabetics with SND (14±10%) than in those without SND (31±12%) (P=0.015) and the healthy volunteers (48±24%) (P<0.05 versus both groups of diabetics) (Figure 1). Coronary vascular resistance index fell only in the diabetics without SND and the healthy volunteers.

In univariate analysis, the predictors of the flow response to CPT were the duration of diabetes (R=0.46, P=0.018), a history of smoking (R=−0.36, P=0.042), HDL cholesterol (R=0.36, P=0.055), and the magnitude of [11C]hydroxyephedrine retention (R=0.68, P<0.001). We then per-
formed a stepwise multiple regression analysis to determine independent predictors of the flow response to CPT, including sex, duration of diabetes, a history of smoking, plasma glucose, glycohemoglobin, total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, urine albumin, vWF antigen level, and the magnitude of $^{11}$C-hydroxyephedrine retention. In the final model, the only significant predictors of the flow response to CPT were the magnitude of $^{11}$C-hydroxyephedrine retention ($R^2$ change=0.45, $F=13.71$, $P=0.002$), the duration of diabetes ($R^2$ change=0.14, $F=5.29$, $P=0.035$), and the vWF antigen level ($R^2$ change=0.10, $F=5.05$, $P=0.04$).

Despite the modest regional differences in sympathetic innervation, no significant differences in flow were noted by coronary artery territory ($P=0.08$) or by anatomic location within each territory ($P=0.60$) in either group of diabetics. Nevertheless, we observed highly significant correlations between the magnitude of $^{11}$C-hydroxyephedrine retention and the flow response to CPT on both a regional ($r=-0.163+2.005x$, $R^2=0.305$, $SE=0.12$, $F=68.554$, $P<0.001$) and a global basis (Figure 2).

### Blood Flow Response to Adenosine Infusion

During hyperemia, blood flow increased and coronary vascular resistance decreased homogeneously and significantly in both groups of diabetics and the healthy volunteers (Table 3). However, peak myocardial blood flow was significantly higher in the healthy subjects than in either group of diabetics. Consequently, estimates of coronary vasodilator reserve were higher in the normal subjects than in the diabetics (Figure 1).

### Brachial Artery Reactivity

The degree of flow increase during reactive hyperemia caused by cuff inflation and release was similar in the diabetics and the healthy volunteers (Table 4). Despite this similar increase in flow, brachial artery dilation in the diabetics was lower than in the healthy volunteers (Table 4). However, flow-mediated brachial artery dilation was similar in diabetics with and without SND. In contrast, nitroglycerin-induced brachial artery dilation was similar in the 3 groups studied. The correlation between the coronary blood flow response to CPT and flow-mediated brachial artery dilation was poor and of marginal statistical significance ($r=0.28$, $P=0.063$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Healthy Volunteers</th>
<th>Diabetics SND− (n=15)</th>
<th>Diabetics SND+ (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myocardial blood flow, mL·min$^{-1}·g^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.90±0.16</td>
<td>0.96±0.17</td>
<td>0.91±0.16</td>
</tr>
<tr>
<td>Cold pressor test</td>
<td>1.39±0.39*</td>
<td>1.26±0.27*</td>
<td>1.03±0.18†</td>
</tr>
<tr>
<td>Adenosine</td>
<td>3.18±0.49‡</td>
<td>2.53±0.61*</td>
<td>2.48±0.43*</td>
</tr>
<tr>
<td>Coronary flow reserve</td>
<td>3.04±0.72§</td>
<td>2.59±0.54</td>
<td>2.50±0.43</td>
</tr>
<tr>
<td>Coronary vascular resistance, mm Hg·mL$^{-1}·min^{-1}·g^{-1}$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>92±20</td>
<td>97±20</td>
<td>100±19</td>
</tr>
<tr>
<td>Cold pressor test</td>
<td>74±15*</td>
<td>84±20*</td>
<td>105±16*</td>
</tr>
<tr>
<td>Adenosine</td>
<td>26±6*¶</td>
<td>36±12*</td>
<td>37±8*</td>
</tr>
</tbody>
</table>

*P<0.05 vs corresponding value at baseline.
†P=0.016 vs healthy volunteers and $P=0.097$ vs diabetics SND−.
‡P=0.001 vs both groups of diabetics.
§P=0.05 vs both groups of diabetics.
¶P=0.004 vs healthy volunteers and $P=0.049$ vs diabetics SND−.
§P=0.05 vs both groups of diabetics.

### Table 2. Systemic Hemodynamics in the Diabetics and the Healthy Volunteers

<table>
<thead>
<tr>
<th>Hemodynamic Measure</th>
<th>Baseline</th>
<th>CPT</th>
<th>Adenosine Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteers (n=11)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>68±9</td>
<td>77±13*</td>
<td>101±10*</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>113±11</td>
<td>129±22*</td>
<td>112±14</td>
</tr>
<tr>
<td>Mean aortic</td>
<td>81±7</td>
<td>94±15*</td>
<td>81±9</td>
</tr>
<tr>
<td>Rate-pressure product</td>
<td>0.76±0.15</td>
<td>10.1±0.30*</td>
<td>11.4±0.23*</td>
</tr>
<tr>
<td>Diabetics SND− (n=15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>74±8</td>
<td>80±6*</td>
<td>99±9*</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>129±16†</td>
<td>147±29*</td>
<td>125±25</td>
</tr>
<tr>
<td>Mean aortic</td>
<td>89±9</td>
<td>101±15*</td>
<td>84±14</td>
</tr>
<tr>
<td>Rate-pressure product</td>
<td>0.96±0.16†</td>
<td>11.8±0.27*</td>
<td>12.3±0.26*</td>
</tr>
</tbody>
</table>

*P<0.05 vs corresponding value at baseline.
†P<0.05 vs healthy volunteers.
Diabetes mellitus has long been known to predispose people to premature atherosclerotic coronary disease. Diabetic autonomic neuropathy has been linked to silent cardiac ischemia and infarction and increased cardiovascular mortality.\(^5,6\)

However, the mechanisms underlying these associations are not well understood. Our findings provide evidence that diabetic autonomic dysfunction affecting cardiac efferent sympathetic signals is an important determinant of impaired coronary blood flow during increased sympathetic stimulation. In this study, sympathetically mediated myocardial blood flow increased by 31\(\pm\)6\% in diabetics without SND and by only 14\(\pm\)10\% in diabetics with SND. This impaired coronary flow response to cold was related to the degree of cardiac SND, as assessed by PET.

Our findings also suggest that even early, preclinical stages of autonomic neuropathy may be associated with abnormalities in cardiac sympathetic function that can result in impaired coronary flow regulation, particularly in response to stress. Indeed, 41\% of patients without clinical autonomic dysfunction based on classic diagnostic criteria had significant abnormalities in cardiac sympathetic innervation, as defined by PET. \[^{1}\]C]hydroxyephedrine imaging allows for a direct and quantitative assessment of the integrity of cardiac sympathetic-nerve fibers that can detect lesions at an earlier preclinical stage, before autonomic neuropathy can be detected by tests that only indirectly reflect autonomic nerve function.\(^2,4,7\)

We have previously reported that the coronary blood flow response to sympathetic stimulation is related to the integrity of cardiac sympathetic pathways and is largely independent of changes in systemic hemodynamics and circulating catecholamines.\(^7\) The potential role of changes in regional contractility causing metabolically mediated coronary vasodilation in response to neurally released norepinephrine (a \(\beta_1\)- and \(\alpha\)-adrenergic agonist) has been addressed previously.\(^7\) Alternatively, sympathetically mediated coronary vasodilation may result from direct stimulation of \(\alpha_2\)-adrenergic receptors in intact endothelial cells and release of nitric oxide. Indeed, norepinephrine and BHT 920 (a selective \(\alpha_2\)-adrenergic receptor agonist) induce dose-dependent increases in nitrate release from human coronary microvessels, which are significantly reduced by blocking nitric oxide synthesis and local bradykinin production.\(^12\)

There are multiple, converging lines of evidence that normal arterial endothelium is an important modulator of coronary vasomotion during increased sympathetic activation.\(^13\) In this study, we showed that diabetics have impaired arterial endothelial function compared with healthy control subjects, consistent with previous data.\(^14\) However, endothelium-dependent arterial dilation was similar among the diabetics with and without SND. In addition, vWF antigen, a marker of endothelial cell damage,\(^15\) was also similar in both groups of diabetics, as were other factors known to modulate endothelial function (Table 1). These data suggest that the differences in sympathetically mediated myocardial blood flow between both groups of diabetics were unlikely to have been caused by differences in endothelial function. Although
precise and elegant, invasive, catheter-based intracoronary investigations of endothelial function were unjustified in our study patients without overt cardiovascular disease. Nevertheless, endothelial dysfunction in the brachial artery appears to correlate well with coronary endothelial physiology. In addition, diabetes causes similar impairment of endothelial-dependent dilation in the coronary and forearm resistance vessels, suggesting that this functional abnormality in diabetics is global.

We also observed that hyperemic flows were 20% lower in the diabetics than in the healthy control subjects, confirming the results of previous studies. However, hyperemic flows were similar in both groups of diabetics and were not limited by the differences in cardiac sympathetic innervation, consistent with previous reports from our group and others. However, this finding differs from the results of Stevens et al, who reported lower hyperemic flows among diabetics with than those without autonomic neuropathy. One possible reason for this difference in the study by Stevens et al may be that patients with autonomic neuropathy had more diffuse coronary atherosclerosis than those without autonomic neuropathy who showed a normal coronary vasodilator reserve, a finding that is at variance with most previous studies.

The reason for the modest impairment in coronary vasodilator reserve in our diabetic patients cannot be determined from this study. One possibility is that occult atherosclerosis might have attenuated the maximal flow response to adenosine. We deliberately studied young asymptomatic diabetics, all of whom had normal maximal stress echocardiography, and none showed regional defects on rest-stress perfusion imaging. These findings argue against flow-limiting epicardial coronary stenoses in our healthy control and diabetic subjects. Although structural abnormalities in the coronary microcirculation in the diabetics may have contributed to the impaired vasodilator response to adenosine, such abnormalities have not been universally observed. Finally, the impaired vasodilator response to adenosine in the diabetics may also be related to the presence of endothelial dysfunction. In humans, blockade of nitric oxide production by L-NMMA during continuous infusion of adenosine reduces forearm blood flow by ~30%.

In conclusion, we have shown that diabetics with evidence of cardiac SND, as assessed by PET, have impaired sympathetically mediated dilation of coronary resistance vessels. Our data suggest that this vasomotor abnormality develops early in the course of diabetic autonomic neuropathy and that its severity is related to the degree of cardiac SND. These findings suggest that cardiac sympathetic signals play an important role in modulating myocardial blood flow during periods of activation of the sympathetic nervous system, such as exercise, cold exposure, and mental stress.

This novel mechanism of impaired myocardial perfusion in diabetics with cardiac SND may have important implications. The inadequate dilator response of resistance vessels can lead to myocardial ischemia and left ventricular dysfunction during periods of increased oxygen demand, even in the absence of overt coronary atherosclerosis. Indeed, there is clinical evidence demonstrating abnormalities in left ventricular function in response to exercise in diabetics without coronary artery disease, particularly among those with autonomic neuropathy. This kind of vascular dysfunction could participate in the pathogenesis of progressive left ventricular dysfunction even in diabetics with angiographically normal coronary arteries. In addition, this vasomotor dysfunction could aggravate the abnormalities caused by endothelial dysfunction and coronary atherosclerosis and contribute to other cardiovascular events in diabetic patients.

**Acknowledgments**

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**References**


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