

Translation Animal Models of Diabetic Kidney Disease: Biochemical and Histological Phenotypes, Advantages and Limitations

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Abstract: Animal models play a crucial role in studying the pathogenesis of diseases, developing new drugs, identifying disease risk markers, and improving means of prevention and treatment. However, modeling diabetic kidney disease (DKD) has posed a challenge for scientists. Although numerous models have been successfully developed, none of them can encompass all the key characteristics of human DKD. It is essential to choose the appropriate model according to the research needs, as different models develop different phenotypes and have their limitations. This paper provides a comprehensive overview of biochemical and histological phenotypes, modeling mechanisms, advantages and limitations of DKD animal models, in order to update relevant model information and provide insights and references for generating or selecting the appropriate animal models to fit different experimental needs.

Keywords: modeling, advanced diabetic kidney disease, diabetic complications, hypertension, atherosclerosis, non-proteinuric diabetic kidney disease

Introduction

Diabetic kidney disease (DKD) is a common microvascular complication of diabetes mellitus (DM) that is clinically characterized by a gradual decline in renal function, with or without proteinuria, due to prolonged hyperglycemia. There are no substantial differences between patients with type 1 DM (T1D) and those with type 2 DM (T2D) in the basic pathophysiological mechanisms leading to nephropathy.¹ The early glomerular pathologic changes in DKD are principally mesangial matrix expansion, glomerular basement membranes (GBM) thickening and compensatory glomerular hypertrophy causing ultrafiltration, which subsequently progresses to diffuse and/or nodular glomerulosclerosis with decreased glomerular filtration rate (GFR) and arterial hyalinosis. In addition, tubulointerstitial injury is a consistent feature of DKD, manifested by interstitial inflammation, tubular basement membrane thickening, tubular atrophy, and interstitial fibrosis. Chronic tubulointerstitial injury may be more severe than diabetic glomerulopathy in patients with T2D-DKD because of the more heterogeneous lesions often associated with atherosclerosis.¹

DKD can be found in 25–40% of diabetes and is one of the leading causes of end-stage renal disease (ESRD).¹ Additionally, its presence poses a significant cardiovascular risk and greatly increases cardiovascular mortality in patients.^{2–4} As a prevalent chronic disease, DM already affects the health of more than 350 million people worldwide. Research has indicated that the number of diabetes is expected to reach 600 million by 2035, by which time more than one in ten adults will have DM,⁵ suggesting that more people may suffer from DKD. Although a combination of glycemic control, anti-hypertension, lipid regulation, and lifestyle interventions has been clinically proven to be effective in slowing down the progression of DKD,⁶ this disease remains incurable or irreversible.

The etiology and pathogenesis of DKD are complex and not well understood, which is a major obstacle to developing clinical control methods. Suitable animal models can provide a basis for clinical treatment and important clues for studying the etiology, pathogenesis, and pathophysiological changes.⁷ There are various animal models of DKD available; however, no single model can represent all the features of human DKD. Therefore, it is crucial to select a suitable animal model based on research needs and purposes. There are three classical categories of animal models of DKD used in laboratories: induced models (chemical, dietary, and surgical induction), spontaneous models and genetic models. DKD, especially T2D-DKD, is not clinically independent and is often accompanied by complications such as hyperlipidaemia, hypertension, and atherosclerosis. In addition, the increasing number of patients with non-proteinuric DKD has raised concerns. Traditional DKD models cannot meet all the needs of studies due to their non-significant comorbid phenotypes. Scientists have been working on further development of new animal models to better mimic human DKD. In recent years, several new translation models, such as models of DKD with stable hypertension, DKD with atherosclerosis, and non-proteinuric DKD, have emerged, offering more possibilities for DKD research. Based on the signs of human DKD and the optimal characteristics proposed by the Animal Models of Diabetic Complications Consortium (AMDCC, <http://www.amdcc.org>), which are expected in a DKD model, this paper reviews translation animal models in terms of metabolic indicators, proteinuria (whether increased >10-fold), early/advanced glomerular pathology, tubulointerstitial pathology, and GFR (whether decreased >50%), as well as their modeling mechanisms, advantages and limitations. The purpose is to update the model information and provide important insights and references for generating or selecting reasonable animal models to fit different experimental needs.

Animal Models of T1D-DKD

Streptozotocin (STZ) Induced Animal Models of T1D-DKD

Using chemical agents – STZ or Alloxan is one of the most popular means to establish animal models of DKD. Compared to Alloxan, STZ is used more frequently in the laboratory because of its higher potency, less tissue toxicity, and stronger model stability.⁸

Model Mechanism

STZ is a 2-deoxy-2(3-(methyl-3-nitrosoureido)-aminoglucose synthesized by the *Streptomyces* chromogene. It is readily transported into pancreatic β -cells via GLUT-2. On the one hand, as an N-acetylglucosamine (GlcNAc) analogue, STZ selectively inhibits the activity of O-linked N-acetylglucosamine hydrolase (O-GlcNAcase, OGA), leading to irreversible O-glycosylation of intracellular proteins.⁹ On the other hand, the generation of NO and reactive oxygen species (ROS) during intracellular metabolism of STZ, as well as its strong alkylation properties, can induce DNA fragmentation and apoptosis of β -cells, ultimately leading to an absolute deficiency of insulin.¹⁰

Species and Strains

Pancreatic β -cells of rats and mice are more sensitive to the cytotoxicity of STZ than those of rabbits, dogs and pigs.^{11,12} Besides, the short generation time and low breeding cost make rodents the most commonly used in STZ-induced DKD experiments. For mouse strains, C57BL/6 (B6), DBA/2, CD1, 129/Sv and KK/HIJ are the most popular, among them, DBA/2, KK/HIJ and CD1 background are sensitive to STZ induction, while B6 are relatively resistant to the development of nephropathy.^{13,14} Even so, B6 mice are still widely used because of the advantage of easy genetic manipulation. For rat strains, the most frequently used are Sprague-Dawley (SD) rats and Wistar rats. In recent years, there has been some progress in STZ-induced DM and related complications in model organisms such as zebrafish and drosophila. High reproduction rate, ease of genetic manipulation and imaging deep tissues *in vivo* are their significant advantages. These models are most likely to be used for genetic or drug screening, however, the findings need to be replicated in mammals because their primitive kidney cells are functionally very different from those of humans.¹⁵

Phenotypes

Male rodents are more often chosen than females as the latter are comparatively insensitive to the toxic effects of STZ, which may be attributed to sexual dimorphism and estrogen.¹⁶ In STZ induced mouse models, male B6 mice show a significant increase in blood glucose with mild proteinuria 2 weeks after diabetes induction, but their renal lesions are

not significant, with only mild mesangial expansion and GBM thickening.^{17–19} STZ-129/Sv mice are characterized by the ability to develop hypertension, but do not perform as well as STZ-B6 mice in terms of hyperglycemia and proteinuria.^{13,17} STZ-DBA/2 mice are an excellent model of T1D-DKD with higher glucose and proteinuria levels and more severe renal lesions, for example, increased mesangial matrix accumulation, podocyte apoptosis, and leukocyte infiltration compared with STZ-B6 mice; moreover, glomerulosclerosis and arterial hyalinization can be observed in these mice.^{17,20} STZ-KK/HIJ mice have similar but less severe renal pathological alterations as STZ-DBA/2 mice.¹⁴ STZ-CD1 mice are advantageous in studying diabetic renal fibrosis; they display renal fibrosis associated with the epithelial–mesenchymal transition (EMT) and abnormal glycolysis at ~4 months after diabetes induction and significant lesions at ~6 months;^{21–23} these mice die because of diabetic complications by 6–7 months after the first STZ injection.¹³ For STZ induced rat models, renal lesions are mild in both SD strain and Wistar strain, although elevated blood glucose and increased urinary protein excretion are present.^{24–28}

Advantages and Limitations

STZ-induced wild-type (WT) models have the advantage of low cost and simple experimental procedures.²⁹ STZ destroys islet β -cells causing absolute insulin deficiency, which is similar to the pathogenesis of T1D in humans. However, these models have some non-negligible limitations: (i) renal lesions are mostly mild in most strains of mice and rats, with few advanced pathological changes such as nodular and/or diffuse glomerulosclerosis and decreased GFR; therefore, STZ induction is often combined with other approaches, such as dietary interventions and surgery, or the use of genetic animals to accelerate the disease processes to obtain the phenotypes desired, which was described in other sections of this review; (ii) at high doses or systemic administration, STZ exhibits non-specific cytotoxic to the kidney, especially tubular cells, and subsequently causes acute kidney injury, which makes it difficult to explain whether the kidney lesions are attributable to hyperglycemia or to the direct toxic effects of STZ.³⁰ So, it is recommended that any morphological and functional assessment of experimental animals should be started at least 2–3 weeks after STZ administration.^{31,32}

Uninephrectomy (UNx) + STZ Induced Animal Models of T1D-DKD

Model Mechanism

The addition of UNx to STZ is an intensive means of inducing T1D-DKD. Different from STZ that gradually develops renal ultrafiltration by inducing hyperglycemia, UNx directly alters renal hemodynamics causing an increase in GFR in the residual kidney. Ultrafiltration is an early manifestation of chronic kidney disease, and it can induce mechanical stretch to cause mesangial cell injury, further leading to glomerular hypertrophy and ECM production.³³ Excessive activation of the renin–angiotensin system (RAS) after UNx can increase blood pressure, as well as the production of pro-fibroblastic cytokines and ROS,^{33–37} all of which contribute to progressive kidney injury and accelerate the development of nephropathy. For strains resistant to DKD development, UNx + STZ is the best approach to induce T1D-DKD.³⁸

Species and Strain

B6 mice, SD rats, Wistar rats, spontaneous hypertension rats (SHR).

Phenotypes

Hyperglycemia, albuminuria, and early glomerular pathological changes such as glomerular hypertrophy, GBM thickening, mesangial matrix expansion and mild glomerulosclerosis can be observed in UNx + STZ treated mice and rats.^{38–43} Furthermore, tubular atrophy, brush border loss, tubular dilatation and tubulointerstitial fibrosis develop in B6 mice at 12th week after the treatment of UNx and multiple low-dose STZ.³⁸ These tubular damages are considered to be caused by glucose toxicity, while they are not evident or present under chemical induction alone.

Advantages and Limitation

In general, UNx + STZ induced models take shorter time to develop more severe kidney disease than those controls with STZ induced alone. However, Wada et al noted that in STZ-sensitive CD1 strain, UNx played a very limited role in accelerating the progression of renal lesions.³⁹ In addition, generating this type of models demands specialized technical

knowledge and complete surgical conditions, as well as reasonable antibiotic and analgesic treatments, to avoid the risk of surgical-related deaths.

Genetic Animal Models of T1D-DKD

Genetic animals are popular in DKD research because they often have more advanced nephropathy manifestations than traditional induced models. Islet-related genetic changes can generate T1D-DKD models, such as Akita mice and OVE26 mice.

Akita Mouse Lines

Model Mechanism

Akita mice carry an autosomal dominant spontaneous point mutation ($Ins2^{C96Y}$) in the pre-insulin gene ($Ins2$). This mutation leads to improper folding of insulin protein, resulting in proteotoxicity to pancreatic β -cells, which causes dysfunction and cell death.⁴⁴

Mouse Strains

B6, DBA/2, 129/SvEv, KK/Ta, Balb/c.

Phenotypes

Akita mice can develop significant and persistent hyperglycemia at 4 week-old, with some degree of elevated blood pressure, then spontaneously progress to DKD.⁴⁵ However, the severity of renal lesions is highly dependent on the mouse strain. The original line, B6- $Ins2^{+/C96Y}$, exhibits moderate albuminuria (~2.8-fold increase over B6-WT) and mesangial expansion. Attempts were made to cross over the variant to strains more susceptible to nephropathy (eg, DBA/2, 129/SvEv and KK/Ta), and new Akita mouse lines have been successfully developed. In contrast to B6- $Ins2^{+/C96Y}$, DBA/2- $Ins2^{+/C96Y}$ mice and 129/SvEv- $Ins2^{+/C96Y}$ mice show more pronounced albuminuria (3-fold and 5-fold relative change, respectively), but no exacerbation in renal pathology.⁴⁴ Besides, male KK/Ta- $Ins2^{+/C96Y}$ mice show progressive nephrotic changes, with a marked increase in urinary albumin excretion started at ~5 weeks old and a more than 600-fold increase in urinary microalbumin/creatinine (ACR) compared to non-diabetic controls after 15 weeks old; an increase in GFR occurred at ~10 weeks old, presenting a glomerular hyperfiltration state, then a decline started at ~20 weeks old, a sign of disease progression to end-stage renal failure;⁴⁶ their renal pathology reveals moderate mesangial dilatation, irregular thickening of GBM, glomerulosclerosis, small arteriolar hyalinosis and focal tubulointerstitial fibrosis.⁴⁷ On the other hand, Yu et al found that induction of two insulin 2 allele mutations ($Ins2^{C96Y/C96Y}$), ie, Akita knockout (KO), in Balb/c background allowed 80% of mice to survive for more than 6 months, unlike the other backgrounds in which most of the homozygotes died perinatally; moreover, Balb/c- $Ins2^{C96Y/C96Y}$ mice likewise develop more severe DKD than the original line, such as decreased GFR, a sustained increase in ACR (3-fold more than non-diabetic controls); the renal pathology is seen as mesangial dilatation, glomerulosclerosis and GBM thickening.⁴⁸

Advantages and Limitations

These mice are advantageous in reflecting progressive nephropathology. Unfortunately, none of the available Akita mouse lines show the characteristic changes of advanced DKD with diffuse and/or nodular glomerulosclerosis and a significant decrease in GFR (~50%).

OVE26 Mice

Model Mechanism

Overexpression (OE) of calcineurin in pancreatic β -cells under the regulation of the rat insulin 2 promoter causes cell damage and inadequate insulin secretion in OVE26 mice.⁴⁹

Mouse Strain

FVB.

Phenotypes

These mice develop DM within the first weeks of life and can survive for more than a year without insulin treatment

while remaining at near-normal weight.⁵⁰ They can also develop DKD at an early age (~2 months old) and reproduce many features of late-stage lesions, such as marked renal hypertrophy, severe progressive albuminuria (70–140 times that of controls at 9 months old), marked thickening of GBM (~87% increase), diffuse and nodular mesangial matrix expansion, progressive decrease in GFR (to 83–53% of controls at 9 months old), and the appearance of PAS-positive glomerular nodules similar to Kimmelstiel-Wilson (KW) disease, the most typical lesion of human nodular glomerulosclerosis; additionally, tubular atrophy, interstitial monocytes infiltration and fibrosis can be seen in these mice.⁵⁰ By performing UNx in 2-month-old OVE26 mice, Zheng et al described late-stage pathologic changes at 10 weeks postoperatively (~4.5-month-old) in the surgical group, comparable to the 8-month-old non-surgical group, implying that surgery greatly shortened the disease process so that the high mortality rate due to aging in older OVE26 mice could be avoided.⁵¹ Furthermore, it has been reported that, unlike most DKD models, female OVE26 mice present more severe nephropathy than males, such as more severe albuminuria, greater podocyte loss, additional fibrosis and significantly more inflammatory cell infiltration, although their blood glucose levels may be lower.⁵² Reduced circulating estradiol and renal estrogen receptors are responsible for this phenomenon.⁵²

Advantages and Limitations

OVE26 mice are considered an accurate model of early-onset T1D with advanced kidney disease and are valuable in studying the mechanisms and treatment of diabetic renal failure. Additionally, they are advantageous in gender studies of DKD and an excellent model to elucidate the potential mechanisms by which females are sensitive to renal injury. Previous studies have shown normal calmodulin expression in their kidney tissue, suggesting that the kidney damage is caused by DM itself, which fits the natural pathogenesis of human T1D-DKD.⁵⁰ However, some limitations of this model cannot be ignored: (i) transgenesis can only be achieved in FVB strain to obtain the desired characteristics; (ii) extreme and persistent hyperglycemia (≥ 600 mg/dl) does not correspond to the clinical reality; and (iii) any extent of arterial hyalinosis is not observed.

Animal Models of T2D-DKD

STZ Induced Animal Models of T2D-DKD

Model Mechanism

To induce T2D-DKD, STZ is often used in combination with nicotinamide (NAD), a derivative of vitamin B3, whose powerful antioxidant capacity protects β -cells partially against the toxic damage from STZ and keeps animals from developing absolute insulin deficiency.¹⁵ The elevated blood glucose in these models is associated with a reduction in β -cell mass and insulin secretion.

Mouse Strains

Wistar rats, B6 mice.

Phenotypes

Both rat and mouse models with NAD + STZ induction develop moderate levels of hyperglycemia and albuminuria. DKD symptoms typically appear after 30–45 days, for example, mild mesangial expansion, stromal hyperplasia and fibrosis.^{53–61} Kishore et al described that after 30 days of NAD + STZ induction, serum total cholesterol (TC), triglyceride (TG), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) significantly increased in DKD rats.⁵⁸ However, the findings of lipid disorders in these models are not consistent.

Advantages and Limitations

These models are less time-consuming and less expensive than that created by genetic engineering or high-calorie diets feeding and have been widely used for testing the therapeutic effects of numerous anti-diabetic or renoprotective materials.⁶² They are non-obese that may provide a unique platform for the study of non-obese DKD. However, these models do not develop insulin resistance and glucose intolerance, which is partly different from the pathogenesis of human T2D-DKD.

Diet Induced Animal Models of T2D-DKD

Model Mechanism

It is widely accepted that T2D is driven by the interaction of genetic factors and poor lifestyle. Unhealthy dietary structure such as high fat (especially referring to high trans fatty acids and low unsaturated fatty acids) and high carbohydrate are important contributors to the development of T2D. Meanwhile, excessive obesity ($IBM \geq 30 \text{ kg/m}^2$) is considered the strongest independent risk factor for T2D.⁶³ Obesity induces cellular stress and metabolic syndrome that includes insulin resistance, glucose intolerance, dyslipidemia and hypertension. Oxidative stress and macrophage interaction-mediated cellular inflammation exacerbate damage to renal lipid metabolism, proteins and DNA, subsequently leading to DKD.⁶⁴ Diet-induced models replicate unhealthy dietary patterns in humans, making it close to the pathogenesis of T2D-DKD. High-calorie diets include various dietary structures, for example, high-fat diet (HFD), high-sugar-high-fat diet (HsuHF), high-sugar diet (HsuD) and high-fat-high-sugar-high-cholesterol diet (HFHsuHC); the first two are the most common among the dietary regimens for inducing T2D-DKD. There is no unified international standard for the exact fat content and composition in HFD. Generally, HFD is considered a diet structure in which 40–60% of total food energy is provided by fat. It should be noted that the fat mainly refers to animal fats and vegetable oils containing ω -6/ ω -9 fatty acids, but not fish oils or vegetable glycerols, because the latter two do not cause obesity, metabolic disorders and insulin resistance and even have certain benefits for animals.^{65,66}

Species and Strains

B6 mice, Chinese Bama minipigs.

Phenotypes

Among rodents, B6 mice are more responsive to HFD. Male HFD-fed (≥ 12 weeks) B6 mice demonstrate metabolic disorders like obesity, hyperglycemia, hyperinsulinemia and dyslipidemia, and develop early nephrotic manifestations such as proteinuria, glomerular hypertrophy, mesangial expansion, thickening of GBM dominated by increased fibronectin and collagen, tubular dilatation and vacuolization, renal lipid deposition and inflammatory infiltration.^{64,67,68} Prolonged dietary interventions have been attempted to induce more severe nephropathy; unfortunately, even when the HFD-feeding time is increased to 32 weeks, advanced pathological changes are still not detected in B6 mice.⁶⁹ In contrast, high-calorie diets (HFD or HSuHF) do not induce significant nephropathy in rats, but only mild renal endothelial dysfunction, mesangial expansion and GBM thickening,⁷⁰ therefore, STZ + HFD or operating on genetic rats (eg, ZDF, ZSF1) would be a better means to generate relevant rat models. Pigs are very promising in DKD research because of their similarity to humans in anatomy, circadian rhythms and metabolism. In addition, omnivorous pigs are more closely resembling humans in terms of diet structure and feeding behavior. Liu et al found that after feeding HSuHF or HFHsuHC (10% lard, 37% sucrose and 2% cholesterol in 51% normal minipig feed) for 5 months, Chinese Bama minipigs developed metabolic syndrome and islet destruction; their kidneys demonstrated ectopic lipid deposition, glomerular hypertrophy, podocyte fusion, basement membrane thickening, mesangial expansion, moderate glomerulosclerosis and tubulointerstitial fibrosis, with production of microalbuminuria.^{71,72} However, severe hyperglycemia is rarely seen in these minipig models.^{73–75}

Advantages and Limitations

These models simulate the pathogenesis of most human T2D-DKD and also are excellent for studying metabolic disorders, potential mechanisms of insulin resistance, as well as dietary intervention therapies. However, the majority of mice and rats are resistant to the development of obesity, DM and associated nephropathy induced by high-calorie diets alone, so a combination with other means is often required to accelerate disease progression, such as: (i) low-dose STZ to partially destroy β -cells and render islets dysfunctional; (ii) use of genetic animals; and (iii) nephrectomy to reduce kidney mass.

STZ + HFD Induced Animal Models of T2D-DKD

Model Mechanism

Insulin resistance and β -cell dysfunction are two major features of T2D-DKD.⁶³ HFD induces metabolic disorders, while STZ reduces β -cell mass, ultimately leading to T2D-DKD in animals.

Species and Strains

SD rats, Wistar rats, B6 mice.

Phenotypes

Male SD or Wistar rats receive a single low-dose STZ (30–35 mg/kg, i.p.) after being fed HFD or HSuHF for 4–6 weeks, and almost all of these rats develop significant hyperglycemia and proteinuria after induction, with early renal pathology such as glomerular hypertrophy, mesangial expansion, extracellular matrix (ECM) deposition and mild glomerulosclerosis.^{76–79} In male B6 mice, T2D-DKD can be induced by HFD-feeding with a single low-dose STZ (40 mg/kg, i.p.), or medium-dose STZ (100–120 mg/kg, i.p.), or multiple low-dose STZ (40–50 mg/kg, i.p., for 5 days).^{80–86} These mice can develop glomerulosclerosis and tubulointerstitial fibrosis, which is not observed in HFD induced alone. However, Glastras et al noted that one dose STZ cannot aggravate the altered renal pathology in B6 mice.⁸⁷

Advantages and Limitations

The addition of STZ to dietary intervention to reduce β -cell mass can further mimic the features as well as accelerate the progression of kidney disease. As many of the key pathological characteristics of human DKD are missing in the rat models induced by HFD alone, the regimen of STZ + HFD is more commonly used. Both rats and mice have more severe hyperglycemia in this regimen, but signs of obesity and insulin resistance (ie, hyperinsulinemia) are almost less pronounced than HFD induction alone, which may be related to STZ-mediated glucose metabolism reduction, protein/lipid catabolism excess and insulin release reduction.^{79,88–90}

UNx + STZ + HFD Induced Animal Models of T2D-DKD

Model Mechanism

The mechanism of UNx, STZ and HFD inducing DKD was described previously. There is no uniformity in the induction sequence and specific contents of these three, where high-fat diet can be used either throughout the experimental cycle or after UNx and/or STZ to induce metabolic disorders and insulin resistance.

Species and Strains

SD rats, Wistar rats, B6 mice.

Phenotypes

Sugano et al found diffuse glomerulosclerosis at week 25 of the experiment in male SD rats treated with a single low-dose STZ (40 mg/kg, i.p.), followed by UNx and HFD intervention after 9 and 23 days, respectively.⁹¹ Similar results were described by Bayrasheva et al in male Wistar rats treated with another procedure, ie, HFD started 3 weeks after UNx, then NAD (230 mg/kg, i.p.) and high-dose STZ (65 mg/kg, i.p.) administered at week 8.³² The renal tissue of these rats showed a significant enlargement of the mesangium and sclerosis in both glomerulus and tubulointerstitium at week 30 of the experiment. Further, it has been suggested that NAD + high-dose STZ results in more stable metabolic syndrome in rats compared to low-dose STZ.^{32,91} Dusabimana et al performed HFD-feeding after UNx on B6 mice, and then administered a single medium-dose STZ (100mg/kg, i.p.) 3 weeks after surgery; at the end of the experiment, these mice developed varying degrees of hyalinosis, glomerular atrophy, capsule shrinkage and chronic glomerulonephritis, with a significant decrease in the number of podocytes, also, they developed serious interstitial injury, including tubular dilatation, loss and necrosis of brush border, ECM accumulation and interstitial fibrosis.⁹²

Advantages and Limitations

The above studies suggest that the protocol of UNx + STZ + HFD accelerates the course of nephropathy in rodents. Nevertheless, the phenotypic findings of these models are not consistent while advanced renal pathological changes are not fully accessible in most other similar researches.

Spontaneous Animal Models of T2D-DKD

Models in this category are established by breeding animals that develop DKD due to genetic abnormalities. These models are reliable because they have similar pathogenesis and renal abnormalities to humans. However, their use in

current DKD research is not as widespread as other types because of relative difficulty in feeding and breeding, long modeling period and high cost. Otsuka Long-Evans Tokushima Fatty (OLETF) rats and Goto Kakizaki (GK) rats are the more popular strains among them.

OLETF Rats

Phenotypes

OLETF rats are a spontaneous T2D-DKD model characterized by (i) diabetic phenotypes inherited only in males; (ii) having a transition from non-insulin-dependent DM (NIDDM) to insulin-dependent DM (IDDM), ie, islet cell proliferation and peripheral blood insulin resistance lead to hyperglycemia and hyperinsulinemia at early stage, while pancreatic β -cells begin to deteriorate and eventually develop hypoinsulinemia and IDDM after 40-week-old. (iii) early mild obesity with hyperphagia and weight loss began after 28-week-old; (iv) hyperlipidemia; (v) DM occurred around 18–25-week-old, and blood glucose maintained above 200 mg/dL after ~32-week-old; (vi) progressive microalbuminuria appeared at ~16-week-old, and renal pathological changes started at ~23-week-old and worsened at ~29-week-old with segmental glomerulopathy, further, mesangial expansion, GBM thickening and glomerulosclerosis emerged above 40-week-old, which fully develop into diabetic glomerulopathy with tuberos scleriosis after 65 week-old, in addition to renal hypertrophy at ~55-weeks-old.^{93–96}

Advantages and Limitations

The progression of DKD and renal pathological changes in OLETF rats highly resemble human T2D-DKD, both exhibiting exudative glomerulopathy characterized by fibrin cap, decreased capsule membrane and aneurysmal dilatation of intra-glomerular vessels.⁹⁵ However, OLETF rats have relatively mild hyperglycemia and late onset of DKD. More importantly, later islet β -cells destruction may require insulin-dependent survival. Thus, OLETF rats are mainly used for early T2D-DKD studies at present.

GK Rats

Phenotypes

GK rats are a non-obese model of early T2D-DKD, produced from Wistar rats by repeatedly selective breeding over many generations using glucose intolerance as an indicator of selection.⁹⁷ DM in GK rats results from a combination of several pathogenic mechanisms, including impaired individual development of islet cells, abnormal insulin release after glucose loading, insulin resistance, basal hyperinsulinemia, and abnormal glucose metabolism.^{98–102} These rats typically develop moderately persistent hyperglycemia at 3–4 weeks old, with a glycemic range of 12.9–17.0 mmol/L.^{103,104} Their renal lesions are mild, with basement membrane thickening and glomerular hypertrophy seen in early and middle life (statistically significant at 35 weeks of age); Interstitial monocyte/macrophage infiltration increases with progression of nephropathy.^{104,105} It is not until late in life (24 months of age) that a mild increase in urinary protein excretion as well as significant renal pathological changes can be observed: segmental glomerulosclerosis, mesangial proliferation, also, tubulointerstitial fibrosis, atrophy and dilatation and inflammatory cell infiltration.¹⁰⁵

Advantages and Limitations

Unlike most other animal models of T2D-DKD, GK rats do not develop obesity, hypertension and hyperlipidemia, thus allowing a more direct study of the relationship between long-term hyperglycemia and DKD without the interference of other risk factors. Since the development of proteinuria in these rats shows an age-related rather than a glycemic correlation in early to middle stage, and the increasing trend of proteinuria is not significant across all ages, this model may be more appropriate for studying early non-proteinuric T2D-DKD in humans.^{103–105} In addition, administration of deoxycorticosterone acetate (DOCA) or angiotensin I (Ang I) + high-salt diet to superimpose a hypertensive phenotype can lead to progressive proteinuria and glomerulosclerosis in these rats.^{103,106} Besides, HFD-feeding can worsen their glucose metabolism.¹⁰⁷ Consequently, GK rats seem to be an ideal model to study the effects of other risk factors, especially hypertension, on diabetic renal function, kidney morphology and pathological progression.

Genetic Animal Models of T2D-DKD

There are various genetic animal models of T2D-DKD, among them, the models correlated with leptin signaling pathway abnormalities, such as *ob/ob* mice, *db/db* mice, Zucker diabetic fat (ZDF) rats and obese ZSF1 rats, as well as some special transgenic models like *KK^A/Ta* mice, are the most frequently used.

Ob/Ob Mice

Model Mechanism

Ob/ob mice are characterized by a single autosomal recessive mutation (*Lep^{ob/ob}*) on the obesity gene encoding leptin, resulting in a complete lack of leptin production and subsequent hyperphagia, obesity, hyperlipidemia, hyperglycemia and insulin resistance.¹⁰⁸

Mouse Strains

For current DKD research, the mutation is mostly induced under the black and tan, brachyuric (BTBR) mouse strain which shows congenital insulin resistance, because other backgrounds, such as B6-*ob/ob* mice and C57BLKS/J (BKS)-*ob/ob* mice, are not ideal in the development of nephropathy, although they can develop hyperglycemia.¹⁰⁹

Phenotypes

Insulin resistance is detectable in these mice at 4 weeks of age, with islet hypertrophy, hyperinsulinemia and persistent hyperglycemia (up to 350–400 mg/dl), followed by podocyte loss and progressive proteinuria at 8 weeks of age, and mesangial expansion at 10 weeks of age; the nephropathy in BTBR-*ob/ob* mice is progressive and comes into advanced stage at ~18–20 weeks of age, manifested by >10-fold increase in proteinuria, diffuse/nodular mesangial sclerosis, focal small arterial hyalinization, mesangial vascular lysis, increased mesangial matrix, GBM thickening and focal mild interstitial fibrosis.¹¹⁰ In addition, high protein diet (HPD, 30% kcal protein) feeding can exacerbate glomerular pathology and interstitial fibrosis, and thus shorten the modeling time.¹¹¹

Advantages and Limitations

BTBR-*ob/ob* mice are precious because they come close to meeting all the proposed criteria of AMDCC and largely reproduce the basic structural changes and functional features of human diabetic glomerular injury, where high mesangial dilatation and interstitial lysis are uncommon in other animal models. Meanwhile, their renal tissues show large aggregates of monocytes/macrophages, similar to humans, which is valid for studying the relationship between inflammation and DKD. Furthermore, continuous infusion of leptin into BTBR-*ob/ob* mice via an osmotic minipump can completely reverse the late-stage lesions, making it possible to consider leptin replacement as a strategy to study the underlying reversible mechanisms of DKD, as well as providing ideas for regression.¹¹² Nevertheless, the greatest limitations of this model are strain dependence and leptin deficiency. BTBR-*ob/ob* mice are sterile, so breeding to sufficient numbers for experimental use requires a lot of time and money. More importantly, pronounced leptin deficiency is not a feature of human diabetes, which limits the overall similarity of these mice to the clinical reality.

Db/Db Mice

Model Mechanism

A single autosomal recessive mutation (*LepR^{db/db}*) in the leptin receptor gene (*LepR*) from Gly to Thr causes defects in the hypothalamic leptin signaling pathway in these mice, followed by obesity, insulin resistance and T2D. Unlike *ob/ob* mice, *db/db* mice exhibit circulating leptin excess and leptin resistance.

Mouse Strains

The mutation was first identified in BKS strain which possesses 84% of the B6 allele and 16% of the DBA/2J allele and is more sensitive to the development of DKD.^{113,114} By far, BKS strain is the most widely used for *db* mutations, followed by B6, DBA/2J and FVB. Recently, *db/db* mice were also successfully established in fibrosis-prone CD1 background.¹¹⁵

Phenotypes

In contrast to B6-*db/db* mice with mild hyperglycemia and marked hyperinsulinemia, BKS-*db/db* mice show severe progressive hyperglycemia and moderate hyperinsulinemia at 8 weeks of age due to atrophy and necrosis of partial islet β -cells and reduced pancreatic hormone secretion, with peak glucose levels up to 44 mM at ~16 weeks of age; their urinary albumin excretion is significantly increased at ~8 weeks of age; renal pathological changes mainly occur after 12 weeks of age, manifested by renal hypertrophy, glomerulomegaly, expansion of the mesangial matrix, loss of podocytes and thickening of GBM; arterial hyaline degeneration is occasionally seen in older mice (~21 weeks of age), in addition, tubular lesions are dominated by vacuolization.^{116–119} Males possess more severe hyperglycemia and nephropathy than females.¹¹⁶ However, due to the slow progression of nephropathy and mild pathological changes in BKS-*db/db* mice, attempts have been made to address this problem by combining other induction modalities or using susceptible strains. It has been shown that HFD (60% kcal fat) leads to renal failure, as well as early death in BKS-*db/db* mice, while HPD (60% kcal protein) significantly exacerbates glomerular and tubular pathological alterations without increasing mortality.¹²⁰ HPD-fed BKS-*db/db* mice develop glomerulosclerosis and tubulointerstitial fibrosis, although the glycemic rise is reduced.¹²¹ Similar pathological results can be found in *db/db* mice post-UNx or in female FVB-*db/db* mice.^{122–124} In contrast, despite higher albuminuria levels, DBA/2J-*db/db* mice do not differ significantly from BKS-*db/db* mice on glucose levels and renal pathology.¹¹⁷ CD1-*db/db* mice are a novel mouse model of T2D-DKD with progressive renal fibrosis and cardiac fibrosis. Males appear to have elevated blood glucose levels and decreased fasting insulin levels compared with those of BKS-*db/db* mice; plasma cystatin C levels tend to be elevated in CD1-*db/db* mice from 16 to 24-weeks-of-age; moreover, males show significantly progressive kidney and heart fibrosis from 16 to 24-weeks-of-age, while weak fibrosis in females; however, these mice do not develop nodular lesions or vascular lysis.¹¹⁵ The CD1 mouse is an outbred strain and therefore could show genetic diversity that could produce an unstable phenotype in the kidney as compared with inbred strains, such as the BKS strain.¹¹⁵

Advantages and Limitations

Db/db mice are one of the most widely used models of T2D-DKD. The relatively consistent high albuminuria levels and significant mesangial matrix expansion make them a good model for studying early T2D-DKD. However, leptin resistance is a rare disorder in human T2D-DKD, also, the presence of immune complex deposits in the glomeruli and the non-persistent increase in albuminuria are not consistent with human phenotypes.^{115,116}

ZDF Rats

Model Mechanism

ZDF rats, also known as *fa/fa* rats, are produced by selective breeding of Zucker rats with leptin receptor mutations (*LepR fa/fa*).

Rat Strain

Zucker (male).

Phenotypes

Only males develop T2D and DKD. Their physiological and metabolic characteristics, such as obesity, dyslipidemia, abnormal glucose tolerance, insulin resistance, hypertension and progressive renal injury, are similar to those of humans.¹²⁵ Meanwhile, their impaired and reduced numbers of β -cells may be caused by glucose/lipid toxicity.^{125,126} The combination of reduced insulin secretion and insulin resistance results in marked DM in male ZDF rats at ~8 weeks of age, with progressive elevation of blood glucose (200–500 mg/dL).^{125,127,128} Significant albuminuria occurs after 12 weeks of age; then, after 20–22 weeks of age, mesangial matrix expansion, focal segmental glomerulosclerosis, tubular atrophy and dilatation, and tubulointerstitial fibrosis can be observed.^{127–130} In addition, HFD (60% kcal fat) can exacerbate nephropathy in these rats and shorten the experimental duration.^{130,131}

Advantages and Limitations

ZDF rats are a widely used model in studies of metabolic syndrome and diabetes and can also develop some degree of nephropathy. However, ZDF rats have obvious shortcomings: (i) as the disease progresses, both sexes develop

unexplained severe hydronephrosis unrelated to human DKD and lead to severe complications including renal abscess, pyelonephritis and necrotizing granuloma, which makes the cause of nephropathy complex and difficult to explain;¹²⁵ (ii) whether animal models show a high degree of similarity to human disease at the molecular levels is one of the criteria for judging their merit, and Siwy et al found that ZDF rats are more consistent with human peptide regulation in cardiovascular disease (CVD) than in chronic kidney disease (CKD) at the molecular levels, suggesting that they may be a more appropriate model for human CVD rather than CKD.¹³²

Obese ZSF1 Rats

Model Mechanism

The obese ZSF1 rat (ZSF1 *fa/fa^{cp}*) is the F1 offspring produced by crossing lean female ZDF (ZDF *+/fa*) rats with lean male spontaneously hypertensive heart failure (SHHF/*Mcc-fa^{cp}*, *+/fa*) rats.¹³³

Rat Strains

Female ZDF × Male SHHF.

Phenotypes

In contrast to ZDF rats, obese ZSF1 rats do not develop hydronephrosis and have more severe hypertension with cardiac hypertrophy and atherosclerosis. They exhibit DM, metabolic syndrome and renal damage at ~8 weeks of age; their renal disease is progressively worse, proceeding to late-stage of DKD after ~44 weeks of age and eventually developing into ESRD; most of them die at 12 months of age.^{133–135}

Advantages and Limitations

Obese ZSF1 rats almost fulfill the standards suggested by AMDCC and have similarities to human DKD in terms of glomerular gene expression changes.^{134,136} Therefore, they are considered as an excellent preclinical translational model of T2D-DKD and a reasonable model to study the effects of comprehensive risk factors (ie, hyperglycemia, obesity, hyperlipidemia and hypertension) on renal function.

KK^{A^y}/Ta Mice

Model Mechanism

KK^{A^y}/Ta mice are established by introducing the yellow obese *A^y* gene into the KK/Ta strain which is congenitally glucose intolerance and insulin resistant. KK/Ta mice spontaneously develop a dominant diabetic state with age or consumption of high calorie diet, manifesting as mild obesity, polyphagia, compensatory hyperinsulinemia, hyperglycemia, urine glucose and microalbuminuria, which are more severe in males.¹³⁷ Therefore, male KK/Ta mice are commonly used in the study of metabolic syndrome and obesity-related DM. Traditional KK/Ta mice with a long period of DKD development and insignificant phenotypic characteristics do not have an advantage among the many models available today, so they are more often used as blank controls or to provide a susceptible genetic background, such as KK/Ta-Akita (described previously) and KK^{A^y}/Ta.

Mouse Strain

KK/Ta.

Phenotypes

KK^{A^y}/Ta mice spontaneously appear obesity, polyphagia, glucose intolerance, insulin resistance, hyperinsulinemia, impaired insulin secretion and hyperlipidemia.^{138–141} They develop hyperglycemia with predominantly random blood glucose (RBG) elevation (22.6–25.9 mmol/L) at ~12 weeks of age; their fasting blood glucose (FBG) is mildly raised and not significantly different from non-diabetic controls after 20 weeks of age; progressive proteinuria appears after 8 weeks of age and increases significantly after 12 weeks of age; renal tissue shows significant pathological changes at ~20 weeks of age with diffuse mesangial expansion, GBM thickening and segmental sclerosis.^{140,141} Moreover, chronic tubulointerstitial injury, for example, varying degrees of interstitial fibrosis and tubular atrophy, nodular glomerular hyaline

degeneration and focal segmental/global glomerulosclerosis, can be seen at ~40 weeks of age.¹³⁹ HFD exacerbates FBG and metabolic disturbances, as well as accelerates the progression of nephropathy in KK^{y}/Ta mice.¹³⁸

Advantages and Limitations

KK^{y}/Ta mice are a powerful polygenic spontaneous model of T2D-DKD; however, their hyperglycemia and lipid metabolic disturbances show some degree of spontaneous recovery after 16 weeks of age; also, proteinuria increases <10-fold and no statistically significant differences in microalbuminuria are observed in these mice.^{138,140} In the absence of additional intervention, these mice do not show the characteristics of advanced DKD.

T2DN/Mcwi (T2DN) Rats

Model Mechanism

The T2DN rat is a transgenic subline of GK rat, developed by introducing the mitochondrial and some passenger loci genes from the Fawn Hooded Hypertensive (FHH) rat into the GK strain.¹⁴²

Rat Strain

T2DN/Mcwi.

Phenotypes

Despite their high genetic similarity (97% at locus 681) and similar glucose and blood pressure, nephropathy manifests differently between T2DN and GK rats. T2DN rats have hypercholesterolemia and hypertriglyceridemia, and develop albuminuria at 6 months of age with renal histological abnormalities such as focal glomerulosclerosis, mesangial matrix expansion, and GBM thickening. These features develop over time, so that almost all T2DN rats show a > 10-fold increase in albuminuria, > 50% decrease in GFR, severe diffuse alloglomerulosclerosis, KW-like nodules, arterial hyalinization, tubular necrosis, interstitial inflammation, and fibrosis at 18 months old.^{142–144}

Advantages and Limitations

The evolution of nephropathy in T2DN rats from 3 to 18 months old resembles the natural progression of human DKD, encompassing almost all histological features, and is, therefore well suited to test the effectiveness of new therapeutic approaches to delay disease progression. Furthermore, T2DN rats are significantly sexually dimorphic, with females showing no insulin resistance, hyperlipidemia, milder glycemetic and nephrotic phenotypes,¹⁴⁵ suggesting that this model may be a valuable tool in the study of sex differences in T2D-DKD.

Complication Models of DKD

Animal Models of DKD with High Blood Pressure

$eNOS^{-/-}Db/Db$ Mice

Model Mechanism

Endothelial nitric oxide synthase (eNOS, NOS III) is closely associated with the development and progression of human DKD. Inhibitory activity or genetic deficiency of eNOS reduces nitric oxide (NO) synthesis, which plays an important role in controlling the integrity of interendothelial junctions (IEJs) and regulating vascular permeability.^{146,147} Increased production of NO synthesized by eNOS in the early-stage of human DKD may lead to renal vasodilation, hyperfiltration, and microalbuminuria.^{148,149} However, with disease progression, there is a decrease in renal eNOS, leading to activation of the renin-angiotensin-aldosterone system (RAAS), elevated tissue factor (TF, factor III) levels and excessive activation of the exogenous coagulation that promotes renal thrombosis.¹⁵⁰ Severe proteinuria, decreased renal function and hypertension are also considered to correlate with progressive NO deficiency.^{148,151} Animal models of DKD have been generated through genetic engineering to induce eNOS deficiency. $eNOS$ KO can make mice more prone to develop typical and severe DKD with a moderate hypertensive phenotype, although not able to have a significant effect on blood glucose levels.^{152–162}

Mouse Strain

BKS.

Phenotypes

The *eNOS*^{-/-}/*LepR*^{db/db} double KO is used to induce late-stage T2D-DKD. This model inherits obesity, high fasting blood glucose (FBG), insulin resistance and hypercholesterolemia of the *db/db* parents, as well as the moderate hypertension and diminished vascular repair of the *eNOS*^{-/-} parents.¹⁵³ Despite having lower FBG than *db/db* mice, *eNOS*^{-/-}/*db/db* mice exhibit higher and gradually increasing levels of urinary albumin at 8 weeks of age and have a progressive glomerular pathology similar to that of human T2D-DKD. The pathology initially involves GBM thickening and mesangial dilatation, followed by mesangial lysis leading to microaneurysms, repeated vascular injury leading to nodular lesions similar to KW lesions, and eventually diffuse/nodular glomerulosclerosis; rare fibrin thrombi in glomerular arteries and occasionally arterial hyaline degeneration are also found in these mice.¹⁵³ Their early elevated GFR decreases significantly at 20 weeks of age¹⁶² and by more than 50% at 26 weeks of age.¹⁵² Their tubular damage is severe with tubular dilatation, cast formation, epithelial flattening, and increased apoptosis, but their tubular fibrosis is relatively mild.^{152,153}

Advantages and Limitations

eNOS^{-/-}/*db/db* mice are a robust animal model of advanced DKD with stable hypertension. On the other hand, Stec's team has confirmed that these mice in the BKS background exhibit urinary metabolome changes similar to those seen in human DKD making them a useful tool for pathological metabolomic studies.¹⁶³ However, *eNOS*^{-/-}/*db/db* mice have a short lifespan and typically die between 24 and 28 weeks of age without insulin treatment.^{152,162}

Animal Models of DKD with Atherogenesis

The development of DM and DKD is closely related to atherosclerosis. Chronic hyperglycemia-induced injuries, such as increased intravascular ROS production, enhanced inflammatory state, lipid oxidation, and compositional changes, including elevated total cholesterol, total triglycerides, chylomicron/VLDL and intermediate density lipoprotein (IDL)/LDL ratios, can not only accelerate endothelial damage, but also promote plaque formation and smooth muscle cell proliferation, subsequently leading to atherosclerotic lesions in diabetic patients.¹⁶⁴ Clinically, the majority of T2D-DKD patients have macroangiopathy as the disease progresses. Atherosclerosis, especially intrarenal atherosclerosis, is one of the main causes of impairing renal function.¹⁶⁵ In addition, the superimposed effect of nephropathy and atherosclerosis greatly increases the cardiovascular risk and mortality of patients. However, the mechanism of interaction between these two conditions is not clear; therefore, animal models with accurate translation of these phenotypes are urgently needed.

IDOL-AAV-DJ/8-Ob/Ob Mice

Model Mechanism

IDOL is a key substance in reducing LDL receptor and thereby increasing LDL cholesterol (LDL-C) and total cholesterol levels. It targets the LDL receptor ubiquitin-mediated lysosomal degradation and increases LDL-C levels when over-expressed, which promotes atherogenesis.¹⁶⁶

Mouse Strain

BTBR.

Phenotypes

After injecting male BTBR-*ob/ob* mice with the AAV-DJ/8 viral genome containing IDOL mutants to overexpress IDOL under the control of the liver-specific human thyroxine-binding globulin promoter, and feeding these animals a semi-purified, high-fat, cholesterol-containing diet (40% kcal fat and 1.25% kcal cholesterol) post-injection to promote atherosclerosis, Bornfeldt's team successfully generated a new T2D-DKD model with atherosclerosis.¹⁶⁶ Compared to BTBR-*ob/ob* controls, this model exhibits higher blood cholesterol (non-HDL-C), plasma triglyceride and urinary albumin levels, more severe glomerular macrophage accumulation and local inflammation; also, it shows early atherosclerosis formation – aortic smooth muscle fibrous cap lesions containing Mac-2-positive macrophages.¹⁶⁶ The rest of phenotypes of this model are similar to those of BTBR-*ob/ob* mice.

Advantages and Limitations

IDOL-AAV-DJ/8-*ob/ob* mice provide a new tool to study the mechanisms of interaction between T2D-DKD and atherosclerosis, as well as to develop new therapeutic strategies. It should be noted that the mortality rate of BTBR-*ob/ob* mice increases after 24 weeks old, and studies should be performed before this age. On the other hand, atherosclerosis in the BTBR strain is relatively mild, and lesions are only observed in the aorta. It remains to be explored whether other strains that are more susceptible to atherosclerosis, such as the B6 strain, can generate relevant models.

Animal Models of Non-Proteinuric DKD

The concept of nonproteinuric DKD was first proposed by Tsalamandris et al.¹⁶⁷ This type of DKD is common in clinical practice and manifests as normal-range proteinuria, ie, ACR < 30 mg/g or UAER < 30 mg/day, but with renal insufficiency. It is now commonly referred to as normoalbuminuric DKD (NADKD), nonalbuminuric diabetic nephropathy, or DKD without albuminuria.¹⁶⁵ The latest diagnostic criteria for DKD proposed by the American Diabetes Association (ADA) in 2015 includes UAER > 30 mg/24h or eGFR < 60 mL/(min·1.73 m²).¹⁶⁸ Therefore, DM patients with normal-range proteinuria and renal insufficiency meet the criteria for DKD. In recent years, numerous studies have shown that atherosclerosis and tubulointerstitial damage (but not glomerular damage) are strongly associated with NADKD.^{165,169}

HFD-Fed ApoE^{-/-} Mice

Model Mechanism

Unlike classical DKD models that revolve around urinary albumin and glomerular lesions, the typical pathologies of the NADKD model should be focus on atherosclerosis, renal insufficiency, and tubulointerstitial damage. ApoE is a polymorphic protein that is an important ligand for apolipoproteins, which have an elimination receptor-mediated role. In the absence of ApoE, cholesterol accumulates in the vessel wall, causing thickening and sclerosis, as well as reduced elasticity and lumen narrowing, further developing into atherosclerosis.¹⁷⁰ HFD not only causes metabolic disturbances in ApoE^{-/-} mice, dominated by significantly elevated levels of triglycerides, total cholesterol, and LDL, but also promotes the development of atherosclerosis.

Mouse Strain

B6.

Phenotypes

Tomita's team crossed 8-week-old female and male ApoE^{+/-} mice to produce ApoE^{-/-} mice. Subsequently, 10-week-old male ApoE^{-/-} mice were selected and fed with HFD (60% kcal fat) for 24 weeks to generate the NADKD model.¹⁶⁹ Compared to normal diet (ND)-fed ApoE^{+/+} mice, HFD-fed ApoE^{-/-} mice exhibit obesity-related hyperglycemia, aortic atherosclerosis, decreased CD31-positive peritubular capillary density and renal blood flow, and renal dysfunction defined by high levels of plasma cystatin-C at ~34-week-old; their Renal pathology reveals glomerular hypertrophy and mesangial expansion with fibrosis; however, podocyte ultrastructure is essentially normal with mild proteinuria.¹⁶⁹ In contrast, these mice have severe proximal tubular epithelial cell damage with follicular alterations, apoptosis, as well as tubulointerstitial fibrosis and inflammation.¹⁶⁹

Advantages and Limitations

HFD-fed ApoE^{-/-} mice are considered to successfully reflect the renal phenotypes of human NADKD. However, renal dysfunction in these mice is mild and it is not clear whether the lesions are progressive. Therefore, it is preferable to use these mice for studies in the early-stage of the disease.

Conclusion

There are various animal models of DKD available, but no single model can represent all the features of human DKD. Therefore, it is essential to choose the appropriate model based on the research needs, as different models develop different phenotypes and indispensably have their limitations. The desired positive results may not be obtained if the animal model does not fit the experiment, which can be avoided in principle. To address this issue, this paper provides a comprehensive overview of the biochemical and histological phenotypes of DKD animal models, based on the key features of human DKD and the criteria of AMDCC (Tables 1–3, respectively). Generally, STZ-induced models and

Table 1 Biochemical and Histological Phenotypes in Translation Animal Models of T1D-DKD

Type	Animal Model	Treatment	Strain	Ob	HBS	HBP	HTG	HC	PRO	Early Glomerular Pathology			Advanced Glomerular Pathology		TIF	GFR (#)	Ref.	
										Mesangial Matrix Expansion	GBM Thickening	Fibrosis or Mild Sclerosis	Nodular/Diffuse Sclerosis	Arterial Hyalinosis				
Chemical	Mice	STZ	B6	-	+	-	NA	NA	+	+	+	-	- / -	-	-	-	[13,14,18,19]	
			DBA/2	-	+	-	NA	NA	+	+ ^b	+	+	- / -	+	-	-		
			CD1	-	+	-	+	-	+	+ ^a	+ ^b	+	+	- / +	-	+		+
			I29Sv	-	+	+	NA	NA	±	+	+	+	-	- / -	-	-		-
			KK/HIJ	-	+	-	NA	NA	+	+	+	+	+	- / -	+	-		-
Rats	STZ	SD, Wistar		-	+	-	NA	NA	+	±	±	-	- / -	-	-	-	[24–28]	
			Zebrafish	STZ		NA	+	NA	NA	NA	-	NA	+	NA	NA	NA	NA	[15]
Surgical + Chemical	Mice Rats	UNx + STZ UNx + STZ	B6	-	+	-	NA	NA	+	+	+	+	- / -	-	+	-	[38]	
			SD, Wistar	-	+	-	NA	NA	+	+	+	+	+	- / -	-	-	-	[40,41]
			SHR	-	+	+	+	+	+	+	+	+	+	- / -	-	NA	NA	[42]
Genetic	Akita mice	Akita KO	B6	-	+	+	-	-	+	+	-	-	- / -	-	NA	-	[44,47]	
			DBA/2, I29/SvEv	-	+	+	NA	NA	+	+	-	-	-	- / -	-	NA	-	[44]
			KK/Ta	-	+	+	+	+	+	+	+	+	+	- / -	+	+	+	[46,47]
			Balb/c	-	+	+	NA	NA	+	+	+	+	+	- / -	-	-	+	[48]
			FVB	-	+	+	NA	NA	+ ^a	+ ^b	+	+	+	+ / -	-	+	+ ^b	[50,51]

Notes: +, positive; -, negative; ±, weakly positive or inconsistent results; NA, not available; a, > 10-fold; b, > 50%; #, vs normal value.

Abbreviations: T1D-DKD, type 1 diabetic kidney disease; Ob, Obesity; HBS, high blood sugar; HBP, high blood pressure; HTG, high triglycerides; HC, high cholesterol; PRO, Proteinuria; GMB, glomerular basement membrane; TIF, tubulointerstitial fibrosis; GFR, glomerular filtration rate; Ref., references; KO, knockout; STZ, streptozotocin; UNx, uninephrectomy.

Table 2 Biochemical and Histological Phenotypes in Translation Animal Models of T2D-DKD

Type	Animal Model	Treatment	Strain	Ob	HBS	HBP	HTC	HC	PRO	Early Glomerular Pathology			Advanced Glomerular Pathology		TIF	GFR (#)	Ref.		
										Mesangial Matrix Expansion	GBM Thickening	Fibrosis or Mild Sclerosis	Nodular/Diffuse Sclerosis	Arterial Hyalinosis					
Chemical	Mice Rats	STZ + NAD	B6	-	+	NA	NA	NA	+	+	+	+	- / -	-	+	-	[53,54]		
		STZ + NAD	Wistar	-	+	NA	±	±	+	+	+	+	- / -	-	+	-	[55-61]		
Dietary	Mice Chinese Bama minipigs	HFD	B6	+	+	+	+	+	+	+	+	-	- / -	-	-	-	[64,67-69]		
		HsuHF or HFHsuHC		+	±	NA	+	+	+	+	+	+	-	- / -	-	+	-	[71,72]	
Chemical + Dietary	Mice Rats	STZ + HFD	B6	±	+	NA	+	+	+	+	+	+	- / -	-	+	-	[80-87]		
		STZ + HFD	SD, Wistar	±	+	NA	+	±	+	+	+	+	- / -	-	-	-	[76-79]		
Surgical + Chemical + Dietary	Mice Rats	UNx + STZ + HFD	B6	NA	+	NA	NA	NA	+	+	+	+	- / -	+	+	-	[92]		
		UNx + STZ + HFD or UNx + STZ + NAD + HFD	SD, Wistar	±	+	±	+	+	+	+	+	+	- / ±	-	±	-	[32,91]		
Spontaneous	OLETF rats GK rats	DOCA or AngI + HSaD HFD	OLETF	+	+	±	+	+	+	+	+	+	+	+	+	-	-	[93-96]	
			GK	-	+	-	-	-	-	±	+	+	+	- / -	-	+	-	[103-105]	
				-	+	+	-	-	-	+	+	+	+	- / -	-	+	-	[103,106]	
				-	+	-	-	-	-	±	+	+	+	- / -	-	+	-	[107]	
Genetic	ob/ob mice	HPD	BTBR	+	+	-	+	+	+	+	+	+	± / +	+	±	-	[110]		
				+	+	-	+	+	+	+	+	+	± / +	+	+	-	[111]		
	db/db mice	HPD	BKS	+	+	-	+	+	+	+	+	+	-	- / -	±	-	-	[116-119,122]	
				+	+	NA	+	+	+	+	+	+	+	- / -	±	+	-	[121]	
	ZDF rats	HFD	UNx	+	+	-	+	+	+	+	+	+	+	- / -	-	+	+	[122,123]	
			DBA/2J	+	+	NA	+	+	+	+	+	+	+	+	- / -	-	-	-	[117]
	ZDF rats	HFD	FVB	+	+	NA	+	+	+	+	+	+	+	- / -	-	+	-	[124]	
			CD1	+	+	-	NA	NA	+	+	+	+	+	+	- / +	-	+	+	[115]
	ZSF1 rats	HFD	ZDF	+	+	±	+	+	+	+	+	+	+	+	- / -	-	+	-	[125,127-130]
				+	+	±	+	+	+	+	+	+	+	+	- / -	-	+	-	[130,131]
	KKA ^l /Ta mice	HFD	ZDF × SHHF	+	+	+	+	+	+	+	+	+	+	- / ±	+	+	+	[133-135]	
			KK/Ta	+	+	-	+	+	+	+	+	+	+	- / -	-	+	-	[138-140]	
	T2DN rats	HFD		+	+	-	+	+	+	+	+	+	+	- / -	-	+	-	[138]	
			T2DN/Mcwi	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	[142-144]

Notes: +, positive; -, negative; ±, weakly positive or inconsistent results; NA, not available; a, > 10-fold; b, > 50%; #, vs normal value.

Abbreviations: T2D-DKD, type 2 diabetic kidney disease; Ob, Obesity; HBS, high blood sugar; HBP, high blood pressure; HTC, high triglycerides; HC, high cholesterol; PRO, Proteinuria; GMB, glomerular basement membrane; TIF, tubulointerstitial fibrosis; GFR, glomerular filtration rate; Ref., references; STZ, streptozotocin; UNx, uninephrectomy; NAD, nicotinamide; HFD, high-fat diet; HsuHF, high-sugar-high-fat diet; HFHsuHC, high-fat-high-sugar-high-cholesterol diet; AngI, angiotensinI; DOCA, deoxycorticosterone acetate; HSaD, high-salt diet; HPD, high-protein diet.

Table 3 Biochemical and Histological Phenotypes in Complication Models of DKD and NADKD

Type	Animal Model	Treatment	Strain	Ob	HBS	HBP	HTG	HC	PRO	Early Glomerular Pathology			Advanced Glomerular Pathology		TIF	GFR (#)	Ref.
										Mesangial Matrix Expansion	GBM Thickening	Fibrosis or Mild Sclerosis	Nodular/Diffuse Sclerosis	Arterial Hyalinosis			
DKD × HBP	<i>eNOS</i> ^{-/-} / <i>db/db</i> mice	eNOSKO	BKS	+	+	+	-	+	+ ^a	+	+	+	+ / +	±	±	+ ^b	[152,153,162]
DKD × Atherosclerosis	IDOL-AAV-DJ/8- <i>ob/ob</i> mice	HFHC	BTBR (<i>ob/ob</i>)	+	+	NA	+	+	+ ^a	+ ^b	+	+	± / +	+	±	-	[166]
NADKD × Atherosclerosis	HFD- <i>ApoE</i> ^{-/-} mice	HFD	B6	+	+	NA	+	+	±	+	NA	+	- / -	-	+	-	[169]

Notes: +, positive; -, negative; ±, weakly positive or inconsistent results; NA, not available; a, > 10-fold; b, > 50%; #, vs normal value.

Abbreviations: DKD, diabetic kidney disease; NADKD, normoalbuminuric diabetic kidney disease; Ob, Obesity; HBS, high blood sugar; HBP, high blood pressure; HTG, high triglycerides; HC, high cholesterol; PRO, Proteinuria; GMB, glomerular basement membrane; TIF, tubulointerstitial fibrosis; GFR, glomerular filtration rate; Ref., references; KO, knockout; HFD, high-fat diet; HFHC high-fat-high-cholesterol diet.

Akita mice are the most commonly used for T1D-DKD studies, while db/db mice for T2D-DKD studies. A few animal models can develop advanced DKD phenotypes, among them, OVE26 mice, T2DN rats, *eNOS*^{-/-} db/db mice appear to be the most potent. Additionally, female OVE26 mice seem to be advantageous in the study of female DKD, while T2DN rats seem to be suitable for exploring sex differences in T2D-DKD. However, it is also important to pay attention to the adaptability of these models, as some classical genetic mutant models of DKD may no longer exhibit severe renal pathological changes due to adaptive changes.

Induction methods are an essential part of DKD modeling. This paper summarizes routine laboratory procedures for DKD (Figures 1 and 2). Standardizing modeling is crucial to improve the success rate and model stability, save resources and make experimental data more reliable. Furthermore, exploration traditional induction means and searching for new induction tools, such as adenovirus (AV), adeno-associated virus (AAV), double-stranded adeno-associated virus (dsAAV), Entranster™-in vivo, etc., could be the focus of future studies.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

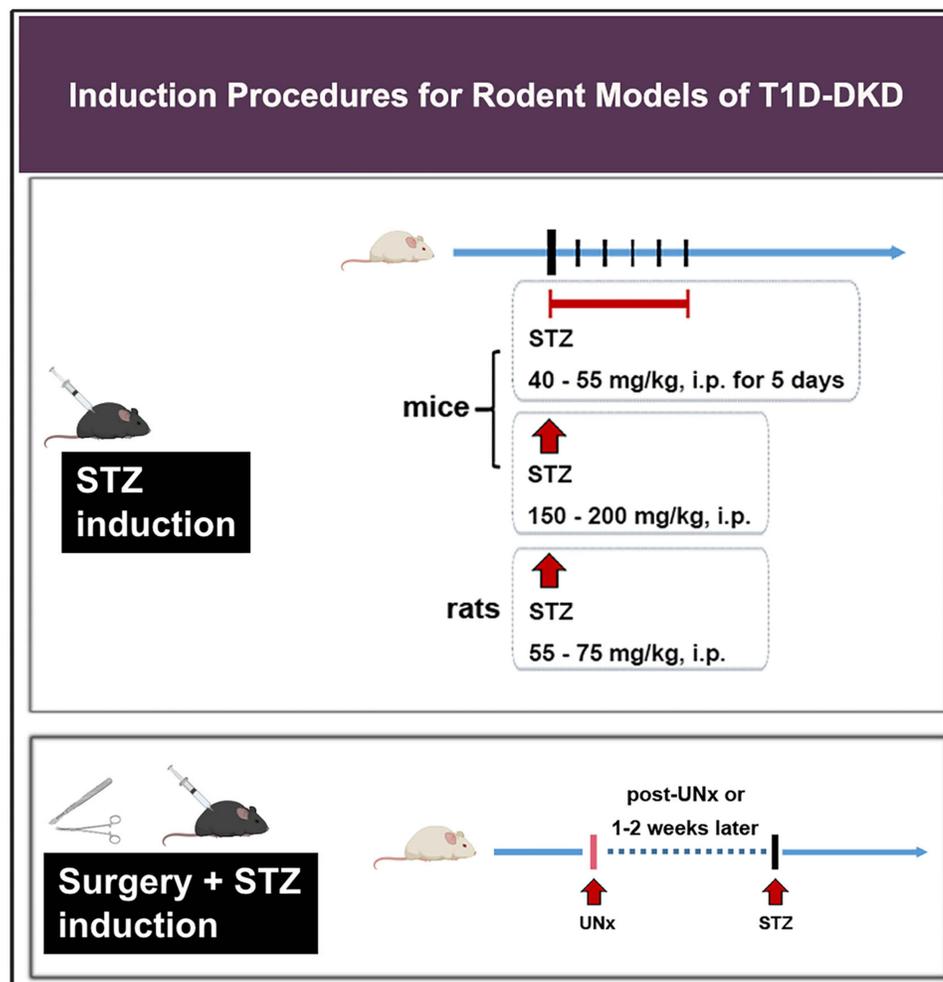


Figure 1 Induction procedures for rodent models of T1D-DKD.

Abbreviations: T1D-DKD, type I diabetic kidney disease; STZ, streptozotocin; UNx, uninephrectomy; i.p. intraperitoneal injection.

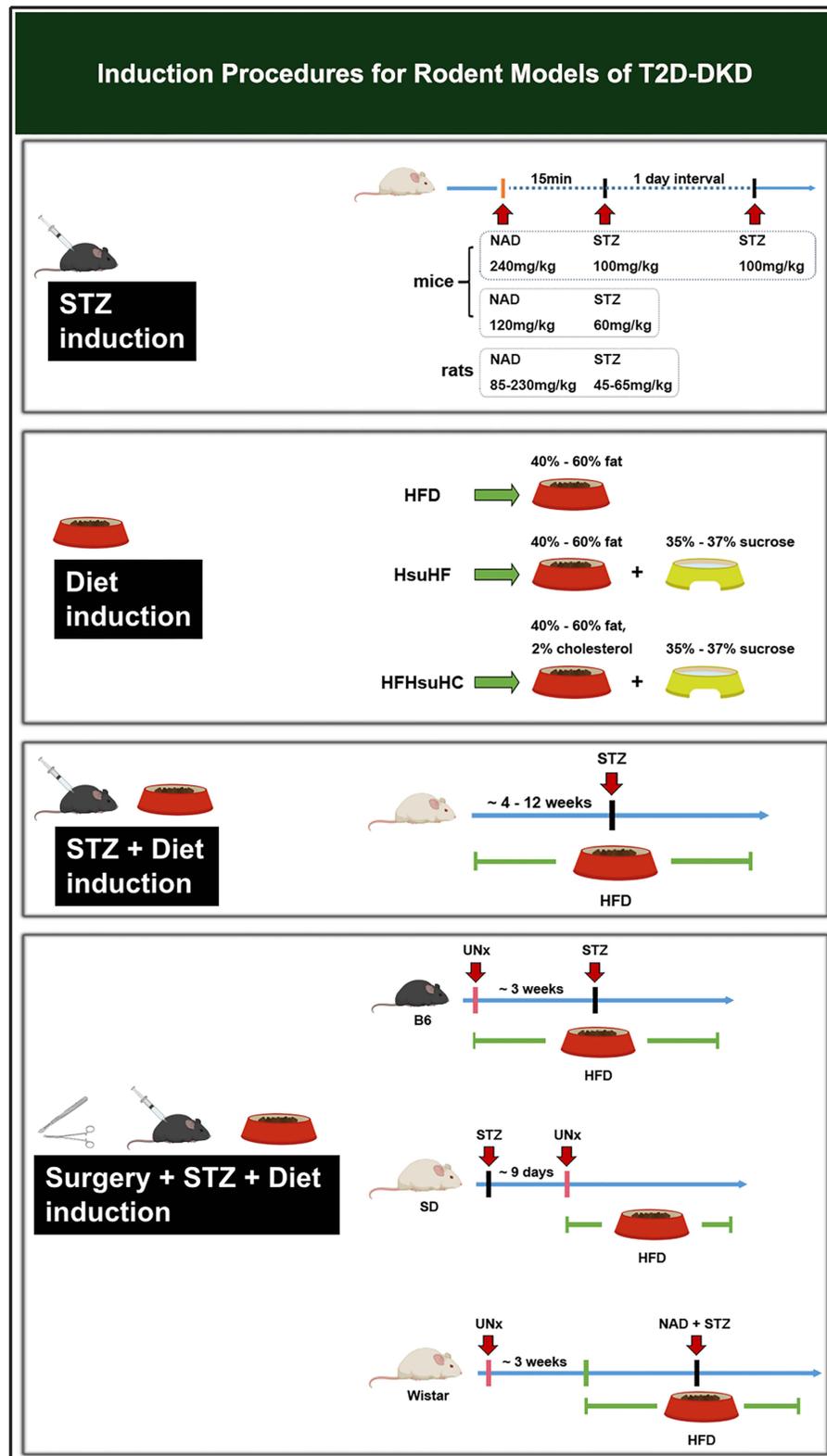


Figure 2 Induction procedures for rodent models of T2D-DKD.

Abbreviations: T2D-DKD, type 2 diabetic kidney disease; NAD, nicotinamide; STZ, streptozotocin; UNx, uninephrectomy; i.p. intraperitoneal injection; HFD, high-fat diet; HsuHF, high-sugar-high-fat diet; HFHsuHC, high-fat-high-sugar-high-cholesterol diet.

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Disclosure

The authors report no conflicts of interest in this work.

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