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Persistent Remodeling of Resistance Arteries in Type 2 Diabetic Patients on Antihypertensive Treatment

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Abstract—We hypothesized that resistance arteries from diabetic patients with controlled hypertension have less remodeling than vessels from untreated hypertensive subjects. Eight normotensive subjects (aged 44±3 years, 3 men; values are mean±SEM), 19 untreated hypertensive subjects (46±2 years, 9 men), and 23 hypertensive subjects with type 2 diabetes mellitus under antihypertensive treatment (58±1 years, 15 men) were studied. Resistance arteries dissected from gluteal subcutaneous tissue were assessed on a pressurized myograph. Most diabetic patients (70%) were being treated with angiotensin-converting enzyme inhibitors. Although systolic blood pressure was still above the normotensive range in these patients (144±2 versus 150±3 mm Hg in hypertensive and 114±4 mm Hg in normotensive subjects), diastolic blood pressure was well controlled (83±2 mm Hg) and significantly lower compared with that in untreated hypertensives (100±1 mm Hg; P<0.001) but higher than in normotensives (76±3 mm Hg; P<0.05). Thus, pulse pressure was higher in diabetic patients (P<0.05). The media-to-lumen ratio of resistance arteries was greater in hypertensives (0.083±0.002) compared with normotensive controls (0.059±0.003; P<0.05) and was even higher in diabetic hypertensive subjects (0.105±0.004; P<0.001 versus normotensive controls). The medial cross-sectional area was greater in diabetic and hypertensive patients compared with normotensive controls (P<0.001). Acetylcholine-induced relaxation was impaired in vessels from hypertensive patients and from patients with both diabetes mellitus and hypertension (P<0.05 versus normotensive controls), whereas endothelium-independent vasorelaxation was similar in all groups. Despite effective antihypertensive treatment, resistance arteries from hypertensive diabetic patients showed marked remodeling, greater than that of vessels from untreated, nondiabetic, hypertensive subjects, in agreement with the high cardiovascular risk of subjects suffering from both diabetes and hypertension. (Hypertension. 2004;43[part 2]:399-404.)

Key Words: diabetes mellitus ■ hypertension, detection and control ■ angiotensin-converting enzyme inhibitors ■ remodeling ■ endothelium

Type 2 diabetes mellitus (DM-2) is a major cardiovascular risk factor. In the UKPDS study, the incidence of complications of diabetes was strongly associated with elevated blood pressure (BP).1 Moreover, tight BP control substantially reduced the risk of macrovascular disease, stroke, and deaths related to diabetes.2 Macrovessels and microvessels of diabetic patients show marked structural remodeling and impaired endothelial function. In a large, population-based cohort study, DM-2 was associated with increased stiffness of large arteries, as assessed by ultrasound.3 Other ultrasound studies of large arteries have confirmed the presence of stiffer vessels in patients with DM-2.4,5 In hypertensive patients, we previously reported that endothelial function of small arteries is correlated with that of large vessels, but ultrasound assessment of large arteries appeared to be less sensitive than in vitro measurements in small resistance arteries.6 Moreover, small-artery remodeling seemed to be the earliest form of end-organ damage in hypertension.7 However, there are few studies addressing the structural and functional alterations of small resistance arteries rather than large vessels in DM-2. Using a wire myograph system, Rizzoni et al8,9 described vascular remodeling and endothelial dysfunction in small resistance arteries, which were qualitatively similar to these parameters in patients with hypertension. However, the media-to-lumen ratio was significantly higher in diabetic patients with hypertension than in patients who had either diabetes or hypertension only. In contrast to hypertensive patients who presented with predominantly eutrophic remodeling and fibrosis, DM-2 patients had hypertrophic remodeling and less fibrosis. Using a more physiologic pressurized myograph system, Schofield et al10 also reported vascular
hypertrophy and endothelial dysfunction, as well as increased myogenic responses of small resistance arteries, in DM-2. In these studies of resistance arteries from patients with DM-2 and hypertension, BP pressure was not controlled (SBP ≈ 160 and DBP 96 to 100 mm Hg).

We have shown in several studies that BP control with angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor antagonists, or calcium channel blockers corrected the structural and/or endothelial alterations in hypertensive patients.\(^{11-16}\) Because hypertension increases cardiovascular risk in diabetes and this increased risk can be reduced by antihypertensive therapy, we tested the hypothesis that DM-2 patients whose BP was controlled to the level of the tight BP control group in the UKPDS study\(^2\) should have fewer alterations of small resistance arteries than untreated nondiabetic, hypertensive patients.

**Methods**

**Patients**

The protocol was approved by the Ethics Committee of the Clinical Research Institute of Montreal. Normotensive subjects (n=8), essential hypertensive patients (n=19), and hypertensive diabetic patients (n=23) provided written, informed consent to participate in the study. Control subjects had BPs <135 mm Hg systolic and <85 mm Hg diastolic. Hypertensive patients had a history of sitting DBP >90 mm Hg on at least 3 occasions. Hypertensive patients had not been exposed to antihypertensive medication for at least the previous 6 months. Ten of them had never received antihypertensive medication. DM-2 patients had a history of diabetes for at least 6 months, as defined by the usual criteria based on basal glycosuria or glucose tolerance test. Diabetes was generally well controlled; only 4 patients had glycated hemoglobin >0.08. All diabetic patients were hypertensive. The absence of secondary forms of hypertension was ascertained by normal serum electrolytes, creatinine, urinalysis, abdominal echocardiogram, and, when indicated, renal scintiscan, renal arteriogram, or computed abdominal tomography. Clinic sitting BP was measured after 15 minutes of rest; diastolic BP was read as phase V Korotkoff sounds. End-organ damage in diabetic patients was assessed by measuring albuminuria and electrocardiographic evidence of left ventricular hypertrophy, the latter obtained as the product of QRS duration time and Cornell voltage combination from 12-lead electrocardiograms, as previously described.\(^{17}\) Gluteral subcutaneous biopsy samples measuring 1.0×0.5×0.5 cm\(^3\) were obtained under local anesthesia (2% lidocaine), as previously described by us.\(^{11-16}\)

**Resistance-Artery Study**

Small arteries (150 to 300 \(\mu\)m) were isolated from subcutaneous tissue immediately after biopsy sample procurement and mounted on a pressurized myograph.\(^{15-16}\) Vessel segments (~2 mm long) were mounted onto 2 glass cannulas, one of which was positioned until the side (10/11002) internal diameters of the normotensive and hypertensive vessels, respectively, and \((D_{\text{remodel}})\) is the remodeled internal diameter. \((D_{\text{remodel}})\) was calculated as \(\{(D_{\text{h}})^2-4CSA_n/\pi\}^{1/2}\), where \((D_{\text{h}})\) is the external diameter of the hypertensive vessels and CSA\(_n\) is the medial cross-sectional area of normotensive vessels. The growth index was calculated as \((CSA_h-CSA_n)/CSA_n\), where CSA\(_n\) and CSA\(_h\) are the medial cross-sectional areas of normotensive and hypertensive vessels, respectively.

**Data Analysis**

Results are presented as mean±SEM. Comparisons were performed by 1-way ANOVA, followed by Bonferroni post hoc testing or 2-tailed Student t test, as appropriate. Regression analysis was performed by the least-squares method. A value of \(P\leq0.05\) was considered statistically significant.

**Results**

The demographics of the normotensive, hypertensive, and diabetic subjects appear in Table 1. The diabetic hypertensive patients were significantly older and heavier than subjects in the other groups. Systolic and diastolic BPs of hypertensive patients were significantly higher than in normotensive controls. BP, particularly diastolic, was lower in the diabetic hypertensive subjects compared with the untreated hypertensive patients. Accordingly, diabetic hypertensive patients exhibited a significantly higher pulse pressure compared with normotensive controls and hypertensive patients. Nevertheless, systolic and diastolic BPs were still higher in diabetic hypertensive patients than in control subjects, despite the fact that the diabetic hypertensive individuals were receiving, on average, 2 antihypertensive medications, one of which was an ACE inhibitor (70% of the patients). Length of known hypertension was greater in the diabetic hypertensive patients than in nondiabetic hypertensive patients. Cholesterol and LDL levels were well controlled in diabetic hypertensive patients, 60% of whom received lipid-lowering therapy with a fibrate (5 patients) or a statin (9 patients). As a result, diabetic subjects had significantly lower LDL and total cholesterol levels than the other groups. Serum creatinine was significantly lower in diabetic hypertensive patients than in nondiabetic subjects. Three diabetic patients had microalbuminuria (>2.5 mg albumin/mmol creatinine), 2 diabetic patients had left ventricular hypertrophy (by electrocardiogram), and 3 diabetic patients had both microalbuminuria and left ventricular hypertrophy.

Resistance arteries exhibited significantly greater media thickness and media-to-lumen ratio in hypertensive than in normotensive patients (Table 2 and Figure 1). The media width and media-to-lumen ratio of vessels from diabetic hypertensive patients were significantly higher than in nondiabetic hypertensive patients. The media CSA was increased in diabetic hypertensive patients, indicating hypertrophic remodeling. Regression analysis showed no correlation between age and media-to-lumen ratio in nondiabetic and diabetic hypertensive patients (\(R^2=0.05\) and 0.029, respectively). There was no correlation between age and maximum relaxation to acetylcholine (\(R^2=0.016\)) in diabetic hypertensive patients. There was also no correlation between duration of hypertension and media-to-lumen ratio in nondiabetic and diabetic hypertensive subjects (\(R^2=0.007\) and 0.013, respectively) nor any correlation between pulse pressure and media-to-lumen ratio in diabetic hypertensive patients (\(R^2=0.061\)).
Endothelial function of resistance arteries tested with acetylcholine was equally impaired in nondiabetic and diabetic hypertensive patients (Table 2 and Figure 2), whereas endothelium-independent vasorelaxation was similar in all groups.

**Discussion**

In this study, diabetic patients on antihypertensive therapy including ACE inhibitors, with controlled hypertension and blood lipids, had similar endothelial dysfunction but a greater media-to-lumen ratio of resistance arteries than did untreated hypertensive patients. With controlled BP and lipid levels, the very elevated media-to-lumen ratio beyond that of untreated, nondiabetic hypertensive subjects was surprising. This is specially so, in light of the fact that the DM-2 patients had the same systolic BP as did patients after successful intervention in the UKPDS study, which showed a substantial lowering of cardiovascular risk.\(^2\) Furthermore, 70\% of DM-2 patients in the present study were being treated with an ACE inhibitor. We previously showed that treatment with an ACE inhibitor corrected remodeling and endothelial function in hypertensive patients.\(^11,12,18\) Our results demonstrate persistent hypertrophic remodeling in hypertensive patients with DM-2.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTRL</th>
<th>HTN</th>
<th>DM-2+HTN</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>8</td>
<td>19</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>3/5</td>
<td>11/8</td>
<td>15/8</td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>44.1±3.4</td>
<td>46.6±2.2</td>
<td>58.1±1.4(^†)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>67.0±6.6</td>
<td>77.3±4.3</td>
<td>85.5±3.0(^†)</td>
<td>0.028</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>23.8±1.3</td>
<td>26.9±1.4</td>
<td>30.0±0.9(^†)</td>
<td>0.010</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>114±4</td>
<td>150±3(^*)</td>
<td>144±2(^*)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>76±3</td>
<td>100±1(^*)</td>
<td>83±2(^†)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>34±3</td>
<td>50±3</td>
<td>61±3(^‡)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Duration of HTN, y</td>
<td>...</td>
<td>8.9±1.9</td>
<td>17.5±1.8</td>
<td>0.001 (t test)</td>
</tr>
<tr>
<td>Duration of DM-2, y</td>
<td>...</td>
<td>...</td>
<td>7.4±1.6</td>
<td></td>
</tr>
<tr>
<td>No. of patients who never received antihypertensive treatment</td>
<td>8</td>
<td>10</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>No. of anthypertensive medication per patient</td>
<td>...</td>
<td>...</td>
<td>2.0±0.2</td>
<td></td>
</tr>
<tr>
<td>No. of patients on ACE-inhibitor</td>
<td>...</td>
<td>...</td>
<td>16 (=70%)</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine, (\mu)mol/L</td>
<td>91.8±3.5</td>
<td>99.9±4.4</td>
<td>75.9±3.0(^†)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum cholesterol, mmol/L</td>
<td>5.6±0.4</td>
<td>5.4±0.2</td>
<td>4.8±0.2</td>
<td>0.050</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.4±0.3</td>
<td>1.3±0.1</td>
<td>2.1±0.4</td>
<td>0.143</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.5±0.1</td>
<td>1.4±0.1</td>
<td>1.4±0.1</td>
<td>0.508</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.3±0.4</td>
<td>3.4±0.1</td>
<td>2.5±0.1(^§)</td>
<td>0.005</td>
</tr>
<tr>
<td>Fasting glucose, mmol/L</td>
<td>...</td>
<td>...</td>
<td>7.8±0.4</td>
<td></td>
</tr>
<tr>
<td>Glycated hemoglobin</td>
<td>...</td>
<td>...</td>
<td>0.070±0.002</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2. Resistance-Artery Data**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CTRL</th>
<th>HTN</th>
<th>DM+HTN</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>8</td>
<td>19</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Media, (\mu)m</td>
<td>11.2±1.3</td>
<td>14.6±2.2(^*)</td>
<td>22.2±0.8(^†)(^§)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lumen, (\mu)m</td>
<td>191±17</td>
<td>177±7</td>
<td>202±7(^†)</td>
<td>0.007</td>
</tr>
<tr>
<td>CSA, (\mu)m(^2)×10(^^)</td>
<td>7.7±1.6</td>
<td>9.1±0.6</td>
<td>17.4±1.2(^‡)(^§)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maximum relaxation to acetylcholine, %</td>
<td>0.95±0.02</td>
<td>0.80±0.3(^*)</td>
<td>0.80±0.04(^*)</td>
<td>0.034</td>
</tr>
<tr>
<td>Remodeling index, %</td>
<td>110</td>
<td>45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth index, %</td>
<td>18</td>
<td>126</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CTRL indicates control subjects; HTN, nondiabetic hypertensive patients. Other abbreviations are as defined in text. Values are mean±SEM. \(^*P<0.05\) vs CTRL; \(^†P<0.001\) vs HTN; \(^‡P<0.05\) vs HTN; \(^§P<0.001\) vs HTN.
rather than the eutrophic remodeling predominantly found in essential hypertension in the absence of diabetes.

These findings confirm and extend those of other studies of small arteries in DM-2 by underlining the vascular risk still persisting in these patients even after their BP is reasonably if not perfectly controlled. The larger lumen in diabetic patients indicates presence of outward hypertrophic remodeling. However, these data might be subject to sampling bias, as already described, and are not as robust or reproducible for intersubject or intrasubject comparisons as the media-to-lumen ratio. Endothelial function was equally impaired in DM-2 and hypertension, which parallels other studies, which did not find an additive effect of DM-2 and hypertension on endothelial function. Schofield et al described a correlation between total cholesterol levels and endothelial dysfunction that was not present in our population. However, lipid levels were well controlled, with 60% of the diabetic patients receiving either a statin or a fibrate. Diabetes in these individuals was predominately treated with oral antidiabetic medication, with good if not optimal success as measured by glycated hemoglobin. Because BP and lipid levels were lower in hypertensive diabetic patients than in nondiabetic hypertensives, other factors seem to be responsible for the persistently increased CSA of the media and media-to-lumen ratio of resistance arteries. Mechanically, increased pulse pressure due to stiffer large arteries might play a role. Pulse pressure has been shown to be increased in diabetic patients. In a previous study, we found that pulse pressure was not a major determinant for increased media-to-lumen ratio in hypertensive patients below the age of 60, whereas in a population with an average age of 75, the media-to-lumen ratio was shown to be closely correlated with pulse pressure. In the present study, we did not find a correlation between pulse pressure and media-to-lumen ratio in small arteries. Humoral and hormonal factors might play a role in remodeling in hypertensive persons with DM-2. Advanced glycation end-products induce vascular transforming growth factor-β gene expression and increase vascular collagen deposition in diabetic rats. Insulin promotes growth of vascular smooth muscle cells. Furthermore, elevated glucose induces oxidative stress. Therefore in DM-2, humoral factors such as chronic hyperglycemia, hyperinsulinemia, oxidative stress, and chronic low-grade inflammation might be involved in the remodeling and hypertrophic processes affecting small arteries beyond the effects of BP and blood lipids or the renin-angiotensin system. This might result in changes that are difficult to reverse, even with therapeutic strategies that are effective in nondiabetic hypertensive patients.

A recent study demonstrated that in hypertensive patients, an elevated media-to-lumen ratio of resistance arteries dissected from gluteal subcutaneous tissue, such as those examined in this study, is a marker of increased cardiovascular risk. It is therefore likely that the remodeling of small arteries also predicts increased cardiovascular risk in diabetic hypertensives. Thus, the fact that even under treatment with ACE inhibitors, which we and others have demonstrated correct remodeling and endothelial dysfunction of small arteries in human essential hypertension, there is a persistent and severe abnormality suggests that other treatment strategies must be superimposed to improve the vascular and high cardiovascular risk in the case of hypertension associated with DM-2. These might include addition of angiotensin receptor blockers, use of peroxisome proliferator activated receptor activators, or other therapeutic approaches. However, the effects of antihypertensive treatment on small-artery structure and function in hypertensive
patients with diabetes might only be unquestionably demonstrated by longitudinal, prospective, intervention studies. The marked vascular remodeling despite reasonably controlled hypertension in our study population emphasizes the need of such studies in these patients, which should be performed under tight BP control according to goals established in current guidelines.

A limitation of this study is the different age of diabetic and nondiabetic subjects, but age and duration of hypertension were not correlated with media-to-lumen ratio in these groups, indicating a minor influence of these parameters on the media-to-lumen ratio of resistance arteries. Also, age was not correlated with maximum vasorelaxation to acetylcholine. These results agree with another study that did not find a correlation between age and vascular resistance in hypertensive and diabetic patients, indicating the involvement of other neurohumoral factors. Although differences in the known duration of hypertension could influence results, these differences are almost unavoidable when diabetic hypertensive subjects treated with antihypertensive agents and untreated hypertensive patients are recruited. Furthermore, small-artery structure was not correlated significantly with duration of hypertension. Lower creatinine levels in the DM-2 patients could be explained in part by smaller muscle mass in older patients; however, hyperfiltration might already be present in some diabetic patients.

Perspectives
The remodeling of resistance arteries from diabetic hypertensive patients was greater than that of nondiabetic hypertensive patients, despite controlled hypertension and blood lipids and reasonable diabetic control. These findings might likely contribute to the increased cardiovascular risk of diabetic patients and underline the difficulties in successfully treating these patients, as well as the need for new approaches to reduce cardiovascular risk in persons with diabetes and hypertension.

Acknowledgments
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