Vascular Structural and Functional Changes in Type 2 Diabetes Mellitus
Evidence for the Roles of Abnormal Myogenic Responsiveness and Dyslipidemia

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Background—To further investigate vascular morphology and function in type 2 (non–insulin-dependent) diabetes mellitus (type 2D), small arteries were examined in vitro from carefully defined cohorts of patients with or without concomitant hypertension and the results compared with those from selected normotensive nondiabetic control subjects and a group of untreated patients with essential hypertension (EH).

Methods and Results—Blood vessels were studied through the use of pressure myography to determine vascular morphology, mechanics, and myogenic responsiveness, together with testing of constrictor and dilator function. Small arteries from patients with EH demonstrated eutrophic inward remodeling and an increased distensibility. Vessels from type 2D patients demonstrated hypertrophy, a further increase in distensibility, and a highly significant loss of myogenic responsiveness compared with patients with EH and control patients. Vasoconstrictor function to norepinephrine was normal in patients with type 2D and type 2D/H and EH. Endothelium-dependent dilation was normal in patients with EH but abnormal in patients with type 2D and type 2D/H. There was a significant correlation between dilator impairment and the degree of dyslipidemia recorded in all groups.

Conclusions—These results demonstrate vascular hypertrophy in small arteries from patients with type 2D. This could be a consequence of impaired myogenic responsiveness, which will increase wall stress for a given intraluminal pressure, which may be a stimulus for vascular hypertrophy. A substantial proportion of endothelial dysfunction can be attributed to an effect of the abnormal lipid profile seen in such patients. (Circulation. 2002;106:3037-3043.)

Key Words: diabetes mellitus • arteries • structure • hypertrophy

North America and Europe are having a rapid rise in the prevalence of type 2 diabetes (type 2D), and in consequence, there is an increase in morbidity and mortality rates as a result of damage to vital organs such as the brain, heart, and kidney. Such problems are seen even more frequently when type 2D is accompanied by hypertension and dyslipidemia. It is becoming accepted that type 2D is a vascular disease best treated by meticulous control of hypertension and any disordered lipid profile.

Against this background, the vasculature of patients with type 2D has been subjected to investigation. In one study, minimum forearm vascular resistance was found to be increased, suggesting that small-vessel structure was altered. However, studies in type 2D are fraught with difficulty, given the recognized associations with hypertension and dyslipidemia, both of which are known to influence small-artery structure and function.

Recently, Rizzoni et al reported that small arteries of patients with type 2D have an increased media thickness to lumen diameter ratio compared with vessels from control subjects. This appeared to be due to a combination of remodeling and hypertrophy. A similar structural change was observed in patients with type 2D and hypertension (type 2D/H), which contrasted with the eutrophic remodeling reported in patients with essential hypertension (EH). Functional changes were also found in type 2D, type 2D/H, and EH subjects with deficient dilation to acetylcholine and bradykinin. Such studies were achieved with the use of wire-mounted segments of small arteries. As such, the vessels are examined isometrically, the effects of pressure on the vascular wall are removed, and myogenic responsiveness cannot be assessed. The pathophysiological relevance is that an abnormality in myogenic responsiveness may impair autoregulation and additionally, impaired myogenic constriction will increase wall stress for a given intraluminal pressure, which may stimulate vascular hypertrophy.

Accordingly, we investigated the morphological and functional characteristics of small arteries from patients with type
Pharmacological Assessment and Passive Structure
After viability assessment with KPSS, each vessel was stimulated as follows: (1) Cumulative addition of norepinephrine, \(10^{-5}, 3 \times 10^{-5}, 10^{-4}, 3 \times 10^{-4}, 10^{-5}, 3 \times 10^{-5}, 10^{-6},\) and \(10^{-7}\) mol/L, with 3 to 5 minutes per concentration. (2) Cumulative response to acetylcholine (Ach) was done by adding serial concentrations (mol/L): \(10^{-4}, 3 \times 10^{-4}, 10^{-3}, 3 \times 10^{-3}, 10^{-2}, 3 \times 10^{-2}, 10^{-1},\) and \(10^{-0}\) to a preconstricted vessel with \(10^{-6}\) norepinephrine. (3) After 30 minutes of incubation with \(5 \times 10^{-3}\) mol/L \(L-N^\text{G}-\text{monomethyl-arginine (L-NMMA)}, a\) inhibitor of nitric oxide synthase, the response to Ach was repeated as in (2). (4) Cumulative response to endothelial independent nitro donor sodium nitroprusside was performed on a preconstricted vessel (mol/L): \(10^{-6}, 3 \times 10^{-6}, 10^{-5}, 3 \times 10^{-5}, 10^{-4}, 3 \times 10^{-4}, 10^{-3},\) and \(10^{-2}\).

Passive structure was determined on each vessel; after the completion of collecting the pharmacological responses, the vessel was superfused for 20 minutes with \(Ca^{2+}\)-free PSS. The lumen pressure was reduced to 3 mm Hg and then increased, allowing time at each pressure step for equilibration of \(\approx 3\) minutes, through the following steps (nm Hg): 5, 10, 20, 30, 40, 50, 70, 100, 120, 150, 180, and 200.

Calculations
Cross-sectional wall area (CSA) was calculated as \(\text{CSA} = \pi (D^2 - 2WT/2)^2 / \pi (D^2/2),\) where \(D\) is the inner diameter and \(W\) the wall thickness/wall/lumen ratio: \(W/L = WT/D/100.\)

The remodeling index is defined as the percentage of the observed difference in the internal diameter of hypertensive and normotensive vessels that could be accounted for by remodeling of the normotensive vessel. It is calculated as follows.

Consider a “normotensive” vessel with internal and external media diameters \((D_i, D_e)\) and a “hypertensive” vessel with internal and external medial diameters \((D^\text{hi}, D^\text{he})\). The corresponding media CSAs are \(\text{CSA}_{\text{n}} = \pi (D_i^2/4 - D^\text{hi}_i^2)\) and \(\text{CSA}_{\text{h}} = \pi (D^\text{he}_e^2/4 - D^\text{hi}_e^2),\) respectively. Remodeling of the normotensive vessel in which the CSA is kept equal to \(\text{CSA}_{\text{n}}\) but its external diameter became \(D^\text{hi}_e\) would then give an internal diameter \(D_i\) remodel given by \((D_i)_{\text{remodel}} = \sqrt{\pi(\text{CSA}_{\text{n}}/\pi).}\)

Therefore, the remodeling index is calculated as \(100 + ((D^\text{hi}_i - (Di)_{\text{remodel}})/(Di)_{\text{n}}).\) If the remodeling index is not equal to 100, then there will have been growth, defined here as Growth index = \(\text{CSA}_{\text{n}} - \text{CSA}_{\text{h}}) / \text{CSA}_{\text{n}}.\)

Data for remodeling indexes are calculated from mean values, so SEM are not provided: Stress = \text{PxD/2WT}, where \(P = 1334\) dyne/cm²; Strain = \(D - D^\text{hi}/D^\text{hi},\) where \(D^\text{hi}\) is the diameter at 3 mm Hg.

Statistical Analysis
Unless otherwise stated, all data are presented as mean ± SEM. Differences between pressure-diameter relations were analyzed as repeated-measures ANOVA; when relations differed, diameters at individual pressures were compared by using the Tukey test. One-way ANOVA with Bonferroni’s correction for multiple comparisons were used to evaluate differences among groups. Linear regression was used for continuous variables and a 2-way ANOVA for repeated measures was used for the cumulative dose-response curves. All analyses were performed with the use of GraphPad Prism software.

Results
Patient Characteristics
The demographic details of the patients and control subjects who took part in the study are shown in Table 1. All three patient groups were slightly older than control subjects, but none was significantly different. The blood pressures were significantly higher in type 2D + H and EH groups compared with control subjects but no different when compared with each other (Table 1). Urinary albumin excretion rates were significantly higher in type 2D and type 2D + H patients. Total cholesterol levels were slightly higher in type 2D and

Myogenic Tone Protocol
To determine myogenic responsiveness, the intraluminal distending pressure was reduced to 20 mm Hg, then increased to 40 mm Hg, and subsequent increments of 40 mm Hg up to 200 mm Hg; at each pressure step, the vessel was allowed to stabilize over 5 to 10 minutes. After this, the pressure was again reduced to 50 mm Hg and the vessel was superfused with \(Ca^{2+}\)-free PSS containing EGTA for 20 minutes. The pressure steps were then repeated to obtain passive pressure-diameter relations.

Pressure Myography
A single subcutaneous gluteal fat biopsy was obtained from each subject by using 3 to 5 mL of \(1\%\) lidocaine, allowing tissue \((2 \times 1.5 \times 1.5 \text{ cm})\) to be harvested and placed immediately in ice-cold physiological saline solution (PSS). Small arteries 65 to 230 \(\mu\)m were dissected from the tissue and carefully cleaned under a dissecting microscope. Isolated vessels were then transferred to an arteriographic bath chamber (Living Systems Instruments) and cannulated as described previously. The chamber was placed on the stage of an inverted microscope and superfused with PSS, gassed with \(5\%\) \(CO_2/95\%\) air \((pH 7.4\) to 7.45) at 37°C for 3 minutes, through the following steps (mm Hg): 5, 10, 20 minutes. The pressure steps were then repeated to obtain passive pressure-diameter relations. We report that myogenic responsiveness is abnormal in type 2D, which may provide the explanation for the structural alterations seen in this disease.

Methods
Twenty-two patients with type 2D, 22 patients with type 2D + H, and 18 healthy nondoabetic normotensive subjects gave full written informed consent and took part in the study, which was approved by the Local Research Ethics Committee (LREC). To standardize treatment, type 2D patients continued their therapy for diabetes, but any treatment for hypertension \((n = 15)\) was stopped for 4 weeks before study. The cohort of hypertensive patients had either never received treatment \((n = 15)\) or had been washed out for 4 weeks \((n = 7).\) The presence of hypertension (diastolic pressure >90 mm Hg on 2 consecutive occasions after being seated for 20 minutes) was accepted according to International Society of Hypertension/World Health Organization guidelines, and type 2 diabetes was diagnosed by using Guidelines of the Expert Committee on the Diagnosis and Classification of Diabetes.

On the day of study, venous blood samples were drawn for biochemistry screening, including renal function, random blood sugar, and lipid profile as well as glycosylated hemoglobin. Also, microalbuminuria was quantified by analysis of overnight urine collections. Blood pressure was measured sitting, after 15 minutes of rest, by a semiautomatic machine (OMRON 705 CP, White Medical), with the mean of 3 readings recorded.

2D, type 2D + H, and EH and compared the results with those obtained from matched control subjects by using pressure myography. We report that myogenic responsiveness is abnormal in type 2D, which may provide the explanation for the structural alterations seen in this disease.
type 2D+H patients, but neither group was significantly different from control subjects. Lipid subfractions are also shown in Table 1: Mean HDL levels did not differ between groups, but mean LDL levels were significantly higher in type 2D and type 2D+H patients compared with control subjects. Mean triglyceride values did not differ in any group when compared with the control group (Table 1). Six patients with type 2D and 9 patients with type 2D+H were receiving treatment with sulfonylurea drugs, but subgroup analyses demonstrated no influence on the findings reported below. Duration of diabetes did not differ significantly between type 2D and type 2D+H. There was a slight increase in complications in type 2D+H, but this did not reach statistical significance compared with type 2D (Table 1).

Small-Artery Morphology
Passive structural properties at a distending pressure of 100 mm Hg are shown in Table 2. The lumen diameter was significantly reduced in vessels from EH but unchanged in type 2D and type 2D+H. Small arteries from patients with EH also had a significantly increased wall thickness to lumen diameter ratio but no increase in medial cross-sectional area. Calculation of the remodeling index\(^1\) indicated that the vessels had undergone eutrophic inward remodeling (Table 2).

Irrespective of the presence or absence of concomitant hypertension, small arteries from patients with type 2D had a normal lumen diameter and a significantly increased wall thickness to lumen diameter ratio and a significantly increased medial cross-sectional area, indicating that a growth response had occurred. Data obtained at 100 mm Hg were representative of the findings across the entire pressure range (not shown).

Small-Artery Mechanics
Strain was increased, as a function of pressure, in arteries from patients with EH (\(P<0.05\)) and further increased equally in arteries from patients with type 2D and type 2D+H.
Compared with controls (Figure 1A), the wall stress–intraluminal pressure relation was unchanged in arteries from patients with type 2D and type 2D/H compared with control patients; whereas wall stress was reduced in arteries from patients with EH at high intraluminal pressure \((P<0.05)\). The stress-strain relation in small arteries from control subjects \((>)\), patients with EH \((\Delta)\), and patients with type 2D \((\bigcirc)\) and type 2D+H \((\bigcirc)\) are shown. \(*P<0.05\), ANOVA, vs control. \(\dagger P<0.05\), ANOVA, vs H. B, Effects of preincubation of preconstricted small arteries with L-NMMA \((5 \times 10^{-5} \text{ mol/L})\) followed by exposure to increasing concentrations of Ach. \(\square\) indicates control subjects; \(\Delta\), EH; \(\bigcirc\), type 2D; \(\bigcirc\), type 2D+H. \(*P<0.05\) vs control. \(\dagger P<0.05\) vs EH.

**Vasoconstrictor Function**

Cumulative concentration-response curves to norepinephrine were obtained from vessels studied from all patients and control subjects. There was no difference in sensitivity or maximum response in any patient group compared with control subjects or each other. The maximum response to norepinephrine as a percentage of that obtained with KPSS was control subjects, 73±2.6%; patients with EH, 75±1.8%; patients with type 2D, 75±1.9%; and patients with type 2D+H, 75±2.2%.
Vasodilator Function

Endothelium-Dependent Dilation
Small arteries preconstricted with norepinephrine and then challenged with cumulative concentrations of Ach demonstrated varied responses (Figure 2A). There was no significant difference observed between maximum dilation in vessels from patients with EH compared with control subjects (79±7.3 versus 86±7.5%, respectively; NS Figure 2A). However, arteries from type 2D patients dilated significantly less well compared with control subjects (62±5.7 versus 86±7.5% of maximum, P<0.01; Figure 2A), and the maximum response was further attenuated in arteries from type 2D+H patients (49±4.6 versus 86±7.5%, P<0.01; Figure 2A). When compared with responses from vessels from patients with EH, those observed in type 2D and type 2D+H patients were significantly impaired (P<0.05, both comparisons; Figure 2A). The results of preincubating vessels with L-NMMA (5×10⁻⁵ mol/L) before constriction with norepinephrine and dilation with Ach are shown in Figure 2B. Arteries from control subjects had a 29% attenuation in maximum response (P<0.05), and those from patients with EH showed a 19% reduction (P<0.05). However, the responses in vessels from type 2D and type 2D+H patients only showed nonsignificant reductions of 10% and 1.2%, respectively. There were significant negative correlations between total cholesterol and percent dilation to Ach observed in arteries from patients with EH (r=-0.71 P<0.01; Figure 3A), type 2D (r=-0.61 P<0.01; Figure 3B), and type 2D+H (r=-0.77 P<0.01; Figure 3C), as well as control subjects (r=-0.56 P<0.01). Because the groups of diabetic patients were slightly older than control subjects, we examined whether age influenced Ach-induced dilation but found no significant association.

Endothelium-Independent Dilation
The responses of small arteries preconstricted with norepinephrine and challenged with sodium nitroprusside are shown in Figure 4. Although similar patterns emerged for each patient group as were seen with Ach, no parameter attained statistical significance.

Myogenic Reactivity
Because the responses from type 2D and type 2D+H patients were identical, the groups were combined. The data over the pressure range 20 to 200 mm Hg are shown in Figure 5. Small arteries from control subjects demonstrated a typical myogenic response over the pressure range and forced dilation >160 mm Hg. The response in patients with EH was shifted to the right: A similar response was observed but at higher pressures. However, vessels from type 2D and type 2D+H patients showed a significant impairment of myogenicity at pressures >50 mm Hg (P<0.05; Figure 5).

Discussion
This is the first study to report measurements of small-artery structure and function from patients with type 2D and type 2D+H using pressure myography. This technique permits assessment of vascular myogenic reactivity, which is not possible with the use of wire-mounted vessels; in this article, we have reported abnormalities of structure, distensibility, myogenicity, and endothelium-dependent dilation. The cohorts of patients investigated were carefully selected: with the exception of age, which was not significantly different, groups were comparable. The important features to emphasize are that the blood pressures were similar in patients with type 2D+H compared with patients with EH and that lipid profiles were abnormal to the same degree in patients with type 2D and type 2D+H. The presence or absence of diabetic complications and the use of oral hypoglycemic drugs or insulin did not influence the results.
Given the similar findings in the current study in vessels from both normotensive and hypertensive diabetic patients, it appears that contraction, at least to norepinephrine, can be regarded as normal in diabetes.

However, endothelium-dependent dilation is abnormal in small arteries from diabetic patients. In type 2D, preconstricted vessels exposed to Ach showed normal sensitivity but significant attenuation of the maximum dilation attained. The response was impaired further in type 2D+H despite the degree of hypertension being similar to our group of patients with EH, in which dilation was not significantly changed. The studies with L-NMMA demonstrate that the failure to dilate is due to an abnormality of nitric oxide–induced dilation as L-NMMA, which inhibits NO production, had no significantly inhibitory effect on the Ach-induced dilation of arteries from diabetic patients. The dissociation of hypertension from this endothelial dysfunction must point to another parameter in the metabolic syndrome of diabetes being responsible. In this article, we report significant correlations between maximum Ach-induced dilation and total cholesterol in all our subject groups, a finding previously documented in hyperlipidemic patients, and in subjects with high-normal lipid profiles.

In this context, it has been shown that hypercholesterolemia reduces the bioavailability of nitric oxide: Potential mechanisms include reduced availability of l-arginine, down-regulation of the guanosine Giα subunit that mediates nitric oxide activation, reduced expression of nitric oxide synthase, and inactivation of nitric oxide by superoxide anions or oxidized lipoproteins. Careful inspection of Figure 4 reveals nonsignificant impairment of endothelium-dependent independent dilation, which worsens in type 2D+H similarly to endothelium-dependent dilation. The implication is that at least part of the mechanism involves the activation of cGMP in vascular smooth muscle. Certainly the activity of guanylate cyclase may be affected directly by hypercholesterolemia as a result of the redox state of the vascular smooth muscle. The evidence supports a role for dyslipidemia contributing to endothelial function in all individuals, and the causes and their relative contributions remain to be determined.

The structural studies of small arteries in diabetes revealed a fundamental abnormality, which needs to be considered against what is known about the effects of hypertension on the vasculature. Morphological measurements of vessels from patients with EH confirmed the previous finding of eutrophic remodeling, which is a small reduction of lumen diameter and an increase in medial thickness without a change in cross-sectional area. However, in vessels from type 2 diabetic patients, whether hypertensive or not, the findings were different: Lumen diameter was unchanged compared with control values, and wall thickness was significantly increased with an increased cross-sectional area. The findings were similar in type 2D and type 2D+H, and calculations of growth and remodeling indexes clearly point to the vessels having undergone hypertrophy, a feature noted in previous investigations in diabetes.

Myogenic tone was assessed across a range of pressures up to 200 mm Hg, and we demonstrated myogenic responses with a rise in intraluminal pressures in vessels from control subjects:
Small arteries from patients with EH showed a similar profile, but responses were shifted to higher pressures, reflecting a shift in the autoregulatory response. However, in type 2D and type 2D+H, myogenic responsiveness was severely impaired across the whole pressure range. Wall stress may increase in the diabetic resistance vasculature as a consequence of an impaired myogenic response, and this may be the stimulus for vascular hypertrophy. This explanation assumes that defects in the myogenic response precede the changes in small-artery structure. This requires further investigation. The hypertrophic response, observed in vessels from diabetic patients, was associated with an increase in distensibility, as indicated by the rightward shift in the stress-strain relation. As this relation is independent of vessel geometry, the data indicate changes in wall composition as well as an increase in wall mass of arteries from diabetic patients, compared with arteries from both control patients and patients with EH.

The pathological consequences of these findings could be extremely important: Impaired endothelial function has been implicated as an early sign of a proatherogenic vascular environment, and diabetics are certainly prone to this upstream in medium-sized arteries. A failure to autoregulate blood flow efficiently might lead to increased high blood pressure flow to target organs, effecting downstream damage, as observed in the kidney and eye, for example.

In summary, we have demonstrated that small arteries in type 2D, irrespective of whether there is concomitant hypertension, show no change in vascular lumen diameter, significant wall hypertrophy, and markedly embarrassed myogenic reactivity. Studies directed at correcting dyslipidemia and investigating whether endothelial dysfunction can be reversed as has been reported in hypercholesterolemic patients, as well as attempting to improve abnormal myogenic tone must be regarded as a priority in diabetes, which is clearly emerging as a disease of disordered vascular structure and function.

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References
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