Diabetic kidney disease in the \textit{db/db} mouse

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Sharma, Kumar, Peter McCue, and Stephen R. Dunn. Diabetic kidney disease in the \textit{db/db} mouse. Am J Physiol Renal Physiol 284: F1138–F1144, 2003; 10.1152/ajprenal.00315.2002.—Diabetic nephropathy is increasing in incidence and is now the number one cause of end-stage renal disease in the industrialized world. To gain insight into the genetic susceptibility and pathophysiology of diabetic nephropathy, an appropriate mouse model of diabetic nephropathy would be critical. A large number of mouse models of diabetes have been identified and their kidney disease characterized to various degrees. Perhaps the best characterized and most intensively investigated model is the \textit{db/db} mouse. Because this model appears to exhibit the most consistent and robust increase in albuminuria and mesangial matrix expansion, it has been used as a model of progressive diabetic renal disease. In this review, we present the findings from various studies on the renal pathology of the \textit{db/db} mouse model of diabetes in the context of human diabetic nephropathy. Furthermore, we discuss shortfalls of assessing functional renal disease in mouse models of diabetic kidney disease.

diabetic nephropathy; creatinine; albuminuria; mesangial matrix

THE \textit{db/db} MOUSE was identified initially in 1966 in Jackson Labs as an obese mouse that was hyperphagic soon on weaning (13). The diabetic gene (\textit{db}) is transmitted as an autosomal recessive trait. The \textit{db} gene encodes for a G-to-T point mutation of the leptin receptor, leading to abnormal splicing and defective signaling of the adipocyte-derived hormone leptin (1, 15). Lack of leptin signaling in the hypothalamus will lead to persistent hyperphagia and obesity with consequently high leptin and insulin levels. The recognition of diabetes initially was recognized in mice from the C57BLKS/J strain. The C57BLKS/J mouse shares 84% of its alleles with the common C57BL/6 strain and 16% with the DBA/2J strain and was initially maintained by Dr. N. Kaliss (KS). The updated nomenclature from Jackson Labs uses the term C57BLKS/J\textit{\textsuperscript{Lcpr}} (KS for Kaliss) to designate the \textit{db/db} mouse in the C57 black Kaliss background (Jackson Labs, http://jaxmice.jax.org/jaxmicedb/html/model_66.shtml). For the purpose of this review, the common name \textit{db/db} will be used.

The natural history of diabetes and the renal manifestations in the \textit{db/db} mice have been described primarily in the C57BLKS/J strain. In the C57BL/6J background, less hyperglycemia is found despite similar degrees of hyperphagia and weight gain (12). This may be attributed to the development of pancreatic islet cell hypertrophy in the C57BL/6J background rather than islet cell degeneration, as seen in the C57BLKS/J background (12). Another obese, diabetic mouse is the \textit{ob/ob} mouse. This \textit{ob/ob} mouse differs from the \textit{db/db} mouse in that it has a deficiency in the production of leptin but intact leptin signaling. Similar to the \textit{db/db} mouse, the \textit{ob/ob} mouse in the C57BLKS/J background develops \textit{\beta}-cell atrophy and severe hyperglycemia, whereas \textit{ob/ob} mice in the C57BL/6J background develop hyperplasia of the pancreatic ducts and only mild hyperglycemia (12). Interestingly, the C57BLKS/J mouse is more susceptible to the effects of the \textit{\beta}-cell toxin streptozotocin compared with the C57BL/6J strain (20). Thus severe susceptibility to diabetes appears to be present in the KS background and may be independent of the underlying trigger for islet dysfunction. The renal disease in \textit{ob/ob} mice primarily consists of diffuse and nodular lipohyaline changes in glomeruli (34). The relative paucity of diabetic renal lesions in the \textit{ob/ob} mouse, compared with the \textit{db/db} mouse, may be due to lack of circulating leptin, as leptin has been found to directly stimulate matrix production (10); however, part of the difference may be due to the different backgrounds of the mice that have been studied.

In the C57BLKS/J \textit{db/db} mouse, hyperinsulinemia is noted by 10 days of age and blood glucose levels are slightly elevated at 1 mo of age (7.2 ± 2.3 mM) (17). After 1 mo of age, the \textit{db/db} mice are distinguished from wild-type and heterozygous mice by the presence of increased fat deposition in the inguinal and axillary regions. The \textit{db/db} mouse develops frank hyperglycemia with glucose values of 9.7 ± 1.6 mM by 8 wk of age and 15.7 ± 4.3 mM at 10 wk of age (17). There is a progressive increase in food and water intake associ-
ated with progressive weight gain until 4–5 mo of age. Food intake averages ~40 g/wk between the ages of 4 and 16 wk of age. Water intake averages ~30 ml/wk before the onset of hyperglycemia and increases to 100 ml/wk with worsening hyperglycemia (17). Progressive hyperglycemia is noted with mean levels of glucose of 28.6 ± 13.2 mM and peak levels reaching as high as 44 mM at 16 wk of age (17, 19). After 5–6 mo of age, the body weight and insulin levels begin to fall in association with pancreatic islet cell degeneration (12). By this time, the mice become so obese that they have difficulty ambulating in the cage and obtaining food and water. The cause of death is not clear, although ketonuria, hematuria, and gastrointestinal bleeding have all been noted during the terminal stage (13).

RENNAL HYPERTROPHY

At the tissue level, several studies measured renal size in the db/db mouse. An important caveat is because a large amount of fat envelops the kidneys, to ensure accurate weight, careful removal of fat tissue is required before weighing of the kidneys. Table 1 lists the results of various kidney parameters that have been reported from several published studies. Evidence of kidney hypertrophy has been noted in db/db mice at the age of 16 wk (6, 11, 35). Surprisingly, the kidney weights in the diabetic mice do not remain significantly increased above control values between the ages of 21 and 25 wk (Table 1) (14). In 22-wk-old male mice, we found that the right kidney is significantly heavier than the left kidney in the db/db mice (right 248 ± 6 vs. left 208 ± 7 mg, P < 0.05; Sharma and Dunn, unpublished observations). On the basis of the above, renal hypertrophy should be evaluated before 16–20 wk of age and both kidneys should be weighed individually.

GLOMERULAR HYPERTROPHY

Glomerular surface area has been measured using standard measurements of glomerular tuft areas using a digital planimeter at various ages of the db/db and db/m mice (Table 1). Cohen et al. (4) found that glomerular surface area in nonperfused kidneys was increased by 23% at 8 wk of age and remained increased by 27% at 16 wk of age. Koya et al. (14) studied glomerular surface area in perfused db/db mouse kidneys at 25 wk of age and also found a significant increase (Table 1). Although the absolute values of the glomerular surface area were over twofold greater in perfused kidneys (14), compared with nonperfused kidneys (4), the degree of glomerular hypertrophy in the db/db group was similar (22 and 27% in both studies). Glomerular hypertrophy at the onset of diabetes may be due to alteration of glomerular hemodynamics as there is evidence of glomerular hyperfiltration in db/db mice during the early stages of diabetes (8). However, there have not been any studies of glomerular capillary pressures in db/db mice and the contribution of capillary loop enlargement and cell hypertrophy of the different glomerular cell compartments has not been evaluated.

GLOMERULAR PATHOLOGY

An in-depth characterization of renal pathology in the db/db mouse was first described by Like and colleagues (21) in 1972. In this initial study, mice were studied at various time intervals, with some mice having their food restricted between weeks 7 and 11 to allow the mice to live to 12–22 mo of age. Gross examination did not reveal any differences in the kidney, although calyceal dilation and flattening of the papilla were notable in diabetic mice older than 5–6 mo of age. Before the onset of sustained hyperglycemia (4–6 wk of age), the glomeruli of mice were not distinguishable from their nondiabetic littermates. By 5–6 mo of age, diabetic mice had larger glomeruli with increased mesangial matrix by periodic acid-Schiff (PAS) staining. By 18–20 mo of age, the mesangial matrix and glomerular enlargement became more pronounced and thickening of the glomerular basement membrane (GBM) was notable. Nodular lesions of the subepithelia basal lamina were also noted, which were PAS positive. It should be noted that the described subepithelial nodules were distinct from the Kimmelstein-Wilson nodules described in human diabetic nephropathy. By electron microscopy (EM), the finding of increased mesangial matrix with frequent foci of vacuolar change and embedded collagen fibrils was found to distinguish older diabetic mice (5–6 mo of age) from nondiabetic littermates. GBM thickening and subepithelial nodularity were noted primarily in diabetic mice older than 12 mo of age. In the oldest diabetic mice studied (16–22 mo of age), strikingly large subepithelial nodular densities were observed along with foot process fusion.

Table 1. Renal parameters in C57BLKS/JLepr (db/db and db/m) mice at various ages

<table>
<thead>
<tr>
<th>Age</th>
<th>Kidney Weight, mg</th>
<th>Glomerular Surface Area, μm²</th>
<th>Mesangial Matrix Area, %glomerular area</th>
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<td>DB</td>
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Values are means ± SE. Kidney weights of the right kidney are shown. *P < 0.05 vs. corresponding value for control group.

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Subsequent studies of diabetic renal pathology were performed in the early 1980s by Lee and colleagues (16–19). By morphometric analysis of glomeruli, mesangial matrix enlargement was consistently noted after the age of 16 wk in the \textit{db/db} mouse. When \textit{db/db} mice were placed on caloric restriction by limiting their time of feeding to 3 h/day, their body weight and the blood glucose levels were only slightly higher than the heterozygous control (\textit{db/m}) mice, and renal pathology was prevented (17).

A reappraisal of renal pathology in \textit{db/db} mice by several groups in the past decade has brought renewed recognition of this mouse model as a useful tool for the study of diabetic nephropathy.

**Mesangial Matrix Expansion**

Diffuse expansion of the mesangial matrix is considered to be the hallmark pathological feature of established diabetic nephropathy in humans (24, 25, 29, 32). Of the many mouse models of diabetes that have been identified, the \textit{db/db} mouse appears to most closely mimic the progressive nature of mesangial matrix expansion seen in human diabetic nephropathy. The time course of mesangial matrix expansion was described recently by Cohen et al. (4) (Table 1). At 8 wk of age, before the onset of severe hyperglycemia, there is no discernible increase in the mesangial compartment of the \textit{db/db} mouse. At 12 wk of age and after 4–6 wk of hyperglycemia, a twofold increase in mesangial matrix was noted. After 16 wk of age, a consistent threefold increase in mesangial matrix expansion was reported by several independent studies (4, 6, 14, 35) (Table 1). Although many of the prior studies were performed without perfusion of the mouse kidneys, the study by Koya et al. (14) showed a similar increase in mesangial matrix expansion in perfused kidneys from \textit{db/db} mice of 25 wk of age.

Selected images of PAS-stained glomeruli from \textit{db/m} and \textit{db/db} male mice of 21 wk of age are presented in Fig. 1. In the normal heterozygous mouse (C57BLKS/J \textit{db/m}), the outer cortical glomerulus is of normal size and configuration (Fig. 1A). Bowman's capsule is of the usual caliber, and there is no epithelial cell proliferation. The capillary tuft is fully expanded with patent capillary loops, and the GBMs appear thin and delicate. The mesangium contains the usual complement of cells and matrix without matrix expansion, inflammation, or sclerosis. In distinction, the most severely affected glomerulus from a \textit{db/db} mouse kidney shown appears dramatically different (Fig. 1B). The visceral epithelial cells are swollen and appear prominent. The glomerular capillary basement membranes appear thickened, and the peripheral capillary loop appears collapsed. The mesangium is diffusely and markedly expanded with PAS-positive matrix material. The overall cellularity is normal without inflammation or necrosis. Approximately 30% of glomeruli have a similar appearance, and the remaining have a lesser degree of mesangial matrix expansion. Thus, by light microscopy, the appearance is very similar to moderate human diabetic nephropathy at the glomerular level. However, there was no evidence of Kimmelstein-Wilson lesions and capsular drop lesions were only rarely seen. By EM analysis, segmental GBM thickening has been reported by several studies (11, 21, 22). However,
formal quantitative studies have not been published to establish the degree of GBM thickening.

The constituents of the mesangial matrix expansion in the db/db mouse kidney consist of increased type IV collagen, fibronectin, and laminin (6, 9, 14, 30, 35). By Northern blot analysis of renal cortex, a two- to eightfold increase in type IV collagen [α1(IV)] gene expression and a fourfold increase in fibronectin gene expression appeared at 16 wk of age (6, 30, 35). By immunostaining, a marked increase in fibronectin and type IV collagen was described in glomeruli of db/db mice at 25 wk of age (14). Laminin isoforms-β1 and α5 were found to be increased in glomeruli of db/db mice by immunostaining; however, the mRNA levels were decreased in the renal cortex at the same time points (9). In human diabetic nephropathy, the increase in mesangial matrix expansion has also been associated with an increase in fibronectin, α1(IV) and α2(IV) collagen chains, and laminins (28). Thus a very similar pattern of the components of mesangial matrix that account for the expansion in human diabetic nephropathy has been noted in the db/db mouse.

IMMUNOFLUORESCENT STUDIES

Several studies observed diffuse uptake of IgG, IgA, IgM, and complement in glomeruli of db/db mice at as early as 8 wk of age (16–19). On the basis of the study by Like et al. (21) with older db/db mice (>8 mo of age), the presence of subepithelial nodular deposits under EM may represent immune complexes. However, such lesions have not been described on EM analysis in the db/db mouse at 16 wk of age (11). It should also be noted that nondiseased mice from various backgrounds may exhibit glomerular binding of immunoglobulins, including IgG, IgM, and IgA (23). The degree of immune complex deposition can be reduced if they are raised in germ-free environments (23). Therefore, the increased glomerular immunoglobulin uptake in the db/db mice may be secondary to increased susceptibility to infections and resulting immune complex formation. To help clarify the role of immune complex deposition in relation to the renal disease of db/db mice, immunofluorescent studies in db/db mice raised in germ-free environments will be necessary.

VASCULAR AND TUBULOINTERSTITIUM

Advanced human diabetic nephropathy often exhibits arteriolar hyalinosis and tubular atrophy coupled with an increase in the interstitial volume (28). These features are largely absent in the db/db mouse kidney at 16 wk of age. At the more advanced age (21 wk), lesions suggestive of arteriolar hyalinosis (Fig. 1C) were noted. However, it has not been characterized whether both afferent and efferent arterioles are similarly affected. The tubular changes noted in the db/db mouse kidney primarily consist of vacuolization of tubular cells (Fig. 1, B and C). No evidence of tubular atrophy, tubulointerstitial fibrosis, or alterations of the medullary structure by light microscopy was discernible. An overall increase in renal collagen content has been reported in db/db mice (22); however, comprehensive and sensitive studies of tubular and vascular damage have not yet been published.

RENAO FUNCTIONAL MEASUREMENTS (CLEARANCE STUDIES)

Given that the db/db mouse exhibits a progressive increase in mesangial matrix expansion in the setting of severe hyperglycemia, it has been considered an appropriate model of progressive human diabetic nephropathy. As human diabetic nephropathy is initially characterized by a supernormal glomerular filtration rate (GFR) in the early stages and a decline in GFR in the later stages, measurements of GFR have also been assessed in db/db mice. GFR was repeatedly measured in conscious C57BL/6J db/db female mice and C57BLKS/J mice between 7 and 24 wk of age using single injections of 51chromium-EDTA (8). As the GFR values were similar in both strains of db/db mice, the data were pooled (8). GFR was found to be elevated in db/db mice as early as 7 wk of age, even though blood glucose levels were only mildly increased. Between 7 and 14 wk of age, the GFR was ~300 µl·min⁻¹·mouse⁻¹ in the control mice, whereas the GFR was ~700 µl·min⁻¹·mouse⁻¹ in the db/db mice. After 17 wk of age, the GFR remained constant in the nondiabetic mice; however, a marked variability in the GFR was observed in the db/db mice, with some mice having GFRs below normal. This study concluded that female db/db mice do hyperfilter at the onset of hyperglycemia and that GFR declines with the duration of diabetes. However, the GFR did not progressively increase in association with the severity of hyperglycemia and was similar in both the BL6 and KSJBL6 mice strains.

Recent studies used endogenous creatinine clearance in the db/db mouse model (4, 35) as an index of renal function. At 8 wk of age, a twofold increase in creatinine clearance in db/db male mice was observed (4). By 16 wk of age, the creatinine clearance was reduced by 30–50% compared with age-matched control heterozygous mice (4, 35). Blood levels of creatinine correspondingly increased by twofold in db/db mice at 16 wk of age (4, 35). Thus it appears that the pattern of creatinine clearance associated with the duration of diabetes in the db/db mouse corresponds well to actual GFR levels as reported by Gartner (8). However, by absolute values the calculated creatinine clearance in normal heterozygous mice was on the order of 60 µl·min⁻¹·mouse⁻¹ (4), whereas the GFR was ~300 µl·min⁻¹·mouse⁻¹ by the 51chromium-EDTA method (8).

The measurement of plasma or serum creatinine is quite problematic in mice (26, 27). Measurement of creatinine levels in normal mouse blood by Jaffe alka-line picrate reaction has yielded values ranging from 17 to 106 µmol/l (0.2 to 1.2 mg/dl). However, the Jaffé reaction has been reported to grossly overestimate the actual plasma creatinine concentration (26, 27). The actual result based on an assay using HPLC provides values that are roughly one-third to one-fifth of the
value found by the Jaffé reaction in normal mice (27). Similar results were found in db/db mice (Sharma and Dunn, unpublished observations). The increased concentration as measured by the Jaffé reaction is thought to be due to the presence of yet unidentified noncreatinine chromagens present exclusively in mouse blood. Although several modifications of the Jaffé reaction have been touted to be more specific (7, 26, 31, 33), these measurements have not been performed compared with HPLC. Presently, HPLC with either spectrophotometric (UV detection) or HPLC coupled to a mass spectrometer appears to be the definitive method to measure creatinine in mouse plasma or serum. Therefore, it is unclear what the endogenous creatinine clearance signifies in relation to renal function. Nevertheless, as prior studies have found that db/db mice do have an increase in serum creatinine after the age of 16 wk (4, 35) and db/db mice older than 17 wk of age do have a decline in GFR (8), it may well prove correct that the true endogenous creatinine clearance reflects renal function. Formal studies comparing creatinine clearance by HPLC-based methods with inulin-based GFR methods are presently underway to address this issue in diabetic mice.

ALBUMINURIA

With the availability of sensitive and specific ELISAs for measuring mouse albumin (6), levels of urinary albumin may easily be followed from mice placed in metabolic or diuresis cages. Several studies established that albumin excretion rates are higher by 8 - 62-fold in db/db mice beginning at the age of 8 wk (Table 2). The range of albuminuria is between 68 and 303 µg/24 h in the db/db male mouse, whereas it is between 4 and 21 µg/24 h in the age-matched heterozygous littermate (4, 14). The degree of albuminuria does not consistently increase with the duration of diabetes as there are similar levels of albuminuria at 8, 16, 21, and 25 wk (4, 9, 14, 35).

Interestingly, when urinary protein was measured by a total protein assay such as the Bradford reaction, no statistical increase in urinary protein was found in the 20-wk-old db/db mouse (30). This would suggest that there is a selective increase in albuminuria in the db/db mouse and thus can only be detected by an assay specific for albumin. Although a head-to-head study has not been performed between db/db females and db/db males, the db/db males generally have twice as much albuminuria compared with females (21) (Sharma and Dunn, unpublished observations). In addition, in unpublished studies, we found that addition of a high-salt (5%) and high-protein (30%) diet led to a 50% increase in 24-h urinary albumin excretion in db/db males.

It is of interest that the increase in urinary albumin excretion has been noted at 8 wk of age (Table 2), before the development of obvious structural evidence of alterations of the GBM or the podocytes. By EM examination, a mild segmental increase in GBM thickening and rare podocyte foot process fusion has been noted at 16 wk of age (11) (Sharma and Dunn, unpublished observations). Measurement of podocyte numbers as well as podocyte-specific proteins in relation to the development of albuminuria in the db/db mouse would be very informative.

BLOOD PRESSURE

In the study by Koya et al. (14), blood pressure was measured by the tail cuff method at the age of 16 wk in male db/m and db/db mice. At this time point, the mean blood pressure was 117 mmHg in db/m mice and 110 mmHg in db/db mice and not significantly different. To our knowledge, no other studies reported blood pressure measurements in the C57BLKS/J db/db mouse.

INSIGHTS FROM RECENT INTERVENTIONAL STUDIES

On the basis of the recognition that the db/db mouse has many similar features of human diabetic nephropathy, several studies were performed in this model to investigate the role of various candidate pathways in the progression of diabetic renal disease. Studies by Cohen et al. (3, 6) found that db/db mice have elevated glycated albumin and that antibodies against glycated albumin attenuate albuminuria, mesangial matrix accumulation, and decline in renal function when given over a course of 8 wk. In addition, an oral inhibitor of Amadori glucose adducts maintained levels of glycated albumin in the normal range and exerted beneficial effects on features of diabetic nephropathy in the db/db mice (5). The role of PKC-β in progressive diabetic kidney disease has also been assessed in this model. Glomeruli from db/db mice at 25 wk of age were found to have a twofold increase in PKC activity (14). In db/db mice given an oral PKC-β inhibitor from week 9 until week 25, normalization of glomerular PKC activity and significant reduction in albuminuria and mesangial matrix expansion were observed (14).

Table 2. Albuminuria in C57BLKS/J-db (db/db and db/m) mice at various ages

<table>
<thead>
<tr>
<th>Age</th>
<th>Albuminuria, µg/24 h</th>
<th>Ref. no.</th>
<th>Albuminuria, µg Alb/µg Crt</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>db/db</td>
<td>db/m</td>
<td></td>
<td></td>
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<tr>
<td>Age 8 wk</td>
<td>247 ± 41*</td>
<td>6 ± 3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Age 12 wk</td>
<td>249 ± 34*</td>
<td>4 ± 2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Age 16 wk</td>
<td>303 ± 30*</td>
<td>21 ± 8</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Age 21–25 wk</td>
<td>214 ± 44*</td>
<td>10 ± 2.5</td>
<td>14</td>
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Values are means ± SE. Alb, albumin; Crt, creatinine. *P < 0.05 vs. corresponding value for control group.
The role of anti-fibrotic inhibitors to inhibit progression of diabetic kidney disease has also been demonstrated using the db/db mouse. The db/db mouse exhibits an increase in glomerular transforming growth factor-β1 (TGF-β1) as well as an increase in the type II receptor for TGF-β (11). Inhibiting TGF-β, with anti-TGF-β antibodies, normalizes mesangial matrix accumulation, gene expression for type IV collagen, and fibronectin, as well as renal functional parameters (35). Interestingly, a recent study by Ziyadeh’s group (2) found that mesangial matrix changes in the db/db mouse appear to be reversible, as treatment of db/db mice with established disease decreased the extent of the lesions. The benefit of anti-TGF-β antibodies was found without a reduction in albuminuria in the treated mice (35), suggesting that an anti-TGF-β approach may be beneficial even without affecting albuminuria. In large part due to the beneficial effect of inhibitors of various pathways in the db/db mice, approaches to block glycated proteins, PKC-β activation, TGF-β action, and renal fibrosis are presently being considered as novel treatment strategies for human diabetic nephropathy.

In summary, the db/db mouse has a long history as a model of human diabetic nephropathy. Key common features with the human condition are renal hypertension, glomerular enlargement, albuminuria, and mesangial matrix expansion. Occasionally, arteriolar hyalinosis is observed in the glomerular arterioles. Features that are not as reproducibly altered in the db/db mouse with respect to the human condition are the increase in GBM thickening in relation to albuminuria and the lack of progressive increase in albuminuria. Features that are not observed in the db/db mouse are the advanced features of diabetic nephropathy, such as nodular sclerosis in the glomeruli, tubulointerstitial fibrosis, and tubular atrophy. Features that may be inconsistent with human diabetic nephropathy include immune complex deposition in the glomerulus and nodular thickening of the subepithelial space. Improved methods to assess kidney function in the db/db mice are required to establish the extent of renal functional deterioration with duration of diabetes. Additional studies to examine the mechanisms underlying the development of glomerular hypertrophy, albuminuria, and mesangial matrix expansion in the db/db mouse could provide important insight and are likely to be of relevance to the development of human diabetic nephropathy.

REFERENCES


