Mechanical properties of mesenteric arteries in diabetic rats: consequences of outward remodeling

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Crijns, Francy R. L., Bruce H. R. Wolfenbuttel, Jo G. R. De Mey, and Harry A. J. Struijker Boudier. Mechanical properties of mesenteric arteries in diabetic rats: consequences of outward remodeling. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1672–H1677, 1999.—Diabetes induces hemodynamic and biochemical changes that can influence mechanical properties of arteries. Structure and mechanics of mesenteric small arteries were investigated in rats with streptozotocin-induced diabetes (duration 7–9 wk). The external diameter of mesenteric artery branches was measured in control (n = 9) and diabetic (n = 7) Wistar Rp rats at baseline and during pressurization in situ (0–150 mmHg) under normal and passive smooth muscle conditions. Mean arterial pressure and mesenteric artery pressure were not significantly different. Baseline mesenteric artery diameter was larger in the diabetes-induced group (439 ± 12 vs. 388 ± 18 µm, P < 0.05). Media cross-sectional area of arteries from diabetic rats was not significantly increased (0.0149 ± 0.0015 vs. 0.0122 ± 0.0007 mm2). Cross-sectional compliance was significantly increased in diabetic rats at intraluminal pressures ranging from 25 to 75 mmHg (P < 0.005), whereas cross-sectional distensibility was not modified. Wall tension and circumferential wall stress were increased in diabetes. These results indicate that mesenteric small arteries of diabetic rats display eutrophic outward remodeling associated with increased wall tension and circumferential wall stress.

compliance; distensibility; wall tension; circumferential wall stress; hyperglycemia

DIABETES MELLITUS is associated with hemodynamic changes and alterations in the regional distribution of cardiac output (10). In rats with streptozotocin (STZ)-induced diabetes, blood flow to the small intestine is markedly increased, whereas skin and skeletal muscle blood flow are significantly reduced (10). Changes in flow and/or pressure can modify structural and mechanical properties of vascular beds and individual vascular segments (6). Arteries respond to changes in blood flow with acute vasomotor responses and with changes in wall structure when flow changes persist (17). Pourageaud and De Mey (23) demonstrated that chronic elevation of blood flow in mesenteric small arteries resulted in an increase in lumen diameter and media cross-sectional area, which normalize wall shear rate and circumferential wall stress. Sustained elevation of transmural pressure, as in chronic hypertension, is in many cases associated with an increase in the arterial wall mass-to-lumen ratio as a result of media hypertrophy or reduction of arterial lumen diameter (8). These chronic alterations in vascular structure are collectively referred to as remodeling and may have important effects on the mechanical properties of vessels such as compliance and distensibility (19). Arterial compliance is defined as the change in lumen volume for a given change in pressure, expressing the buffering capacities of the arterial system (32). This compliance depends on the size of the vascular lumen and on the elastic properties of the vascular wall. Distensibility is compliance corrected for the size of the vascular lumen and depends on the mass and intrinsic elastic properties of the vascular wall (32).

In diabetes, in addition to flow-induced changes, hyperglycemia by itself can lead to structural and functional vascular changes. High concentrations of D-glucose stimulate smooth muscle cell proliferation and growth (7). Furthermore, formation of advanced glycation end products, as a consequence of chronic hyperglycemia, can influence vascular properties. These products accumulate on tissue proteins with slow degradation rates and have been implicated in many complications associated with diabetes. Increased advanced glycation end products cause a diminished vascular elasticity and greater permeability of the vascular wall in diabetes (12). Moreover, glycated collagen has been proven to abolish the inhibitory effect of collagen on smooth muscle cell proliferation and may thus induce an increase in vascular wall mass (13).

The present study was performed to investigate to what degree the diabetes-induced hemodynamic and biochemical changes affect mechanical and morphological properties of mesenteric small arteries in rats, using an in situ model (24) to assess these properties.

MATERIALS AND METHODS

Animals. Sixteen male Wistar Rp rats (TNO-Repgo, Rijswijk, The Netherlands) were housed individually in temperature-controlled rooms and had free access to standard rat chow and water. At an average body weight of 250 g (approximate age of 2 mo) diabetes was induced in seven animals by intraperitoneal injection of 70 mg/kg STZ (Sigma, St. Louis, MO) in citrate buffer (pH 4.5). The remaining rats received the vehicle. The experimental procedures were performed according to institutional guidelines and were approved by the Ethical Committee for the Use of Experimental Animals.
of the Maastricht University (The Netherlands). Between 7 and 9 wk after diabetes was induced in the rats, we performed the mesenteric artery experiments.

Mesenteric artery preparation. The experimental setup of the in situ mesenteric artery is an adaptation from a previously described method (24). Animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg). An arterial catheter connected to a pressure transducer (CP-01, Century Technology, Inglewood, CA) was introduced into the left femoral artery to measure mean arterial blood pressure. A second catheter was introduced into the left femoral vein for continuous infusion of pentobarbital sodium (30 mg·kg\(^{-1}·\)h\(^{-1}\)) to maintain anesthesia during the whole experiment. A blood sample of the nonfasted rats was taken via the arterial catheter to measure levels of glucose and glycated hemoglobin (HbA\(_{1c}\)) by a hexokinase method and HPLC, respectively. HbA\(_{1c}\) levels reflect the amount of adducts of glucose to hemoglobin and estimate the long-term glycemic control.

A median laparotomy was performed, and the most distal loop of small intestine was exteriorized. This tissue was exposed and irrigated with a buffered Tyrode solution (pH 7.4) maintained at 38°C. The mesenteric arteries and arcades were neither fixed nor stretched. The preparation was translucent. A short segment of mesenteric artery (3–3 mm) of a second-generation branch (diameter 300–500 µm) was gently freed of surrounding tissue under a binocular lens (Wild M5A, Heerbrugg, Switzerland). A video camera (CCD, Sony, The Netherlands) was mounted on the binocular lens. The final magnification was \(\times 100\). All arterial branches downstream of the exposed arterial segment, except one, were ligated. A removable microclamp was placed on the mesenteric artery upstream of the isolated segment. A polyethylene catheter filled with Tyrode solution containing albumin (4%) was introduced into the unligated branch for local mesenteric artery pressure measurements using a microswitch pressure transducer (150 PC Flow-Thru pressure sensor, Honeywell). The same catheter was connected via a three-way tap to a manometer with adjustable pressure levels. After a removable microclamp was placed proximally on the observed segment, the mesenteric artery was exposed to different pressure levels, while external diameter was measured with an image-shearing monitor (model 908, IPM, San Diego, CA). The artery was exposed to pressure steps of 25 mmHg from 0 to 150 mmHg at 2-min intervals. Video recordings of the last 20 s of each pressure step were used for the diameter measurements.

Study protocol. After the arterial segment was exposed to atmospheric pressure for 30 min, mean arterial pressure, mean mesenteric artery pressure, and external diameter of the mesenteric artery were measured. Mesenteric arterial pressure-diameter relationships were obtained during superfusion with normal Tyrode solution (control conditions). Subsequently, the mesenteric artery was flushed and superfused with Tyrode solution containing potassium cyanide (100 mg/l). A 30-min incubation period was sufficient to poison the smooth muscle and totally abolish smooth muscle tone without a significant effect on vessel wall morphology (2). The mesenteric arterial diameter-pressure relation was then determined in this fully relaxed state.

At the end of the experiment, the mesenteric artery was flushed and filled with formaldehyde (4%). The vessel was maintained for 20 min under these conditions at its individual baseline mesenteric artery pressure. The fixed vessel was then excised and processed for morphometrical analysis. Media cross-sectional area, defined as the area enclosed by the internal and external elastic laminae, and media thickness were determined by semiautomated morphometry (JAVA 1.21, Jandel Scientific, Corte Madera, CA) on 4-µm sections stained with Lawson’s solution, a classic elastin stain. Media-to-lumen ratio was calculated as two times media thickness divided by the lumen diameter.

Data calculation. With the use of the measured values of the “in situ” arterial external diameter (\(D_e\), µm) over the pressure range of 0–150 mmHg and the media cross-sectional area (CSA, µm\(^2\)) measured after the experiment, the following parameters were calculated, using SI units. Wall thickness (h, µm) was calculated as: \(h = D_e/2 - (D_e/4 - CSA/A)^{1/2}\) on the basis of the assumption of a noncompressible arterial wall; internal diameter (\(D_i\), µm) as: \(D_i = D_e - 2h\); internal lumen area (\(A_i\), µm\(^2\)) as: \(A_i = \pi D_i^2/4\); cross-sectional compliance (\(C_{cs}\), mm\(^2\)/kPa) as: \(C_{cs} = \Delta A_i/\Delta P\), in which \(\Delta A_i\) is the change in internal lumen area induced by a pressure change (\(\Delta P\)); cross-sectional distensibility (\(D_{cs}\), kPa\(^{-1}\)) as: \(D_{cs} = C_{cs}/A_i\); wall tension (WT, N/m) as: \(WT = D_i^2/2\) on the basis of Laplace’s law; and circumferential wall stress (WS, kPa) as: \(WS = WT/h\). The various parameters were determined for each pressure applied.

Statistical analysis. Results are expressed as means ± SE. Statistical significance of differences was evaluated by Student’s t-test or two-way ANOVA followed by the Student-Newman-Keuls post hoc test for multiple comparisons. P values <0.05 were considered statistically significant.

RESULTS

Table 1 summarizes the general characteristics of the control and diabetic animals. Glucose levels were significantly elevated in the diabetes group compared with the control group, whereas body weight was significantly lower (\(P < 0.001\), t-test). The increased HbA\(_{1c}\) levels in the diabetic rats (\(P < 0.01\), t-test) reflect long-term exposure to high blood glucose levels. Mean aortic and mesenteric artery pressures were slightly but not significantly lower in the diabetes group compared with the controls. External diameter of mesenteric arteries at baseline was larger in the diabetes group (\(P < 0.05\), t-test). Morphological properties of the mesenteric arteries, measured on cross sections of formaldehyde-fixed vessels, are shown in Table 2. Media cross-sectional area was not significantly increased in the diabetic rats (\(P = 0.09\), t-test). Media thickness and media-to-lumen ratio were comparable in both groups.

Figure 1A shows the pressure-diameter relationships under basal conditions and after inactivation of the smooth muscle. At all pressure levels and under both basal and passive smooth muscle conditions, the
diabetic animals exhibited a larger external arterial diameter than their nondiabetic littermates \( (P < 0.001, \text{two-way ANOVA}) \). Although smooth muscle poisoning seemed to have more effect in the control than in the diabetic rats, two-way ANOVA showed no statistically significant difference between basal and passive diameters within each group. In both groups, cross-sectional compliance and distensibility reached the highest level in the pressure range of 25–75 mmHg (Fig. 1, B and C). In this range, compliance was significantly larger in the diabetes group than that in the control group \( (P < 0.005, \text{t-test}) \), whereas distensibility was comparable between groups. In the lower pressure range (25–75 mmHg) of control animals, cross-sectional compliance was higher under passive conditions compared with basal, whereas in the higher pressure range opposite effects were obtained. Comparable to the effect on external diameter, smooth muscle poisoning had only minor effects on compliance of arteries of diabetic rats. Pressure-wall tension and pressure-circumferential wall stress relationships (Fig. 2) were steeper for diabetic rats compared with controls \( (P < 0.001, \text{two-way ANOVA}) \). Similar findings were obtained in the presence of potassium cyanide (not shown).

**DISCUSSION**

The present study shows that mesenteric small arteries of STZ-induced diabetic rats display an increased diameter. This outward remodeling is observed over a broad range of intraluminal pressures and persists after abolition of smooth muscle tone. Mesenteric artery compliance is increased at low pressure levels in diabetic rats, whereas distensibility is not modified. On the other hand, wall tension and circumferential wall stress in mesenteric arteries of diabetic rats are enhanced compared with mesenteric arteries of nondiabetic control rats.

The STZ-induced diabetic rat is a widely used model for type 1 diabetes. Numerous studies are performed with this model, proving its usefulness in studying diabetic complications. Histological studies and studies using insulin-treated diabetic rats have clearly demonstrated that STZ is selectively toxic to the pancreatic \( \beta \)-cells (28).

Most studies of arterial mechanics in diabetes have been performed in large vessels, such as the aorta and carotid arteries. They describe increased arterial stiffness, indicated by reduced distensibility (15, 21). Few reports demonstrate that the mechanical properties of small arteries and arterioles are affected as well (9, 14).

Arterial mechanical properties can be influenced by humoral and metabolic factors on the one hand and hemodynamic forces and structural factors on the other hand. In diabetes, accumulation of advanced glycation end products in the vascular wall is a major feature that can contribute to a diminished elasticity of arteries, as demonstrated by Huijberts et al. (12), who studied carotid artery properties of aminoguanidine (AG)-treated rats. Rumble et al. (25) showed that the formation of advanced glycation end products is present in the mesenteric vascular bed of STZ-induced diabetic rats as well. Nevertheless, we demonstrate in this study that in mesenteric small arteries of diabetic rats no change in distensibility occurs, whereas compliance is even increased at low pressure levels. The latter

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<th>Control ((n = 9))</th>
<th>Diabetes ((n = 7))</th>
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<tbody>
<tr>
<td><strong>CSA, mm(^2)</strong></td>
<td>0.0122 ± 0.0007</td>
<td>0.0149 ± 0.0015</td>
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<tr>
<td><strong>MT, mm</strong></td>
<td>0.0129 ± 0.0010</td>
<td>0.0145 ± 0.0010</td>
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<td><strong>M/L</strong></td>
<td>0.091 ± 0.006</td>
<td>0.094 ± 0.003</td>
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Values are means ± SE; \( n \), number of rats. CSA, media cross-sectional area mesenteric artery; MT, media thickness; M/L, media-to-lumen ratio.
is in contradiction with earlier reports on arterial mechanical properties in diabetes. A reduced arterial compliance and distensibility has been found by several authors who studied mechanical properties of arteries or arterioles in diabetes (9, 14, 15, 21). However, none of these studies investigated mechanical properties in mesenteric arteries. The enhanced compliance, as described in our study, results from changes in mesenteric artery dimensions that occur in experimental diabetes. We observed larger external mesenteric artery diameters of diabetic rats at baseline and at imposed pressures ranging from 0 to 150 mmHg. The enhanced mesenteric artery diameter at baseline can be caused by several factors. Korhuis et al. (16) demonstrated that blood-borne or humoral agents that are associated with diabetes mellitus, e.g., hyperosmolarity, hyperglycemia, and elevated plasma glucagon, induce vasodilation of the intestinal vascular bed. Furthermore, insulin is known to induce vasoconstriction in mesenteric arteries (31). Because the insulin concentration is extremely low in STZ-diabetic rats, these arteries may be dilated compared with mesenteric arteries of control rats. Our observation that the enhanced diameter in diabetic arteries persists after perfusion of the arteries with Tyrode solution and under passive smooth muscle conditions indicates, however, that diameter changes in the mesenteric arterial bed following long-term diabetes involve other processes than vasomotor mechanisms. An increased flow to the small intestine of diabetic rats due to marked hyperphagia (3, 10) is a plausible stimulus for the outward remodeling (23) that occurs in mesenteric arteries of diabetic rats. An increase in flow or wall shear stress has been shown to lead to an acute vasodilatation involving endothelium-derived nitric oxide (NO) and prostacyclin (22). Fujii et al. (4) demonstrated that flow-induced dilatation is, unlike acetylcholine-induced endothelium-dependent relaxation, well preserved in the basilar artery of diabetic rats. Our study suggests that chronic flow-induced outward remodeling, which has been demonstrated to depend on the endothelium in certain settings (29), is preserved in mesenteric arteries of diabetic rats as well.

In addition to outward remodeling, chronic increases in blood flow are accompanied by wall hypertrophy to normalize circumferential wall stress that increases during the expansion of the vessels (23). However, in the present study we could not detect a statistically significant hypertrophy of small mesenteric arteries. This is in contrast with earlier findings (1, 30). Nevertheless, these studies were performed in Sprague-Dawley rats on arteries and arterioles of different sizes than those used in our study. In addition, the diabetes duration in the rats was different (3 wk and 6 mo). This might explain why these authors observed a significant hypertrophy and we did not. Although the exact mechanism of vascular wall hypertrophy accompanying hyperperfusion is not yet elucidated, an upregulation of growth factor genes in response to elevated shear stress has been demonstrated (20). In hyperglycemic circumstances, the mitogenic activity of basic fibroblast growth factor is markedly reduced due to cytosolic protein modification by advanced glycation end products (5). It may be speculated that, in diabetes, intracellular advanced glycation end products interfere in this way with the wall hypertrophy that normally accompanies flow-induced remodeling. One possibility to test this hypothesis would be to study vascular wall hypertrophy in AG-treated diabetic rats, because AG is known to inhibit the formation of advanced glycation end products (5). We performed morphological measurements on mesenteric arteries of rats that were diabetic for 8 wk and chronically treated with AG (250 mg/l in drinking water). This group of rats supported our hypothesis that glycation products interfere with the hypertrophic response to flow. Mesenteric arteries of AG-treated rats showed indeed a significantly enhanced hypertrophy of the media compared with nondiabetic control rats (media cross-sectional area = 0.0174 ± 0.0015 mm² in AG-treated diabetic rats vs. 0.0122 ± 0.0071 mm² in control rats; P < 0.05). However, AG is also known to act as an NO synthase inhibitor (18). Because NO has antimitogenic effects (26), reduction of NO production by AG treatment might increase vascular smooth muscle growth. Therefore, the AG data cannot be used as a definite proof of our hypothesis. We have to await the availability of newly developed drugs, acting specifically on the formation or degradation of glycation products, to obtain a definite answer to the question whether flow-induced hypertrophy is inhibited by the formation of advanced glycation end products in diabetes or not.

As a result of the outward remodeling of the mesenteric small arteries without significant wall hypertro-
phy, wall tension as well as circumferential wall stress was elevated in the diabetic rats. Apparently, the structural response of the arteries of diabetic rats was insufficient to normalize circumferential wall stress. This is in contrast with earlier findings in hypertension where the pressure-related hypertrophy of the vascular wall results in a normalization of the arterial circumferential wall stress (24, 27). The elevated circumferential wall stress in mesenteric arteries of diabetic rats can therefore be deleterious for the functional integrity of the vascular wall (6) and may contribute to enhanced permeability of blood vessels in diabetes (11, 12).

In the first years after the onset of diabetes in humans, blood flow has been found to be increased in several organs and tissues, e.g., in the kidney and retina. Taking into account the experimental findings of the present study, outward remodeling of arteries may lead to an elevated wall tension and circumferential wall stress and may affect these tissues in a similar way and contribute to the typical complications such as diabetic retinopathy and nephropathy.

In conclusion, this study demonstrates that, in STZ-induced diabetic rats, mesenteric small arteries display eutrophic outward remodeling. Despite these structural changes, which could develop as a consequence of increases in blood flow to the small intestine, mesenteric arteries show an elevated circumferential wall stress. We hypothesize that advanced glycosylation of proteins that are involved in normal control of arterial wall stress and may affect these tissues in a similar way and contribute to the typical complications such as diabetic retinopathy and nephropathy.

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