Diabetic nephropathy in a nonobese mouse model of Type 2 diabetes mellitus

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Mallipattu SK, Gallagher EJ, Le Roith D, Liu R, Mehrotra A, Horne SJ, Chuang PY, Yang VW, He JC. Diabetic nephropathy in a nonobese mouse model of Type 2 diabetes mellitus. Am J Physiol Renal Physiol 306: F1008–F1017, 2014. First published March 5, 2014; doi:10.1152/ajprenal.00597.2013.—A large body of research has contributed to our understanding of the pathophysiology of diabetic nephropathy. Yet, many questions remain regarding the progression of a disease that accounts for nearly half the patients entering dialysis yearly. Several murine models of diabetic nephropathy secondary to Type 2 diabetes mellitus (T2DM) do exist, and some are more representative than others, but all have limitations. In this study, we aimed to identify a new mouse model of diabetic nephropathy secondary to T2DM in a previously described T2DM model, the MKR (MCK-KR-hIGF-IR) mouse. In this mouse model, T2DM develops as a result of functional inactivation of insulin-like growth factor-I receptor (IGF-1R) in the skeletal muscle. These mice are lean, with marked insulin resistance, hyperinsulinemia, hyperglycemia, and dyslipidemia and thus are representative of nonobese human T2DM. We show that the MKR mice, when under stress (high-fat diet or unilateral nephrectomy), develop progressive diabetic nephropathy with marked albuminuria and meet the histopathological criteria as defined by the Animal Models of Diabetic Complications Consortium. Finally, these MKR mice are fertile and are on a common background strain, making it a novel model to study the progression of diabetic nephropathy.

diabetic nephropathy; leptin; insulin-like growth factor-I receptor; Type 2 diabetes mellitus

DIABETIC KIDNEY DISEASE (DKD) is the main cause of chronic kidney disease (CKD) worldwide. In fact, it accounts for nearly 50% of patients entering dialysis yearly (23). Combined with the rising health care costs of this patient population, targeted therapy is essential for this disease. Although DKD contributes to the majority of CKD and end-stage renal disease, many questions remain unanswered regarding the progressive nature of the disease. To address these questions, various murine models of diabetic nephropathy have been proposed and utilized for more than 15 years. Although some of these mouse models have shown promise, none have completely satisfied the criteria for a progressive mouse model of DKD as proposed by the National Institute of Health funded Animal Models of Diabetic Complications Consortium (AMDCC) (1, 4).

Recent reviews have clearly illustrated the limitations in the current mouse models of diabetic nephropathy from Type 1 DM as well as Type 2 DM (1, 4). Although diabetic nephropathy can result from both Type 1 DM and Type 2 DM, the majority of patients with diabetic nephropathy have Type 2 DM. Furthermore, of the many mouse models of diabetic nephropathy, only a few exhibit most of the representative features of diabetic nephropathy secondary to Type 2 DM. For example, mouse models involving the dysregulation of leptin production (db/db mouse model), or leptin signaling pathway (black and tan, brachyuric, and BTBR ob/ob mouse model), have shown encouraging results in modeling progressive diabetic nephropathy secondary to Type 2 DM. However, in the db/db mouse model, mesangial expansion develops rather slowly with no evidence of renal insufficiency (22). The BTBR ob/ob mice develop glomerular lesions with progressive albuminuria relatively sooner than the db/db mice (9, 16); however, one of the major concerns of the BTBR ob/ob mouse model is that these mice are infertile resulting in difficult breeding strategies (16). Furthermore, Alpers and Hudkins (1) suggest that since BTBR is not a commonly used background strain to introduce genetic mutations, it makes it difficult to study molecular pathways involved in the progression of diabetic nephropathy. Nevertheless, the BTBR ob/ob mice and the db/db are currently the most promising mouse models of diabetic nephropathy from Type 2 DM. In recent years, endothelial nitric oxide synthase deficiency in the db/db mouse proved to be quite a representative model of advanced diabetic nephropathy. However, since its initial description in 2006 (27), the use of endothelial nitric oxide synthase knockout mice in understanding the mechanisms in the progression of diabetic nephropathy has been limited. Similar to the speculation provided by previous groups, this may potentially be a result of infertility and/or complicated and time-consuming breeding strategies to introduce multiple mutations in these mice (16).

In this study, we aimed to identify a viable and representative mouse model of diabetic nephropathy secondary to Type 2 DM. We employed the previously described murine model of Type 2 DM, the MKR (MCK-KR-hIGF-IR) mouse (6). In this mouse model, Type 2 DM is a result of functional inactivation of insulin-like growth factor-I receptor (IGF-1R) specifically in the skeletal muscle (6). The expression of MCK-KR-hIGF-IR results in the formation of hybrid receptors between the mutant and the endogenous IGF-I and insulin receptors, thereby leading to receptor dysfunction. The loss of normal receptor function leads to insulin resistance, impaired glucose uptake, glucose homeostasis, and eventual pancreatic β-cell dysfunction (6). In contrast to many other murine models of Type 2 DM, the MKR mice are lean with marked insulin resistance, hyper-
glycemia, hyperinsulinemia, and dyslipidemia, features that are representative of nonobese human diabetes (8, 26). Here, we demonstrate that the MKR mouse under stress is a novel murine model of progressive diabetic nephropathy secondary to Type 2 DM by fulfilling the majority of the criteria proposed by the AMDCC.

METHODS

Genotyping of MKR mice. The Mount Sinai School of Medicine Institutional Animal Care and Use Committee approved all animal studies and the National Institutes of Health Guide for the Care and Use of Laboratory Animals was followed strictly. All mice used in these studies were male on an FVB/n background. Derivation and characterization of the transgenic mouse line MCK-KR-hIGF-IR (MKR) mice that bears a dominant-negative IGF-1R specifically targeted in the skeletal muscle have been previously described (6). Wild-type (WT) FVB/n mice were used as the controls in the studies. Genotyping by tail biopsy and PCR were performed at 2 wk of age as previously described (6).

High- and low-fat diet feeding. In the high-fat diet (HFD) and low-fat diet (LFD) diet mouse model, 12-wk-old WT and MKR mice were housed in cages, maintained on a 12-h light/dark cycle, and fed HFD (45% of kilocalories from fat) or LFD (10% of kilocalories from fat) from Research Diets (New Brunswick, NJ) for a 24-wk period. Urine, serum, and body weight were checked monthly. At the end of 24-wk period, blood pressure, urine, serum, body weight, and kidney weight were measured before being killed.

Unilateral nephrectomy. Twenty-four-week-old WT and MKR mice underwent either unilateral nephrectomy (UNX) or sham operation on standard Chow diet (25% of kilocalories from fat). After shaving off the overlying fur, UNX was performed in anesthetized mice through dorsal skin. An incision was made in the left psoas muscle wall. The left renal pedicle was identified and ligated, followed by total removal of left kidney. Then, the left psoas muscle and overlying skin were sutured and mice were warmed and monitored until full recovery from anesthesia. Sham-operated mice had the left renal pedicles manipulated without ligation. Urine, serum, and body weight were checked biweekly. Ten weeks post-UNX or postsham operation, blood pressure, urine, serum, body weight, and kidney weight were measured before animals were killed.

Blood pressure monitoring. Blood pressure was measured using the CODA® programmable noninvasive tail-cuff® sphygmomanometer (Kent Scientific, Torrington, CT) on conscious mice as previously described (12). Mice were initially subjected to acclimation period of five cycles before blood pressure assessment. Subsequently, blood pressure was measured in each mouse for 60 continuous cycles and an average of systolic blood pressure (SBP) and diastolic blood pressure (DBP) quantified as previously described (12).

Measurement of urine albumin and creatinine. Urine albumin was quantified by ELISA using a kit from Bethyl Laboratory (Houston, TX). Urine creatinine levels were measured in the same samples using QuantiChrom Creatinine Assay Kit (DICT-500; BioAssay Systems, Hayward, CA) according to the manufacturer’s instructions. The urinary albumin excretion rate was expressed as the ratio of albumin to creatinine (13).

Measurement of glomerular filtration rate. Glomerular filtration rate (GFR) in mice was determined using the clearance of fluorescein isothiocyanate-inulin (FITC-inulin) as described in the AMDCC and Rieg (19). Briefly, 10-wk-old anesthetized mice were injected retroorbitally with diazylzed 5% FITC-inulin (2 μg/g body wt). Blood was collected from the tail at 3, 5, 7, 10, 15, 35, 56, and 75 min after injection. Separated serum was buffered in 0.5 mol/l HEPES pH 7.4, and fluorescence was measured as described previously (19). GFR was calculated using the two-compartment clearance model with the following equation:

$$GFR = \frac{I}{A + \frac{B}{\alpha + \beta}}$$

where I is the total amount of FITC-inulin delivered in the bolus injection retroorbitally, A is the Y-intercept of the rapid phase of elimination, B is the Y-intercept of the slow phase of elimination, α is the decay constant for elimination, and β is the decay constant for distribution.
Serum creatinine, serum blood urea nitrate, serum cholesterol, and blood glucose measurements. Initially, 200 µl of blood were collected by submandibular vein puncture at time of death and serum was extracted as previously described (14). First, 25 µl of mouse serum were used to measure serum creatinine by high-performance liquid chromatography with ultraviolet detect at 225 nm (Hitachi, Foster, CA) as previously described (25). Serum blood urea nitrogen (BUN) was determined by the colorimetric method using the modified Jung method as per manufacture’s (BioAssay Systems) protocol (Hayward, CA). Also, serum cholesterol was quantified enzymatically using cholesterol esterase and oxidase with the Point Scientific Assay Kit (Canton, MI). Blood glucose was measured from the tail vein using One Touch glucometer (Lifescan, Milpitas, CA) after mice were fasted for 6 h, as previously described (14).

Bright-field light microscopy and morphometric studies. Mice were perfused with HBSS, and the kidneys were fixed in 10% phosphate buffered formalin overnight and switched to 70% ethanol before processing for histology. Kidney tissue was embedded in paraffin by American Histolabs (Gaithersberg, MD), and 3-µm thick sections were stained with periodic acid-Schiff (Sigma-Aldrich, St. Louis, MO). Quantification of mesangial area and glomerular volume was performed as previously described (3, 20). In brief, digitized images were scanned and profile areas were traced using ImageJ 1.26t software.

Table 2. Physiological parameters of wild-type and diabetic MKR mice post-dietary change

<table>
<thead>
<tr>
<th></th>
<th>WT + LFD</th>
<th>%BWΔ (from baseline)</th>
<th>WT + HFD</th>
<th>%BWΔ (from baseline)</th>
<th>MKR + LFD</th>
<th>%BWΔ (from baseline)</th>
<th>MKR + HFD</th>
<th>%BWΔ (from baseline)</th>
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<tbody>
<tr>
<td>Baseline</td>
<td>140.0 (7.0)</td>
<td>33.8 (1.0)</td>
<td>150.0 (15.4)</td>
<td>32.8 (1.5)</td>
<td>534.8 (13.0)</td>
<td>27.2 (0.3)</td>
<td>426.5 (36.9)</td>
<td>28.0 (1.3)</td>
</tr>
<tr>
<td>Post 6 wk</td>
<td>144.0 (4.4)</td>
<td>36.5 (0.8)</td>
<td>145.0 (7.5)</td>
<td>42.7 (1.1)†</td>
<td>30.3 (2.6)†</td>
<td>30.6 (0.8)</td>
<td>12.2 (2.6)</td>
<td>34.4 (1.1)†</td>
</tr>
<tr>
<td>Post 12 wk</td>
<td>143.7 (6.2)</td>
<td>37.6 (0.9)</td>
<td>161.0 (8.0)</td>
<td>45.7 (0.9)†</td>
<td>39.5 (3.6)†</td>
<td>30.6 (0.8)</td>
<td>11.8 (1.8)</td>
<td>23.1 (2.9)†</td>
</tr>
<tr>
<td>Post 18 wk</td>
<td>141.3 (2.8)</td>
<td>39.9 (0.9)</td>
<td>172.0 (13.0)</td>
<td>47.6 (1.1)†</td>
<td>45.3 (3.3)†</td>
<td>29.5 (0.7)</td>
<td>13.8 (0.2)</td>
<td>32.4 (2.6)†</td>
</tr>
<tr>
<td>Post 24 wk</td>
<td>145.3 (15.3)</td>
<td>38.0 (0.3)</td>
<td>186.0 (10.0)</td>
<td>48.3 (0.3)†</td>
<td>47.3 (5.8)†</td>
<td>33.6 (0.8)</td>
<td>13.7 (0.2)</td>
<td>32.5 (2.1)†</td>
</tr>
</tbody>
</table>

Values are median (IQR); n = 4. BW, body weight; WT, wild type; LFD, low-fat diet; HFD, high-fat diet. *P < 0.05 vs. all WT mice. †P < 0.05 vs. all groups. §P < 0.05 vs. WT + HFD and MKR + LFD.
Mean glomerular tuft volume (GV) was determined from mean glomerular cross-sectional area (GA) by light microscopy. GA was calculated based on average area of 30 glomeruli in each group and GV was calculated based on the following equation:

\[ GV = \frac{\beta}{\kappa} \times GA^{3/2} \]

where \( \beta = 1.38 \), the shape coefficient of spheres (the idealized shape of glomeruli), and \( \kappa = 1.1 \), the size distribution coefficient.

Mesangial expansion was defined as periodic acid-Schiff-positive and nuclei-free area in the mesangium. Quantification of mesangial expansion was based on 25 glomeruli cut at the vascular pole in each group.

Quantification of podocyte number per glomerulus was determined using WT-1 stained podocytes. Kidney sections from these mice were initially prepared in identical fashion. Subsequently, 4-μm thick sections were stained with rabbit anti-WT-1 (Novus Biologicals, Littleton, CO) as previously described (21). Counting of podocytes and measurement of glomerular area and volume was performed using ImageJ and by the method described by AMDCC (20).

Transmission electron microscopy and morphometric studies. Initially, mice were perfused with HBSS and immediately fixed in 2.5% glutaraldehyde for electron microscopy. Sections stained with uranyl acetate and lead citrate were mounted on a copper grid and photographed under a Hitachi H7650 electron microscope using Hitachi imaging software at the department of pathology (Mount Sinai School of Medicine).

The quantification of podocyte effacement was performed as previously described (11). In brief, negatives were digitized, and images with a final magnitude of approximately at \( \times 5,000 \) and \( \times 15,000 \) were obtained. ImageJ was used to measure the length of the peripheral glomerular basement membrane (GBM), and the number of slit pores (National Institutes of Health: rsb.info.nih.gov/ij).

Fig. 3. Diabetic MKR mice on high-fat diet (HFD) exhibit an increase in albuminuria and kidney weight. WT and MKR mice were subjected to high-fat diet and low-fat diet (LFD) at 12 wk of age. Urine was collected at baseline before dietary change and urine albumin/creatinine ratio was measured. A: urine albumin/creatinine ratio at 24 wk post-dietary change (\( n = 4 \) per group; *\( P < 0.01 \) vs. all other groups). B: kidney weight/body weight (g/g) ratio at 24-wk post-dietary change is shown (\( n = 4 \) per group; *\( P < 0.01 \) vs. WT mice). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured on conscious mice before being killed. Also, serum was collected before the time of death via submandibular vein injection. Measurements for serum cholesterol (C; \( n = 4 \); *\( P < 0.01 \)), SBP (D), and DBP (E) were measured.

Fig. 4. Diabetic MKR mice on HFD exhibit no change in renal function. Serum was collected in WT and MKR mice on HFD and LFD before the time of death via submandibular vein injection. Measurements for serum blood urea nitrogen (BUN; A; mg/dl) and serum creatinine (B; mg/dl) were measured (\( n = 4 \) per group).
overlying this GBM length was counted. The arithmetic mean of the foot process width (WFP) was calculated using the following equation:

$$WFP = \frac{\pi}{4} \times \frac{\Sigma \text{GBM length}}{\Sigma \text{slits}}$$

where $\Sigma \text{GBM length}$ indicates the total GBM length measured in one glomerulus, $\Sigma \text{slits}$ indicates the total number of slits counted, and $\frac{\pi}{4}$ is the correction factor for the random orientation by which the foot processes were sectioned (11).

Quantification of GBM thickness was performed as previously described (18). The thickness of multiple capillaries was measured in five to seven glomeruli per mouse. A mean of 579 measurements was taken per mouse (from podocyte to endothelial cell membrane) at random sites where GBM was displayed in the best cross section.

**Statistical analysis.** Since we could not assume normality of the data due to the small sample size, nonparametric statistical tests were performed using the Mann-Whitney test to compare data between two groups and Kruskal-Wallis test with Dunn’s posttest to compare data between more than two groups. Data are expressed as median (interquartile range). Our sample size allowed us 80% power to detect a difference of 200 μg/g in albumin/creatinine, 20 mmHg in SBP, 10 mmHg in DBP, 10 mg/dl in serum BUN, 0.15 mg/dl in serum creatinine, and 10% in morphometric measurements (GBM thickness, glomerular volume, mesangial expansion, podocyte number, and FP effacement). All experiments were repeated a minimum of three times, and representative experiments are shown. Statistical significance was considered when $P < 0.05$. All statistical analysis was performed using GraphPad Prism 5.0a.

**RESULTS**

**Diabetic MKR mice exhibit significant albuminuria and glomerular injury.** Fasting blood glucose was measured in the WT and MKR mice. Consistent with our previous findings, the adult MKR mice demonstrated marked hyperglycemia compared with control mice (Table 1). We then examined the effect of the diabetic phenotype on renal function on standard chow diet. At 30 wk of age, the MKR mice exhibited significantly greater albuminuria and larger kidney weight, relative to body weight, compared with WT mice (Fig. 1, A and B). Furthermore, the diabetic MKR mice exhibited glomerular hyperfiltration, as shown with an increase in GFR at 10 wk of age (Fig. 1C). Histological changes by light and electron microscopy (low- and high-power magnification) at 10 and 30 wk of age reveal an increase in mesangial expansion and GBM thickening in the MKR mice (Fig. 2).

**HFD feeding accelerates glomerular injury in the MKR mice.** To determine if MKR mice are susceptible to progressive diabetic nephropathy, WT and MKR mice were subjected to HFD and LFD feeding. Both WT and MKR mice on the HFD
The MKR mice demonstrated a significant increase in glomerular creatinine, compared with LFD-fed mice (Figs. 3, A, and B). Significant differences in SBP, DBP, serum BUN, or serum creatinine were observed in the MKR groups. Also, serum cholesterol was elevated in the MKR mice compared with WT mice, but no changes were observed between the MKR HFD and LFD groups (Table 2). After 24 wk of HFD feeding, MKR mice exhibited a significant increase in albuminuria than LFD-fed MKR mice (Fig. 3C). Kidney weight/body weight (Fig. 3B) remained elevated in both LFD and HFD MKR mice compared with WT mice, but no difference in relative kidney weight was observed between the MKR groups. Also, serum cholesterol was elevated in the MKR mice compared with WT mice, but no changes were observed with change in diet (Fig. 3C). HFD feeding led to no significant differences in SBP, DBP, serum BUN, or serum creatinine, compared with LFD-fed mice (Figs. 3, D and E, and 4, A and B). Histological changes by light and electron microscopy (low- and high-power magnification) at 24-wk post-dietary change demonstrate an increase in mesangial expansion, foot process effacement, and GBM thickening (Fig. 5). Further, morphometric studies revealed a significant increase in foot-process effacement and a significant decrease in podocyte number in HFD-fed MKR mice (Fig. 6, A and B). Also, the MKR mice demonstrated a significant increase in glomerular volume, mesangial expansion, and GBM thickness regardless of dietary change (Fig. 6, C–E). These results demonstrate that the diabetic MKR mice exhibit glomerular injury at baseline and that HFD feeding accelerates the glomerulopathy observed in the MKR mice, in the absence of blood pressure changes.

Glomerular injury is accelerated in diabetic MKR mice with uninephrectomy. To determine if the MKR mice are susceptible to progressive diabetic nephropathy in the absence of dietary change, WT and MKR mice were subjected to UNX or a sham operation. MKR mice post-UNX remained hyperglycemic but gained less body weight than the WT mice that were subjected to UNX or the sham-operated MKR mice (Table 3). At 10 wk postsurgery, MKR mice with UNX exhibited a significant increase in albuminuria and kidney weight/body weight (Fig. 7, A and B). In addition, serum cholesterol was elevated in the MKR mice compared with WT mice at baseline, but no significant differences were observed with UNX (Fig. 7C). Also, SBP and DBP were significantly increased in MKR post-UNX, compared with all other groups (Fig. 7, D and E). Furthermore, serum BUN was significantly increased.

Table 3. Physiological parameters of wild-type and diabetic MKR mice with and without uninephrectomy

<table>
<thead>
<tr>
<th>Diet</th>
<th>WT + Sham</th>
<th>Fasting blood glucose, mg/dl</th>
<th>BW, g</th>
<th>%BWΔ</th>
<th>MKR + Sham</th>
<th>Fasting blood glucose, mg/dl</th>
<th>BW, g</th>
<th>%BWΔ</th>
<th>WT + UNX</th>
<th>Fasting blood glucose, mg/dl</th>
<th>BW, g</th>
<th>%BWΔ</th>
<th>MKR + UNX</th>
<th>Fasting blood glucose, mg/dl</th>
<th>BW, g</th>
<th>%BWΔ</th>
</tr>
</thead>
<tbody>
<tr>
<td>LFD</td>
<td>156.6 (21.5)</td>
<td>33.2 (0.7)</td>
<td>27.2 (0.3)</td>
<td>407.0 (64.2)</td>
<td>29.2 (0.4)</td>
<td>306.2 (52.3)</td>
<td>30.2 (0.5)</td>
<td>3.3 (2.2)</td>
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</table>

Values are median (IQR). UNX, uninephrectomy. *P < 0.05 vs. all WT mice. †P < 0.05 vs. all groups. ‡P < 0.05 vs. WT mice.
in both WT mice and MKR mice post-UNX compared with sham operated WT mice (Fig. 8A). However, only the MKR mice post-UNX exhibited a significant increase in serum creatinine compared with the sham operated mice (Fig. 8B). Histological changes by light and electron microscopy (low- and high-power magnification) at 10-wk post-surgical intervention demonstrate an increase in mesangial expansion, foot process effacement, and GBM thickening (Fig. 9). Further, morphometric studies revealed a significant increase in foot process effacement, glomerular volume, mesangial matrix expansion, and GBM thickness in MKR mice post-UNX (Fig. 10, A–D). Also, we observed a significant decrease in podocyte number in MKR mice post-UNX (Fig. 10E). Compared with the HFD/LFD diet model, the diabetic MKR mice subjected to UNX exhibited a more rapid progression to glomerulopathy, associated with increased SBP and DBP compared with control mice.

**DISCUSSION**

The AMDCC proposes that the following criteria should be used to validate the ideal mouse model of progressive diabetic nephropathy: 1) greater than a 50% decline in renal function over the lifetime of animal, 2) more than a 10-fold increase in albuminuria compared with strain- and gender-matched controls, and 3) key histopathological features of diabetic nephropathy. These histopathological features include advanced mesangial matrix expansion (with or without nodular sclerosis and mesangiolysis), any degree of arteriolar hyalinosis, greater than 50% thickening of GBM compared with baseline, and tubulo-interstitial fibrosis (4). Although not a guideline, additional histopathological features defining progressive diabetic nephropathy were also highlighted by the AMDCC in 2009 (4).

In this study, we demonstrated that MKR mouse under conditions of stress, such as a high-fat dietary change and UNX, serves as a representative model of progressive diabetic nephropathy by addressing the majority of the criteria proposed by the AMDCC. Similar to the other murine models of diabetic nephropathy secondary to Type 2 DM (1, 9, 22, 27), the MKR mice under stress demonstrates progressive mesangial matrix expansion, podocyte loss, and GBM thickening with increasing albuminuria. The MKR mice also exhibit marked foot process effacement with an increase in glomerular volume and kidney weight. In addition, MKR mice are fertile with a rather simple breeding strategy. Furthermore, these lean MKR mice, at baseline, exhibit severe insulin resistance, hyperglycemia, hyperinsulinemia, and dyslipidemia (8, 26), features typically representative of a Type 2 DM population. Also, MKR mice are more susceptible to hypertension compared with WT mice in the setting of UNX, which is likely a result of the combination of UNX and diabetes, rather than diabetes alone. Further studies are required to elucidate the mechanism underlying this process. Although WT mice and MKR mice had a significant increase in serum BUN post-UNX, only the MKR mice post-UNX exhibited a significant increase in serum creatinine. This
were measured (Measurements for serum BUN (A; mg/dl) and serum creatinine (B; mg/dl) uninephrectomy before the time of death via submandibular vein injection.

Fig. 8. Diabetic MKR mice post-UNX exhibit deterioration in renal function. Serum was collected in WT and MKR mice subjected in sham operation or uninephrectomy before the time of death via submandibular vein injection. Measurements for serum BUN (A; mg/dl) and serum creatinine (B; mg/dl) were measured (n = 4; *P < 0.01 vs. sham-operated mice).

suggests the change in renal function was likely a combination of prerenal etiology and early changes observed with loss in nephron mass. Since we did not observe tubulointerstitial disease in the MKR mice post-UNX, we speculate that the significant increase in serum creatinine maybe secondary to early tubular dysfunction (5). Further studies will be required to determine if a longer follow up in the MKR mice post-UNX will result in worsening renal function secondary to tubulointerstitial disease. Finally, the FVB background strain is relatively common and a more susceptible strain for diabetic glomerulopathy, compared with resistant strains such as C57BL/6 (4).

As with other murine models of diabetic nephropathy, some limitations do exist in the MKR model. First, there is mild glomerulopathy and albuminuria with slow progression in MKR mice at baseline. Second, the degree of interstitial fibrosis was not remarkable in the MKR mice with and without stressors (HFD or UNX), which is different from late-stage DKD observed in humans. However, as highlighted by the AMDCC, not all the criteria have to be fulfilled to be considered a viable model of progressive diabetic nephropathy (4). Finally, the addition of HFD as well as UNX was able to accelerate diabetic nephropathy in the MKR mice, suggesting that, although these mice do not develop overt diabetic nephropathy at baseline, they are highly susceptible to progressive glomerulopathy. In addition to the severe hyperglycemia in the MKR mice (6), marked dyslipidemia and hyperinsulinemia (8, 26) may also contribute to the progression in glomerulopathy observed in these studies. Furthermore, previous studies have characterized the critical role of dyslipidemia in the progression of glomerulosclerosis (24). However, the high serum cholesterol levels in the MKR mice did not worsen in the setting of HFD or UNX, suggesting that dyslipidemia was not the major contributing factor to the progressive glomerulopathy observed in the MKR mice. Nonetheless, we observed that a change in diet, from standard chow diet to LFD, reduced albuminuria. Additional studies will be required to determine if the switch to LFD can abrogate the albuminuria observed in the older MKR mice. Other than the mechanism by which hyperglycemia induces diabetic glomerulosclerosis (17), the role of hyperinsulinemia in the progression of diabetic nephropathy needs to be further studied in this murine model. We speculate that the hyperinsulinemia observed in the MKR mice (6) interferes with the systemic and intrarenal renin-angiotensin system, thereby leading to previously described mechanism of insulin-induced activation of angiotensin II induced mesangial contraction (2). In addition, the potential role of persistent hyperinsulinemia in reducing renal nitric oxide production has to be further characterized in the MKR mice (10). Similarly, the critical role of hyperinsulinemia in the generation of oxygen free radicals and oxidative stress may also contribute to the increased susceptibility to glomerulopathy observed in the MKR mice. Furthermore, future studies will focus on the mechanism by which these metabolic derangements may up-regulate proinflammatory pathways (15), thereby accelerating diabetic nephropathy. Nonetheless, in combination with the common background strain and normal fertility of these mice, the MKR model could be widely used to examine the cellular and molecular mechanisms mediating the progression of diabetic nephropathy secondary to Type 2 DM (7).

In summary, we demonstrate a new model of diabetic nephropathy secondary to Type 2 DM that fulfills a majority of the criteria proposed by the AMDCC. The MKR model, a mouse model of insulin resistance, hyperinsulinemia, and hyperglycemia without obesity and the inflammation associated with obesity, exhibits some key features of human diabetic nephropathy under stress. Our studies suggest that these MKR mice are also highly susceptible to progressive glomerulopathy. Therefore, we speculate that the MKR mice will serve as a valuable model to study the progression of diabetic nephropathy secondary to Type 2 DM.

ACKNOWLEDGMENTS
We thank the training received for performing the uninephrectomy from the Vanderbilt O’Brien Mouse Kidney Injury Workshop.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Fig. 9. Diabetic MKR mice post-UNX exhibit an increase in glomerular injury. Paraffin embedded sections were stained with PAS and images were taken at low power (×20) and high power (×40). Ultrastructural changes are shown at ×5,000 and ×15,000 by transmission electron microscopy.


REFERENCES

Fig. 10. Quantification of glomerular injury in diabetic MKR mice postsurgery. Quantification of foot process width (A; \( n = 20 \) glomeruli per group; \( *P < 0.01 \) vs. WT + sham and MKR + UNX; \( **P < 0.01 \) vs. all other groups), glomerular volume (B; \( n = 120 \) glomeruli per group; \( *P < 0.01 \) vs. all other groups), mesangial matrix expansion (C; \( n = 100 \) glomeruli per group; \( *P < 0.01 \) vs. all other groups), GBM thickness (D; \( n = 20 \) glomeruli per group; \( *P < 0.01 \) vs. all other groups), and podocyte number/glomerulus are shown (E; \( n = 100 \) glomeruli per group; \( *P < 0.01 \) vs. WT + sham and MKR + UNX; \( **P < 0.01 \) vs. all other groups).