Uncontrolled Hypertension, Uncompensated Type II Diabetes, and Smoking Have Different Patterns of Vascular Dysfunction*

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Study objectives: We evaluated the vascular reactivity in healthy subjects, heavy smokers, uncompensated type II diabetics, and patients with uncontrolled essential hypertension. Plasma nitrite/nitrate, cyclic 3',5'-guanosine monophosphate (cGMP), and thromboxane (TX)-B2 levels were measured.

Participants: One hundred participants were classified into four groups: normal control subjects (n = 25), heavy smokers (n = 25), uncompensated type II diabetics (n = 25), and patients with uncontrolled essential hypertension (n = 25).

Interventions: The brachial artery diameter was measured by a high-resolution ultrasound technique before and after reactive hyperemia and glyceryl trinitrate (GTN), 0.4 mg, administration. Plasma nitrite/nitrate, cGMP, and TX-B2 levels were also measured.

Results: Heavy smokers, uncompensated type II diabetics, and uncontrolled hypertensive patients showed impaired endothelium-dependent, nitric oxide (NO) flow-mediated vasodilation (8.0 ± 2.5%, 5.8 ± 2.7%, and 7.2 ± 3.3%, respectively [mean ± SD]) when compared to the control subjects (12.6 ± 3.6%; p < 0.01). Smokers had a normal endothelium-independent function induced by NO donor (GTN) [25.0 ± 7.3% vs 25.3 ± 8.5% for control subjects]. Uncompensated type II diabetics and patients with uncontrolled hypertension had impaired endothelium-independent responses (17.7 ± 7.1% and 16.8 ± 6.9%, respectively, vs 25.3 ± 8.5 for normal control subjects; p < 0.05). Plasma levels of cGMP and TX-B2 were not significantly different in the four groups, but nitrate/nitrate concentrations were increased in diabetics compared to the control subjects (266 ± 47 µmol/L vs 98 ± 18 µmol/L, p < 0.05).

Conclusion: Both uncontrolled hypertension and type II diabetes mellitus, but not smoking, are associated with impaired vascular smooth-muscle reactivity induced by NO donors. However, only uncompensated type II diabetics showed an increase in plasma nitrite/nitrate levels, suggesting an association with excessive production and/or inactivation of NO.

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Key words: diabetes mellitus; endothelial function; nicotine; nitric oxide; thromboxane; vascular remodeling

Abbreviations: cGMP = cyclic 3', 5'-guanosine monophosphate; GTN = glyceryl trinitrate; LDL = low-density lipoprotein; NO = nitric oxide; TX = thromboxane

Cigarette smoking, type II diabetes mellitus, and arterial hypertension increase the risk of atherosclerosis and premature cardiovascular diseases.1,2 Several studies3–6 have shown an altered endothelial function in these disorders. Since the endothelium of conduit and resistance vessels is the target for many lesions, early endothelial dysfunction in these condi-

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tions has been shown to result in atherosclerosis.7–9 In this context, the loss of endothelium-dependent vasodilatation may be related to reduced nitric oxide (NO) activity.10–15 Loss of this dilatation can be measured in the peripheral circulation by using changes in vessel endothelial function,16,17 and changes in blood flow as an index of resistance vessel endothelial function. Endothelium-independent vascular function can be assessed by evaluating the vasodilatory responses to a NO donor such as glyceryl trinitrate (GTN).18

Although there is substantial evidence that endothelium-dependent vasodilatation is impaired in smokers,19–21 type II diabetics,22–24 and in subjects with essential hypertension,25–27 most of the patients enrolled in these investigations had either compensated type II diabetes mellitus or were patients with controlled or mild essential hypertension. In the present study, we examined the extent of endothelium-dependent, NO flow-mediated vasodilatation and endothelium-independent vasodilatation induced by NO donors, in heavy smokers, uncompensated type II diabetics, and uncontrolled patients with essential hypertension compared with normal subjects. The vascular measurements were obtained from the brachial artery using high-resolution ultrasound, which is an accurate tool for noninvasive evaluation of vasoreactivity.17

Since many investigators have demonstrated that NO is essential for endothelium flow-mediated vasodilatation,28 we also measured the plasma levels of nitrite/nitrate (stable metabolites of NO) to assess the biosynthesis of NO, irrespective of whether a decrease or increase in NO synthesis may underlie these disorders.29,30 The simultaneous measurement of circulating cyclic 3′,5′-guanosine monophosphate (cGMP), a second messenger produced by soluble guanylate cyclase following stimulation by NO, allowed differentiation between impaired NO synthesis and oxidative inactivation.31 Finally, we measured the plasma concentrations of thromboxane (TX)-B2, the stable product resulting from the breakdown of TX-A2 to compare the circulating levels of this potent vasoconstrictor and platelet-aggregating substance32 among the four experimental groups.

Materials and Methods

Study Participants

The volunteers in this study were as follows: (1) control group, 25 apparently healthy subjects with no familial history of coronary artery disease or arterial hypertension (BP < 140/90 mm Hg), and who were nonhypercholesterolemic (low-density lipoprotein [LDL] cholesterol < 129 mg/dL, according to the National Cholesterol Education Program III guidelines), nondiabetic (overnight fasting glycemia < 126 mg/dL, according to the American Diabetes Association), and nonsmokers; (2) smokers, 25 heavy smokers (> 20 cigarettes per day for > 10 years) who were nondiabetic (overnight fasting glycemia < 126 mg/dL), nonhypercholesterolemic (LDL cholesterol < 129 mg/dL, according to National Cholesterol Education Program III guidelines), and normotensive (BP < 140/90 mm Hg); (3) type II diabetes mellitus group, 25 patients with uncompensated noninsulin-dependent diabetes mellitus (overnight fasting glycemia > 126 mg/dL and hemoglobin A1c > 7.0%, according to the American Diabetes Association) who were normotensive (BP < 140/90 mm Hg); and (4) hypertensive group, 25 individuals with uncontrolled essential hypertension (VI Joint National Committee criterion) recruited from cases diagnosed in our outpatient clinic.

Patients with secondary forms of hypertension, cardiac or cerebral ischemic vascular disease, impaired renal function, or other major pathologies were excluded from the study. According to the institutional guidelines, all patients were aware of the investigative nature of the study and gave informed written consent for their participation. The study was approved by the faculty ethics committee on the use of human subjects in research.

Experimental Procedures

All studies were initiated at 8 AM after overnight fasting, with the subjects in a supine position in a quiet, air-conditioned room (22 to 24°C).

Study Protocol

Baseline blood samples levels of all subjects were collected in tubes containing ethylenediamine tetra-acetic acid for the at rest measurement of nitrite/nitrate, cGMP, and TX-B2. Nitrite and nitrate levels are used clinically as markers for the activity of NO synthase and NO biosynthesis. The assay is based on the determination of nitrite using the Griess reaction. Nitrate was measured as nitrate after enzymatic conversion by nitrate reductase as described by Green et al.20 Plasma cGMP and TX-B2 levels were measured using commercial enzyme immunoassays (Cayman Chemical; Ann Arbor, MI). The plasma was separated by centrifugation and stored at −20°C until assayed. Prior to the assay, the plasma samples were extracted with ethanol, centrifuged, and the supernatants were then dried by evaporation under a stream of nitrogen. The dried samples were reconstituted with enzyme-linked immunosorbent assay buffer and assayed according to the instructions of the manufacturer.

Vascular responses in the brachial artery were studied by noninvasive, high-resolution ultrasound scans, using a modification of the technique described by Celermajer.10 Arterial endothelial and vascular smooth-muscle function were assessed by examining brachial artery responses to endothelial-dependent (shear stress-induced) and endothelial-independent (glyceryl trinitrate [GTN]–mediated) stimuli. The subjects rested quietly for 15 min before the first scan and remained in the supine position throughout the study.

The brachial artery was scanned longitudinally 5 to 10 cm above the elbow, and the center of the artery was identified when the clearest picture of the anterior and posterior arterial walls was obtained. When a satisfactory transducer position had been found, a special probe holder designed specifically for the study was fixed around the arm to secure the ultrasound transducer, and it was held in the same position throughout the study.
Arterial diameter measurements were made at end-diastole (R-wave peak of the ECG) using electronic calipers. Four cardiac cycles were analyzed, and an average of the measurements was taken. Blood flow was calculated as the product of the velocity (R-wave peak of the ECG) using electronic calipers. Four cardiac observations of Sorensen et al.17 were 1.9% and 1.6%, respectively. These values agreed with the observations of Sorensen et al.17

Age, yr 43.3
Weight, kg 76.5
Height, cm 168.2
Body mass index 25.5
Glycemia, mg/dL 89.6
Glycosylated hemoglobin, % 5.6
Total cholesterol, mg/dL 195.6
LDL cholesterol, mg/dL 110.5
Triglycerides, mg/dL 160.5

Statistical Analysis

Descriptive data were expressed as the mean ± SD. The sample size for shear stress-mediated vasodilatation was calculated as a power of 0.80. Variance analysis was used with the least significant difference test (α level of 0.05) to determine significant differences among the four groups. Multiple comparison tests were used when appropriate. One-way analysis of variance was used to compare endothelium-dependent and endothelium-independent responses among the four groups.

Demographic data of the four groups were compared by variance analysis, and changes in BP during the study period were assessed by repeated measurements. All calculations were made using the Sigma Plot 3.0 (Jandel Scientific; San Rafael, CA) on a personal computer.

Results

The clinical characteristics of the four groups are shown in Table 1. There were no significant differences among the four groups in relation to the total cholesterol, LDL cholesterol, triglyceride levels, and the body mass indexes (one-way analysis of variance, p > 0.05). The endothelium-dependent and GTN-induced vasodilatation results are summarized in Table 2.

Shear Stress-Induced (Endothelium-Dependent) Vasodilatation

Heavy smokers, uncompensated type II diabetics, and patients with uncontrolled essential hypertension had lower shear stress-induced changes in the brachial artery diameter compared to the control subjects (8.0 ± 2.5%, 5.8 ± 2.7%, and 7.2 ± 3.3% vs 12.6 ± 3.6%, respectively; p < 0.001; Fig 1). There were no significant differences in the changes in arterial blood flow among the four groups (data not shown).

Nitroglycerin-Induced (Endothelium-Independent) Vasodilatation

In contrast to endothelium-dependent responses, smokers showed endothelium-independent responses

Table 1—Clinical Characteristics of the Study Participants (n = 25)*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control Subjects</th>
<th>Smokers</th>
<th>Diabetics</th>
<th>Hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>43.3 ± 12.1</td>
<td>43.9 ± 8.1</td>
<td>53.1 ± 9.7</td>
<td>52.8 ± 9.7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76.5 ± 18.4</td>
<td>75.5 ± 14.4</td>
<td>76.4 ± 17.2</td>
<td>88.7 ± 20.5</td>
</tr>
<tr>
<td>Height, cm</td>
<td>168.2 ± 7.2</td>
<td>163.5 ± 11.5</td>
<td>163.1 ± 10.7</td>
<td>169.7 ± 6.4</td>
</tr>
<tr>
<td>Body mass index</td>
<td>25 ± 5.5</td>
<td>29.0 ± 2.7</td>
<td>26.0 ± 3.4</td>
<td>30.0 ± 3.3</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>126 ± 13</td>
<td>127 ± 15</td>
<td>133 ± 13</td>
<td>159 ± 10</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>80 ± 4</td>
<td>81 ± 12</td>
<td>83 ± 10</td>
<td>107 ± 8</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>80 ± 13</td>
<td>70 ± 10</td>
<td>84 ± 18</td>
<td>76 ± 15</td>
</tr>
<tr>
<td>Smoking, cigarettes/d</td>
<td>0</td>
<td>22.3 ± 6.2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Glycemia, mg/dL</td>
<td>89.6 ± 11.2</td>
<td>91.6 ± 16.3</td>
<td>225.7 ± 42.7</td>
<td>103.7 ± 14.2</td>
</tr>
<tr>
<td>Glycosylated hemoglobin, %</td>
<td>5.6 ± 0.4</td>
<td>5.5 ± 0.3</td>
<td>9.2 ± 1.0</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>195.6 ± 24.1</td>
<td>189.1 ± 34.0</td>
<td>210.8 ± 43.0</td>
<td>199.2 ± 28.9</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>110.5 ± 14.7</td>
<td>93.5 ± 26.4</td>
<td>122 ± 39.8</td>
<td>115 ± 17.4</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>160.5 ± 74.1</td>
<td>160.0 ± 70.4</td>
<td>169.4 ± 83.8</td>
<td>126.4 ± 74.2</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD.
similar to those observed in the control subjects (25.0 ± 7.3% vs 25.3 ± 8.5%, respectively; p > 0.05). However, patients with uncompensated type II diabetes mellitus and subjects with uncontrolled essential hypertension had lower GTN-induced changes in brachial artery diameters (17.7 ± 7.1% and 16.8 ± 6.9%, respectively; p < 0.05) when compared to the control subjects (Fig 2). There were no significant differences in the changes in arterial blood flow among the four groups (data not shown).

### Plasma Nitrite/Nitrate, cGMP, and TX-B2 levels

Nitrite/nitrate levels in smokers and subjects with essential hypertension were similar to the control subjects. In contrast, higher nitrite/nitrate levels were found in diabetic subjects compared to the control subjects (p < 0.05; Table 3, Fig 3). There were no significant differences in the plasma TX-B2 and cGMP levels among the four groups.

### Discussion

Heavy smokers, uncompensated type II diabetics, and patients with uncontrolled essential hypertension showed similarly impaired shear stress-induced endothelium-dependent vasodilation in our investigation, whereas endothelium-independent vasodilation was preserved only in the heavy smokers. Uncompensated type II diabetics had nitrate/nitrite plasma levels higher than the control subjects, and there were no significant changes in the TX-B2 and cGMP plasma concentrations among the four groups.

The heavy smokers group in this study had abnormal endothelium-dependent responses to NO in shear stress-induced vasodilation, as was previously reported by Celermajer et al.\(^1\) Similarly, the preserved response to GTN in heavy smokers agreed with the report by Motoyama et al.\(^2\) We have also

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**Table 2—Brachial Artery Dilatation in Response to Changes in Flow and GTN**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Subjects</th>
<th>Smokers</th>
<th>Diabetics</th>
<th>Hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow-mediated vasodilation</td>
<td>12.6 ± 3.6</td>
<td>8.0 ± 2.5†</td>
<td>5.8 ± 2.7†</td>
<td>7.2 ± 3.3†</td>
</tr>
<tr>
<td>GTN vasodilation</td>
<td>25.3 ± 8.5</td>
<td>25.0 ± 7.3</td>
<td>17.7 ± 7.1†</td>
<td>16.8 ± 6.9†</td>
</tr>
<tr>
<td>Hyperemic flow</td>
<td>122.6 ± 70.0</td>
<td>86.7 ± 50.1</td>
<td>65.2 ± 58.4</td>
<td>47.0 ± 27.2</td>
</tr>
<tr>
<td>GTN flow</td>
<td>49.8 ± 45.7</td>
<td>39.1 ± 33.5</td>
<td>22.6 ± 14.1</td>
<td>30.2 ± 25.1</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD % change.
†p < 0.001 vs control subjects.
‡p < 0.05 vs control subjects.

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**Figure 1. Flow-mediated vasodilation (percentage change) in the four groups.**
previously demonstrated that smokers have a reduced venodilation response to bradykinin but a normal response to sodium nitroprusside, and that smoking cessation was associated with a complete recovery of endothelial dysfunction in human hand veins.33,34

Smoking-induced impairment of endothelium-dependent vasodilatation is most probably related to a decreased availability of NO. Studies35,36 strongly suggest a role for oxygen-derived free radicals that can affect the endothelial function directly or indirectly in decreasing the metabolic end products of NO. Indeed, antioxidants, especially vitamin C, improve the impaired endothelium-dependent vasodilatation in chronic smokers.39 Endothelial dysfunction in smokers with a normal vascular smooth-muscle cell reactivity is related to the toxic effects of nicotine, carbon monoxide, and oxygen that may increase oxidative stress and decrease the availability of NO. Nicot ine can also affect the expression of adhesion molecules and monocyte migration through the formation of cyclooxygenase-dependent endothelium-derived contraction factors (mainly TX),37 and can alter DNA synthesis/repair, cell proliferation, and cytotoxicity.38

Plasma concentrations of NO decreased in heavy smokers who ceased smoking 24 h before blood sampling.36 The normal endothelium-independent response to GTN seen in the smokers studied here probably reflected the lack of structural changes in the vessel wall, which could prevent the penetration of NO to vascular smooth-muscle cells.

Endothelial dysfunction seen as a reduction in endothelium-dependent vasodilatation is a common feature in conduit and resistance arteries in diabetics. This evidence derives largely from animal models with alloxan-induced diabetes.39–41

In our study, patients with uncompensated type II diabetes mellitus showed attenuated shear stress-induced endothelium-dependent vasodilatation, and a blunted endothelium-independent response. These findings were consistent with the report by

Table 3—Baseline Plasma Levels of Nitrite/Nitrates, TX-B2, and cGMP in the Four Groups*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control Subjects (n = 25)</th>
<th>Smokers (n = 25)</th>
<th>Diabetics (n = 25)</th>
<th>Hypertensives (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrites/nitrates, μmol/L</td>
<td>98 ± 18</td>
<td>125 ± 22</td>
<td>266 ± 47†</td>
<td>82 ± 12</td>
</tr>
<tr>
<td>TX-B2, pg/mL</td>
<td>422 ± 70</td>
<td>624 ± 208</td>
<td>550 ± 142</td>
<td>456 ± 129</td>
</tr>
<tr>
<td>cGMP, pg/mL</td>
<td>2.1 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>1.9 ± 0.4</td>
<td>1.9 ± 0.3</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD.
†p < 0.05 vs control subjects.
McVeigh et al,\textsuperscript{42} who demonstrated impaired vasodilatation to acetylcholine and GTN in patients with type II diabetes mellitus. These authors interpreted the abnormal response to GTN as reflecting tolerance to this drug and speculated that the response to a direct acting NO donor such as nitroprusside would not be attenuated.\textsuperscript{23} In contrast, Goodfellow et al\textsuperscript{22} reported greatly impaired, endothelium-dependent, shear stress-induced vasodilatation in type II diabetic patients, whereas endothelium-independent responses induced by GTN were similar to those in normal subjects.

The plasma nitrite/nitrate levels were increased in our type II diabetic patients, a finding in agreement with the suggestion that oxidative stress may explain the abnormal endothelial responses in uncompensated type II diabetic patients.\textsuperscript{43,44} Hyperglycemia results in oxidative stress and the increased formation of advanced glycosylation end products, both of which increase the inactivation of NO and enhance the oxidative modification of lipoprotein particles. The latter results in the production of abnormalities in the lipoprotein metabolism, the most significant being hypertriglyceridemia which is associated with increased plasma concentrations of small dense LDL and low levels of high-density lipoprotein.\textsuperscript{45} By elevating endothelial cell calcium, hyperglycemia also stimulates the synthesis of NO, but in the presence of O$_2^-$, NO is converted into the highly potent oxidant molecule peroxynitrite,\textsuperscript{46} which potentiates the oxidative stress and contributes to the decrease in vascular reactivity in this group. Our data suggest that the vascular dysfunction in uncompensated type II diabetic patients in this study results from a loss of the protective properties of NO, and this could contribute to the increased incidence of vascular disease in these individuals. We reinforce the need of further studies in diabetics in other clinical conditions.

Our patients with uncontrolled essential hypertension showed a markedly reduced response to endothelium-dependent vasodilatation compared to the control subjects, but no significant difference when compared to the other two groups. A similarly reduced dilatation in the brachial artery was reported in patients with controlled essential hypertension,\textsuperscript{47,48} but there was no significant difference in the response to GTN in these patients.

The plasma NO levels of this group were similar among the control subjects, heavy smokers, and hypertensive individuals, all of which were lower than in uncompensated type II diabetics (see earlier). The plasma cGMP levels were similar in the four groups. These findings suggest that the defective response to shear stress was a consequence of the reduced availability of NO in the endothelial cells (or other relaxing factors). These results cannot exclude the possibility that there may be an increase in the release of constricting factors in patients with hypertension.\textsuperscript{49}

The plasma TX-B$_2$ levels in the four groups contrasts with a previous study\textsuperscript{43} showing that smok-
ers have increased urinary excretion of TX-A₂. This discrepancy may reflect the methodologic limitations of quantifying this eicosanoid in plasma samples.\(^{30}\)

**CONCLUSION**

A similar degree of impairment in endothelium-dependent, shear stress-induced vasodilatation was observed in heavy smokers, uncompensated type II diabetics, and patients with uncontrolled hypertension. According to our investigation, there is a suggestion that uncontrolled hypertension and uncompensated type II diabetes mellitus, but not smoking, result in impaired vascular smooth-muscle reactivity to NO donors. However, only diabetics showed an increase in plasma nitrite/nitrate levels, suggesting an association with excessive production and/or inactivation of NO.

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