

# MOUSE MODELS OF DIABETIC NEPHROPATHY

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## **ABSTRACT**

Mice provide an experimental model of unparalleled flexibility for studying mammalian diseases. Inbred strains of mice exhibit substantial differences in their susceptibility to the renal complications of diabetes mellitus. Much remains to be established regarding the course of diabetic nephropathy in mice as well as defining those strains and/or mutants most susceptible to renal injury from diabetes mellitus. Through the use of the unique genetic reagents available in mice (including knockouts and transgenics), the validation of a mouse model reproducing human diabetic nephropathy should significantly facilitate our understanding of the underlying genetic mechanisms contributing to the development of diabetic nephropathy. Establishment of an authentic mouse model of DN will undoubtedly facilitate testing of translational diagnostic and therapeutic interventions in mice prior to testing in humans.

## **INTRODUCTION**

Diabetic nephropathy (DN) is the major single cause of end stage renal disease (ESRD) in the United States (1) with costs for care of these patients projected to be \$12 billion/year by the 2010 (1). Despite the high prevalence of DN, only 20-40% of all diabetic patients are prone to developing kidney failure, and family based studies suggest a significant genetic component conferring risk for DN (2-4). Studies of diabetic mice suggest that, like people, mice exhibit differential susceptibility to diabetes, renal, and cardiovascular diseases (5-7). In mice, identification of a strain prone to disease provides the relevant discriminator, rather than identification of an individual at risk for diabetic complications. However in contrast to people, each inbred

mouse strain represents a genetically homogenous and readily replenished resource that is amenable to repeated experimental study.

Biomedical experimentation in mice affords significant advantages over experimentation in other species. These advantages include the development of diverse and unique genetic resources including completion of a detailed map of murine genomic sequence that is freely available through the internet, as well as over 450 inbred strains of mice that have been generated over the past century, with each strain genetically being homogenous and offering a unique array of phenotypes (8, 9). The availability of murine embryonic stem cells has also provided the ability to disrupt the expression and function of specific pre-selected genes (10-12). Finally repositories of mice bearing multiple mutations altering function in each known gene, are gradually being assembled (13-15). These unique resources have the potential to significantly facilitate studies into the pathogenesis of disease in mice. Through the use of the unique genetic reagents available in mice (including knockouts and transgenics), the identification of a robust mouse model of diabetic nephropathy should significantly facilitate understanding of the underlying genetic mechanisms contributing to the development of diabetic nephropathy in people and as well as mice. Establishment of a valid mouse model of DN should also facilitate testing of translational diagnostic and therapeutic interventions in mice prior to testing in humans.

#### **A WORKING DEFINITION OF DIABETIC NEPHROPATHY:**

In humans diabetic nephropathy manifests as a clinical syndrome comprised of albuminuria, progressively declining glomerular filtration rate (GFR) and increased risk for cardiovascular disease (16, 17). The occurrence of cardiovascular disease is an integral component of DN and underscores the systemic nature of this disorder of which nephropathy is only one aspect. Another key aspect of DN in humans is that it is a late complication of diabetes, occurring progressively in susceptible people only after 15-25 years of diabetes (18-20). Because of issues of cost and convenience, most studies of diabetic nephropathy in mice have focused on the earlier harbingers of diabetic nephropathy including the development of albuminuria and histopathologic changes and have not explicitly used renal insufficiency as an endpoint (21, 22).

Diabetic albuminuria in humans is associated with the development of characteristic histopathologic features including thickening of the glomerular basement membrane (GBM) and mesangial expansion. As albuminuria progresses and renal insufficiency ensues, glomerulosclerosis, arteriolar hyalinosis, and tubulointerstitial fibrosis develop (23). These latter pathological features correlate well with glomerular filtration rate in human diabetics with kidney disease (24, 25) and would be important features in a robust mouse model of diabetic nephropathy.

#### **CHARACTERIZATION OF DIABETIC NEPHROPATHY IN MICE:**

**Deficiencies:** The major deficiency in animal models of diabetic nephropathy is the absence of renal failure. While animal models of diabetic kidney disease exhibit albuminuria and some of the characteristic pathological changes, reports of renal failure

resulting from diabetes in mice or rats are lacking. Furthermore, associated increased risk for cardiovascular disease, neuropathy, and retinopathy have been poorly characterized. Whether the inability to detect renal failure in mice is simply a consequence of studying mice with established diabetes for an insufficient time period, or whether the absence of renal failure reflects an intrinsic resistance to nephropathy of the strains studied thus far, remains uncertain.

In contrast to the absence of renal failure, several of the more short-term consequences including the development of glomerular hyperfiltration, increased albuminuria and some of the characteristic **histopathologic** changes can be detected in animal models. Human diabetic nephropathy proceeds through several distinct **pathophysiologic** stages including an early stage of glomerular hyperfiltration, followed by the so-called silent phase where GFR returns to normal (26). This is followed by the sequential development of micro-albuminuria, dipstick positive proteinuria, and then a progressive decline in glomerular filtration rate leading to end stage renal disease (20, 27, 28). **An** optimistic view is that the detection of the early functional and histopathologic changes in mouse models of diabetes reflects inadequate duration of hyperglycemia prior to the study of these models.

### **ASSESSMENT OF HYPERGLYCEMIA IN MICE:**

In humans the degree of hyperglycemia is a critical determinant of risk for developing diabetic nephropathy (29, 30). Similar studies have not been systematically performed in mice, but in small studies nephropathy appears to correlate with the severity of

**hyperglycemia.** Measurement of glucose control in mice is generally achieved by determination of fasting blood glucose or determination of glycosylated hemoglobin.

**Fasting blood glucose:** Because mice are nocturnal feeders, an overnight fast before measuring blood glucose usually translates into a more prolonged fast of ~24 hours. This **24-hour** fast can activate several physiologic counter-regulatory mechanisms that obscure the reliability of glucose readings, due to this significant distress. Because of this fasting should be started on the morning of blood sampling. The NIH has established mouse metabolic phenotyping centers ([www.mmpc.org](http://www.mmpc.org)) that have developed a standard protocol comprised of a fast between 7am and 1pm with blood drawn at 1pm. This protocol has been adapted by the AMDCC (Animal Models of Diabetic Complications Consortium, [www.amdcc.org](http://www.amdcc.org)), another NIH consortium whose goal it is to identify and establish mouse models of diabetic complications.

**Glycosylated hemoglobin (including HgbA<sub>1c</sub>):** When carbohydrates become non-enzymatically linked to hemoglobin these species can be separated from normal adult hemoglobin (designated HgbA<sub>0</sub>) either by altered charge or by formation of a stable complex between the coplanar cis-diol groups of glycated hemoglobin in an affinity column are collectively designated as HgbA<sub>1</sub> (31, 32). These hemoglobins can be further separated into HgbA<sub>1a</sub>, HgbA<sub>1b</sub>, and HgbA<sub>1c</sub> among which HgbA<sub>1c</sub> is the main glycated form in humans (33). HgbA<sub>1c</sub> has been widely used to assess glucose control in people and by extrapolation it has been widely used to assess glucose control in diabetic mice. Studies in diabetic mice are consistent with the validity of the use of

HgbA<sub>1c</sub> to assess glucose control(34, 35) although levels of the various glycosylated hemoglobin assays have not been rigorously correlated to fasting blood glucose values in mice.

While HgbA<sub>1c</sub> correlates well with glucose control in people (36), there appear to be some human populations that exhibit substantially different HgbA<sub>1c</sub> values despite similar levels of glucose control (37). This observation has been attributed to differences in red cell life span rather than rates of glycation (38). Another intriguing possibility is that people with different endogenous rates of glycation exist despite similar levels of hyperglycemia and that “high glycaters” exhibit greater susceptibility to diabetic nephropathy and retinopathy (39). Variability in red cell life-span among strains of mice seems likely and may significantly impact interpretation of HgbA<sub>1c</sub> levels (35, 40, 41).

## **ASSESSMENT OF RENAL FUNCTION IN MICE**

**Glomerular Filtration rate:** A serious issue impeding the evaluation of renal function in mice has been the lack of a simple, reproducible method to estimate glomerular filtration rate in conscious mice. As in humans, blood urea nitrogen levels are extremely sensitive to extra-cellular fluid volume depletion, and may be artificially elevated in the diabetic from volume depletion resulting from the osmotic diuresis of hyperglycemia. The use of serum creatinine as a tool to evaluate renal function in mice has also been called into question since Meyer et. al. reported that creatinine levels in plasma of mice measured by the Jaffé alkaline picrate method yielded significantly higher levels (3-5 times) than those measured by high performance liquid

chromatography (HPLC) (42). These studies concluded that the Jaffé method greatly overestimated the plasma creatinine levels, attributing this increase to non-creatinine chromagens. Other studies have found similar assay dependent variability in the accuracy of creatinine as a measure of renal function in mice (43, 44) and in humans (45).

The impact of these cross-reacting chromagens on apparent levels of plasma creatinine may be significantly affected by keto-acidosis (i.e. as occurs in diabetes) (46, 47). Since absolute creatinine levels as measured by HPLC are substantially lower in mice ( $0.128\pm 0.026$  to  $0.207\pm 0.012$  mg/dl) than in people (48, 49) the impact of these chromagens on apparent creatinine values may be significantly greater in normal mice than in normal humans. . In mice with normal renal function, the picric acid based method routinely overestimates HPLC determined serum creatinine by two to five fold, yielding apparent serum creatinine values of 0.6 to 1.0 mg/dl (48, 49). Conversely, enzymatic creatininase methods coupled to a peroxidase indicator more closely agree with HPLC methods, providing another potentially useful method for determination of serum creatinine in mice (44). Despite the availability of this information regarding the inaccuracy of serum creatinine measured by the Jaffé reaction, the assay remains widely used by the biomedical research community to assess renal function in mice. Thus, artifactual increases in serum creatinine likely contribute to reports of decreased creatinine clearance in many studies examining diabetic nephropathy in mice.

With the increasing use of mice as an experimental model of diabetic kidney disease awareness of these deficiencies must be heightened. Several approaches have been adopted to re-address this problem. New methods to accurately measure plasma creatinine measurements by HPLC have been established and have confirmed significant over-estimation of plasma creatinine using **picrate-based** methods. When compared to inulin clearance, creatinine clearance determinations using the Jaffé reaction underestimates GFR by greater than 50% **(48, 49)**.

Measurement of glomerular filtration rate using inulin clearance, the traditional gold-standard measurement, has also been adapted for use in mice. Clearance of fluorescein (FITC) labeled-inulin has been measured in both anesthetized and conscious mice **(50, 51)**. Serial measurements of inulin clearance can be determined in conscious mice over a period of months using an intravenous bolus FITC inulin and measuring the decay rate of inulin in plasma **(50)**. Using these techniques substantial differences in GFR between mice of different strains and genders have been reported **(50, 52, 53)**. Consideration of the effects of surgical preparation and/or anesthesia is appropriate, when GFR determination is performed in anesthetized mice. It remains to be determined how well HPLC determined creatinine clearance correlates with FITC inulin clearance in individual mice. Nevertheless, previously published studies in which GFR has been estimated in mice using plasma creatinine values determined by picrate based methods should be carefully re-evaluated in light of the aforementioned problems.

## **BLOOD PRESSURE:**

There are several proven methods for monitoring blood pressure in mice that can be applied to the study of diabetic mouse models. Indirect measurements using tail cuff manometry systems are simple and non-invasive. Their reliability and reproducibility have been well documented across a number of experimental systems (54). These systems measure blood pressure in conscious mice and they are inexpensive and adaptable to most laboratory settings. On the other hand, they lack the precision and sensitivity of systems that directly measure intra-arterial pressure. This approach is also complicated by physiologically apparent stress in tested mice that can affect the level of blood pressure but this can be minimized by adequately training mice beforehand. In our experience, tail cuff manometry is a useful approach to screen for alterations of blood pressure in mouse models, realizing that very modest differences may be missed because of the relative insensitivity of this method. Using this approach in studies under the auspices of the MMDC, we have found that blood pressures tend to be reduced in most strains of STZ-treated mice when significant hyperglycemia is present (Gurley et al., manuscript in preparation). Tail cuff measurements have also been reported by others in models of diet-induced diabetes and does not appear to be dramatically affected by diabetes (55) and genetically altered mice treated with STZ (56, 57).

Intra-arterial pressures can also be measured directly in conscious mice. These measurements can be accomplished by cannulating arteries with catheters that are tunneled under the skin, exteriorized, and connected to external transducers and monitors via a tether apparatus. Although this approach requires significant surgical

and technical proficiency, it is relatively inexpensive and accessible to most laboratories, providing a reasonable method for verifying blood pressure differences that have been detected by indirect methods. However, it is difficult to maintain line patency for recording blood pressures beyond 1-2 weeks after catheter placement. The recent development of radio-telemetry units for intra-arterial blood pressure measurements in mice has provided a more suitable approach for chronic measurement of intra-arterial pressures in conscious, unrestrained animals (58). These units provide the capacity to obtain direct and continuous measurements of intra-arterial pressure 24-hours per day over a period of 2-3 months. Accordingly, they should prove very useful for correlating levels of blood pressure with the extent of kidney injury in experimental models. The major disadvantage of this system is expense. The initial capital outlay for a small-scale telemetry unit is typically twice that of most tail-cuff monitors. Furthermore, individual transmitters cost approximately \$3,000 each and they must be refurbished periodically for an additional fee.

**Albuminuria:** Prior to developing renal failure, humans with overt diabetic nephropathy typically develop nephrotic range albuminuria (albumin excretion rates exceeding 3g/24hours). **Preceding** this, albumin excretion rates gradually and progressively increase over the course of years through a phase of microalbuminuria (between 30-300mg/24hours that is negative by urine dipstick), to albuminuria greater than 300mg/24hours, a phase that is characterized by an overtly positive urine dipstick (19, 59, 60). *A priori*, it is expected that mice developing diabetic nephropathy should

progress through similar phases, so that the amount of albuminuria should roughly correlate with the severity of diabetic nephropathy.

Albuminuria has been quantified in approximately 100 studies of diabetic nephropathy in mice, and in the absence of accurate measures of GFR, probably remains the best functional surrogate for severity of diabetic nephropathy. However, comparisons of the severity of nephropathy in the diverse models of diabetes reported **are confounded** by use of diverse methodologies (e.g. quantifying total urine protein, anti-rat antibodies or anti-human antibodies to measure mouse albumin on ELISA), differences in units of reporting (e.g. ng/min,  $\mu\text{g}/24\text{h}$ ,  $\mu\text{g Alb}/\text{mg Cre}$ ;  $\mu\text{gAlb}/\mu\text{Mol Creatinine}$ , total Urine Protein  $\mu\text{g}/24$  hours,  $\mu\text{gAlb}/\text{ml}$  urine) and non-standardized methods of urine collection.

Quantification of proteinuria in mice by dipstick is probably inaccurate (unpublished results). The exact reasons for this are not firmly established, however the inaccuracy of the dipstick proteinuria test in mouse urine may be a consequence sensitivity of this assay to abundant major urinary proteins (MUP complex), normally present in mouse urine (61, 62). Albuminuria determined by an immunoassay generally provides a reliable assessment of proteinuria due to glomerular injury. Use of antibodies raised against non-murine albumin may be particularly problematic as mouse albumin amino acid sequence is only 89% identical to rat albumin and 73% identical to human albumin, allowing for differences in the sensitivity of immunoassays. The AMDCC has chosen to use an anti-mouse albumin ELSIA kit and report albuminuria as  $\mu\text{g}/24$  hour urine

collection or  $\mu\text{g}$  Albumin/mg Creatinine. A standardized assay utilized by consortium members is available at the AMDCC website ([www.amdcc.org](http://www.amdcc.org)).

The values of urine albumin excretion rates in mice that signify glomerular disease have not been firmly established. Furthermore it is likely that significant variation in albumin excretion rates exist between inbred mouse strains. In the absence of well-defined normal values, a first approximation of abnormal albuminuria would be to extrapolate from human disease and test for values 10-fold greater than non-diabetic controls as reflective of incipient disease. Urine albumin excretion rates 100 to 1000-fold more than controls should reflect established diabetic renal disease. We have attempted to compare the severity of diabetic albuminuria in various strains and diabetic models studied by limiting our analysis to those studies performed on a 24 hour urine collection and using assays specific for mouse albumin (Figure 1). A single study in KK-Ay mice fed a high cholesterol diet suggests a 1000 fold increase in albuminuria may be achieved in this model, however whether renal failure ensues in this case, remains to be determined (63).

**Histopathology:** In human DN, renal histopathologic findings include diffuse thickening of the glomerular basement membrane (GBM) between the endothelium and the podocyte, together with prominent mesangial expansion. This is accompanied by diffuse and sometimes nodular mesangial sclerosis, as well as arteriolar hyalinosis and tubulointerstitial fibrosis (24, 25, 64). The best **histopathologic** correlate of renal function in diabetic patients is fractional volume of the mesangium, an index of

mesangial expansion (24, 25). Tubulointerstitial volume also correlates well with renal function.

Given the absence of documented renal failure in mouse models of DN, whether specific pathological criteria are associated with or predictive of renal insufficiency from diabetic nephropathy cannot be readily formulated. GBM thickening has been reported in several but not all mouse models of DN (65-68). In mice, nodular glomerular sclerosis is generally absent as is arteriolar hyalinosis, two features generally only seen in people with more advanced renal disease (24). The reproducible identification of these latter two lesions in mouse models of DN remains a benchmark yet to be achieved.

## MODELS OF TYPE 1 DIABETES MELLITUS

**High dose Streptozotocin:** Streptozotocin (STZ) induced type 1 diabetes has been widely used as a model for diabetic nephropathy, however interpreting results in this model may be complicated by non-specific toxicity of STZ. STZ was originally identified as an antibiotic (69) comprised of a glucosamine-nitrosourea. It was soon recognized to cause diabetes mellitus in animals secondary to pancreatic  $\beta$  cell failure (70, 71). STZ is presumed to be especially toxic for pancreatic  $\beta$ -cells because its glucose moiety is avidly transported into  $\beta$ -cells by GLUT2 (72), however it may be toxic to a variety of other tissues. Given the relative resistance of mice to single doses of STZ (requiring ~150-200 mg/kg to obtain chronic hyperglycemia) substantial collateral tissue toxicity may occur (73-75). This model of type 1 diabetes typically develops albuminuria (66, 76,

77), even on the relatively sclerosis resistant C57BL/6J background (see below), however the potential for collateral tissue toxicity complicates the interpretation of the etiology of this albuminuria. Evidence for robust oxidative stress in STZ diabetes but not other hyperglycemic models of diabetes (e.g. *db/db* and KK mice) has been reported (78). Mice receiving high dose STZ develop more albuminuria than mice receiving the low dose STZ regimen (see Figure 1, and text below), despite exhibiting similar levels of hyperglycemia, also consistent with an effect independent of hyperglycemia. Nevertheless, it should be noted that the aforementioned potential for non-specific renal toxicity has not been rigorously proven, and that the high dose STZ model of diabetes mellitus is still widely accepted and commonly used. It also should be noted reversibility of diabetic lesions following treatment of mice with insulin to normalize plasma glucose, would provide another means of excluding non-specific toxicity due to high dose STZ.

**Low dose STZ:** In order to mitigate non-specific cytotoxicity, multiple low-dose STZ injections have been used to induce diabetes mellitus, causing repetitive low-grade  $\beta$ -cell damage accompanied by lymphocytic infiltration of the pancreatic islets (79, 80). This regimen typically calls for daily i.p. injections of 40-50mg/kg STZ for five days (71, 81). It is notable that inbred strains of mice exhibit substantial differences in susceptibility to both  $\beta$ -cell toxicity (82) and to non-specific toxic effects of low dose STZ (71) so dose adjustments should be made. In general, when administered in strain-appropriate doses, the low dose STZ regimen produces comparable levels of hyperglycemia as those obtained using the high dose regimen. It is notable, that the levels of albuminuria (56, 83) are generally lower than with high dose STZ, possibly

reflecting reduced direct renal toxicity (Figure 1). In addition, we have confirmed that there is no loss of podocyte markers such as WT-1 or apparent decrement in podocyte number 2 weeks after completion of the low dose STZ regimen in C57BL/6J mice (Siu B, et al, submitted) suggesting that this regimen has no toxic effects of on podocytes.

Our experience using low-dose STZ in mice has shown hyperglycemia occurs within two weeks of the low dose STZ regimen (for the regimen used see [www.AMDCC.org](http://www.AMDCC.org)). Albuminuria develops to a variable degree (depending on strain of mice –see below) within five weeks. At this early time point, the major histological feature is glomerular hypertrophy, so it is likely that hemodynamic changes contribute to the development of this early albuminuria. As time progresses, mesangial expansion and in some strains, mesangial sclerosis develops. Only a minority of mouse strains (e.g. KK) develop evidence of arteriolar hyalinosis or nodular glomerulosclerosis. These changes occur later (15-30 wks of hyperglycemia). At these later time points the diabetic mice may exhibit significant weight loss, possibly due to catabolic effects of insulin deficiency and well as volume depletion associated with the osmotic diuresis. This may significantly impact on subsequent survival and more long-term studies. To circumvent this occurrence intermittent treatment with insulin, sufficient to reverse weight loss but maintain hyperglycemia, may be desirable.

**Ins2<sup>Akita</sup>**: To circumvent the potential non-specific tissue toxicity occurring in the STZ model of type 1 diabetes one may utilize the recently described insulin-2 *Akita* (Ins2<sup>Akita</sup>) mouse mutant model of type 1 diabetes mellitus (84-86). These mice develop pancreatic  $\beta$ -cell failure due to  $\beta$ -cell selective proteotoxicity resulting from misfolding of insulin2 (87) and are commercially available through the Jackson Laboratories. Although

originally reported to represent a model of MODY with insulin resistance, subsequent studies clearly show that this model is insulin responsive. Islets from *Ins2<sup>Akita</sup>* mice are depleted of  $\beta$  cells and those remaining  $\beta$ -cells release very little mature insulin. This finding together with the finding that mutant mice respond to exogenously administered insulin (84), indicates that *Ins2<sup>Akita</sup>* mice should serve as a substitute for mice rendered insulin dependent diabetic by treatment with alloxan or streptozotocin.

The *Ins2<sup>Akita</sup>* mutation is autosomal dominant. Mice heterozygous for the Akita spontaneous mutation (*Ins2<sup>Akita</sup>*) are viable and fertile. Mice homozygous for the *Ins2<sup>Akita</sup>* allele exhibit failure to thrive and die within 1-2 months. Symptoms in heterozygous mutant mice include hyperglycemia, hypo-insulinemia, polydipsia, and polyuria beginning around 3-4 weeks of age.

At present this mutation exists on the C57BL/6 and C3H/He strains (88). Studies of *Ins2<sup>Akita</sup>* mice on the C57BL/6J background show the hyperglycemia is sexually dimorphic, with the hyperglycemia being substantially worse in male mice than female mice. As seen with other models of diabetic nephropathy in the C57BL/6J strain, albuminuria is not a prominent feature (10.23 $\pm$ 2.73 $\mu$ g/24hours in diabetic mice vs. 13.9 $\pm$ 8.8 $\mu$ g/24 hours in controls) (89). Renal immunopathological studies show significant deposition of IgA in the glomeruli of Akita mice (88), however given the association of IgA deposition in glomeruli of up to 20% of humans with diabetes (90) and rat models of diabetes mellitus, the significance of these findings is uncertain (91-93). Overall the severity of diabetic nephropathy in the C57BL/6J *Ins2<sup>Akita</sup>* mouse does not appear to be robust.

**The Non-obese diabetic mouse (NOD):** The Non-obese diabetic mouse (NOD): The genetic mouse model of type 1 diabetes that has been most thoroughly studied is the NOD mouse (94-96). These animals develop spontaneous autoimmune destruction of beta cells around 5 months of age although the precise age of onset of diabetes is somewhat variable. However, unlike STZ mice, insulin treatment is required to maintain NOD mice for any extended time after the onset of hyperglycemia indicating more complete insulin deficiency. Moreover, unlike enhanced susceptibility in males for STZ diabetes, there is a female predominance in the NOD line with a female: male ratio of ~4:1. The characteristics of autoimmune disease contributing to  $\beta$ -cell failure in NOD mice have been studied extensively and the model has a number of similarities with features of human IDDM (97-99). These include: (1) inheritance of specific MHC class II alleles and many non-MHC loci as polygenic susceptibility loci, (2) transmission of the disease by hematopoietic stem cells, (3) the development of an intra-islet inflammatory infiltrate (insulinitis) with anti-islet cell antibodies, and (4) a strict dependence of disease on T cells.

Despite the intensive study of the immuno-pathogenesis of islet cell destruction in the NOD mouse, very little work has been done to study complications of DM in this model. In part, this is because of the late and somewhat variable age of onset of diabetes and the requirement for more intensive management including daily administration of exogenous insulin. In addition, the genetics of the NOD model is complex. The line was initially developed from an out-bred ICR mouse line (94-96). Moreover, there are 6 or 7 background loci that must be retained for development of disease. Accordingly, identifying an appropriate non-diabetic control strain has been a

problem. NON mice were derived from non-diabetic progeny in the original crosses that generated the NOD line. However, NON mice contain a diabetes resistant MHC haplotype ( $K^b$ ,  $I-A^{nb1}$ ,  $I-E^k$ ,  $D^b$ ) that is distinct from the NOD haplotype ( $K^d$ ,  $I-A^{g7}$ ,  $I-E^{null}$ ,  $D^b$ ) associated with diabetes susceptibility (100). Although some studies have used the NON animals as controls for the NOD mouse, NON mice appear to develop spontaneous renal disease of uncertain etiology (101). Thus, their utility as controls for studies of diabetic nephropathy is questionable.

As discussed above, there have been relatively few studies of kidney disease in the NOD line. Nonetheless, those few studies indicate that albuminuria develops in hyperglycemic NOD animals. The levels of albuminuria, assessed by albumin-to-creatinine ratio, are roughly seven-fold higher than in NOD mice prior to development of hyperglycemia (102). Furthermore, studies of diabetic nephropathy using this model have supported roles for TGF- $\beta$ , and advanced glycosylation end products (AGEs) in the pathogenesis of mesangial proliferation and sclerosis (103-106). Because of such results along with the pathogenic similarities between NOD mice and humans with type 1 DM, this strain is likely to have utility for developing new models of diabetic complications. To circumvent the problems posed by its complicated genetics, AMDCC is working to generate ES cell lines from the NOD line to facilitate applications of transgenesis and gene targeting to our efforts at model development.

## MOUSE MODELS OF TYPE 2 DIABETES MELLITUS

**High fat diet:** High fat diet provides a commonly used approach to induce obesity and insulin resistance in C57BL6 mice (107-109) and is particularly useful for study of

accelerated atherosclerosis (104, 110, 111). The effect of high fat diet appears to depend on the strain of mouse studied with A/J mice being relatively resistant to this effect (107). Although one study suggested this syndrome was associated with the development of hypertension, a subsequent study failed to confirm this and further found no change in glomerular filtration rate (55, 112). To our knowledge the effects of high fat on albuminuria in these models has not been reported, although one study examined its effect on albuminuria in the KK-Ay mouse and found a rather dramatic effect (63).

**LepR<sup>db</sup>/LepR<sup>db</sup>:** The *db/db* mutation on the C57BLKS background has been intensively investigated and exhibits many features similar to human diabetic nephropathy. Details regarding the identification and nomenclature regarding the *db/db* mouse may be found in a recent review (22). The diabetic gene (*db*) is transmitted as an autosomal recessive trait. The *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 (113, 114). This obese and diabetic mutant (*db*) was initially recognized in the C57BLKS/J strain and was subsequently also backcrossed to a pure C57BL/6J background. 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).

In the C57BLKS/J *db/db* mouse, hyper-insulinemia is noted by 10 days of age and blood glucose levels are slightly elevated at 1 mo of age ( $7.2 \pm 2.3$  mM) (115). The *db/db* mouse develops frank hyperglycemia with glucose values of  $9.7 \pm 1.6$  mM by 8 wk

of age and  $15.7 \pm 4.3$  mM at 10 wk of age (115). Progressive hyperglycemia is noted with mean levels of glucose of  $28.6 \pm 13.2$  mM at 16 wk of age (115, 116). After 5-6 mo of age, the body weight and insulin levels begin to fall in association with pancreatic islet cell degeneration (117).

By 2-4 mo of age, diabetic mice have a 20-30% increase in glomerular size (118). 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 (119). After 16 weeks of age there is a very consistent 3-fold increase in mesangial matrix expansion based on several independent studies (reviewed in (22)). The range of albuminuria is between 68 and 600  $\mu\text{g}/24$  h in the *db/db* male mouse (22, 118, 120-122), whereas it is between 4 and 21  $\mu\text{g}/24$  h in the age-matched heterozygous littermate (118, 122). The degree of albuminuria does not consistently increase with the duration of diabetes as there are similar levels of albuminuria between 8 and 25 wk (118, 122-124). Arteriolar hyalinosis has been described in this model, however there is virtually no evidence of advanced tubulointerstitial fibrosis.

The progression of diabetes in *db/db* mice on the C57BL/6 background the diabetic phenotype is less severe than that in C57BLKS/J, and as these mice age, plasma glucose appears to normalize (22, 125-129). More recently some investigators have reported observing a subset of ~50% of C57BL/6J *db/db* mice develop more persistent hyperglycemia (130, 131). In these mice more robust albuminuria and renal histopathologic diabetic changes have been reported (130, 131). Unfortunately, the factors causing this sub-group of C57BL/6J *db/db* mice to develop persistent

hyperglycemia remain to be elucidated. Nevertheless, although the leptin mutation may not produce as robust a model for diabetic nephropathy on the C57BL/6 background as it does on the C57BLKS/J background, it does provide a clear advantage for genetic studies since most transgenic and knockout strains are available on this background and can cleanly introgressed onto this strain.

**Ob/ob:** As opposed to the *db/db* mutants, the *ob/ob* recessive obese mouse carries a mutation in leptin, the ligand for the leptin receptor (132, 133). The  $Lep^{ob}$  mutation exists on DBA2/J and C57BL/6J strains (132, 134). Renal structure and function in C57BL/6J *ob/ob* mice is said to be relatively mild (21) but primary studies supporting this statement are obscure.

**Agouti mutation:** The agouti gene product encodes a 131-amino-acid protein containing a signal sequence. It is produced in the hair follicle where it acts in a paracrine manner as a high-affinity antagonist of the MSH receptor on melanocytes to inhibit alpha-MSH-induced eumelanin production causing their yellow coat color (135). Similarly an agouti-related protein is a potent and selective antagonist of melanocortin receptors 3 and 4 (Mc3r and Mc4r), receptor subtypes, which are expressed in the hypothalamus and implicated in weight regulation (136). Mice carrying the dominant homozygous lethal mutations in agouti ( $A^y$ ) or viable ( $A^{vy}$ ) exhibit a complex phenotype of obesity and insulin resistance in addition to yellow fur. The unifying molecular feature of all yellow obese Agouti mutations is that they confer ubiquitous and strong expression of the wild-type agouti protein through use of an alternative transcriptional

promoter (137, 138). Reports of albuminuria in diabetic KK-Ay mice suggest this mutation may be useful for the study of nephropathy (see below and Figure 1) (139, 140).

**New Zealand Obese mice (NZO):** These mice have polygenically inherited form of obesity and type 2 diabetes (141-143). They may be prone to autoimmune disease as the kidneys exhibit light microscopic features of both diabetic and lupus nephropathies: glomerular proliferation, mesangial deposits, mild basement membrane thickening, glomerulosclerosis (144). Eosinophilic nodules may be seen in some glomeruli, with occasional hyalinization of the glomerular arterioles, and healing arteriolar inflammation (144).

From the preceding discussion, it should be apparent that diverse models of both type 1 and type 2 diabetes mellitus are available in mice. While renal changes have been reported in many of these models, renal failure has not as yet been identified as a consequence of diabetes in any of these models. Whether this is a result of inadequate length of diabetes prior to study or underlying resistance of specific strains to nephropathy, remains to be addressed.

### **INBRED MICE -- STRAIN DEPENDENCE OF DIABETIC NEPHROPATHY**

Studies have identified certain strains (e.g. 129, ROP, NON, and KK/HIJ) as more prone to glomerulosclerosis, than some more commonly studied strains (e.g. C57BL/6, FVB/NJ) that appear relatively resistant to renal disease. It is noteworthy that studies of

diabetic nephropathy have been reported in fewer than 5% of the available mouse strains. The following section overviews the literature regarding the sensitivity of the kidney to diabetes mellitus in the major strains studied so far.

**C57BL6:** Although C57BL/6 is the most widely used inbred strain, studies of kidney disease in this strain suggest it is relatively resistant to renal injury (145, 146) (other than a possible sub-group, mentioned below, regarding a cohort of this strain that may be more susceptible to DN in the db/db model). The C57BL/6J strain provided the DNA source for the first high quality draft sequence of the mouse genome. This strain is used in a wide variety of research areas including cardiovascular biology, developmental biology, diabetes and obesity. Overall, C57BL/6 mice breed well, are long-lived, and have a low susceptibility to tumors. Other characteristics include: a high susceptibility to diet-induced obesity, type 2 diabetes, and atherosclerosis. C57BL/6J mice fed a high-fat diet develop obesity, hyperglycemia, and hyper-insulinemia (55, 112), however this does not appear to impact renal function (112). In contrast to their resistance to glomerulosclerosis, C57BL/6J mice provide a reasonably robust strain for studying of atherosclerosis when fed an atherogenic diet (111). In the experience of the AMDCC and others, the leptin receptor (*db/db*) mutation in this background often results in relatively mild and transient hyperglycemia and modest albuminuria (22, 147) when compared to the presence of this mutation in the C57BLKS strain (see below).

**C57BLKS** (formerly C57BL/KsJ) This strain was originally derived from C57BL/6J mice maintained by Dr. N. Kaliss (Ks) and may be somewhat more susceptible to renal

disease than the parental C57BL/6. Genomic analysis shows that 84% of the alleles in C57BLKS are shared with C57BL/6 and 16% are shared with DBA/2J, consistent with genetic contamination of the C57BL/6J progenitors early in the strain's history (148). Studies indicate that on the C57BLKS background type 2 diabetic *db/db* mice have lesions consistent with diabetic nephropathy (thickened basement membrane, mesangial expansion) whereas they are resistant to DN on a C57BL/6 background (22, 149-151). Although these models have not been completely characterized, this difference suggests two possibilities: either the worse severity of hyperglycemia that develops in C57BLKS causes this (111) (a combination of peripheral insulin resistance and insulin deficiency develops in C57BLKS $Lep^{db}$ , while C57BL/6 $Lep^{db}$  have only peripheral insulin resistance) or C57BLKS express modifier genes that predispose to diabetic nephropathy. These mice appear to be relatively prone to atherosclerosis (111) and are also develop myocardial disease (152). In support of the former possibility is the general experience including that of the AMDCC that fasting blood glucoses are sustained at much higher levels in *db/db* mice on this background than in *db/db* mice on a C57BL/6J background.

**DBA/2J** In contrast to C57BL/6 this strain appears to be prone to diabetic albuminuria (153, 154) but this remains to be rigorously tested. DBA/2J are relatively resistant to the development of atherosclerosis on a semi-synthetic high fat diet (155) and are **hypo-responsive** to diets containing high levels of fat and cholesterol (156). Spontaneous calcified heart lesions progress with age and dystrophic cardiac calcification may be related to disturbed **most** calcium metabolism (157, 158).

**129/SvJ**: Multiple sub-strains of 129 exist and care must be taken to determine the precise genetic composition of the **specific** 129 **sub-strain** in use for appropriate interpretation of data. This strain of mice has been found to be more susceptible to elevated blood pressure, nephrosclerosis (both glomerular and tubulointerstitial) and albuminuria in the setting of DOCA-salt hypertension than the C57BL/6 strain (159). In addition, 129/Sv mice develop more significant glomerulosclerosis, proteinuria, increases in blood pressure and apparent renal failure than do C57BL/6 mice after 5/6 nephrectomy (145). This susceptibility to renal injury and hypertension makes 129/Sv a potentially attractive strain for diabetic nephropathy studies. No reports to date have compared renal or blood pressure effects of diabetes in animals on this background to C57BL/6J mice or other strains. Unfortunately, the 129/J strains of mice with the *db/db* mutation are resistant to hyperglycemia (40).

**ROP**: This strain of mouse originated from a heterogeneous stock being heterozygous for three mutant alleles -- *Ra/+* (Ragged), *Os/+* (osteosyndactylism) and *Pt/+* (pintail). Studies of *Os/+* ROP mice show they possess approximately 50% fewer nephrons in

the Os/+ mice than in the +/+ mice (160). In general wild-type mice (i.e. Os<sup>+/+</sup>; Ra<sup>+/+</sup>; Pt<sup>+/+</sup>) of ROP background appear to be more prone to glomerulosclerosis than C57BL/6 mice following a reduction in renal mass (161-163). Mice on the ROP background, in the absence of Os, Ra, or Pt mutant alleles may also exhibit increased propensity for glomerulosclerosis following combined nephron mass reduction and diabetes mellitus (146).

**FVB** FVB/NJ was derived from **out bred** Swiss mice at NIH inbred for the *fv1b* allele, which confers sensitivity to the Friend leukemia virus B strain. Due to the prominent pronuclei in their fertilized eggs and the large litter size, FVB/NJ are commonly used for transgenic injection. In this context, FVB/N mice transgenic for a calmodulin minigene regulated by the rat insulin II promoter been shown to develop type 1 diabetes (164, 165). Initial studies characterizing the severity of diabetic nephropathy in this line (designated OVE26) suggest that while they develop glomerular capillary basement membrane thickening, they do not develop significant albuminuria (68, 166). The LepR<sup>db</sup> mutation has also been backcrossed onto the FVB/NJ strain and the kidneys from these mice exhibit mesangial sclerosis, although albuminuria and GFR has not been reported (134).

**Non-obese nondiabetic mice (NON):** This strain is closely related to NOD mice and developed as a “control” for NOD mice. The name was derived from "non-Obese non-diabetic"; however, NON/LtJ mice are not "normal" as they exhibit tendencies toward autoimmune diseases. NON/LtJ mice harbor genes predisposing to type 2 diabetes, as

evidenced by early, impaired glucose tolerance in males and females, and by the development of moderate maturity-onset obesity in the presence of low plasma insulin levels.

The renal phenotype in these mice is not well characterized but is reported to develop spontaneous glomerular lesions resembling nodular glomerulosclerosis, although they are not overtly diabetic (101). The susceptibility of NON mice to diabetic nephropathy using STZ or other models of diabetes mellitus has not been reported.

**KK mice:** Although exhibiting only mild insulin resistance, the KK mouse appears to be predisposed to development of renal lesions very reminiscent of diabetic nephropathy (167, 168). These mice were originally established from inbreeding a Japanese mouse by Kondo (169). The severity of hyperglycemia and insulin resistance is exacerbated by introduction of the agouti (Ay) allele into the KK background (170). Of note is that both KK and KK-Ay mice develop nodular glomerulosclerosis and mesangial proliferation (171) which are improved by glycemic control (167, 172, 173). Albuminuria progressively increases with age, however reports of renal insufficiency or failure have not been forthcoming.

### **MONOGENIC MUTATIONS & TRANSGENIC MICE:**

Despite the preceding caveats regarding the difficulties in phenotyping renal function in mice, the use of transgenic mouse models has provided a better understanding of the factors that exacerbate diabetic nephropathy. The following review is not meant to be comprehensive, but only to overview some of the more recent developments in the field.

The reader is referred to other recent manuscripts for additional perspectives on this topic (174).

**ApoE:** Although control of hyperglycemia can decrease the development of diabetic nephropathy, there is evidence suggesting a genetic susceptibility to development of DN is related to abnormalities in lipid metabolism. Hypertriglyceridemia is among the main phenotypic features distinguishing those people prone to nephropathy (175).

Apolipoprotein (apo) E is a protein of 34 kDa that circulates on plasma lipoproteins at a concentration of 3-5 mg/dl (176, 177). In the human population, the ApoE gene, located on chromosome 19q13.2, has three common alleles: 2, 3, and 4, coding for the 3 main isoforms of the ApoE protein: E2, E3, and E4 (178). ApoE isoforms differ in their ability to bind to LDL receptors, with E4 having the greatest binding capacity and E2 having defective binding and decreased triglyceride clearance (179). Many, but not all studies in patients with either type 1 or type 2 diabetes, have implicated the presence of the E2 allele as a risk factor for development and/or progression of diabetic nephropathy (180-190). The underlying mechanism(s) that might be involved in the development of DN in patients with the ApoE2 polymorphism have not yet been determined. It is possible that the associated hypertriglyceridemia may predispose to renal injury. It has also been suggested that ApoE may play a role in tissue growth and/or repair following injury separate from its effects on maintenance of lipoprotein homeostasis (191).

In mice with targeted disruption of the **ApoE** gene locus, no tissues or cells synthesize or secrete **ApoE**, so **ApoE** is absent from the plasma. As a consequence, the clearance of remnant lipoproteins is blocked, and serum cholesterol and triglycerides increase to levels of about 450 and 250 mg/dl, respectively, similar to levels in human type III hyperlipoproteinemia due to **ApoE** deficiency. ApoE deficiency causes increased susceptibility to atherosclerosis and represents a murine model of spontaneous (non diet-induced) atherosclerosis (192). Of note, fasting glucose and insulin levels are not elevated in **ApoE<sup>-/-</sup>** mice on a normal chow diet compared to C57BL/6J mice. Interestingly, a recent study in low dose Streptozotocin treated C57BL6 mice showed deletion of the ApoE allele was associated with more severe nephropathy as assessed by histopathology and albuminuria (which increased approximately 5 fold, albeit, still less than 100µg/24 hours) (193).

**eNOS:** Endothelial dysfunction is present in diabetes and is associated with impaired vascular NO synthesis. A number of polymorphisms in the endothelial nitric oxide synthase gene (**eNOS**), located on human chromosome 7, have been linked to vasculopathies (194-198). Recent studies have reported an association between **eNOS** polymorphisms that lead to decreased **eNOS** expression and development of advanced nephropathy in type 1 (199-201) and type 2 diabetic patients (202, 203). Other studies have found association of these polymorphisms with **ESRD** (204, 205). However, not all studies have detected any potential association of these **eNOS** polymorphisms with diabetic nephropathy (206-210).

Although direct examination of development of diabetic nephropathy has not yet been undertaken in mice with gene deletion of eNOS, it is noteworthy that studies of the phenotype of mice with combined eNOS and ApoE deficiency indicate accelerated atherosclerosis, hypertension and progressive renal dysfunction characterized by smaller kidney weights and glomerular lipid deposits (211-214)

**Advanced Glycosylation End products (AGEs):** Generation of advanced glycation end products (AGE), nonenzymatically glycosylated protein derivatives resulting from prolonged hyperglycemic exposure, is a cardinal feature of the diabetic milieu, and early and advanced steps of the complex process of reactions leading to glycation of proteins are now well-understood (215). Human diabetic kidney (and other tissues) are characterized by glomerular accumulation of AGE, in particular carboxymethyl lysine (CML) modified adducts of proteins (216). AGE may perturb cell function through receptor-independent mechanisms and through receptor-mediated signaling pathways. Several cell surface receptors/binding proteins have been shown to bind AGE, including the macrophage scavenger receptor (MSR) type II, OST-48, 80K-H, galectin-3, CD36, and receptor for AGE (RAGE) (reviewed (216)). While some of these interactions may function to remove/scavenge AGE, AGE binding to RAGE, a signal transduction receptor of the immunoglobulin superfamily, mediates diverse cellular responses. For example, Oldfield et al. demonstrated that AGEs mediate tubular epithelial to myofibroblast transdifferentiation through RAGE in vitro, and that this effect is dependent upon the pro-sclerotic cytokine TGF- $\beta$  (217). Thus, an AGE-dependent pathway may play a role in the development of tubulointerstitial fibrosis in the diabetic

kidney. RAGE may activate diverse signaling mediators in endothelial cells and macrophages, including p21<sup>ras</sup>, ERK1/2 MAP kinases, **Nf-κB**, and NADPH oxidase **species** (216, 218). However, in the diabetic kidney in human and mice, RAGE is expressed principally by glomerular visceral epithelial cells (podocytes) (219, 220). Interestingly, neutralizing soluble RAGE ameliorated glomerular damage in the hyperglycemic *db/db* mouse (220).

Recently, Yamamoto et al. reported an interesting new transgenic model that provides further evidence for a role of RAGE in acceleration of diabetes-induced glomerular lesions (221). In this model, the human RAGE gene is **over expressed** specifically in endothelial cells under control of an endothelial cell-specific mouse *flk-1* promoter (*flk1-RAGE*) on an inbred CD-1 genetic background. Persistent hyperglycemia was induced in *Flk1-RAGE* mice by interbreeding with diabetic insulin-eNOS transgenic mice, and *flk1-RAGE/ins-eNOS* double transgenic mice exhibited increased hemoglobin A<sub>1c</sub> and serum AGE levels similar to *ins-eNOS* diabetic controls. Kidney enlargement, and glomerular lesions, including albuminuria, mesangial expansion, glomerular hypertrophy, and glomerulosclerosis, were detectable at younger age (4 months) in *flk1-RAGE/ins-eNOS* mice, compared with diabetic *ins-eNOS* mice (6 months). Thus, aberrant endothelial expression of RAGE accelerated a glomerular phenotype in diabetic mice that mimics features of diabetic glomerulopathy in humans, including albuminuria, mesangial expansion, and glomerular hypertrophy. However, cardinal **histopathologic** features of progressive diabetic nephropathy in humans, including arteriolar hyalinosis and tubulointerstitial fibrosis, and progressive renal insufficiency (see section on Working Definition of Diabetic Nephropathy), have not been described

in this model to date. In collaboration with H. Yamamoto, in-depth phenotype examination and validation using AMDCC standards is underway.

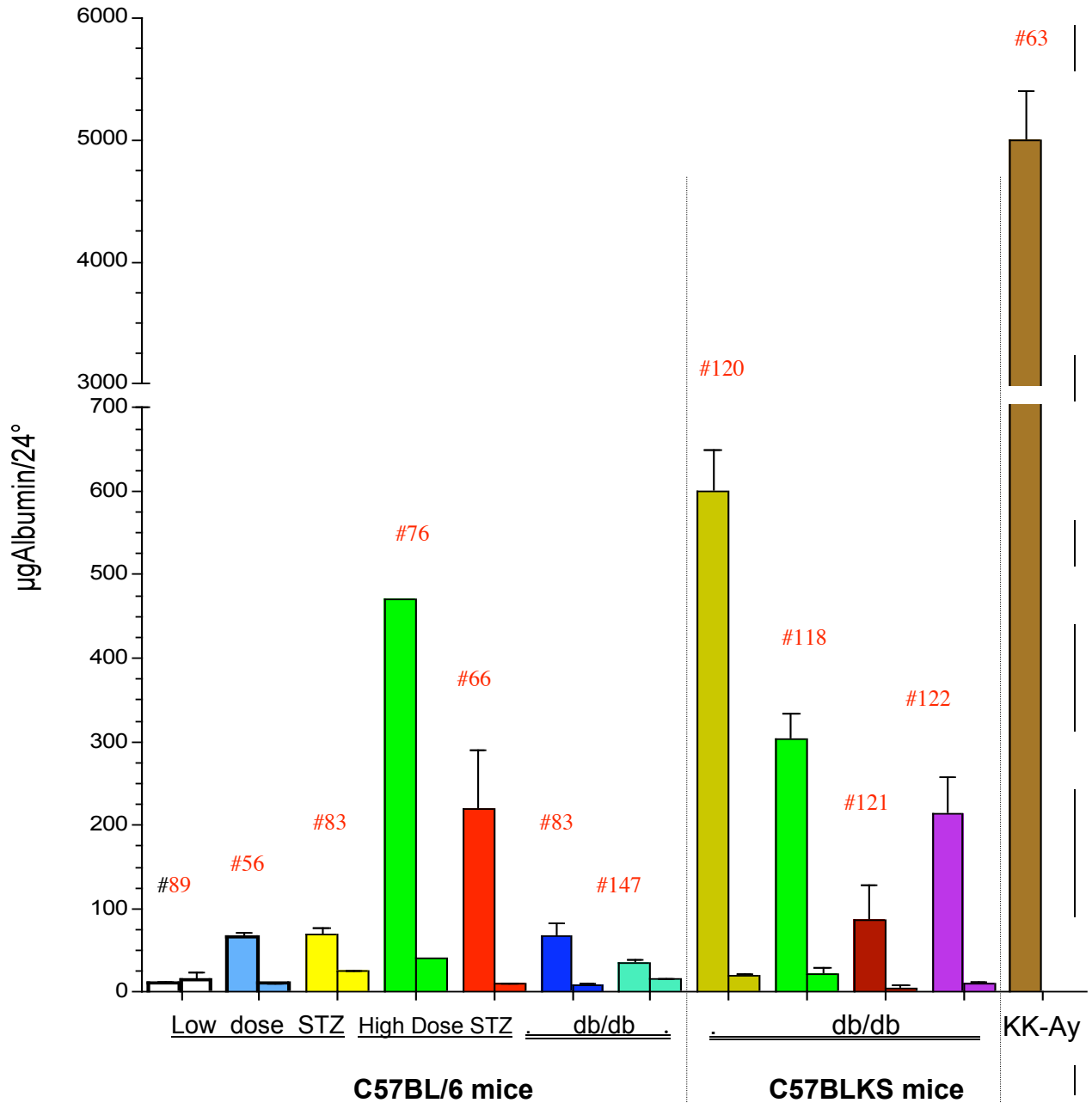
**GLUT1** A role for the GLUT1 glucose transporter in the pathogenesis of diabetic nephropathy has been suggested over the past decade (222, 223). This transporter has been identified as a major glucose transporter of the glomerulus (224), and high glucose exposure or diabetes, increase its expression in cultured mesangial cells and whole glomeruli (225, 226). Presumably, increased plasma membrane expression of GLUT1 in susceptible cells in the kidney would lead to increased glucose metabolic flux, and glucotoxicity perhaps due to enhanced generation of reactive oxygen species. In cultured mesangial cells GLUT1 over expression leads to enhanced PKC activation and fibronectin accumulation whereas suppression of GLUT1 levels reduces PKC activation and fibronectin accumulation (225, 226). Similar results in transgenic mice have been presented in preliminary form. Normoglycemic GLUT1 transgenic mice develop increased mesangial expansion and albuminuria, whereas mice with an anti-sense GLUT1 transgene that reduce glomerular GLUT1 expression are protected from these changes despite the presence of hyperglycemia (225, 227). Thus, differences in GLUT1 expression could explain part of the variable susceptibility to diabetic nephropathy in different strains of mice. Such a possibility is now being explored.

**Conclusions:** Mice provide an experimental model of unparalleled flexibility for studying mammalian diseases, including diabetic nephropathy. Inbred strains of mice exhibit substantial differences in the renal effects of diabetes mellitus. Much remains to

be established regarding the course of diabetic nephropathy in this species and defining those strains and/or mutants most susceptible to renal injury from diabetes mellitus. It will be especially important to determine whether renal function declines in any of these mouse models of diabetic nephropathy. Technical refinements in the measurement of plasma creatinine, glomerular filtration rate, and uniform reporting of albuminuria and histopathology, should facilitate progress in this field and allow better comparisons of results obtained by different laboratories. Together with the unique genetic reagents available in mice, the identification of a mouse model of diabetic nephropathy that closely mirrors human disease will significantly enhance our understanding of diabetic nephropathy and accelerate our progress towards a treatment for this disease.

## **ACKNOWLEDGEMENTS**

The authors acknowledge support of the Juvenile Diabetes Research Foundation (JDRF), NIDDK and NHLBI for funding of U01DK61018 (to MDB) NIH UO1-DK60994 (to FCB). U01 DK060995 (to EPB) U01 HL070523 (to TMC) and bioinformatics and web site creation and maintenance ([www.amdcc.org](http://www.amdcc.org)) from Rick McIndoe at Medical College of Georgia U01 DK060966



**Figure 1. Summary of studies in the literature examining 24 hour albumin excretion in mouse models of diabetes mellitus. (# = reference number)** For each study a pairs of bars represents mean  $\pm$ s.e. $^{24^{\circ}}$  albumin excretion in the diabetic cohort on the left and the non-diabetic to the right. From left to right are: albuminuria in C57BL/6 mice treated with multiple low dose streptozotocin (STZ); C57BL6 high dose STZ and C57BL6 db/db mice, a model of type 2 diabetes. Four studies measuring albuminuria in the C57BLKS db/db strain, show generally higher albuminuria. A single study examining 24hour albuminuria in the KK-Ay strain shows robust albuminuria.

**TABLE I: Some mouse models of diabetes mellitus studied for diabetic nephropathy.**

Mouse model (Ref #)	Strains reported (Ref #)	Diabetic type	Advantages	Disadvantages
Streptozotocin(71, 81, 146, 228-232)	C57BL/6J, C57BLKS, Balb/c, ICR, DBA2, ROP	Type 1	<ul style="list-style-type: none"> <li>Well established</li> <li>Reproducible timing</li> </ul> May be established in strains both resistant and susceptible to diabetic nephropathy.	<ul style="list-style-type: none"> <li>Potential for non-specific toxicity</li> <li>Strain dependent dosing necessary</li> <li>Biohazard-potential mutagen</li> </ul>
Encephalomyocarditis virus D variant (EMC-D) (153, 233, 234)	DBA Balb-C	Type 1	<ul style="list-style-type: none"> <li>May reproduce viral causes of type I diabetes in man</li> <li>DBA may be prone to diabetic nephropathy</li> </ul>	<ul style="list-style-type: none"> <li>Potential for non-specific renal effects</li> <li>Strain dependent dosing necessary</li> <li>Biohazard</li> <li>Not widely studied</li> <li>Renal functional effects not characterized</li> </ul>
Ins2 Akita(84, 88, 89)	C57BL/6 C3H	Type 1	<ul style="list-style-type: none"> <li>Commercially available (JAX)</li> <li>Autosomal dominant mutation</li> </ul>	<ul style="list-style-type: none"> <li>Presently only C57BL/6 commercially available</li> <li>C57BL/6 relatively resistant to nephropathy</li> <li>Hyperglycemia in females is mild.</li> </ul>
NOD(101, 102, 235, 236)	Inbred line derived from ICR (out-bred line)	Type1	<ul style="list-style-type: none"> <li>Spontaneous development of <math>\beta</math>-cell failure may mimic pathophysiology of disease in humans(99)</li> <li>Commercially available</li> </ul>	<ul style="list-style-type: none"> <li>Unpredictable timing of development of diabetes</li> <li>No appropriate control strain</li> <li>Needs insulin therapy to survive long periods</li> <li>Multigenic etiology DM precludes easy intercrosses.</li> </ul>
Db/db(22, 134)	C57BL/6,C57BLKS DBA, FVB 129, CBA (237)	Type 2	<ul style="list-style-type: none"> <li>Available on multiple strains</li> <li>Commercially available</li> </ul>	<ul style="list-style-type: none"> <li>Infertile</li> <li>Autosomal recessive</li> <li>Mutation in leptin receptor is a very rare cause of obesity and type 2 DM in humans</li> </ul>
ob/ob(21)	C57BL/6(238) FVB/N(238) DBA2(239, 240)	Type 2	<ul style="list-style-type: none"> <li>Exists on diverse inbred strains.</li> <li>Nephropathy uncharacterized</li> <li>Commercially available</li> </ul>	<ul style="list-style-type: none"> <li>Infertility- can be circumvented with leptin</li> <li>Autosomal recessive</li> <li>Nephropathy uncharacterized</li> <li>Mutation in leptin receptor is a very rare cause of obesity and type 2 DM in humans</li> </ul>
Agouti (Ay)	KK(170, 241, 242) C57BL(243) C3H(243) FVB(244)	Type 2	<ul style="list-style-type: none"> <li>KK strain is susceptible to renal injury with significant albuminuria.</li> <li>Autosomal dominant</li> <li>Commercially available</li> </ul>	<ul style="list-style-type: none"> <li>Hyperglycemia moderate and males &gt;females.</li> <li>Onset of diabetes is not well defined.</li> <li>Nephropathy may not be robust in strains other than KK</li> </ul>
High fat diet (112)	C57BL/6 is main susceptible strain (245)	Type 2	Onset can be determined by the investigator	<ul style="list-style-type: none"> <li>Only C57BL/6 reported thus far as susceptible</li> <li>Hyperglycemia not prominent</li> </ul>

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