Original Article

Characterization of Susceptibility of Inbred Mouse Strains to Diabetic Nephropathy

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Differential susceptibility to diabetic nephropathy has been observed in humans, but it has not been well defined in inbred strains of mice. The present studies characterized the severity of diabetic nephropathy in six inbred mouse strains including C57BL/6J, DBA/2J, FVB/NJ, MRL/MpJ, A/J, and KK/HlJ mice. Diabetes mellitus was induced using low-dose streptozotocin injection. Progression of renal injury was evaluated by serial measurements of urinary albumin excretion, glomerular filtration rate (GFR), and terminal assessment of renal morphology over 25 weeks. Despite comparable levels of hyperglycemia, urinary albumin excretion and renal histopathological changes were dramatically different among strains. DBA/2J and KK/HlJ mice developed significantly more albuminuria than C57BL/6J, MRL/MpJ, and A/J mice. Severe glomerular mesangial expansion, nodular glomerulosclerosis, and arteriolar hyalinosis were observed in diabetic DBA/2J and KK/HlJ mice. Glomerular hyperfiltration was observed in all diabetic strains studied except A/J. The significant decline in GFR was not evident over the 25-week period of study, but diabetic DBA/2J mice exhibited a tendency for GFR to decline. Taken together, these results indicate that differential susceptibility to diabetic nephropathy exists in inbred mice. DBA/2J and KK/HlJ mice are more prone to diabetic nephropathy, whereas the most widely used C57BL/6J mice are relatively resistant to development of diabetic nephropathy. *Diabetes* 54:2628–2637, 2005

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ACR, albumin-to-creatinine ratio; FITC, fluorescein isothiocyanate; GBM, glomerular basement membrane; GFR, glomerular filtration rate; STZ, streptozotocin; UAE, urinary albumin excretion rate.

Diabetic nephropathy is an insidious and lethal complication of diabetes mellitus leading to renal failure in a substantial fraction of patients with diabetes mellitus. Epidemiological studies in humans suggest that the lifetime risk for development of diabetic nephropathy is ~35% (1,2). Significant racial differences have been reported including increased incidence in native Americans and Asian populations, indicating differential susceptibility to diabetic nephropathy exists in humans (1,3–6). In cohorts susceptible to diabetic nephropathy, patients progressively increase urinary albumin excretion (UAE) followed by a decline of glomerular filtration rate (GFR). These functional changes are accompanied by characteristic renal pathological features including mesangial expansion, glomerulosclerosis, and tubulointerstitial fibrosis (5). In contrast, cohorts resistant to diabetic nephropathy do not develop severe renal histopathological lesions or albuminuria despite comparable levels of hyperglycemia (2,7). Genetic factors have been suggested to contribute to this differential susceptibility to diabetic nephropathy in humans (2–4,8). Despite substantial effort, mapping of susceptibility genes in people has been hindered because of the genetic heterogeneity in human populations as well as the diversity of environmental factors including treatment.

Inbred mice have been wildly used to model human diseases not only because they facilitate gene manipulation, but also because they share high genetic homology with humans (9–11). Many mouse models of diabetes have been established including type 1 diabetes due to streptozotocin (STZ) treatment and genetic models (e.g., C57BL/6-*Ins2*^{Akita}/J) (12–14). Models of type 2 diabetes include diet-induced and inherited deficiency in the leptin receptor (i.e., *db/db*) mice (15–17). However, nephropathy has been studied in only a limited number of strains, and none of these models develops renal lesions that fully resemble advanced human diabetic nephropathy (18). The majority of studies have been performed using C57BL/6J mice; however, this strain does not typically develop robust renal histopathological changes or marked albuminuria (18–21). These deficiencies have hindered the use of the unique genetic murine reagents to dissect the mechanistic underpinnings of diabetic nephropathy.

An inbred mouse strain is established by sibling mating for 20 or more consecutive generations (available at

http://www.jax.org). Thus mice within an inbred strain are genetically identical, whereas mice from other inbred strains are genetically distinct from the index strain (22,23). As in human populations, differential susceptibility to some renal diseases has been demonstrated among inbred mice (21,24,25); however, the susceptibility of inbred mice to diabetic nephropathy has not been defined. Characterization of this susceptibility may not only help establish more robust mouse models of diabetic nephropathy, but also provide a basis for mapping of the underlying genes predisposing to the development of diabetic nephropathy. The present studies compared the development and severity of three major phenotypic features of diabetic nephropathy—albuminuria, histopathology, and GFR—in six commonly used inbred strains of mice.

RESEARCH DESIGN AND METHODS

The inbred male mice used in the present studies were purchased from The Jackson Laboratory (Bar Harbor, ME) at 6–8 weeks of age. STZ and fluorescein isothiocyanate (FITC)-inulin were obtained from Sigma-Aldrich (St. Louis, MO). The enzymatic immunoassay kits for determining urinary albumin and creatinine were purchased from Exocell (Philadelphia, PA). All protocols were approved by the institutional animal care and use committee of Vanderbilt University and recommended by the Animal Models of Diabetic Complications Consortium (available at http://www.amdcc.org).

Induction of diabetes in inbred mice. At 10 weeks of age, inbred mice received daily STZ injections intraperitoneally (40 mg/kg for DBA/2J and 50 mg/kg for all other strains, made freshly in 0.1 mol/l citrate buffer, pH 4.5) for 5 consecutive days. The onset of diabetes was evaluated by measuring fasting blood glucose and HbA_{1c} (A1C). Blood glucose was measured biweekly using a B-Glucose Analyzer (HemoCue, Lake Forest, CA) on samples obtained after a 6-h fast starting at 6:00 A.M. A1C levels were determined monthly using a commercially available analysis kit (DCA 2000; Bayer, Elkhart, IN). Blood was collected in conscious mice via the saphenous vein as described previously (26).

Measurement of UAE. Excretion of urinary albumin was determined using albumin-to-creatinine ratio (ACR) on morning spot urine and UAE in 24-h urine collections. Spot urine collection was conducted monthly using a custom-made mouse urine collection station that used a 96-well enzyme-linked immunosorbent assay plate as the floor. Mice were allowed to roam freely on this 96-well plate until they spontaneously urinated. Urine was obtained in the wells without contamination by feces, using a pipette. Twenty-four–hour urine was collected using metabolic cages (Braintree Scientific, Braintree, MA). The concentration of albumin and creatinine in the urine were determined using a commercially available kit (Exocell).

Measurement of GFR in conscious mice. Renal function in diabetic mice was evaluated by serial determination of GFR before and 5, 15, and 25 weeks after the onset of hyperglycemia. FITC-inulin clearance was determined as described previously (26). Briefly, 3% FITC-inulin was injected retro-orbitally, followed by collection of ~20 μl of saphenous vein blood at 3, 7, 10, 15, 35, 55, and 75 min after the FITC-inulin bolus injection in conscious mice. Plasma fluorescence concentration at each time point was determined using a Fluoroscan Ascent FL (Labsystems, Helsinki, Finland) with 485-nm excitation and read at 538-nm emission. The decay in plasma fluorescence levels was fit to a two-phase exponential decay curve using nonlinear regression (GraphPad Prism; GraphPad Software, San Diego, CA.). GFR is calculated using the equation: $GFR = I/(A/\alpha + B/\beta)$, where I is the amount of FITC-inulin delivered in bolus injection; A and α are the y-intercept and the decay constant of the rapid (initial) decay phase, respectively; and B and β are the y-intercept and the decay constant of the slow decay phase, respectively (26).

Renal histopathology. Mice were killed after hyperglycemia was established for at least 25 weeks. Under anesthesia induced with phentobarbital sodium injection (50 mg/kg body wt i.p.), the left renal artery and vein were clipped with a hemostatic forceps. The left kidney was removed, and the weight was measured. The right kidney was perfused with PBS (pH 7.0) through a butterfly 23-gauge needle inserted into left ventricle at 160–170 mmHg for ~5 min. This is followed by 4% paraformaldehyde for another 5 min. The perfused right kidney was removed and routinely processed. Four-micrometer sections were stained with periodic acid Schiff. A semiquantitative score was used to evaluate the degree and extent of glomerulosclerosis as described previously (27). Mesangial matrix expansion occupying <25, 25–50, 50–75, or >75% of tuft was scored 1, 2, 3, and 4+, respectively, and no mesangial expansion was scored as 0. A whole kidney average sclerosis index was obtained by averaging scores from all glomeruli on one section. On average, more than 100 glomeruli were assessed per mouse. Tubules and interstitium were also evaluated by light microscopy. Electron microscopic examination was conducted in the Research Electron Microscopy Core of Vanderbilt University. Samples were fixed in 2.5% glutaraldehyde in 0.1 mol/l cacodylate buffer (pH 7.4). After fixation, samples were dehydrated through a graded series of ethanol and embedded in Spurr resin. The sections (80–100 nm) were viewed using a FEI/Philips CM12 transmission electron microscope operated at 80 KeV.

Statistics. All data are expressed as means ± SE. ANOVA and t test were used for data analysis. $P < 0.05$ was considered significant.

RESULTS

STZ-induced diabetes in inbred mouse strains. All strains of mice tested developed stable hyperglycemia 1 week after low-dose STZ injection (Table 1). A1C was elevated in all studied mice compared with either each baseline or age-matched controls (Table 1). Strain-dependent susceptibility to STZ was observed. KK/HlJ mice appeared relatively resistant to STZ-induced hyperglycemia with lower levels of blood glucose. Without the insulin supplementation, most diabetic mice survived more than 25 weeks. After that, the mortality increased in most strains studied, especially in DBA/2J, KK/HlJ, and A/J strains (data not shown). In contrast, C57BL/6J mice appear to tolerate persistent hyperglycemia well with a group of diabetic C57BL/6J mice surviving longer than 45 weeks despite fasting glucose levels of 300–600 mg/dl. Compared with age-matched controls, body weight was lower in diabetic mice except in FVB/NJ and MRL/MpJ strains (Table 1). Substantial loss of body weight was especially evident in diabetic DBA/2J mice.

Albuminuria in diabetic inbred mice. Albuminuria is a central manifestation of diabetic nephropathy (2,5,16,28). Spot urine ACR and 24-h UAE were examined in the diabetic and control mice in the present studies. Spot urine ACR was serially measured and 24-h UAE was determined in mice after 25 weeks of hyperglycemia. A significant correlation between spot ACR and 24-h UAE was observed in C57BL/6J, DBA/2J, and KK/HlJ mice (Figs. 1 and 2), but not in FVB/NJ mice (Fig. 2).

Despite persistent hyperglycemia, no significant increase in ACR was observed in C57BL/6J and FVB/NJ mice (Table 1 and Fig. 2*A*). A separate group of diabetic C57BL/6J mice were followed for 45 weeks, and these mice also failed to develop albuminuria (fasting blood glucose, 446.0 ± 103.1 mg/dl; ACR, 19.3 ± 5.8 μl/mg; n = 6). In contrast, diabetic DBA/2J and KK/HlJ mice exhibited a significant increase in ACR compared with their age-matched controls or other strains of diabetic mice (Table 1 and Fig. 2). Hyperglycemic A/J and MRL/MpJ mice intermittently increased ACR, but the levels were significantly less than DBA/2J and KK/HlJ mice.

Consistent with the spot ACR results, 24-h UAE was significantly greater in diabetic DBA/2J and KK/HlJ mice than diabetic C57BL/6J, A/J, and MRL/MpJ mice after ~25 weeks of hyperglycemia (Fig. 2*B*). In contrast, diabetic FVB/NJ mice exhibited a marked increase in 24-h UAE despite an unchanged spot urine ACR (Fig. 2*B*). Further analysis of these mice showed that diabetic FVB/NJ mice significantly increased 24-h urinary creatinine excretion after ~25 weeks of hyperglycemia (1.52 vs. baseline at 0.37 mg/24 h, $P < 0.001$). In contrast, other diabetic mouse

TABLE 1
Fasting blood glucose and UAE in diabetic and control mice

Strain	Glycemic status	Before STZ	Weeks after STZ or vehicle		
			5	15	25
C57BL/6J					
FBS (mg/dl)	DM	170.0 ± 11.2 (10)	424.9 ± 33.9 (9)*†	502.5 ± 31.2 (21)*†	506.6 ± 46.0 (18)*†
	CN	170.0 ± 3.7 (10)	170.4 ± 7.1 (5)	164.8 ± 4.7 (5)	157.4 ± 8.1 (5)
A1C (%)	DM	3.8 ± 0.2 (3)	NA	8.2 ± 0.4 (21)*†	9.4 ± 0.6 (11)*†
	CN	3.6 ± 0.1 (5)	NA	4.1 ± 0.1 (5)*	3.8 ± 0.1 (5)
BW (g)	DM	21.3 ± 0.4 (10)	25.6 ± 0.6 (9)*	25.6 ± 0.7 (21)*†	26.9 ± 0.8 (18)*
	CN	20.3 ± 0.2 (5)	26.7 ± 1.2 (5)*	28.9 ± 0.9 (5)*	29.2 ± 1.4 (5)*
ACR (μg/mg)	DM	36.9 ± 7.6 (9)	35.4 ± 5.7 (9)	29.5 ± 12.4 (16)*	72.1 ± 20.4 (16)
	CN	45.6 ± 12.4 (5)	20.1 ± 1.6 (5)	48.11 ± 16.5 (5)	53.2 ± 24.3 (5)
DBA/2J					
FBS (mg/dl)	DM	160.5 ± 8.6 (15)	485.8 ± 31.3 (19)*†	485.3 ± 27.4 (21)*†	511.3 ± 75.6 (6)*†
	CN	165.2 ± 16.2 (5)	127.4 ± 15.5 (5)*	148.6 ± 8.5 (5)	152.6 ± 4.5 (5)
A1C (%)	DM	2.9 ± 0.1 (3)	NA	6.7 ± 0.1 (21)*†	13.4 ± 0.8 (6)*†‡
	CN	2.8 ± 0.1 (5)	NA	2.8 ± 0.1 (5)	2.7 ± 0.2 (5)
BW (g)	DM	23.4 ± 0.6 (15)	22.3 ± 0.7 (19)†	20.8 ± 0.7 (21)*†	18.7 ± 0.9 (10)*†
	CN	22.2 ± 0.9 (6)	28.2 ± 0.8 (4)*	29.5 ± 1.2 (4)*	30.1 ± 0.9 (5)*
ACR (μg/mg)	DM	26.6 ± 6.6 (29)	424.4 ± 89.4 (24)*†	608.0 ± 220.8 (15)*†§	421.4 ± 167.3 (11)*†§
	CN	19.7 ± 5.1 (5)	48.7 ± 7.0 (9)*	71.1 ± 15.2 (7)*	65.8 ± 13.2 (7)*
A/J					
FBS (mg/dl)	DM	154.4 ± 4.4 (15)	360.8 ± 28.0 (10)*†	505.6 ± 31.4 (22)*†	391.6 ± 50.6 (16)*†
	CN	159.8 ± 7.8 (5)	146.0 ± 13.4 (4)	157.0 ± 4.0 (4)	171.0 ± 2.1 (4)
A1C (%)	DM	2.6 ± 0.03 (9)	NA	5.1 ± 0.1 (22)*†¶	4.1 ± 0.2 (16)*†¶
	CN	2.6 ± 0.03 (5)	NA	2.6 ± 0.04 (5)	2.6 ± 0.04 (5)
BW (g)	DM	23.4 ± 0.6 (15)	18.3 ± 0.6 (10)*†	20.5 ± 0.8 (27)*†	23.2 ± 0.7 (16)†
	CN	22.9 ± 0.6 (5)	26.8 ± 0.8 (4)*	28.2 ± 0.9 (4)*	30.6 ± 1.3 (5)*
ACR (μg/mg)	DM	26.6 ± 12.2 (23)	119.5 ± 21.1 (27)*	174.0 ± 48.2 (17)*†	64.0 ± 12.6 (15)*
	CN	18.7 ± 2.2 (5)	54.5 ± 14.2 (5)*	88.4 ± 25.8 (4)*	81.2 ± 8.8 (4)*
FVB/NJ					
FBS (mg/dl)	DM	141.8 ± 12.4 (12)	576.0 ± 43.0 (8)*†	549.4 ± 52.1 (10)*†	610.0 ± 23.3 (5)*†
	CN	134.0 ± 19.8 (4)	169.5 ± 2.5 (4)	148.3 ± 19.8 (4)	151.0 ± 12.1 (4)
A1C (%)	DM	3.0 ± 0.1 (7)	NA	5.8 ± 0.2 (10)*†	5.0 ± 0.3 (5)*
	CN	NA	NA	3.0 ± 0.2 (4)	NA
BW (g)	DM	27.9 ± 0.8 (12)	26.8 ± 0.3 (8)	27.4 ± 0.6 (10)	33.0 ± 0.5 (5)*
	CN	26.7 ± 2.0 (4)	29.0 ± 2.2 (4)	30.0 ± 2.1 (4)	33.1 ± 1.8 (4)
ACR (μg/mg)	DM	61.6 ± 13.2 (7)	6.1 ± 1.4 (9)*†	88.0 ± 21.3 (12)	89.3 ± 27.7 (7)
	CN	45.4 ± 18.5 (4)	74.1 ± 21.7 (4)	NA	63.0 ± 31.0 (4)
MRL/MpJ					
FBS (mg/dl)	DM	120.8 ± 6.6 (5)	434.8 ± 22.7 (19)*†	493.5 ± 21.9 (17)*†	428.0 ± 45.1 (5)*†
	CN	140.5 ± 4.8 (4)	165.0 ± 3.7 (4)	155.0 ± 12.4 (4)	153.5 ± 20.2 (4)
A1C (%)	DM	3.0 ± 0.1 (5)	5.3 ± 0.1 (19)	6.9 ± 0.2 (8)*†	6.1 ± 0.2 (5)*†
	CN	3.0 ± 0.1 (4)	NA	3.6 ± 0.2 (4)	3.3 ± 0.4 (4)
BW (g)	DM	37.1 ± 0.4 (5)	34.0 ± 0.8 (19)*	36.7 ± 0.6 (17)	37.0 ± 1.4 (5)
	CN	35.7 ± 1.4 (4)	36.5 ± 2.6 (4)	38.6 ± 3.7 (4)	39.2 ± 4.3 (4)
ACR (μg/mg)	DM	74.4 ± 17.0 (14)	95.2 ± 43.4 (20)	122.5 ± 32.7 (18)†	87.7 ± 22.3 (6)†
	CN	39.5 ± 14.2 (4)	73.2 ± 14.5 (4)	18.0 ± 7.5 (4)	43.2 ± 14.0 (4)
KK/HlJ					
FBS (mg/dl)	DM	160.6 ± 7.4 (15)	297.5 ± 30.6 (15)*†‖	321.3 ± 23.0 (19)*†‖	330.0 ± 32.8 (13)*†‖
	CN	142.0 ± 5.2 (5)	157.4 ± 9.3 (10)	158.8 ± 6.8 (10)	156.8 ± 10.9 (10)
A1C (%)	DM	4.5 ± 0.1 (15)‡	NA	6.4 ± 0.4 (19)*†	6.4 ± 0.5 (13)*†
	CN	4.7 ± 0.2 (5)	NA	4.8 ± 0.2 (10)	4.0 ± 0.1 (8)*
BW (g)	DM	31.4 ± 1.0 (25)	29.8 ± 0.7 (15)	30.9 ± 0.6 (19)†	32.3 ± 0.8 (13)†
	CN	31.4 ± 1.5 (7)	33.8 ± 1.5 (5)	36.6 ± 1.4 (10)*	35.9 ± 1.1 (13)*
ACR (μg/mg)	DM	72.9 ± 12.1 (13)	509.9 ± 87.6 (19)*†	586.9 ± 84.9 (19)*†§	635.8 ± 122.0 (13)*†§
	CN	72.8 ± 11.4 (14)	177.0 ± 50.7 (10)	224.4 ± 80.8 (10)*	349.1 ± 39.7 (10)*

Data are means ± SE (no. of animals). *$P < 0.05$ vs. baseline at same group; †$P < 0.05$ vs. age-matched controls at same time point; ‡$P < 0.05$ vs. all other diabetic strains at same time point; §$P < 0.05$ vs. diabetic C57BL/6J, A/J, FVB/NJ, and MRL/MpJ mice; ¶$P < 0.001$ vs. A1C in all other diabetic strains except FVB/NJ strain at same time point; ‖$P < 0.05$ vs. FBS in diabetic C57BL/6J, DBA/2J, and FVB/NJ mice at same time point. BW, body weight; CN, control mice; DM, diabetic mice; FBS, fasting blood glucose; NA, not available.

strains exhibited no change (e.g., A/J and KK/HlJ mice) or a decrease (e.g., DBA/2J mice) in 24-h urinary creatinine excretion. Urine volume in hyperglycemic FVB/NJ mice (12.96 ± 3.06 ml/24 h, $n = 7$) was also significantly greater than in other diabetic strains including C57BL/6J (2.74 ± 0.74 ml/24 h, $n = 13$), DBA/2J (2.56 ± 0.38 ml/24 h, $n = 14$), A/J (0.45 ± 0.10 ml/24 h, $n = 6$), or KK/HlJ mice (1.95 ± 0.91 ml/24 h; $n = 11$). In contrast to previous studies

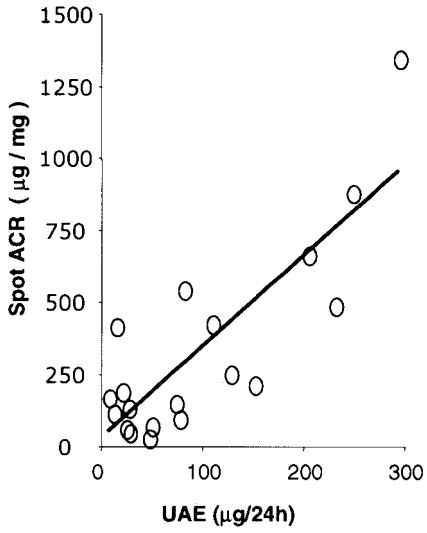

FIG. 1. Relationship between morning spot urine ACR and 24-h UAE in diabetic mice. Data were obtained from diabetic and control C57BL/6J (n = 4), DBA/2J (n = 6), and KK/HlJ (n = 9) mice. Spot urine was collected for 3 consecutive days from each mouse and average spot ACR was used for the correlation analysis. R^2 = 0.69. P < 0.0001.

examining a transgenic diabetic model with FVB/N background (OVE26) (29), we did not observe hydronephrosis in STZ-induced hyperglycemic FVB/NJ mice, despite the presence of polyuria.

Moderate albuminuria was also observed in non–STZ-injected control KK/HlJ mice (Table 1 and Fig. 2), despite lack of increased fasting blood glucose or A1C (Table 1). However, albuminuria in control KK/HlJ mice was significantly less than in STZ-injected hyperglycemic KK/HlJ mice.

Development of albuminuria in C57BL/6-Ins2^{Akita}/J mice. To confirm the resistance of diabetic C57BL/6J mice, we examined the UAE in a second model of type 1 diabetes, the C57BL/6-*Ins2*^{Akita}/J mouse. These mice express a mutation in the insulin 2 gene and develop hyperglycemia because of misfolding of insulin and proteotoxicity of β-cells (14,30,31). As in STZ-injected diabetic model, male C57BL/6-*Ins2*^{Akita}/J mice did not develop significant albuminuria despite 20 weeks of hyperglycemia (at 30 weeks of age: fasting blood glucose, 631.6 ± 55.2 mg/dl, and ACR, 29.2 ± 4.2 μg/mg; n = 8).

Renal morphology in diabetic inbred mice. To examine the relationship between albuminuria and renal histopathological lesions, renal morphology was studied by light and electronic microscopy. Consistent with albuminuria, diabetic DBA/2J and KK/HlJ mice exhibited the most dramatic histopathological changes after 25–35 weeks of hyperglycemia (Fig. 3). Significantly greater mesangial expansion was observed in diabetic DBA/2J and KK/HlJ kidneys with some glomeruli developing nodular glomerular sclerosis and arteriolar hyalinosis (Figs. 3 and 4). Diabetic C57BL/6J mice also exhibited increased mesangial expansion scores compared with age-matched controls, but the scores were significantly less than in diabetic DBA/2J and KK/HlJ mice. In contrast, despite developing

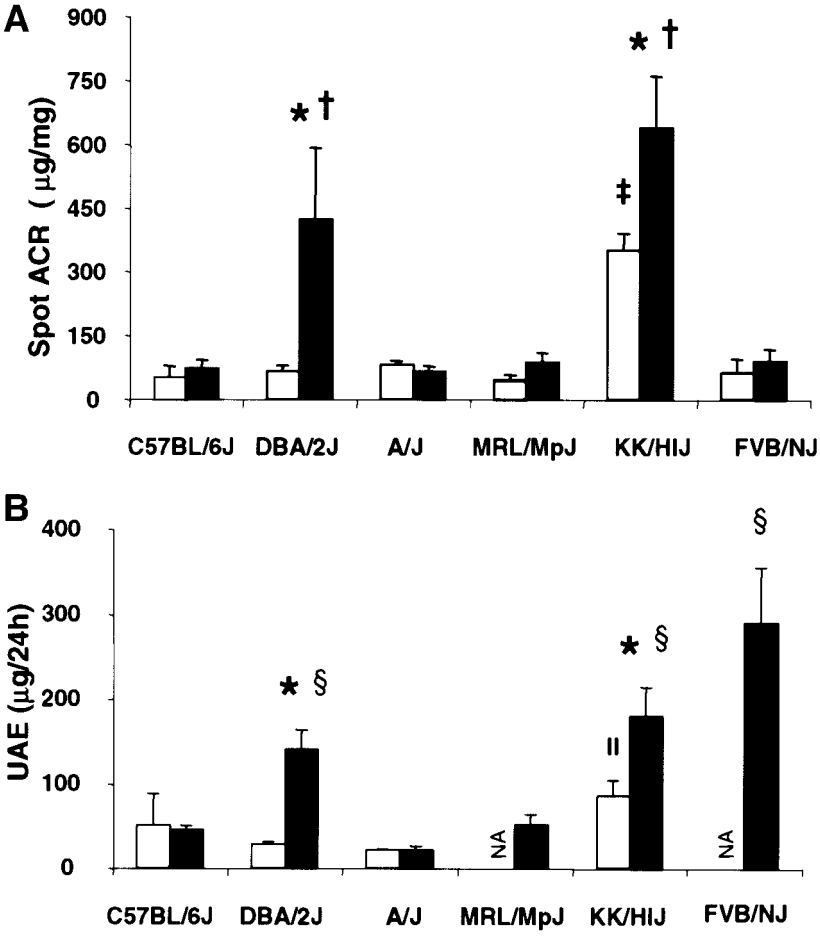

FIG. 2. Development of albuminuria in STZ-induced diabetic inbred mice after ~25 weeks of hyperglycemia. *A*: Spot urine ACR in diabetic mice (■) and age-matched controls (□). *P < 0.05 (t test) vs. respective age-matched controls; †P < 0.05 (ANOVA) vs. diabetic C57BL/6J, A/J, MRL/MpJ, and FVB/NJ mice; and ‡P < 0.001 (ANOVA) vs. controls in other strains. *B*: 24-h UAE in diabetic mice (■) and age-matched controls (□). Age-matched controls for MRL/MpJ and FVB/NJ strains were not available (NA) for studying. *P < 0.05 (t test) vs. age-matched controls; §P < 0.05 (ANOVA) vs. diabetic C57BL/6J, A/J, and MRL/MpJ strains; and ‖P < 0.05 (ANOVA) vs. controls in other studied strains.

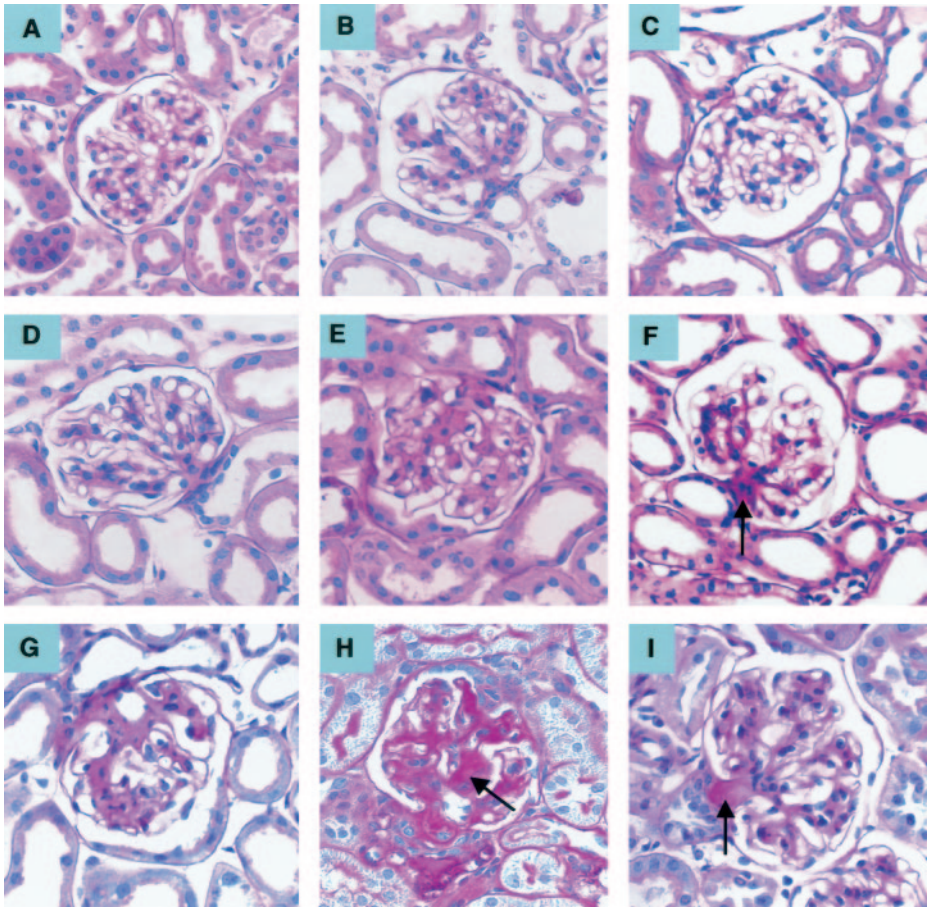

FIG. 3. Representative glomerular histopathology of perfuse-fixed diabetic mouse kidneys (periodic acid Schiff, ×400). *A*: C57BL/6J mice (35 weeks after STZ). *B*: A/J (32 weeks after STZ). *C*: MRL/MpJ (35 weeks after STZ). *D*: FVB/NJ (25 weeks after STZ). *E*: DBA/2J (25 weeks after STZ). *F*: Arteriolar hyalinosis (arrow) in diabetic DBA/2J mice. *G*: KK/HlJ mice (35 weeks after STZ). *H*: Nodular glomerulosclerosis (arrow) in diabetic KK/HlJ mice. *I*: Arteriolar hyalinosis (arrow) in diabetic KK/HlJ mice.

albuminuria, diabetic FVB/NJ mice exhibited significantly less mesangial expansion than diabetic DBA/2J, KK/HlJ, or C57BL/6J mice. Glomerular mesangial expansion in nonhyperglycemic KK/HlJ mice was significantly greater than control DBA/2J and C57BL/6J mice but less than STZ-induced hyperglycemic KK/HlJ mice. These differential levels of glomerulosclerosis are consistent with their relative levels of albuminuria. Interstitial fibrosis or tubular atrophy was not observed in any strain of diabetic mouse within the 25 weeks of study.

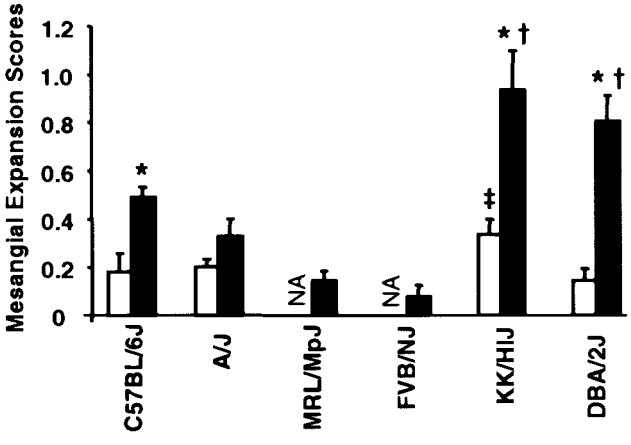

FIG. 4. Glomerular mesangial expansion scores in diabetic inbred mice. The scores were determined using light microscopy at ×400 in perfuse-fixed diabetic mouse kidneys as described in RESEARCH DESIGN AND METHODS. **P* < 0.05 (*t* test) vs. age-matched controls; †*P* < 0.05 (ANOVA) vs. diabetic C57BL/6J, A/J, MRL/MpJ, and FVB/NJ mice; and ‡*P* < 0.05 vs. controls in other strains. Age-matched controls for diabetic FVB/NJ and MRL/MpJ were not available (NA).

Glomerular ultrastructure was further examined using an electronic microscope. Diabetic DBA/2J and KK/HlJ mice developed the most dramatic thickening of glomerular basement membrane (GBM) among the strains studied. Diabetic C57BL/6J mice also exhibited increased GBM width, but the level was significantly less than diabetic DBA/2J and KK/HlJ mice (Figs. 5 and 6). No electronic dense deposits were observed in the basement membrane. Fragmental podocyte foot process effacement was observed in some glomeruli of diabetic KK/HlJ and DBA/2J strains.

Kidney weight in diabetic inbred mice. Renal hypertrophy is observed in type 1 diabetic patients with early-stage diabetic nephropathy (32,33). In the present studies, we examined kidney weight–to–body weight ratio in diabetic and age-matched controls. As shown in Fig. 7, a significant increase in kidney weight was observed in all studied strains of diabetic mice except A/J strains (the age-matched controls for diabetic FVB/NJ mice were not available). Diabetic DBA/2J mice exhibited the highest kidney weight–to–body weight ratio among the studied strains of diabetic mice (Fig. 7).

GFR in diabetic inbred mice. GFR was serially examined in diabetic inbred mice and age-matched controls using FITC-inulin clearance (26). GFR increased in all strains of diabetic mice compared with age-matched controls except for A/J (Fig. 8). Among the strains studied, diabetic DBA/2J and FVB/NJ exhibited the highest GFR at

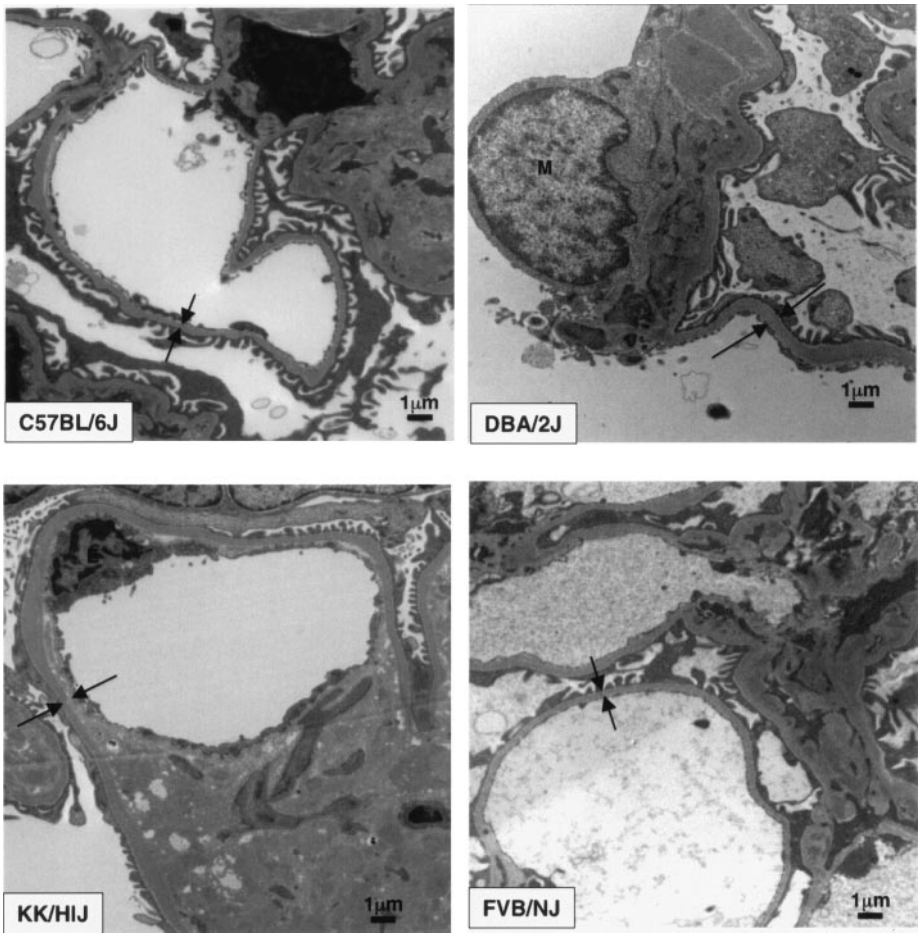

FIG. 5. Electronic microscopic images of glomeruli from diabetic mice after 25 weeks (DBA/2J and FVB/NJ strains) to 35 weeks (C57BL/6J and KK/HlJ strains) of hyperglycemia. Opposed double arrows indicate GBM. E and M designate endothelial cell and mesangial cell, respectively.

15 and 25 weeks after hyperglycemia ($P < 0.05$), respectively. A significant decline in GFR was not observed in these diabetic mice within the 25-week period of study compared with their baseline. Nevertheless, a tendency for GFR to decline was observed in diabetic DBA/2J mice (Fig. 8). The GFR calculated per mouse was listed in Table 2.

DISCUSSION

The progression of diabetic nephropathy in humans has been suggested to be significantly affected by genetic factors accounting for the heterogeneous susceptibility to diabetic nephropathy seen in diabetic patients (2–4,8,34). The present studies addressed whether genetic background also significantly affects the susceptibility to diabetic nephropathy in genetically homogenous inbred mouse strains. These studies compared the severity of the three major criteria for the diagnosis of diabetic nephropathy: albuminuria, GFR, and histopathological changes (5,18).

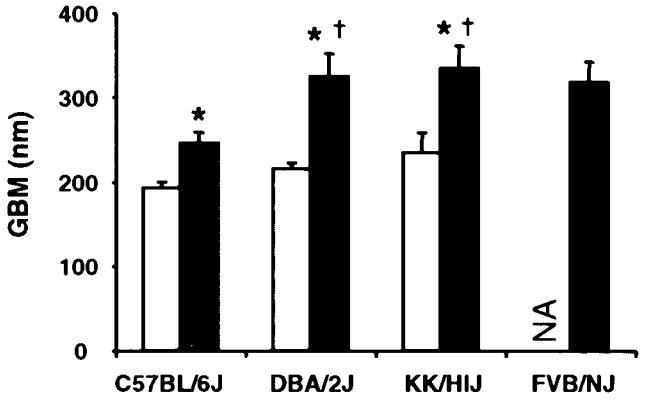

FIG. 6. GBM width in diabetic inbred mice after ~25 weeks of hyperglycemia (■) and age-matched controls (□). *$P < 0.05$ (t test) vs. age-matched controls; †$P < 0.05$ (ANOVA) vs. diabetic C57BL/6J mice. The age-matched controls in FVB/NJ strain were not available (NA).

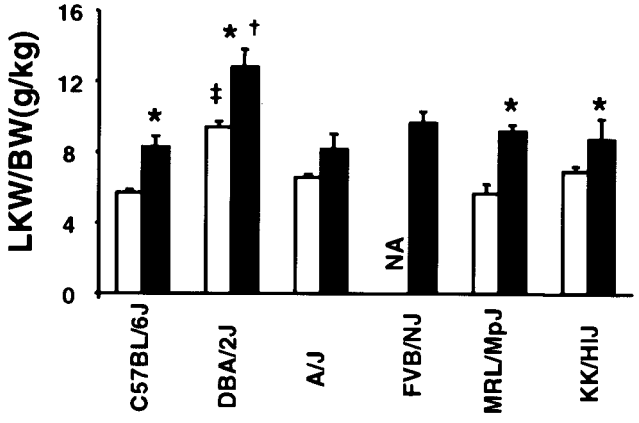

FIG. 7. Left kidney–to–body weight ratio in diabetic inbred mice after ~25 weeks of hyperglycemia (■) and age-matched controls (□). *$P < 0.05$ (t test) vs. age-matched controls; †$P < 0.05$ (ANOVA) vs. all studied strains of diabetic mice, and ‡$P < 0.05$ (ANOVA) vs. controls in other studied strains. The age-matched controls in FVB/NJ strain were not available.

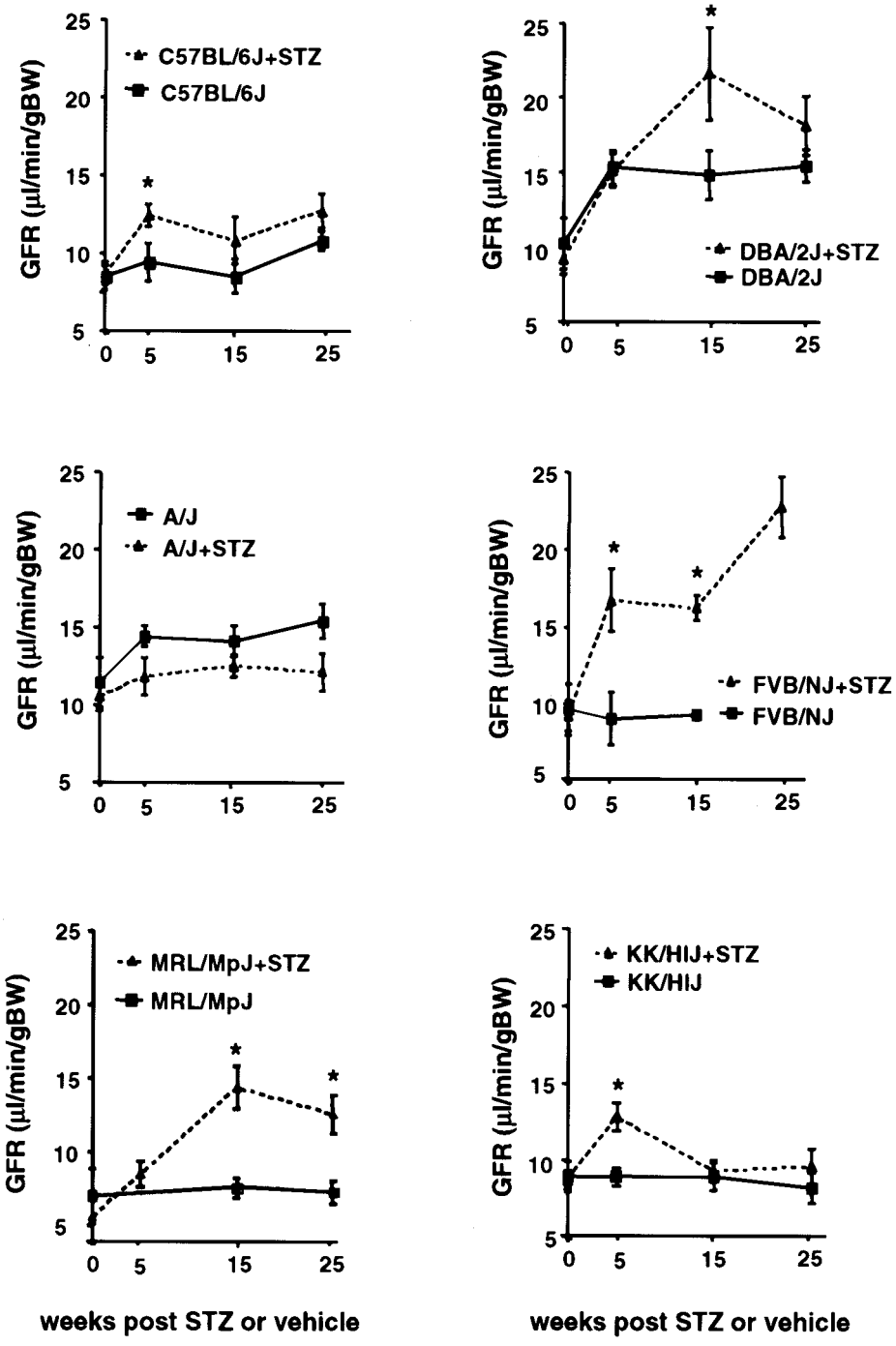

FIG. 8. GFR in diabetic and age-matched control mice. *$P < 0.05$ vs. age-matched controls at same time point.

Diabetes was induced using multiple low-dose STZ injection, an established model for generating type 1 diabetes in mice (12,18). In contrast to single high-dose STZ, which causes massive necrosis of the pancreatic β-cell mass and has potential toxicity to other organs, multiple low doses of STZ initiate an insulitis similar to that observed in type 1 diabetes and moderate hyperglycemia (12,35). After 5 consecutive days of low-dose STZ injection, hyperglycemia developed in all inbred strains of mice. As previously observed (36), the average fasting blood glucose and A1C in response to low-dose STZ were strain dependent. KK/HlJ mice developed relatively low levels of hyperglycemia and A1C compared with other studied strains. In contrast, DBA/2J mice appear to be susceptible to STZ-induced diabetes, developing comparable levels of hyperglycemia with only 40 mg · kg^{-1} · day^{-1} STZ, whereas other strains required 50 mg · kg^{-1} · day^{-1}. To maintain hyperglycemia and to accelerate the development of diabetic nephropathy, we avoided the superimposed effects of insulin treatment on the progression of diabetic nephropathy (37) and did not administer exogenous insulin to these mice. Although most mouse strains survived for >25 weeks with blood glucose levels from 300 to 600 mg/dl, those mouse strains not developing significant albuminuria appeared to exhibit a greater survival rate (data not shown). For example, a group of diabetic C57BL/6J mice lived longer than 45 weeks after the onset of hyperglycemia. In contrast, the mortality was markedly

TABLE 2
Temporal pattern of GFR in STZ-induced diabetic and control inbred mice

Strain	Before STZ	Weeks after STZ or vehicle		
		5	15	25
C57BL/6J				
Diabetic	217.2 ± 13.7 (18)	300.6 ± 24.2 (13)*	275.4 ± 31.7 (12)*	345.5 ± 24.5 (9)*
Control	207.5 ± 18.7 (12)	306.0 ± 47.2 (8)*	244.3 ± 37.9 (4)	325.9 ± 17.6 (5)*
DBA/2J				
Diabetic	220.6 ± 22.7 (20)	365.5 ± 30.4 (23)*	451.9 ± 62.8 (8)*	369.9 ± 43.0 (12)*†
Control	246.3 ± 35.1 (8)	457.5 ± 23.2 (3)*	452.2 ± 121.5 (3)*	473.9 ± 29.4 (12)*
A/J				
Diabetic	261.2 ± 23.6 (16)	246.7 ± 27.3 (15)†	288.7 ± 20.6 (10)†	287.5 ± 28.6 (15)
Control	271.5 ± 47.4 (4)	378.5 ± 32.8 (4)	393.6 ± 13.8 (4)	301.0 ± 80.6 (4)
FVB/NJ				
Diabetic	254.4 ± 26.3 (16)	479.3 ± 64.8 (11)*	562.8 ± 40.0 (4)*†	822.6 ± 84.9 (7)*‡§
Control	260.2 ± 43.9 (7)	245.7 ± 58.8 (4)	302.4 ± 15.2 (4)	NA
MRL/MpJ				
Diabetic	239.8 ± 72.9 (7)	309.6 ± 36.2 (14)	491.5 ± 69.3 (8)*‡	479.1 ± 49.9 (7)*†‡
Control	300.3 ± 118.6 (4)	NA	302.3 ± 31.7 (4)	272.3 ± 25.6 (4)
KK/HlJ				
Diabetic	269.3 ± 28.9 (12)	353.6 ± 24.6 (17)*	285.6 ± 19.5 (15)‡†	315.4 ± 37.2 (10)
Control	261.4 ± 32.6 (8)	286.0 ± 26.8 (4)	483.0 ± 64.6 (6)*‡	307.7 ± 39.3 (17)§

Data are means ± SE (μl/min) (no. of animals). NA, not available. *$P < 0.05$ vs. GFR before STZ injection; †$P < 0.05$ vs. controls at same time point; ‡$P < 0.05$ vs. GFR at 5 weeks after STZ; §$P < 0.05$ vs. GFR at 15 weeks after STZ.

increased in diabetic DBA/2J mice after 25 weeks of hyperglycemia, with ~40% of these mice dying by this time. Whether the high mortality in diabetic mice with albuminuria is due to high incidence of infection, diabetic wasting, or cardiovascular events as in humans remains to be determined (38).

Albuminuria is a hallmark of diabetic nephropathy (5,6). In the present studies, we evaluated the development of albuminuria in diabetic inbred mice using ACR (obtained on morning spot urine) and UAE (obtained from a 24-h urine collection). Daily UAE has been a gold standard for diagnosis of albuminuria. However, environmental stress imposed on mice housed in metabolic cages, together with incomplete collection due to evaporation of urine on the cage walls may substantially influence the accuracy of urine collection in this species (26,39). Thus validation of alternative markers for 24-h UAE should facilitate studies on diabetic nephropathy and other kidney diseases using mouse models. Correlation between ACR and 24-h UAE rate as demonstrated in the present studies suggest that morning spot urine ACR can be used as an alternative for 24-h UAE in C57BL/6J, DBA/2J, A/J, MRL/MpJ, and KK/HlJ strains. This does not hold true for FVB/NJ mice.

Inbred mice exhibited distinct differences in the development of diabetic albuminuria. Diabetic DBA/2J and KK/HlJ mice appear to develop significantly more albuminuria than diabetic C57BL/6J and MRL/MpJ mice (Table 1 and Fig. 2). Although diabetic DBA/2J mice might be exposed to higher levels of blood glucose at 25 weeks after hyperglycemia (as reflected by A1C), the development of albuminuria in this strain could not be solely attributed to blood glucose level because a significant increase in albuminuria was observed after 5 weeks of hyperglycemia. At that time point and in the following weeks, fasting blood glucose and A1C were comparable with other strains. Furthermore, diabetic KK/HlJ developed albuminuria despite lower levels of A1C and hyperglycemia, suggesting that genetic factors play an important role in the development of albuminuria in these two strains. A transient increase in albuminuria was also observed in A/J and MRL/MpJ mice. This finding could correspond to human cohorts that exhibit regression of microalbuminuria to normal levels (34).

It is also significant that the widely studied C57BL/6J strain is relatively resistant to development of albuminuria despite exhibiting moderate mesangial expansion and GBM thickening. The resistance of this strain to albuminuria was not only seen in low-dose STZ-induced diabetic mice, but also in type 1 diabetes resulting from mutation of insulin 2 gene, C57BL/6-Ins2^{Akita}/J mice (14). These results are consistent with previous studies showing that C57BL/6J mice are relatively resistant to the development of albuminuria or severe glomerulosclerosis in either STZ-induced diabetes or 5/6 nephrectomy (18–21,24,40). These previously reported levels for 24-h UAE in STZ-induced hyperglycemic C57BL/6J mice were uniformly less than 100 μg/24 h (18–21), similar to the present studies. Although C57BL/6J strain has been routinely used for studying the effects of gene disruption in mice, their utility as a model for severe diabetic nephropathy may be limited.

The KK strain has previously been proposed to represent a model of diabetic nephropathy (17,41–43). The present finding of moderate albuminuria in nondiabetic KK/HlJ mice (Fig. 2) is consistent with previous studies reporting that KK mice develop albuminuria after ~10 weeks of age (17). The factors contributing to albuminuria in KK/HlJ mice have not been fully elucidated, but spontaneous glucose intolerance and hyperinsulinemia have been previously reported (17,41,44). Regardless, STZ-induced diabetes produced a further increase in albuminuria in KK/HlJ mice, demonstrating that this strain is also susceptible to diabetic albuminuria. Previous studies have also reported albuminuria in KK-A^{y} mice, a model of type 2 diabetes (17).

Diabetic DBA/2J and KK/HlJ mice exhibited significantly

more glomerulosclerosis (Figs. 3 and 4) and GBM thickening (Figs. 5 and 6) compared with other diabetic strains. Diabetic renal lesions seen in advanced stages of human diabetic nephropathy including nodular glomerulosclerosis and arteriolar hyalinosis (5) were observed in diabetic KK/HlJ and DBA/2J mice (Fig. 3). Age-matched nonhyperglycemic control KK/HlJ mice developed less severe glomerulosclerosis than their hyperglycemic counterparts. In humans, interstitial fibrosis and tubular atrophy also develop in advanced diabetic nephropathy (5,45), however, we did not detect these lesions in the present studies.

Altered GFR represents another important indicator of the progression of diabetic nephropathy (5,46). Glomerular hyperfiltration is an early sign of renal involvement in diabetic patients (5). This is followed by a late progressive loss of renal function reflected by a decline in GFR, typically occurring after 20–25 years of type 1 diabetes in humans (5). Using a recently established approach for measuring GFR in conscious mice, we now provide the first systemic survey of GFR in inbred mouse strains over time. Glomerular hyperfiltration was observed in diabetic mice developing albuminuria including DBA/2J, KK/HlJ, and FVB/NJ mice, and in strains without significant albuminuria including C57BL/6J and MRL/MpJ mice (Fig. 8). These results suggest that hyperfiltration is not invariably linked to albuminuria. Renal hyperfiltration was associated with an increased kidney–to–body weight ratio in diabetic C57BL/6J, DBA/2J, MRL/MpJ, and KK/HlJ mice (Figs. 7 and 8).

Reduced GFR is an essential, albeit late, manifestation of diabetic nephropathy in humans. Despite severe albuminuria and renal morphological changes in diabetic DBA/2J and KK/HlJ mice, we did not detect a significant decline in GFR within the 25-week period of study compared with the baseline. In type 1 diabetic patients with nephropathy, GFR decline occurs after 15–25 years of accumulated renal injury (5). The time course of diabetic nephropathy in inbred mice remains to be defined. In a recently published study, Zheng et al. (29) reported a transgenic diabetic mouse model with FVB/N background (OVE26) developing significantly decreased GFR after ~35 weeks of hyperglycemia (9 months). Whereas those mice developed hydronephrosis, this was not observed in the present studies. Nevertheless, a tendency for GFR to decline was observed in diabetic DBA/2J mice after 25 weeks of hyperglycemia (Fig. 8 and Table 2). Thus it is conceivable that a decline in GFR might be detected in diabetic DBA/2J and KK/HlJ mice after a more prolonged period of diabetes. Unfortunately, because of limitations in the survival of these mice, studies at later time points could not be performed.

Diabetic FVB/NJ mice were atypical in several ways. First, although 24-h UAE was significantly increased, no increase in spot ACR was observed (Fig. 2). The reason behind the disassociation between spot ACR and 24-h UAE in this strain remains to be determined; however, it is notable that these mice exhibited dramatically more polyuria than other strains, with 24-h urine volume exceeding 10 ml. For these urine samples, the concentration of albumin and creatinine was relatively low even using nondiluted samples. Second, they developed significantly greater glomerular hyperfiltration after hyperglycemia than other diabetic strains. Furthermore, we did not observe marked renal morphological changes in STZ-induced hyperglycemic FVB/NJ mice within this 25-week period. Taken together, these results suggest that renal hemodynamic changes may contribute to the development of albuminuria in STZ-induced diabetic FVB/NJ mice, rather than intrinsic glomerular diseases.

In summary, the present studies provide evidence for differential susceptibility to diabetic nephropathy in inbred mice. DBA/2J and KK/HlJ mice represent strains prone to diabetic nephropathy, developing more albuminuria and correspondingly more robust renal morphological changes including mesangial expansion, nodular glomerulosclerosis, and arteriolar hyalinosis. In contrast, MRL/MpJ, A/J, and C57BL/6J mice appear to be relatively resistant to diabetic nephropathy. They did not develop significant albuminuria or more severe renal lesions over the period of this study. Although diabetic FVB/NJ mice develop robust albuminuria, they do not develop dramatic histopathological changes over 25 weeks of hyperglycemia. Renal hypertrophy and hyperfiltration were observed in diabetic strains developing albuminuria and in diabetic mice without significant albuminuria. More prolonged hyperglycemia may be required to observe the expected decline in GFR associated with diabetic nephropathy in mice. Further studies should help establish the utility of mouse models to map genes for susceptibility to diabetic nephropathy.

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