Predominance of hemodynamic rather than metabolic factors in the pathogenesis of diabetic glomerulopathy

(glomerular filtration and perfusion/glomerular sclerosis/renal hemodynamics/proteinuria/chronic renal failure)

ROBERTO ZATZ*, TIMOTHY W. MEYER*, HELMUT G. RENNKE†, AND BARRY M. BRENNER*‡

Departments of *Medicine and †Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115

Communicated by Eugene Braunwald, May 17, 1985

ABSTRACT Six groups of Munich-Wistar rats underwent micropuncture study 2–10 weeks and morphologic studies 11–13 months after induction of streptozotocin diabetes or after sham treatment. Diabetic rats received diets containing 6% (group D6), 12% (D12), or 50% protein (D50) and were maintained under similar conditions of moderate hyperglycemia by daily injections of ultralente insulin. Age- and weight-matched normal control rats were also given 6% (Group N6), 12% (N12), or 50% protein (N50). Kidney weight, whole-kidney and single-nephron glomerular filtration rate, glomerular plasma flow, and mean glomerular transcapillary hydraulic pressure difference were higher in D50 rats than in all other groups and predisposed this group to marked and progressive albuminuria. Likewise, histological examination of the kidneys disclosed areas of sclerosis in 19.6% of glomeruli in D50 rats; the frequency of such lesions was <2.5% in all other groups. These findings indicate that the metabolic disorder seen in stable, moderately hyperglycemic diabetic rats does not lead to glomerulopathy as long as elevations in glomerular pressures and flows are prevented.

Increases in glomerular capillary pressures and flows have been implicated in the initiation and progression of diabetic nephropathy (1). In both patients and animals with diabetes, a state of glomerular hyperfiltration has been observed prior to the development of glomerular injury (2–8). In diabetic rats the elevation of glomerular filtration rate (GFR) in individual nephrons was shown first by Hostetter et al. (6) and subsequently by others (9, 10) to result from an augmentation of both glomerular plasma flow rate ($Q_A$) and local transcapillary hydraulic pressure difference ($\Delta P$).

Glomerular morphologic changes resembling those seen in diabetes have been associated with increases in capillary pressures and flows in other experimental forms of renal disease, including ablation of renal mass (11–13), high-protein diet (14), and some models of hypertension (15, 16). Furthermore, the extent of glomerular lesions in diabetic rats can be enhanced by maneuvers known to increase glomerular capillary pressures and flows, such as contralateral renal arterial clipping (17), ablation of the contralateral kidney (18), or high-protein feeding (19).

Studies to date have not dissociated the effects of glomerular hypertension and hyperperfusion from those of chronic hyperglycemia per se (20–22). In the present study, glomerular hemodynamic function was deliberately varied in rats with streptozotocin (STZ)-induced diabetes while stable moderate hyperglycemia was maintained by daily exogenous insulin. This approach made it possible to relate the early alterations in glomerular pressures and flows in experimental diabetes to the subsequent development of glomerular injury.

METHODS Ninety-three adult male Munich-Wistar rats (220–260 g each) were studied. Forty-three rats were made diabetic by a single intravenous injection of STZ (60 mg/kg). Morning blood glucose concentration was determined biweekly. Rats received daily evening injections of ultralente insulin (NOVO Industries, Copenhagen) (23) in doses to maintain blood glucose concentration (BG) between 200 and 400 mg/dl. Diabetic rats were maintained on diets containing 50% (group D50, n = 15), 12% (D12, n = 15), or 6% casein (D6, n = 13). Normal control rats matched for initial body weight received the same diets containing 50% (group N50, n = 18), 12% (N12, n = 16), or 6% casein (N6, n = 16). The composition of the diets was 82% (wt/wt) casein plus carbohydrate, 5% lipid, 1% phosphate, 1% calcium, and 11% other components.

Twenty-five diabetic rats and 24 control rats underwent micropuncture study 2–10 weeks (mean 4.2) after STZ injection. Rats were anesthetized with Inactin (100 mg/kg) and prepared for micropuncture in standard fashion (24). Euvolemia was maintained by iso-oncotic plasma replacement (25). Inulin (10 g/dl in isotonic saline solution) was also infused at a rate of 1.2 ml/hr. Tubule fluid was collected from superficial proximal tubules for determinations of flow rate and inulin concentration (26). Blood samples from surface efferent arterioles were evaluated for total protein concentration (24, 27), and hydraulic pressures were measured by the servo-null technique (24).

Some rats in each group were not subjected to micropuncture but were maintained at stable moderate hyperglycemia by daily ultralente insulin treatment for 11–13 months after induction of diabetes. Twenty-four-hour urinary albumin excretion rates were determined at 3, 6, 9, and 12 months (28). Soon thereafter, glycosylated hemoglobin levels were measured (29), and kidneys were fixed by perfusion for 5 min with 1.25% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After fixation, a mid-coronal section from each kidney was further processed through paraffin embedding for light microscopic evaluation of renal lesions. Three-micrometer-thick sections were stained with hematoxylin/eosin or periodic acid/Schiff reagent (PAS) and examined by light microscopy for segmental glomerular lesions. For quantitative analysis, the number of glomeruli with segmental collapse of capillaries was determined on two coronal sections per animal and expressed as a percent of total number of glomeruli examined; the average number of glomeruli examined per animal was 443. Epoxy-resin-embedded fragments of renal cortex were sectioned at 1 μm, stained with toluidine

Abbreviations: STZ, streptozotocin; GFR, whole kidney glomerular filtration rate; SNGFR, single-nephron glomerular filtration rate; $Q_A$, glomerular plasma flow rate; $P_{OC}$, mean glomerular capillary hydraulic pressure; $\Delta P$, mean glomerular transcapillary hydraulic pressure difference.

§1734 solely to indicate this fact.
Table 1. Glomerular hemodynamic studies in diabetic (2–10 weeks) and normal control rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>SNFGFR</th>
<th>QA</th>
<th>RA</th>
<th>RE</th>
<th>(P_{\text{GC}})</th>
<th>PT</th>
<th>(\Delta P)</th>
<th>CA</th>
<th>CE</th>
<th>Kc</th>
</tr>
</thead>
<tbody>
<tr>
<td>D50</td>
<td>9</td>
<td>77.4 ± 5.0</td>
<td>274 ± 25</td>
<td>0.78 ± 0.12</td>
<td>0.98 ± 0.09</td>
<td>63 ± 11</td>
<td>52 ± 3</td>
<td>5.8 ± 0.1</td>
<td>8.3 ± 0.3</td>
<td>56 ± 6</td>
<td></td>
</tr>
<tr>
<td>D12</td>
<td>8</td>
<td>57.1 ± 6.7</td>
<td>204 ± 21</td>
<td>1.32 ± 0.20</td>
<td>1.08 ± 0.17</td>
<td>52 ± 2</td>
<td>11 ± 1</td>
<td>41 ± 2</td>
<td>5.7 ± 0.1</td>
<td>7.8 ± 0.2</td>
<td>71 ± 15</td>
</tr>
<tr>
<td>D6</td>
<td>6</td>
<td>34.9 ± 3.4</td>
<td>104 ± 13</td>
<td>2.57 ± 0.46</td>
<td>2.27 ± 0.41</td>
<td>50 ± 1</td>
<td>11 ± 0.3</td>
<td>39 ± 1</td>
<td>5.6 ± 0.2</td>
<td>8.2 ± 0.3</td>
<td>39 ± 6</td>
</tr>
<tr>
<td>N50</td>
<td>10</td>
<td>52.7 ± 6.8</td>
<td>182 ± 19</td>
<td>1.67 ± 0.02</td>
<td>1.15 ± 0.10</td>
<td>50 ± 2</td>
<td>11 ± 1</td>
<td>40 ± 1</td>
<td>5.7 ± 0.1</td>
<td>8.1 ± 0.8</td>
<td>70 ± 10</td>
</tr>
<tr>
<td>N12</td>
<td>7</td>
<td>37.3 ± 2.3</td>
<td>129 ± 12</td>
<td>2.12 ± 0.15</td>
<td>1.60 ± 0.41</td>
<td>51 ± 2</td>
<td>13 ± 1</td>
<td>37 ± 1</td>
<td>5.8 ± 0.1</td>
<td>8.3 ± 0.2</td>
<td>71 ± 10</td>
</tr>
<tr>
<td>N6</td>
<td>7</td>
<td>30.3 ± 2.0</td>
<td>108 ± 11</td>
<td>2.75 ± 0.30</td>
<td>1.73 ± 0.19</td>
<td>47 ± 1</td>
<td>11 ± 1</td>
<td>36 ± 1</td>
<td>5.0 ± 0.1</td>
<td>7.0 ± 0.2</td>
<td>37 ± 5</td>
</tr>
</tbody>
</table>

\(R_A\) and \(R_E\) are, respectively, afferent and efferent arteriolar resistances. \(C_A\) and \(C_E\) are the systemic and the efferent arteriolar protein concentrations. \(K_c\) is the glomerular capillary ultrafiltration coefficient.

*Millimeter of mercury; 1 mm Hg = 133.3 pascals.

Table 2. Probabilities (P) from pairwise comparisons of the data in Table 1.

<table>
<thead>
<tr>
<th>SNFGFR</th>
<th>QA</th>
<th>RA</th>
<th>RE</th>
<th>(P_{\text{GC}})</th>
<th>PT</th>
<th>(\Delta P)</th>
<th>CA</th>
<th>CE</th>
<th>Kc</th>
</tr>
</thead>
<tbody>
<tr>
<td>D12 vs. D50</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>D6 vs. D50</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>D50 vs. N50</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D6 vs. N6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D12 vs. N12</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>N6 vs. N50</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>N12 vs. N50</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.001</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

NS, not significant (P > 0.05).

RESULTS

At 2–10 weeks after induction of diabetes or sham treatment, average body weight was slightly lower in groups D12 (252 ± 5 g, mean ± SEM) and D50 (264 ± 8 g) as compared to groups N12 (291 ± 6 g) and N50 (301 ± 16 g), whereas groups D6 (211 ± 3 g) and N6 (234 ± 5 g) showed further limitation in body mass. Blood glucose concentration measured at this time was similar in the three diabetic groups (=325 mg/dl), averaging about 3 times higher than control levels. No differences in mean arterial pressure or hematocrit were observed. Left kidney weight was increased 21–38% in diabetic groups as compared to corresponding normal groups. Increasing dietary protein content also increased left kidney weight, average values for the D50 and N50 groups being 60% and 80% higher than for the D6 and N6 groups, respectively. GFR was significantly higher in D50 (2.1 ± 0.1 ml/min) and D12 (1.5 ± 0.1) groups as compared to N50 (1.50 ± 0.2) and N12 (1.2 ± 0.1). Moderate protein restriction significantly reduced GFR in the D12 group as compared to D50 rats without limiting body growth. GFR averaged 1.1 ± 0.1 ml/min in D6 and 0.8 ± 0.1 ml/min in N6.

Tables 1 and 2 and Fig. 1 summarize results of glomerular microcirculatory function. As with left kidney weight and GFR, mean values for single-nephron GFR (SNFGFR) were nearly 50% higher in D50 and D12 as compared to N50 or N12 groups. SNFGFR values tended to vary in proportion with glomerular plasma flow (QA). Increasing QA reflected increasing renal arteriolar vaso-dilation, most pronounced in the D50 group. In this group, afferent arteriolar resistance (RA) was lowered to a proportionately greater extent than was efferent resistance (RE). As a result the D50 group, and only this group, had values of mean glomerular capillary hydraulic pressure \(P_{\text{GC}}\) and \(\Delta P\) that were markedly increased above normal. Little differences in values for proximal tubule hydraulic pressure (PT) were found among groups. Elevation in \(\Delta P\), in association with elevation in QA, accounted for the marked elevation in SNFGFR observed in D50 rats. Dietary

Fig. 1. Mean values for SNFGFR, QA, and \(\Delta P\) 2–10 weeks after induction of diabetes or sham treatment. Open bars represent normal rats and solid bars represent insulin-treated diabetic rats. The stippled horizontal bands indicate normal values (±1 SEM) observed in rats fed 24% protein chow (31). Asterisks denote significant differences from normal. Note that only D50 rats had significant increases in all three hemodynamic parameters.
protein restriction prevented increases in $P_{\text{GC}}$ in both the D6 and D12 groups; the elevation of $Q_a$ was likewise completely prevented in the D6 and partially prevented in the D12 group (31, 32). Except for mild reductions in systemic ($C_v$) and efferent arteriolar ($C_E$) protein concentrations in the N6 group, values for these quantities were similar in the other groups studied. A unique value for the glomerular capillary ultrafiltration coefficient, $K_f$, could be calculated in virtually every experiment. No significant alteration in this parameter was observed as a consequence of diabetes or dietary protein manipulation, though numerically lower mean $K_f$ values were found in the groups subjected to severe protein restriction. Thus, the observed differences in SNGFRI among the diabetic groups were due largely to variations in glomerular pressures and flows.

In rats sacrificed 11–13 months after induction of diabetes or sham treatment, body weight was significantly lower in D50 ($365 \pm 24 \text{ g}$) and D12 ($355 \pm 10 \text{ g}$) rats as compared to N50 ($442 \pm 12 \text{ g}$) and N12 ($408 \pm 10 \text{ g}$). No differences in body weight were found between D6 and N6. As in the short-term studies, left kidney weight was increased in diabetics as compared to corresponding normal control animals; likewise, left kidney weight increased with increasing dietary protein in both normal and diabetic rats.

As depicted in Fig. 2, moderate hyperglycemia was maintained over the duration of the long-term study, and mean blood glucose values obtained prior to sacrifice were similar in the three diabetic groups. Equivalence of glycemic control in long-term diabetic rats was confirmed with the demonstration of similar levels of glycosylated hemoglobin in the three diabetic groups: $16.4 \pm 1.2\%$ in group D50, $19.0 \pm 0.8\%$ in D12, and $16.8 \pm 3.4\%$ in D6. In normal groups the level of glycosylated hemoglobin averaged $5.7\%$ or less.

At 11–13 months, D50 rats exhibited marked proteinuria, urinary albumin excretion averaging 1–2 orders of magnitude higher than in any other group (Fig. 3). The tendency toward greater albuminuria in the D50 group was already apparent at 3 months of diabetes. Although albumin excretion exhibited a steep increase with time in D50, only a slight elevation above baseline was observed in D6 and D12 rats after 1 year of study.

Renal structural alterations were limited almost exclusively to animals in the D50 group. The most notable glomerular alterations consisted of segmental collapse of the capillary tuft, often accompanied by hyaline deposition and adhesion of the tuft to Bowman’s capsule (Fig. 4a). Sclerotic lesions were observed on average in $19.6 \pm 3.9\%$ of glomeruli in D50 but were seen much less frequently ($P < 0.001$) in all other groups (D12, $2.3 \pm 0.4\%$; D6, $1.8 \pm 0.6\%$; N50, $2.1 \pm 1.4\%$; N12, $0.7 \pm 0.2\%$; N6, $0.4 \pm 0.2\%$). Expansion of the mesangial area, the magnitude of which appeared to be related to the development of segmental lesions, was likewise more prominent in D50 than in any other group (Fig. 4b and c). Tubule lesions characterized by vacuolation of distal segments of the nephron were occasionally present in diabetic animals regardless of the protein content of the diet. Focal tubule atrophy, cast formation, and interstitial inflammation were observed in D50 animals, the frequency of these changes being approximately proportional to the glomerular involvement in any given animal.

**DISCUSSION**

Early stages of type I clinical diabetes and experimental diabetes mellitus are characterized by sustained glomerular hyperfiltration (2–5). In diabetic rats, this increase in GFR has been shown to result from elevations in $Q_a$ and $\Delta P$ (6, 9, 10). A number of observations have suggested that, as in the experimental model of reduction of renal mass, these glomerular hemodynamic alterations contribute to the development of progressive glomerular sclerosis in diabetes (1). Steffes *et al.* (18) showed that after 3 months of diabetes, the extent of glomerular injury was greater in STZ-induced diabetic rats subjected to uninephrectomy than in diabetic rats with two kidneys. In another study (17) from the same laboratory, STZ was used to induce diabetes in rats with two-kidney, one-clip Goldblatt hypertension. After 4 months, a striking intensification in glomerular lesions was observed in the unclipped kidney of diabetic rats, as compared to either nonhypertensive diabetic or hypertensive nondiabetic rats, whereas the clipped kidney was relatively protected. Furthermore, glomerular injury is attenuated in STZ diabetic rats maintained on a low-protein diet and exacerbated in diabetic rats fed a protein-rich diet (19). Taken together, these previous studies suggest that the increases in glomerular capillary pressures and flows that characterize early experimental diabetes contribute to the subsequent development of glomerular injury. They have not established, however, to what extent normalization of capillary pressures and flows can prevent glomerular injury in the face of sustained hypertension.

![Fig. 2. Serial average blood glucose values reveal stable moderate hyperglycemia in all three groups of diabetic rats. Percent protein in diet is given above each graph.](image-url)

![Fig. 3. Urinary albumin excretion ($U_a/V$) as a function of time in long-term studies. Marked and progressive albuminuria occurred only in the D50 group. Percent protein in diet is given above each pair of graphs for matched diabetic and control groups.](image-url)

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hyperglycemia. The present study was designed to dissociate glomerular hemodynamic alterations in diabetes from the metabolic derangements that characterize this disorder. Diabetic rats maintained on a 50% protein diet exhibited marked glomerular capillary hypertension and hyperperfusion similar to that described previously in diabetic rats on a standard (24% protein) diet (6, 31), whereas these alterations were essentially absent in diabetic rats maintained on diets of lower protein content.

D50 rats exhibited a marked increase in urinary albumin excretion with time, reaching nearly 200 mg/24 hr by 1 year. Morphologic examination disclosed a high incidence of glomerular lesions in these rats, with nearly 20% of glomeruli showing significant sclerosis. Only low-grade albuminuria and minimal glomerular sclerosis were observed in the other groups, presumably due to normal aging (33). Since the same degree of blood-glucose control was imposed in each of the diabetic groups, the divergent patterns of albuminuria and glomerular histology cannot be ascribed to major differences in the metabolic derangements associated with hyperglycemia among the groups.

The mechanism(s) underlying the vasodilatory effect of diabetes on the renal microcirculation is largely unknown. Previous studies have implicated vascular hyporesponsiveness to angiotensin II and catecholamines (34, 35), increased production of prostaglandins (36, 37), and elevated levels of blood glucose (38), glucagon (39), and growth hormone (40). The mechanism(s) responsible for the renal vasodilatory effects of high-protein feeding, seen in this and other studies (32, 41), is likewise unclear. Comparison of the results obtained in D50 and N50 rats, however, suggests that the renal hemodynamic effects of high-protein feeding and diabetes, like the effects on the kidney of partial nephrectomy in combination with diabetes (42), are additive. Further, the finding of normal or subnormal glomerular pressures and flows in diabetic rats subjected to dietary protein restriction suggests that some aspect of protein or amino acid metabolism is crucial to the expression of the renal vasodilatation of early diabetes.

Irrespective of the mechanism(s) operative in the genesis of glomerular injury in diabetes, as an increasing number of glomeruli undergo progressive destruction, an additional burden is imposed on the remaining, less affected glomeruli. As in rats subjected to surgical ablation of renal mass, it has been proposed that this process leads to further renal arteriolar dilitation and consequently additional increases in glomerular pressures and flows, closing a positive feedback loop and perpetuating a process that results ultimately in end-stage renal failure (43). Although the present study was terminated before end-stage renal failure occurred, the exponential increase in albuminuria observed with time in the D50 group suggests that increasingly rapid glomerular injury was occurring in these animals and that, in a study of longer duration, severe loss of renal function would very likely have eventuated.

**FIG. 4.** High-power light micrographs illustrating representative glomerular lesions observed in diabetic animals. (a) A glomerulus showing a characteristic segmental lesion, with collapse of about 40% of the capillary tuft, adhesion of the tuft to Bowman's capsule, and hyaline deposition. Such lesions were present in 19.6% (average) of all glomerular profiles in D50 animals but in less than 2.5% in all other groups. (b) A glomerulus showing moderate expansion of the mesangial compartment, a qualitative alteration observed almost exclusively in D50 animals. Compare to c. (c) A glomerulus from a D12 animal; the mesangium appears normal. Glomeruli in D6 and all control groups were nearly always similar in appearance to the glomerulus shown in c. (Periodic acid/Schiff stain; × 310.)
J. L. Troy, J. L. Noddin, A. W. Nunn, and D. Sandstrom provided expert technical assistance. R.Z. received an International Research Fellowship Award from the Fogarty International Center of the National Institutes of Health (TWO3263-01SI) and a grant from the State of Sao Paulo Foundation for Research Support, Sao Paulo, Brazil (83/2570-1). Additional support for these studies was provided through grants from the National Institutes of Health (AM30410) and The Kroc Foundation.