Development of Late-Stage Diabetic Nephropathy in OVE26 Diabetic Mice

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OVE26 mice are a transgenic model of severe early-onset type 1 diabetes. These mice develop diabetes within the first weeks of life and can survive well over a year with no insulin treatment, and they maintain near normal body weight. To determine whether OVE26 mice provide a valuable model of chronic diabetic nephropathy (DN), OVE26 diabetic mice were compared with their nondiabetic littermates for functional and structural characteristics of DN. OVE26 mice exhibited pronounced polyuria and significant albuminuria by 2 months of age (305 μg/24 h in OVE26 vs. 20 μg/24 h in controls). Albumin excretion rate increased progressively with age and exceeded 15,000 μg/24 h at 9 months of age. The profound loss of albumin led to hypoalbuminemia in some diabetic animals. Albuminuria coincided with an elevation in blood pressure as measured by tail cuff. The glomerular filtration rate (GFR) in OVE26 mice measured using fluorescein isothiocyanateulin clearance demonstrated that GFR increased significantly from 2 to 3 months of age and then decreased significantly from 5 to 9 months. GFR in 9-month-old diabetic mice was significantly lower than that of 9-month-old control mice. The decline in GFR coincided with a significant increase in renal vascular resistance. Structural studies showed an almost twofold increase in kidney weight between 2 and 5 months. Diabetic mice also showed progressively enlarged glomeruli and expanded mesangium with diffuse and nodular expansion of mesangial matrix. Tubulointerstitial fibrosis was also observed in these mice. Glomerular basement membrane was thickened in OVE26 mice. In summary, OVE26 mice demonstrate that most of the characteristics of human DN can be produced by chronic hyperglycemia in a murine model. This model will be useful for improved understanding and treatment of DN. Diabetes 53:3248–3257, 2004

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease in the U.S. (1) and the largest contributor to the total cost of diabetes care. The basic mechanisms of DN are not well understood. An animal, especially a mouse model amenable to genetic manipulation, would be of great advantage. A variety of experimentally induced or spontaneously hyperglycemic animals are used as models of human diabetes, such as streptoztocin-induced diabetic rats, NOD mice, and db/db mice. The kidney disease in many of these animals has been characterized (2,3), but none display the full array of features characteristic of human DN. In fact, the current mouse models primarily display features consistent with the earliest phase of DN, such as microalbuminuria (4,5). This is not surprising since these mouse models typically suffer from diabetes for several months, while the complete pattern of human DN requires decades to develop.

In the current article, we follow the development of DN in a transgenic model of insulinopenic diabetes, the OVE26 mouse (6). The advantages of this model for the study of complications are straightforward: direct damage is limited to the β-cell, diabetes develops early, and very severe diabetes lasts for >1 year. Our results show that with respect to albuminuria, mesangial matrix accumulation, glomerular filtration rate (GFR), and interstitial fibrosis, OVE26 mice are significantly closer to advanced human DN than other available mouse models.

RESEARCH DESIGN AND METHODS

OVE26 mice on the FVB background have been maintained for 14 years in our laboratory. Male heterozygous OVE26 diabetic mice were bred with female wild-type FVB mice. All animals had free access to food and tap water, and no insulin therapy was given. For the purpose of the identifying endothelial cells, the double transgenic line OVE26–green fluorescent protein (GFP) was produced by crossing male OVE26 mice with female tyrosine kinase 2 (TIE2) promoter GFP mice ([TgN{TIE2GFP})287Sato]; The Jackson Lab, Bar Harbor, ME), which express GFP in vascular endothelial cells under the regulation of the endothelial-specific TIE2 promoter (7). All animal procedures adhered to the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the University of Louisville Institutional Animal Care and Use Committee.

Western blot and immunohistochemistry analysis of calmodulin overexpression in islet and kidney. Islets were isolated from FVB and OVE26 mice by expansion of the pancreas and collagenase digestion as we have previously described (8). Kidneys were collected after anesthesia and frozen at −80°C and tissue powder prepared. Both the islet and kidney were treated with the tissue lysis buffer (60 mmol/L Tris, 3% SDS, 0.02% butylated hydroxytoluene, and 2 mmol/L DETAPAC [diethylenetriaminepentaacetic acid]). Samples (10 μg total protein) were subjected to SDS-PAGE (12% gel). After SDS-PAGE, resolved proteins were transferred to nitrocellulose membranes.
and blocked with Tris-buffered saline with 0.1% Tween plus 5% nonfat milk powder. The nitrocellulose blots were incubated overnight with rabbit- raised calmodulin antibody (1:1,000) (Zymed Laboratories, South San Francisco, CA) and then with the horseradish peroxidase–conjugated anti-rabbit IgG (1:2000) for 2 h. Bound antibody was visualized using the enhanced chemiluminescence Western blotting protocol (Amersham). Immunohistochemistry examination of calmodulin expression was carried out with cryostat sections of pancreas and kidney. Calmodulin antibody was used at 1:100 dilution and visualized with donkey anti-rabbit IgG conjugated with Cy3 (1:500).

**Urinary protein assay.** Urine was collected from individually caged mice during a 24-h period in metabolic cages (Nalgene; Braintree Scientific, Braintree, MA). Mice had free access to standard mouse diet. To obtain sufficient urine volume, particularly from nondiabetic mice, 10% liquid diet (Glucerna; Abbott Laboratories) was added to the feeding water, which increased liquid intake. At the end of each collection period, the urine was centrifuged at the Mouse Animal House and the supernatant was used.

**Renal hemodynamic studies.** Studies were performed as previously described (9). Briefly, control mice were anesthetized with separate intraperitoneal injections of ketamine (50 mg/kg body wt) followed by Inactin (190 mg/kg body wt) and oxygen. Mice were cannulated for collection of urine. Blood pressure and heart rate were monitored and recorded using a MacLab data acquisition system. Renal function was determined from the clearance of inulin and para-aminomuoric. Immediately following surgery, a 3-μl/μg body wt bolus of 1% fluorescein isothiocyanate (FTTC) inulin and 3% para-aminomuoric acid sodium salt (PAH) in isotonic saline was administered. The same solution was infused at a rate of 0.15 μl·min⁻¹·g body wt⁻¹ for the remainder of the experiment. After a 30-min equilibration period, baseline renal function was measured with two readings, and consecutive readings were averaged. For evaluating the effect of anesthesia on renal function, donor blood was replaced at a rate of 10–20 additional minutes of each collection period. To replace lost blood volume, donor blood was immediately administered after all blood samples. At the end of the second collection, another blood sample was obtained for electrolyte determination.

Blood samples were obtained for FTTC inulin (10) and PAH determinations. The PAH assay was a modification of the method used by Waugh and Beall (11) with a modification for use on a microcomputer (12). Glomerular filtration rate (GFR) was calculated from inulin and PAH clearance and was used as an index of effective renal plasma flow. Renal vascular resistance was calculated by dividing mean arterial pressure (MAP) by PAH clearance divided by 1 hematocrit. Effective renal plasma flow was calculated as the product of Mf×VG. Four mice were analyzed per group, and 20 glomeruli were measured per mouse. The cross-sectional area of renal cortical tubules was determined by outlining the inner and outer circumference of the tubules. The area of the inner diameter of the tube was subtracted from the total tubule cross-sectional area. Vascularity in glomeruli was determined by examination of endothelial markers in kidneys. Heterozygous TIE2-GFP mice with or without the OVE26 transgene were anesthetized and perfused with 10% formalin in PBS. GFP expressed from the TIE2 promoter was visualized by green fluorescence. The identity of endothelial cells was also confirmed by double staining for CD31 (platelet-endothelial cell adhesion molecule-1; Pharming, San Diego, CA).

**Tissue preparation and transmission electron microscopic technique.** Mice were anesthetized with sodium pentobarbital (100 mg/kg) and fixed with vascular perfusion at a flow rate of 10 ml/min through the left ventricle. Perfusates included half-strength Karnovsky’s (19) fixative with 1% procaine hydrochloride and 0.1 M sodium cacodylate (pH 7.4) followed by full-strength fixative, pH 7.4. Renal cortical samples were prepared as previously described (20).

Cortical tissues were minced to 1 mm (3) and further fixed 3 h to overnight at 4°C. They were postfixed in 1% OsO 4 at 4°C (90 min) and dehydrated through graded ethanols and propylene oxide. Fixed tissue blocks were embedded in Epon/Araldite (21). Thick (1 μm) sections were cut to determine tissue position and orientation. Thin sections were mounted on 200 mesh naked copper grids and stained with lead citrate (22) and uranyl acetyl (4% in absolute ethanol).

**Statistical analysis.** Results are expressed as means ± SE, unless otherwise specified. Comparisons between multiple groups were performed by one- or two-way ANOVA. Comparisons between two groups were performed by Student’s t test. P ≤ 0.05 was considered statistically significant.

**RESULTS**

**General condition of OVE26 mice.** Diabetes in OVE26 mice has been previously described (6). In this study, we found that blood glucose levels were more than twofold above normal by 20 days after birth (Table 1). Blood glucose continued to rise in diabetic mice until at least 60 days of age, when values usually exceeded 600 mg/dl. Frequent testing of other animals indicated that blood glucose remained extremely high for the remainder of the animal’s life span (frequently 12–15 months). Despite the early hyperglycemia, weight gain was normal, though distribution was not. On autopsy, liver and kidney were enlarged, but there was an obvious loss of fat tissue by 5 months of age. Distended bladders were noted in many

### Table 1: Body weight and blood glucose levels in OVE26 transgenic mice before 2 months of age

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Genotype</th>
<th>Body weight (g)</th>
<th>Blood glucose (mg/dl)</th>
<th>BG &gt;600†</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
</tr>
<tr>
<td>20</td>
<td>FVB</td>
<td>9.9 ± 0.6</td>
<td>170 ± 10</td>
<td>10</td>
</tr>
<tr>
<td>30</td>
<td>FVB</td>
<td>19.2 ± 1.2</td>
<td>180 ± 16</td>
<td>13</td>
</tr>
<tr>
<td>40</td>
<td>FVB</td>
<td>20.9 ± 1.2</td>
<td>172 ± 16</td>
<td>7</td>
</tr>
<tr>
<td>50</td>
<td>FVB</td>
<td>22.0 ± 0.7</td>
<td>165 ± 20</td>
<td>6</td>
</tr>
<tr>
<td>60</td>
<td>FVB</td>
<td>26.6 ± 3.6</td>
<td>180 ± 29</td>
<td>8</td>
</tr>
<tr>
<td>20</td>
<td>OVE26</td>
<td>9.5 ± 0.9</td>
<td>373 ± 45</td>
<td>10</td>
</tr>
<tr>
<td>30</td>
<td>OVE26</td>
<td>18.7 ± 1.0</td>
<td>498 ± 84</td>
<td>15</td>
</tr>
<tr>
<td>40</td>
<td>OVE26</td>
<td>20.7 ± 1.0</td>
<td>571 ± 32</td>
<td>7</td>
</tr>
<tr>
<td>50</td>
<td>OVE26</td>
<td>21.1 ± 1.9</td>
<td>577 ± 35</td>
<td>6</td>
</tr>
<tr>
<td>60</td>
<td>OVE26</td>
<td>24.7 ± 2.1</td>
<td>594 ± 11</td>
<td>8</td>
</tr>
</tbody>
</table>

Data are means ± SD. *Blood glucose levels were measured by one-touch glucometer. (The maximum level for the measurement is 600 mg/dl. Greater values are registered as “high” and were registered as 600 mg/dl when doing the analysis.) †The number of mice with blood glucose levels >600 mg/dl. At each age, blood glucose was significantly higher in OVE26 mice (P < 0.05). Body weight was not significantly different at any of these ages.
OVE26 mice >5 months. OVE26 mice received normal housing and food and did not receive insulin injections.

We reported in 1989 (6) that overexpression of calmodulin in pancreatic β-cells of OVE26 mice produced early-onset diabetes. To establish that the transgene had no direct effects on the kidney, we examined calmodulin expression in OVE26 kidneys. The Western blot analysis in Fig. 1A shows that calmodulin levels were normal in kidneys from two OVE26 mice, despite being markedly elevated in OVE26 pancreatic islets. Immunohistochemistry was performed on two additional mice to rule out the possibility that a subpopulation of kidney cells overexpressed calmodulin. As shown in Fig. 1E, diabetic kidney did not show an increase in calmodulin staining compared with normal kidney in Fig. 1D. The absence of calmodulin overexpression in kidney has been confirmed in all six OVE26 transgenic animals that have been examined, either by Western blot and immunohistochemistry analysis, as shown here, or by measuring RNA expression ([6] and P.N.E., unpublished results).

**Albuminuria in OVE26 mice.** UAE was measured by enzyme-linked immunosorbent assay (ELISA) for mouse albumin in control and diabetic mice from 1 to 9 months of age (Fig. 2). The very large differences between groups and the high variation within some groups required that statistical analysis be performed on the log values of UAE (Fig. 2B). UAE was significantly elevated by 2 months of age and continued to remarkably rise with increasing age. By 9 months of age, average albumin excretion exceeded 15 mg/24 h. To confirm these ELISA results, PAGE was performed on the equivalent of 10 s of urine from a 24-h collection. The density of the albumin band in the PAGE gel corresponded closely with the ELISA results and confirmed the extreme albuminuria in older OVE26 mice.

**Morphologic changes in OVE26 kidney.** The most obvious effect of diabetes was enlargement of the kidney (Table 2). As shown in Fig. 3A and B, the increase in kidney size was evident in both the renal cortex and medulla. At least part of this increase was due to expansion of the tubules. Average cortical tubule cross-sectional area (minus the lumen) was 34% greater in 9-month-old OVE26 mice (5, 733 ± 363 μm² for OVE26 vs. 4, 290 ± 250 μm² for FVB; P < 0.01), as determined on at least 60 tubules selected at random from six mice per group. Glomerular volume, mesangial fraction, and mesangial volume measured on PAS-stained sections were also significantly enlarged in OVE26 kidneys (Fig. 3C–E, respectively). Glomerular volume, mesangial fraction, and mesangial volume all increased at the greatest rate in diabetic mice during the age range of 2.5–6 months (Fig. 3).
Hypertension and bladder stasis were observed in many diabetic mice >5 months of age. Hypertension is a common finding in rodent models exhibiting polyuria (9,23,24).

The glomeruli of diabetic mice were assessed with several stains by light microscopy (Fig. 4). Both diffuse and nodular glomerulosclerotic lesions could be shown by obvious accumulation of eosinophilic PAS-positive material in the OVE26 glomeruli (Fig. 4E) compared with control glomeruli (Fig. 4D). Nodules in OVE26 mice were similar to Kimmelsteil-Wilson lesions in size (>40 μm in diameter) and strongly stained with eosin and PAS. As in typical Kimmelsteil-Wilson lesions, the nodules were acellular (25). However, the peripheral displacement of

### TABLE 2

Kidney hemodynamic and filtration function data obtained from FITC inulin and PAH clearance assays

<table>
<thead>
<tr>
<th></th>
<th>2 months</th>
<th>3 months</th>
<th>5 months</th>
<th>9 months</th>
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<tr>
<td>n</td>
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<tr>
<td>Body weight (g)</td>
<td>FVB</td>
<td>FVB</td>
<td>FVB</td>
<td>FVB</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>OVE26</td>
<td>OVE26</td>
<td>OVE26</td>
<td>OVE26</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Kidney/body weight (%)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Kidney/body weight (%)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Urine (μL/min)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
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<tr>
<td>GFR (μL/min)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
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<tr>
<td>Effective renal plasma flow (μL/min)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Effective renal blood flow (μL/min)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Filtration fraction</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
<tr>
<td>Renal vascular resistance (mmHg · ml⁻¹ · min⁻¹)</td>
<td>FVB</td>
<td>OVE26</td>
<td>FVB</td>
<td>OVE26</td>
</tr>
</tbody>
</table>

Data are means ± SE. P < 0.05 vs. 2 months, †vs. 3 months, ‡OVE26 vs. FVB at the same age, and §vs. 5 months. Determinations for these values are described in RESEARCH DESIGN AND METHODS.
mesangial and endothelial cell nuclei sometimes seen in human DN was not identified in the mice. Double staining of endothelial cells for GFP and the endothelial marker CD31 illustrated that there were capillary-free areas in glomeruli corresponding to nodules. In many glomeruli, the capillary-free area accounted for one- to two-thirds of total glomerular area (Fig. 4G–I). Fibrosis was examined by trichrome staining (Fig. 4J–L). At 5 months of age, fibrosis was significant in OVE26 kidneys (Fig. 4J) but essentially absent in control kidneys (Fig. 4J). Fibrosis increased up to age 9 months (Fig. 4L) but was variable between animals of the same age and type. Tubulointerstitial changes in OVE26 kidneys also included dilation of the ducts, atrophy of tubular cells, protein casts in the duct lumen, and prominent infiltration of mononuclear cells.

Electron microscopy (Fig. 6) showed that glomeruli appeared normal in diabetic mice up to 2 months of age. However, in older animals, mesangial matrix was increased, consistent with our light microscopy observations. Electron microscopy also revealed thickened and irregular basement membrane that became more pronounced in 9-month-old diabetic mice. These increases in basement membrane thickness seen in the current study are consistent with our previous quantitative analysis that revealed a 40% increase in basement membrane thickness in OVE26 glomeruli by 6 months of age (20) and an 87% increase by 10–12 months of age (26).

GFR and renal hemodynamics were determined in anesthetized mice from 2 to 9 months of age (Table 2 and Fig. 7) by standard techniques using FITC inulin and PAH clearance. GFR did not change with age in nondiabetic FVB mice. In OVE26 mice, GFR increased significantly from 2 to 3 months of age and then decreased significantly from 5 to 9 months. GFR in 9-month-old diabetic mice was significantly lower than that of 9-month-old control mice. Renal vascular resistance, calculated from renal plasma flow and blood pressure, was significantly elevated in diabetic mice compared with same-age control mice at 3 months of age. The highest renal vascular resistance was measured in the oldest group of diabetic mice.

Blood pressure. Diastolic blood pressure was elevated in conscious diabetic mice at 3 months of age (Fig. 7), as measured with a tail cuff. By 8 months of age, the elevation in blood pressure had increased and was apparent for both diastolic and systolic measurements. In marked contrast, blood pressure of OVE26 mice was significantly reduced when recorded under anesthesia by intravascular cannulation (Table 2). To determine whether these divergent results were due to anesthesia or to the method of measurement, blood pressure was measured in conscious and anesthetized mice by the tail-cuff procedure. As shown in Fig. 7C and D, OVE26 blood pressure was more sensitive to anesthesia than FVB blood pressure. OVE26 values

FIG. 3. Kidney and glomerular enlargement in OVE26 mice. Near midline sagital sections of female 7-month-old diabetic (A) and control (B) kidneys. Pictures were taken at ×7. The bar in panel B designates 1.6 mm. C–E shows the progressive increase in glomerular volume, mesangial fraction, and mesangial volume in OVE26 mice at 2.5, 6, and 10 months of age. *Indicates P < 0.01 for FVB vs. OVE26. Morphometry was performed as described in RESEARCH DESIGN AND METHODS on at least 20 glomeruli from each of four mice per group.
DISCUSSION

In patients, DN is characterized by albuminuria, progressive renal failure, and characteristic pathologic changes in the kidney. Current animal models of diabetes do not display many of the essential characteristics of DN, such as profound albuminuria and progressive decline in GFR (3,4). Development of a suitable mouse model of DN is considered to be a critical priority, as emphasized by the report of the National Institutes of Health Organized Animal Models of Diabetes Complications Consortium (5). A major obstacle to modeling DN in rodents is that diabetes complications require years to develop in patients. Most models of diabetes either have a relatively short life span and/or do not suffer from severe diabetes. We developed the OVE26 mouse model of type 1 diabetes (6), which sustains β-cell–specific damage due to a calmodulin transgene regulated by the insulin promoter. Fortuitously, the β-cell impairment in OVE26 mice results in sustained blood glucose levels well over 600 mg/dl, but there is sufficient insulin secretion to allow these mice to live without treatment for >1 year (20). In this study, we observed the development and progression of kidney problems in OVE26 mice over a period of 9 months. Our results demonstrate...
that the OVE26 mouse has important advantages for studying the pathology of clinical DN compared with other rodent models of diabetes.

Microalbuminuria is the first clinical manifestation of nephropathy in human diabetes. In our previous analysis of OVE26 mice (20), we failed to find albuminuria because we used a nonspecific protein assay. A similar error in protein assay method has caused albuminuria to be overlooked in the db/db mouse model (4). In the current study, albuminuria was measured by a specific ELISA for mouse albumin, and results were confirmed by PAGE analysis. The ELISA demonstrated that OVE26 mice have early-
onset, persistent, and highly progressive albuminuria. Significant albuminuria (5- to 10-fold above control) was first seen at 2 months of age. Albuminuria continued to increase up to at least 9 months of age. At this age, urine albumin was typically elevated >150-fold above controls. Preliminary studies on four diabetic animals indicate that serum albumin was reduced by >30% in older diabetic mice. Occasional diabetic animals exhibited a >500-fold increase in albuminuria that was associated with extreme hypoalbuminemia (serum albumin levels <4 mg/ml), indicating development of the nephrotic syndrome. Patients with DN usually exhibit albuminuria that increases progressively over time to >100-fold above normal. In contrast, the most widely used rodent models of diabetes such as Akita, db/db, or streptozotocin-induced diabetic rat typically demonstrate only a 10-fold increase in albuminuria that does not exceed 300 µg/day (5). This relatively small increase appears to provide a suitable model for the early microalbuminuria of patients, which may later develop into DN. Since most OVE26 animals produced albuminuria exceeding 7,000 µg per day by 9 months of age, which is about 70- to 140-fold greater than control mice, they provide the first mouse model of advanced DN. A rat model with combined hypertension and prolonged severe hyperglycemia has just been reported with a similar degree of albuminuria (27).

Structural features of human DN include renal hypertrophy, glomerular pathology with basement membrane thickening, mesangial expansion, glomerular sclerosis with or without Kimmelstiel-Wilson nodules, and tubulointerstitial fibrosis (25,28). Renal hypertrophy was obvious in all of our diabetic mice by 3 months of age. By 5 months, relative kidney weight peaked at 200% of normal and remained at approximately this level. Tubulointerstitial fibrosis has long been recognized as an important histological parameter that correlates with chronic renal failure in a variety of renal diseases including DN (29). Most OVE26 kidneys >5 months of age exhibited focal regions with tubulointerstitial lesions, such as tubular atrophy, interstitial mononuclear cell infiltration, and fibrosis. Tubulointerstitial lesions increased dramatically in the few animals that developed extreme albuminuria, exceeding 20 mg/day. Similar tubulointerstitial lesions have not been reported in other rodent models of diabetes.

Glomeruli of OVE26 mice demonstrated obvious thickening of basement membrane. This was consistent with our previous report (26) in which we described an 87% increase in 10- to 12-month-old OVE26 mice. Most glomeruli exhibited diffuse and nodular mesangial matrix expansion. By 6 months of age, the calculated mesangial volume was three times higher in OVE26 mice compared with FVB mice. Areas of matrix expansion were devoid of markers for endothelial cells, indicating an absence of vascular perfusion and capillary occlusion. This process will ultimately reduce renal blood flow and increase renal vascular resistance. The most pathognomonic lesions of nodular glomerulosclerosis in human diabetes are Kimmelstiel-Wilson lesions (25), which are nodular accumulations of acellular PAS-positive material (25) sometimes exhibiting a ring of peripheral nuclei. OVE26 glomeruli displayed striking PAS staining nodules, but a peripheral ring of nuclei was never observed. Another distinction between human and OVE26 DN was our failure to identify hyaline arteriolas associated with OVE26 glomeruli.

Human DN is characterized by a prolonged and gradual decline in GFR, culminating in end-stage renal disease (25,28). In the OVE26 mouse, GFR declined gradually from 3 to 9 months of age. At 9 months, average GFR was significantly reduced to 83% of control. Some diabetic animals exhibited GFR values as low as 53% of control. While these reductions do not approach the final reductions seen in DN, it is, to our knowledge, the first rodent model of DN to demonstrate significantly reduced GFR by the inulin clearance method. Inulin clearance is considered to be the gold standard for measurement of GFR (10,12). Measurements based on creatinine clearance determined with picric acid-based procedures are now recognized to be highly inaccurate in mice and rats (30). The development of reduced GFR in OVE26 mice coincided with structural changes such as nodular sclerosis and loss of capillary loops. These structural changes may have contributed to the 60% increase in calculated renal vascular resistance of 9-month-old diabetic mice. This increase in resistance was associated with and may have contributed to the decline in GFR. Renal vascular resis-

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**FIG. 6. Age-dependent changes in renal function of FVB and OVE26 mice.** A: GFR measured by inulin clearance. In OVE26 mice, there was a significant increase in GFR from 2 to 3 months and a significant decrease from 5 to 9 months. At 9 months, GFR in FVB mice was significantly greater than in OVE26 mice. No significant changes were observed in GFR of FVB mice as a function of age. \( P < 0.05 \) for 2 vs. 3 months in OVE26. \( P < 0.05 \) for 9 vs. 3 and 5 months in OVE26. \( P < 0.05 \) for OVE26 vs. FVB at 9 months. B: Renal vascular resistance (RVR) is higher in OVE26 diabetic mice, and diabetic RVR rises from 5 to 9 months of age. There is no difference among the four age-groups of FVB mice. \( P < 0.05 \) for 5 vs. 9 months in OVE26 mice. \( n = 4 \) or 5 at 2 and 3 months of age, \( n = 8–10 \) at 5 and 9 months of age.
tance can be measured more directly than by the calculated procedures used here (31). This should be done to confirm our resistance values. The reduction in GFR of OVE26 mice occurred after only 9 months of diabetes. This is a relatively short time compared with the years required for GFR to decline in humans. Since OVE26 mice frequently live >15 months, it is likely that a more striking decline in GFR can be measured in older animals.

Hypertension is the most important risk factor for progression of DN (25). In OVE26 mice, conflicting blood pressure results were obtained depending on the method of measurement and anesthesia. Conscious OVE26 mice were hypertensive when tested with a tail-cuff monitor. Anesthetized OVE26 mice were hypotensive when measured with an intravascular cannula. This discrepancy could be explained, at least in part, by the fact that anesthesia reduced blood pressure more in OVE26 mice than in nondiabetic mice. This has been observed in other hypertensive models (32). Because conscious diabetic mice were hypertensive, it is likely that the predominant blood pressure effect is hypertension.

Accurate modeling of diabetes complications in rodents is innately difficult due to the fact that it takes longer for patients to develop complications than the full life span of rodents. It is generally believed that complications are caused by the effects of hyperglycemia acting over time. To compensate for the shorter time available in rodents, animal models with very high blood glucose are used. OVE26 mice maintain higher blood glucose levels for a longer period than any other available model. Therefore, it is reasonable that their complications should be more severe. With respect to albuminuria, morphology, and renal function, that appears to be true. However, it is necessary to consider the limitations of this model. Sustained blood glucose levels >600 mg/dl do not occur in humans, and insulinoopenic diabetic patients will always receive insulin treatment. Thus, the OVE26 mice in this study differed from human patients with respect to both extreme hyperglycemia and severe inulinopenia, either of which may have contributed to the nephropathy. Also, it is not possible to rule out the potential contribution of infectious agents, since our animals were not maintained in a pathogen-free environment. Despite these limitations, OVE26 mice provide a significantly more accurate model of advanced DN, which should be valuable for testing new hypotheses on the mechanism and treatment of diabetic renal failure.

ACKNOWLEDGMENTS
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