

## REVIEW

# Progress in experimental models to investigate the in vivo and in vitro antidiabetic activity of drugs

Yasodha Krishna Janapati<sup>1</sup>  | Sunil Junapudi<sup>2</sup>

<sup>1</sup>School of Pharmacy & Health Sciences, United States International University-AFRICA (USIU-A), Nairobi, Kenya

<sup>2</sup>Department of Pharmaceutical Chemistry, Geethanjali College of Pharmacy, Keesara, India

**Correspondence**

Yasodha Krishna Janapati, School of Pharmacy & Health Sciences, United States International University-AFRICA (USIU-A), Thika Road, P.O. Box 14634-00800, Nairobi, Kenya.  
Email: [yjanapati@usiu.ac.ke](mailto:yjanapati@usiu.ac.ke), [krishna.yasodha@gmail.com](mailto:krishna.yasodha@gmail.com)

**Funding information**

Not applicable.

**Abstract**

Diabetes mellitus is one of the world's most prevalent and complex metabolic disorders, and it is a rapidly growing global public health issue. It is characterized by hyperglycemia, a condition involving a high blood glucose level brought on by deficiencies in insulin secretion, decreased activity of insulin, or both. Prolonged effects of diabetes include cardiovascular problems, retinopathy, neuropathy, nephropathy, and vascular alterations in both macro- and micro-blood vessels. In vivo and in vitro models have always been important for investigating and characterizing disease pathogenesis, identifying targets, and reviewing novel treatment options and medications. Fully understanding these models is crucial for the researchers so this review summarizes the different experimental in vivo and in vitro model options used to study diabetes and its consequences. The most popular in vivo studies involves the small animal models, such as rodent models, chemically induced diabetogens like streptozotocin and alloxan, and the possibility of deleting or overexpressing a specific gene by knockout and transgenic technologies on these animals. Other models include virally induced models, diet/nutrition induced diabetic animals, surgically induced models or pancreatectomy models, and non-obese models. Large animals or non-rodent models like porcine (pig), canine (dog), nonhuman primate, and Zebrafish models are also outlined. The in vitro models discussed are murine and human beta-cell lines and pancreatic islets, human stem cells, and organoid cultures. The other enzymatic in vitro tests to assess diabetes include assay of amylase inhibition and inhibition of  $\alpha$ -glucosidase activity.

**KEYWORDS**

animal models, diabetes mellitus type I, diabetes mellitus type II, in vitro and in vivo models

## 1 | INTRODUCTION

Diabetes mellitus (DM), a noncommunicable, long-term, degenerative metabolic disease, has become a serious health issue for the global population. Chronic hyperglycemia is a feature of DM. The

primary cause of type 1 diabetes, observed mainly in children, is the loss of pancreatic beta cells.<sup>1</sup> Insulin resistance and a failure of the beta-cells to compensate are the two main contributing factors to type 2 diabetes, found mainly in obese people.<sup>2</sup> Diabetes is also seen in lean people, where it is known as fibrocalculous

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2024 The Author(s). *Animal Models and Experimental Medicine* published by John Wiley & Sons Australia, Ltd on behalf of The Chinese Association for Laboratory Animal Sciences.

pancreatic diabetes and classified as type 3 diabetes.<sup>3</sup> The long-term effects of DM include cardiovascular problems, retinopathy, neuropathy, nephropathy, and vascular alterations in both macro and microblood vessels.<sup>4</sup> The International Diabetic Federation has estimated that 1 in 10 adults, or 537 million people globally, have diabetes. According to their projections, there will be 643 million adults worldwide who have diabetes by 2030, and 784 million, that is, 1 in 8 individuals, by 2045. In 2021, diabetes contributed to 6.7 million fatalities, or 1 every five seconds. Over 240 million patients with diabetes are believed to go undiagnosed. Global health spending on diabetes was predicted to be USD 966 billion in 2021 (a 316% increase over the previous 15 years).<sup>5-7</sup> The two countries with the greatest prevalence of the disease are China with 141 million people<sup>8</sup> and India with 77 million people.<sup>9</sup> Diabetic people are more likely to contract the virus COVID-19 and are likely to experience more significant complications. Patients with comorbid conditions like diabetes and heart disease are more likely to experience problems arising from the recent world-wide COVID-19 epidemic.<sup>10</sup>

Diabetes poses an important threat to people's health and burdens society financially,<sup>6</sup> and one of the most popular areas of research currently is the management and treatment of DM. In particular, appropriate animal and advanced in vitro research is crucial for the establishment of innovative, efficient methods of treating conditions like diabetes.<sup>11</sup> More generally, use of animal models helps researchers create more effective treatments for many disorders and diseases. Humans and other mammals share many biologically related organs, including the heart, lungs, kidneys, liver, and other organs. They are genetically quite similar as well. For instance, the genes of mice and humans are almost identical.<sup>12</sup> All new medications must first pass legal testing on rodents (often mice or rats) and a bigger nonrodent mammal (typically a dog, pig, or monkey) before being administered to humans. This is done because unfavorable effects in either species frequently point to comparable reactions in people, and if a dose is toxic in both rodent and nonrodent species, it is probably also going to be toxic in people.<sup>13</sup> However, recently the FDA changed the legislation originally passed in 1938 on animal studies to state that they "no longer require drugs to be tested on animals".<sup>14</sup>

## 2 | METHODOLOGY

This review article is based on the databases PubMed, Cochrane, Virtual Health Library, High Wire, Science Direct, Web of Science, Elsevier, Wiley, and academic Google, etc. The databases were systematically searched for articles published in English from 1922 to 2023 with keywords like diabetes animal models, genetically modified rodent models, chemically induced models, surgical induced models, nonrodent models for type II diabetes, diabetic animal models like canine (dog), porcine (pig) models, feline (cat), obese rhesus monkey, virally induced diabetic type I animal models, transgenic/knock-out diabetic type I animals, and the cell line models.

## 3 | OBJECTIVE

In this this review article, we discuss diabetes complications, diabetes around the world, and diabetes models, including in vivo models and in vitro models for DM.

## 4 | ANIMAL MODELS FOR DIABETIC RESEARCH

To accomplish diabetic research, scientists have relied on animal models. Pioneering animal studies on DM in dogs were conducted by Nobel laureates Ivan Pavlov, Fedrick Banting, and Charles Best early last century.<sup>15</sup> Recently small animals like rodents (mice and rats) are more often exploited for diabetic research,<sup>16,17</sup> with the ability to delete or overexpress a specific gene by knockout and transgenic technologies making them popular models.<sup>18</sup> Large animals like porcine (pig) models,<sup>19</sup> canine (dog) models,<sup>20</sup> and nonhuman primate models,<sup>21</sup> as well as Zebrafish models<sup>22</sup> are outlined in Table 1.

### 4.1 | In vivo models for type 1 diabetes

Type 1 diabetes is a condition involving beta cells in the pancreas,<sup>1</sup> and therefore diabetic models are created using chemical induction,<sup>26,27</sup> genetically derived or spontaneously diabetic animals,<sup>28</sup> or genetically or virally induced animals,<sup>29,30</sup> in which the functions of pancreatic beta cells in the experimental animals are ultimately destroyed or modified, eventually leading to hyperglycemia, weight loss, hyperphagia etc.<sup>31,32</sup>

#### 4.1.1 | Chemically induced diabetes type 1 model

Chemical agent-induced diabetes in lab animals is the most prevalent option. Among the agents used are streptozotocin (STZ) and alloxan (ALX), both of which achieve a rapid outcome, resulting in an experimental model useful for elucidating the causes of human DM.<sup>17,33,34</sup> The toxic effects are only specific to pancreatic beta cells, other organs are spared, mortality is low and doses of these diabetogens are specified and have been optimized by many researchers.<sup>26,35</sup> Due to the rapid rate of beta cell regeneration, therapy is less durable and reversible.<sup>36</sup> The details of these chemicals such as chemical structure, IUPAC naming, chemical properties, mechanism of action, etc. are given in Table 2. Chemically induced experimental models are frequently chosen to test new diabetes medications and insulin formulations.<sup>37-39</sup> Other diabetogens used in experimental models are dithizone,<sup>40</sup> cyclosporine, tacrolimus,<sup>41</sup> dehydroascorbic acid, dehydroisoascorbic acid,<sup>42</sup> sodium diethyl dithiocarbonate,<sup>43</sup> potassium xanthate, uric acid, and lithium.<sup>44</sup>

TABLE 1 Animal models of type 1 and type 2 DM.

Animal models	Type 1 DM (non-obese models)	Type 2 DM (obese models)
Chemically induced	<ul style="list-style-type: none"> <li>• Streptozotocin (STZ)</li> <li>• Alloxan (ALX)</li> <li>• Ferric nitrilotriacetate</li> <li>• Dithizone</li> </ul>	<ul style="list-style-type: none"> <li>• Gold thioglucose (GTG) treated obese mice</li> </ul>
Genetically derived or spontaneous diabetic animals	<p>Rodent models</p> <ul style="list-style-type: none"> <li>• NOD (non-obese diabetic) mouse</li> <li>• BB (Bio Breeding) rat</li> <li>• LETL (Long-Evans Tokushima Lean) rat</li> <li>• KDP (Komeda diabetes-prone) rat</li> <li>• Lewis-IDDM (Lewis-insulin dependent diabetes mellitus) rat</li> </ul> <p>Non-rodent models</p> <ul style="list-style-type: none"> <li>• New Zealand rabbit</li> <li>• Keeshond dog</li> <li>• Chinese hamster</li> <li>• Macaca nemestrina</li> <li>• Fascicularis</li> <li>• Nigra papio hamadryas</li> </ul>	<p>Rodent models</p> <ul style="list-style-type: none"> <li>• ob/ob (obese) mouse</li> <li>• db/db mouse</li> <li>• KK (Kuo Kondo) mouse</li> <li>• KK/Ay (Kuo Kondo/Ay) mouse</li> <li>• NZO (New Zealand Obese) mouse</li> <li>• NONc/New Zealand obese 10 mouse</li> <li>• TSOD (Tsumara Suzuki Obese diabetes) mouse</li> <li>• M16 mouse</li> <li>• Zucker fatty rat</li> <li>• ZDF (Zucker diabetic fatty) rat</li> <li>• WDF (winter fatty) rat</li> </ul> <p>Non-rodent models</p> <ul style="list-style-type: none"> <li>• Obese rhesus monkey</li> <li>• Feline (cat)</li> </ul> <p>Non-obese models</p> <ul style="list-style-type: none"> <li>• Goto kakizaki (GK) rats</li> <li>• Cohen diabetic rat (CDR)</li> <li>• Spontaneously Diabetic Torii (SDT) rat</li> <li>• Alloxan susceptible Leiter mouse (ALS/Lt)</li> <li>• Alloxan-resistant Leiter mouse (ALR/Lt)</li> <li>• Human Islet Amyloid Polypeptide (hIAPP) mice</li> </ul>
Transgenic/knock-out diabetic animals	<ul style="list-style-type: none"> <li>• Insulin receptor substrate-1,2, glucose transporter-4, peroxisome proliferator activated receptor knockout mouse</li> <li>• Glucokinase knockout mouse</li> </ul>	<ul style="list-style-type: none"> <li>• Beta-3 receptor knockout mouse</li> <li>• Uncoupling protein (UCP1) knockout mouse</li> </ul>
Other models	<ul style="list-style-type: none"> <li>• Virally induced</li> <li>• Coxsackie B virus</li> <li>• Encephalomyocarditis virus</li> <li>• Kilham rat virus</li> <li>• Lymphocytic choriomeningitis virus (LCMV) under insulin promoter</li> <li>• Rubella</li> <li>• Mumps virus</li> </ul> <p>Surgical induced or pancreatectomy</p> <ul style="list-style-type: none"> <li>• Non-rodent animals like pigs dogs<sup>23,24</sup> and primates<sup>24,25</sup> had hyperglycemia after having a pancreatectomy</li> </ul>	<p>Diet or nutrition induced diabetic animals</p> <ul style="list-style-type: none"> <li>• C57/BL 6J mouse</li> <li>• Desert gerbil</li> <li>• Sand rat</li> <li>• Spiny mouse</li> <li>• Nile grass rat</li> </ul>

#### 4.1.2 | Genetically derived or spontaneous diabetic type 1 animals

The most commonly used animals for genetically derived type 1 DM are NOD mouse,<sup>52</sup> BB rat,<sup>53</sup> LETL rat,<sup>54</sup> KDP rat,<sup>54</sup> and LEW-IDDM rat.<sup>55</sup> Other animal models less frequently used are New Zealand rabbit,<sup>56</sup> Keeshond dog,<sup>57</sup> Chinese hamster,<sup>58</sup> and different monkeys such as *Macaca nemestrina*, *Fascicularis*, and *Nigra papio hamadryas*.<sup>59</sup> Genetic mutations that are naturally occurring frequently exhibit an isomorphic phenotypic resemblance between the diabetic animal and the diabetic person, and animals with these mutations are utilized in DM research.<sup>60</sup> The contrast between the more frequently used animals and humans are detailed in Table 3.<sup>29,44,54</sup> These animal models are generally monogenic and demonstrate distinct mechanisms of action,

whereas the human ADME system is much more complicated.<sup>61,62</sup> In addition, these animal models are naturally rare and post-diabetes care aimed at maintaining the animals' health is difficult.<sup>63,64</sup>

#### 4.1.3 | Transgenic/knock-out diabetic type 1 animals

Powerful techniques for determining the role of particular genes in glucose metabolism and the etiology of diabetes include knock-out and transgenic mice.<sup>29</sup> Pronuclear microinjection produces transgenic animals that often overexpress the transgene, while gene targeting produces animals with an endogenous target gene deleted or altered (knockout/knockin).<sup>65</sup> This method can elucidate which

TABLE 2 Correlation between alloxan and streptozotocin.<sup>34,45-51</sup>

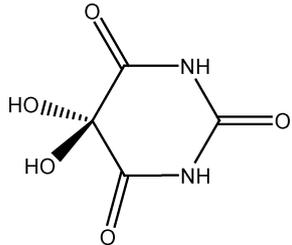
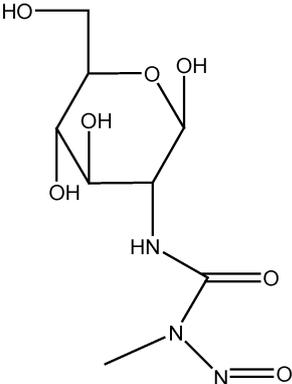
	Alloxan (ALX)	Streptozotocin (STZ)
Basic structure	Pyrimidinetrione	D-Glucopyranose
Chemical structures		
IUPAC name	5,5-Dihydroxypyrimidine-2,4,6(1H,3H,5H)-trione	3-(Tetrahydro-2,4,5-trihydroxy-6-(hydroxymethyl)-2H-pyran-3-yl)-1-methyl-1-nitrosourea
Chemical properties	Very hydrophilic, beta cell-toxic glucose analogue (partition coefficient -1.8); weak acid. Chemically unstable ( $t^{1/2}$ of 1.5 min at pH 7.4 and 37°C, decomposing to alloxanic acid); stable at acid pH	Hydrophilic, beta cell-toxic glucose analogue. Relatively stable at pH 7.4 and 37°C (at least for up to 1 h). Stable for 3 years if stored in refrigerator
Route of administration	Subcutaneous (SC)/intravenous (IV)/intraperitoneal (IP)	Subcutaneous (SC)/intravenous (IV)/intraperitoneal (IP)
Dose (mg/kg)	Rat 40–200 Mice 50–200 Rabbit 100–150 Dog 50–75	Rat 35–65 Mice 100–200 Rabbit 65 mg Dog 20–30
Mechanism of action	$\beta$ Cell toxicity by free radical generation	$\beta$ Cell toxicity by alkylation process
Acute hyperglycemia	45 min	120 min
Depletion of glycogen	Faster	Slow
Hypoglycemia	Less severe	More severe
Sensitivity to insulin	Yes	Yes
Reversibility	After 3 months	Irreversible
Mortality rate	37%	8%

TABLE 3 Characteristics of difference between humans and genetically derived or spontaneous diabetic animals.

Characteristics	Humans	NOD mouse	BB rat	KDP rat	LEW-IDDM rat
Diabetes development	Adolescence	24–30 weeks	8–16 weeks	12–16 weeks	8–12 weeks
MHC associated gene	Human leukocyte antigen—DR, DQ isotype	Unique I-Ag7	At least RT1 B/Du haplotype	At least RT1 B/Du haplotype	At least RT1 B/Du haplotype
Changes in the animal	Hyperglycemia, ketoacidosis	Hyperglycemia and leukocytic invasion of the pancreatic islet of Langerhans are characteristics of the polygenic type 1 diabetes model	Promptly undergo hyperglycemia and ketoacidosis	Spontaneous animal model with nonsense mutation in the Cblb and is a model of autoimmune type 1 diabetes	Develops insulin-dependent autoimmune diabetes on its own because of pancreatic cell death

transcription factor is responsible for pancreatic development and the signaling pathways of insulin.<sup>66–70</sup> The various animals in this category include insulin receptor substrate-1,2 (IRS-1,2) knockout

mouse,<sup>71</sup> glucose transporter-4 (GLUT4) knockout mouse,<sup>25</sup> peroxisome proliferator activated receptor (PPAR) knockout mouse,<sup>72</sup> and glucokinase knockout mouse.<sup>73</sup>

#### 4.1.4 | Virally induced diabetic type 1 animal models

Type 1 diabetes development has been attributed to viral infections.<sup>30</sup> Consequently, beta-cell destruction has been initiated using viruses in several animal models. Direct infection of the beta cell or the start of an autoimmune reaction against the beta cell can both result in destruction.<sup>74</sup> The various viruses used to induce DM are Coxsackie B virus,<sup>75</sup> encephalomyocarditis virus,<sup>76</sup> Kilham rat virus,<sup>77</sup> lymphocytic choriomeningitis virus (LCMV) under insulin promoter,<sup>78</sup> rubella,<sup>79</sup> and the mumps virus.<sup>80</sup>

The virus-induced approach can be challenging because the result depends on the virus replicability as well as the time of the infection.<sup>29</sup> Indeed, research has revealed that, depending on the circumstances, viruses can both cause and prevent autoimmunity.<sup>81</sup> While viruses have been connected to some type 1 diabetes cases in humans, the extent of the role they play in the disease's development is unknown.<sup>30,82</sup>

#### 4.1.5 | Surgically induced models (SIM) or pancreatectomy type 1 diabetic animal models

Non-rodent animals like pigs,<sup>83,84</sup> dogs,<sup>23,24</sup> and primates<sup>24,25</sup> have hyperglycemia after having a pancreatectomy. This model is a trusted way to cause hyperglycemia when a highly skilled and qualified surgeon is involved. However, the animal undergoes a fairly invasive procedure that raises the risk of hypoglycemia and causes pancreatic exocrine insufficiency.

### 4.2 | In vivo models for type 2 diabetes

Insulin resistance and the beta cell's failure to produce insulin to compensate are hallmarks of type 2 diabetes.<sup>2</sup> Consequently, types of animal models for type 2 diabetes include models of beta

cell loss and/or insulin resistance.<sup>44,50,51</sup> Obesity is prevalent in animal models of type 2 diabetes, mimicking the human scenario where obesity is directly associated with the development of type 2 diabetes.<sup>29</sup>

#### 4.2.1 | Genetically derived or spontaneous diabetic type 2 animals (obese model)

The most commonly used animals for type 2 DM are ob/ob (obese) mouse,<sup>85</sup> db/db mouse,<sup>86</sup> KK (Kuo Kondo) mouse,<sup>87</sup> KK/Ay (Kuo Kondo/Ay) mouse,<sup>88</sup> NZO (New Zealand Obese) mouse,<sup>89</sup> NONc/New Zealand obese 10 mouse,<sup>90</sup> TSOD (Tsumara Suzuki Obese diabetes) mouse,<sup>91</sup> M16 mouse,<sup>92</sup> Zucker fatty rat,<sup>93,94</sup> ZDF (Zucker diabetic fatty) rat,<sup>95</sup> and WDF (Wistar diabetic fatty) rat.<sup>96</sup> In the above models, development of diabetes is spontaneous and shares many characteristics with typical human type 2 DM. The majority of inbred animal models are homogeneous and under environmental control, which makes genetic analysis simple. Minimum outcome variability necessitates a small sample size.<sup>18</sup> The characteristics of some of these animals are described in Table 4.

#### 4.2.2 | Genetically derived or spontaneous diabetic type 2 animals (non-obese model)

Lean animal models of type 2 diabetes must also be explored because not all people with DM type 2 are obese. These include models with inadequate beta cells, which eventually results in overt type 2 diabetes in humans (Table 5).<sup>99,100</sup> These models, which include Goto Kakizaki (GK) rats,<sup>101</sup> Cohen diabetic rat (CDR),<sup>102</sup> spontaneously diabetic torii (SDT) rat,<sup>102</sup> Alloxan susceptible/Lt mouse,<sup>103</sup> human islet amyloid polypeptide (hIAPP) mice are rare.<sup>104</sup>

TABLE 4 Characteristics of genetically derived or spontaneous diabetic animals (obese model).

Animal	Diabetes development	Cause of DM	Physiological changes
ob/ob (obese) mouse <sup>85</sup>	3–4 weeks	Leptin deficiency	Hyperinsulinemia or insulin resistance, hyperglycemia, hyperlipidemia, obesity
db/db mouse <sup>86</sup>	4–8 weeks	Leptin deficiency	Hyperinsulinemia or insulin resistance, hyperphagic, obesity
KK (Kuo Kondo) mouse <sup>87</sup>	4–5 months	Antagonizing the melanocortin receptor 4 (MCR4) mouse	Hyperinsulinemia or insulin resistance, obesity
NZO (New Zealand obese) mouse <sup>89</sup>	9–12 weeks	Leptin resistance	Hyperinsulinemia or glucose tolerance, insulin resistance, hyperphagic, obesity
TSOD (Tsumara Suzuki obese diabetes) mouse <sup>91</sup>	2 months	Impaired GLUT4 translocation	Polydipsia, polyuria, hyperinsulinemia or insulin resistance, hypertrophy of pancreatic cells, obesity
M16 mouse <sup>92</sup>	3–6 weeks	Heper leptin	Hyperinsulinemia, weight gain, hyperleptinemia and hypercholesterolemia
Zucker fatty rat <sup>93,94</sup> and ZDF (Zucker diabetic fatty) rat <sup>95–98</sup>	4 weeks	Defect in leptin receptor signaling	Hyperinsulinemia, hyperlipidemia, glucose tolerance, hypertension, proteinuria, and renal failure

### 4.2.3 | Diet/nutrition induced diabetic type 2 animals

In these animal models, diabetes is not induced by chemicals or by genetic changes.<sup>12</sup> Due to insufficient islet compensation, high fat intake can result in obesity, insulin resistance, and impaired glucose homeostasis.<sup>110,111</sup> Examples of animals in this category are C57/BL 6J mouse, desert gerbil or sand rat,<sup>12,112</sup> spiny mouse, and Nile grass rat. The characteristics of these animal models are given in Table 6.

### 4.2.4 | Non-rodent models for type 2 diabetic animal models

Non-rodent animal models includes cats and obese rhesus monkeys. In many ways, feline diabetes mellitus is very similar to human T2DM, including the development of islet amyloid deposits, and complications in a number of organ systems, such as peripheral polyneuropathy and retinopathy.<sup>120-122</sup>

The rhesus monkey (*Macaca mulatta*), a non-rodent model of T2DM, offers the most comparable representation of metabolic problems in diabetes. If kept on an ad libitum laboratory diet, especially fructose, it develops obesity, hyperinsulinemia, and insulin resistance. Over several years, it proceeds to necrosis of beta cells, a sharp drop in insulin levels, and hyperglycemia.<sup>59,123,124</sup>

The Zebrafish model is an attractive model system for the study of metabolic abnormalities. Zebrafish have preserved energy balance and cholesterol metabolism. They are the perfect model for studying lipid metabolism and also, when given an abundance of laboratory nutrients, zebrafish are shown to have hepatic steatosis and higher plasma triglyceride levels. Its fully sequenced genome, ease of genetic manipulation, and greater fertility rates makes it a very versatile model.<sup>125-128</sup>

## 5 | ANIMAL MODELS FOR DIABETIC COMPLICATIONS

Diabetes mellitus is a chronic, sapping metabolic condition that can cause an enormous long-lasting increase in blood sugar levels. The resulting hyperglycemia plays a key role in the development of diabetic complications, such as damage to organs, both structural and functional, resulting in damage to the kidneys (diabetic nephropathy), eyes (diabetic retinopathy), and nerves (diabetic neuropathy).<sup>129,130</sup> It is also linked to chronic macrovascular problems such as peripheral vascular disease, coronary heart disease, and stroke (diabetic cardiomyopathy). It has also been discovered that the primary mechanism behind the pathogenesis of such diabetic complications is the generation of oxygen free radical species (ROS).<sup>11,131</sup> The animal models that are used to analyze these complications are listed in Table 7, along with their characteristics.

TABLE 5 Characteristics of genetically derived or spontaneous diabetic animals (non-obese model).

Animal	Diabetes development	Cause of DM	Physiological changes
Goto Kakizaki (GK) <sup>101</sup> rats	2-8 weeks	Inadequate pancreatic growth factors and compromised insulin sensitivity in the liver, skeletal muscle and adipose tissues	Hyperglycemia, retinopathy, nephropathy, decreased immune markers <sup>105</sup>
Cohen diabetic rat (CDR) <sup>102</sup>	2 months	Diet changes, reduced insulin secretion	Retinopathy, nephropathy, reduced fertility, testicular degeneration, <sup>106</sup> hyperglycemia can be retrieved by adjusting diet <sup>39</sup>
Spontaneously Diabetic Torii (SDT) rat <sup>107</sup>	20 weeks	Insulin resistance	Hyperinsulinemia or insulin resistance, ocular issues such as cataract, retinopathy, <sup>108</sup> gastropathy <sup>109</sup>
Alloxan susceptible/Lt mouse it is used to study both DM I & II <sup>103</sup>	6-8 weeks	Free radical stress	Hyperinsulinemia, impaired glucose tolerance <sup>103</sup>

TABLE 6 Characteristics of diet/nutrition induced diabetic type 2 animals.

Animal	Diabetes development	The diet used to induce DM	Physiological changes
Desert gerbil or sand rat ( <i>Psammomys obesus</i> ). <sup>113,114</sup>	16-24 weeks	High energy nutrition or laboratory chow	Hyperglycemia, ketoacidosis
Spiny mouse ( <i>Acomys cahirinus</i> ). <sup>115,116</sup>	1-2 weeks	High-energy rodent lab chow	Gain weight and exhibit marked pancreatic beta cell hyperplasia, hypertrophy, increased pancreatic insulin, and ketoacidosis
Nile grass rat <sup>117-119</sup>	8-10 weeks	High-energy rodent lab chow	Obesity, dyslipidemia, hyperglycemia, atherosclerosis, liver stenosis

TABLE 7 Experimental models for diabetic complications.<sup>11,129–131</sup>

Diabetic complications	Animal model	Characterization
Diabetic nephropathy <sup>129–132</sup>	Aldose reductase (ALR2) knockout mice ( <i>Aldor1</i> <sup>-/-</sup> )	Development of polyuria, polydipsia and diabetes insipidus
	BB rat	Enhanced GFR, thickening of glomerular basement membrane (GBM)
	C57BL/6	Albuminuria and reduced renal function
	Fat-fed STZ rat	Albuminuria and pathological changes
	Fructose-fed rats	Arteriopathy, renal hypertrophy and glomerular hypertension
	GK rat	Thickening of glomeruli leading to glomerular hypertrophy
	Goto-Kakizaki (GK)	Glomerular hypertrophy, GBM thickening. Segmental glomerulosclerosis, tubulointerstitial fibrosis
	NOD mice	Enlarged glomeruli and mesangial sclerosis
	MKR mice	Increased GFR, exhibit significant albuminuria
	Zebrafish	Overexpression of CIN85/RukL causing edema
Diabetic retinopathy <sup>130,133–137</sup>	Zucker diabetic fatty rat	Glomerulosclerosis, tubulointerstitial fibrosis and renal hypertrophy
	Alloxan induced model	Microaneurysms with increased acellular capillaries
	Akita mice	Decreased number of amacrine and ganglion cells
	AR deficient ( <i>AR</i> <sup>-/-</sup> ) mice	Retinal cell necrosis by leukocytosis.
	Diabetic Torii rats	Retinal thickening, Increased retinal leukostasis, massive hemorrhage.
	db/db mouse	Reduced number of retinal ganglion cells, and thickened retina
	Otsuka Long-Evans	Enhanced leukocyte, reduced retinal and retinal nerve fiber layer thickness twisted arteries and veins in the eye
	Tokushima fatty rats	Deterioration of retinal capillaries, and elevated generation of superoxide by the retina
Diabetic neuropathy <sup>130,138–140</sup>	Wild-type (WT; C57BL/6J)	Deterioration of retinal capillaries, and elevated generation of superoxide by the retina
	Zucker diabetic fatty rats (ZDF)	BM thickening, loss of endothelial cells (ECs) and pericytes, acellular capillaries, increased capillary hypercellularity
	Zebrafish	Degradation and thinning of the retina
	BKS-db/db	Increased thermal latency, lower tail-flick response to heat stimulus, decreased sensory nerve fiber velocity, axonal transport, and neurotransmitter levels.
	Spontaneous	Absence of myelinated fiber loss, shrinkage, and breakdown of the myelin sheath
	B6-ob/ob	Hypoalgesia, tactile allodynia, mechanical response and fiber loss. Increased PARP, immunofluorescence in the sciatic nerve, and spinal cord
	Spontaneous	
	C57BL/6J a diet high in fat	Increased thermal and mechanical latencies, hypoalgesia, hyperplasia allodynia. Peroxynitrite injury in peripheral nerve and dorsal root ganglion neurons
	C57BL/KS (db/db) mice	Decreased sensory nerve conduction velocity and density of intraepidermal nerve fibers (IENF)
	Chinese Hamster	Decreased nerve conduction velocity
Diabetic cardiomyopathy <sup>141–144</sup>	STZ induced rat model	Reduced fiber size of the peroneal nerve and axon than that of the myelin sheath with impaired motor function
	Zucker diabetic fatty rats (ZDF)	Reduced motor sensory, and sciatic blood stream. Structural variation in myelinated axons, which causes sensory loss. Elevated nerve sorbitol levels, thermal hyperalgesia
	Alloxan induced mode	Formation of advanced glycation end products leading to oxidative stress
	BB rats	Reduced calcium-stimulated ATPase activity and cardiac contractility
	GK rats	Hyperglycemia, hyperlipidemia and cardiac cell death
	OLETF rats	Alteration in left ventricular diastolic function
	STZ induced mode	Fibrosis and apoptosis leading to myocardial damage

## 6 | IN VITRO TECHNIQUES FOR ASSESSING DIABETES MELLITUS

In vitro (cell or tissue culture)<sup>145</sup> diabetic models are frequently employed by pharmaceutical companies in the search for new treatments. In vitro models can be employed initially for the screening

of test materials or to characterize the cellular or molecular actions of lead chemical substances in advanced phases of development. In vitro models of diabetes are also used in some basic pharmacological research to identify new treatment targets and gain a better understanding of the cellular and molecular mechanisms underlying the illness.<sup>146</sup> The primary tissues implicated in the

TABLE 8 Advantages and disadvantages of in vitro models for human diabetes research.

In vitro model	Advantages	Disadvantages
Murine beta-cell lines <sup>159,160</sup>	Simple to culture. There are numerous cell types readily available. A good opportunity to research cell physiology and test medications	The mouse cell line can be challenging to select because of differences from humans. Vascularization and cell-to-cell contact are absent
Human beta-cell lines <sup>161</sup>	Simple to culture. Established human beta-cell lines permit progress in human diabetes research and clinical applicability	Stable human cell lines are hard to make, and there aren't many of them. Genetic flaws are present in the majority of human cell lines. Grow slowly or respond poorly to glucose. Vascularization and cell-to-cell contact are absent
Murine pancreatic islets <sup>162,163</sup>	Can be isolated more quickly and inexpensively than human islets. Simple to genetically modify	Human islets have different islet morphology, vascularization, and blood flow to murine pancreatic islets
Human pancreatic islets <sup>164,165</sup>	Maintain the islet structure. Used to study the biology of the human pancreas	Few donors supply. Don't allow long functional studies. Heterogeneity in their characteristics: size, genetics
Human stem cells <sup>166,167</sup>	A renewable source of beta-cells. Can be genetically modified. Allow longer studies than pancreatic islets	To obtain them, a long and expensive process is needed
Organoid cultures <sup>168,169</sup>	Resemble the diseased organ architecture better than traditional 2D cultures	Don't have vascularization

pathophysiology of diabetes include the pancreas, liver, muscle, and adipose tissue. These tissues are typically employed to create in vitro models of diabetes used in the drug development process. In vitro models include primary cell cultures generated from normal, diabetic, transgenic animals, or cell lines derived from normal, or transgenic animals.<sup>130,131,147</sup> The advantages and disadvantages of in vitro models for human diabetes research are specified in Table 8. The other enzymatic in vitro tests to assess diabetes include assay of amylase inhibition and inhibition of  $\alpha$ -glucosidase activity.<sup>148-150</sup> Diabetic complications like diabetic nephropathy, and neuropathy can be assessed by optical fluorescence imaging of the structure of the kidney and nerves. Western blot, ELISA, and PCR can be used to analyze gene expression of inflammatory and oxidative markers. Flow cytometry can be used to investigate the degree of retinal endothelial cell death,<sup>150</sup> and concurrently it can enumerate overall beta islet cell health and beta cell glucose sensitivity.<sup>151</sup> Insulin secretion can be determined by ELISA.<sup>152</sup> The luciferase base assay<sup>153,154</sup> and the glucose uptake assay<sup>155</sup> can be done using radiolabeling methods.<sup>156,157</sup> Reporter gene assays can identify PPAR $\gamma$  and GLUT-4.<sup>158</sup>

## 7 | CONCLUSIONS

Studying the pathophysiology and clinical aspects of diabetes mellitus in humans is important because 537 million people are suffering from the disease and 240 million people remain undiagnosed. In addition, the health expenditure incurred in 2021 was 966 billion USD and is expected to increase to 1 trillion USD by 2045. To overcome this endemic condition many new drugs have been introduced into the market after testing in both in vivo and in vitro models. These models

of diabetes mellitus are very helpful research tools for testing any new synthetic or herbal drug. In this review, we have summarized the development of various models, including those induced by alloxan and streptozotocin, various models using small animal such as rodents, models involving deletion or overexpression of a specific gene using knockout and transgenic animals (immunogenic), and virally induced diabetic models. We also summarize obese and non-obese models, and diet or nutrition-induced models, as well as non-rodent models which are unique for assessing type 2 DM. The Zebrafish model is considered the most appropriate and advanced model for the screening of diabetes and its complications, that is microvascular complications and retinopathy, but it cannot be used for assessing diabetic nephropathy because of primitive renal cells. Diabetes and diabetic neuropathy can be tested best using rodent models due to their similarity structurally, molecularly, and functionally to humans. These models are also the cheapest and mostly easily available, and are easy to handle and maintain compared to other models. The use of large animals like pigs, monkeys, cats, and dogs is considered for pharmacological screening of diabetes induced by chemicals like alloxan, streptozotocin and even by pancreatectomy, but cost, handling and maintenance are some of the issues to be considered. In vitro models like murine and human beta and pancreatic islet cell lines, human stem cells and organoid culture are also discussed, with their advantages and disadvantages. Although no known animal species closely resembles human diabetes, each model serves as a vital tool for research into genetic, endocrine, metabolic, and morphologic changes, and underlying etiopathogenic changes. The study's design will determine the animal model to use. More suitable animal models could be used if the subsets of type 1 and type 2 diabetes are better understood. The wide range of disease manifestations in either type 1 or type 2 diabetes makes it unlikely that one animal model (or one treatment) will fit. Therefore, it is important

to be aware of the advantages and shortcomings of current models (in vivo and in vitro) and to conduct studies bearing in mind that no single model captures all the features or symptoms of disease. Selecting the right animal model can yield crucial information about the pathophysiology process underlying the disease, and this review emphasizes the appropriate use of a variety of animal models whenever possible.

## AUTHOR CONTRIBUTIONS

Yasodha Krishna Janapati: Wrote the manuscript, and corresponding author, conceptualization: conceived and designed the experiments, edited the manuscript and analyzed the data. Sunil Junapudi: Conceptualization, conceived and designed the experiments, edited the manuscript, and critically reviewed the article.

## ACKNOWLEDGMENTS

The authors are thankful to the School of Pharmacy and Health Sciences, USIU-A and Geethanjali College of Pharmacy for providing all the necessary tools and sources for writing this review.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## ETHICS STATEMENT

Not applicable.

## ORCID

Yasodha Krishna Janapati  <https://orcid.org/0000-0002-0151-0470>

## REFERENCES

- Roep BO, Thomaidou S, van Tienhoven R, Zaldumbide A. Type 1 diabetes mellitus as a disease of the  $\beta$ -cell (do not blame the immune system?). *Nat Rev Endocrinol*. 2021;17(3):150-161. doi:10.1038/s41574-020-00443-4
- Wysham C, Shubrook J. Beta-cell failure in type 2 diabetes: mechanisms, markers, and clinical implications. *Postgrad Med*. 2020;132(8):676-686. doi:10.1080/00325481.2020.1771047
- Unnikrishnan R, Anjana RM, Mohan V. Diabetes mellitus and its complications in India. *Nat Rev Endocrinol*. 2016;12(6):357-370.
- Wang M, Liang Y, Chen K, et al. The management of diabetes mellitus by mangiferin: advances and prospects. *Nanoscale*. 2022;14(6):2119-2135. doi:10.1039/D1NR06690K
- <https://diabetesatlas.org/>
- Bommer C, Heesemann E, Sagalova V, et al. The global economic burden of diabetes in adults aged 20–79 years: a cost-of-illness study. *Lancet Diabetes Endocrinol*. 2017;5(6):423-430. doi:10.1016/S2213-8587(17)30097-9
- Zhang P, Gregg E. Global economic burden of diabetes and its implications. *Lancet Diabetes Endocrinol*. 2017;5(6):404-405. doi:10.1016/S2213-8587(17)30100-6
- [https://www.statista.com/topics/6556/diabetes-in-china/#topicHeader\\_\\_wrapper](https://www.statista.com/topics/6556/diabetes-in-china/#topicHeader__wrapper)
- Pradeepa R, Mohan V. Epidemiology of type 2 diabetes in India. *Indian J Ophthalmol*. 2021;69(11):2932-2938. doi:10.4103/ijo.IJO\_1627\_21
- <https://Diabetes.Org/>
- Kottaisamy CPD, Raj DS, Prasanth Kumar V, Sankaran U. Experimental animal models for diabetes and its related complications—a review. *Lab Anim Res*. 2021;37(1):23. doi:10.1186/s42826-021-00101-4
- <https://www.genome.gov/10001345/importance-of-mouse-genome>
- Archibald K. *Animal Experimentation: Working Towards a Paradigm Change*. Brill;2019. <https://brill.com/display/book/edcoll/9789004391192/BP000023.xml>
- Hernandez J. *The FDA No Longer Requires All Drugs to Be Tested on Animals Before Human Trials*; 2023. <https://www.npr.org/2023/01/12/1148529799/fda-animal-testing-pharmaceuticals-drug-development>
- Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA. Pancreatic extracts in the treatment of diabetes mellitus. *Can Med Assoc J*. 1922;12(3):141-146.
- Etuk EU. Animal models for studying diabetes mellitus. *Agric Biol JN Am*. 2010;1(2):130-134.
- Rees DA, Alcolado JC. Animal models of diabetes mellitus. *Diabet Med J Br Diabet Assoc*. 2005;22(4):359-370. doi:10.1111/j.1464-5491.2005.01499.x
- Eddouks M, Chattopadhyay D, Zeggwagh NA. Animal models as tools to investigate antidiabetic and anti-inflammatory plants. *Evid Based Complement Alternat Med*. 2012;2012:142087. doi:10.1155/2012/142087
- Renner S, Blutke A, Clauss S, et al. Porcine models for studying complications and organ crosstalk in diabetes mellitus. *Cell Tissue Res*. 2020;380(2):341-378. doi:10.1007/s00441-019-03158-9
- Pöppel AG, de Carvalho GLC, Vivian IF, Corbellini LG, González FHD. Canine diabetes mellitus risk factors: a matched case-control study. *Res Vet Sci*. 2017;114:469-473. doi:10.1016/j.rvsc.2017.08.003
- Pound LD, Kievit P, Grove KL. The nonhuman primate as a model for type 2 diabetes. *Curr Opin Endocrinol Diabetes Obes*. 2014;21(2):89-94. doi:10.1097/MED.0000000000000043
- Salehpour A, Rezaei M, Khoradmeh A, Tahamtani Y, Tamadon A. Which hyperglycemic model of zebrafish (*Danio rerio*) suites my type 2 diabetes mellitus research? A scoring system for available methods. *Front Cell Dev Biol*. 2021;9:652061. doi:10.3389/fcell.2021.652061
- Adin CA, Gilor C. The diabetic dog as a translational model for human islet transplantation. *Yale J Biol Med*. 2017;90(3):509-515.
- Heeley AM, O'Neill DG, Davison LJ, Church DB, Corless EK, Brodbelt DC. Diabetes mellitus in dogs attending UK primary-care practices: frequency, risk factors and survival. *Canine Med Genet*. 2020;7(1):6. doi:10.1186/s40575-020-00087-7
- Wang TN, Hu XG, Chen GX. Uses of knockout, knockdown, and transgenic models in the studies of glucose transporter 4. *World J Meta-Anal*. 2022;10(1):1-11. doi:10.13105/wjma.v10.i1.1
- Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. *Diabetologia*. 2008;51:216-226. doi:10.1007/s00125-007-0886-7
- Ledoux SP, Wilson GL. Effects of streptozotocin on a clonal isolate of rat insulinoma cells. *Biochim Biophys Acta BBA—Mol Cell Res*. 1984;804(4):387-392. doi:10.1016/0167-4889(84)90064-8
- Lenzen S, Tiedge M, Elsner M, et al. The LEW.1AR1/Ztm-iddm rat: a new model of spontaneous insulin-dependent diabetes mellitus. *Diabetologia*. 2001;44(9):1189-1196. doi:10.1007/s001250100625
- King AJ. The use of animal models in diabetes research: animal models of diabetes. *Br J Pharmacol*. 2012;166(3):877-894. doi:10.1111/j.1476-5381.2012.01911.x
- van der Werf N, Kroese FGM, Rozing J, Hillebrands JL. Viral infections as potential triggers of type 1 diabetes. *Diabetes Metab Res Rev*. 2007;23(3):169-183. doi:10.1002/dmrr.695
- Xiao Y, Karam C, Yi J, et al. ROS-related mitochondrial dysfunction in skeletal muscle of an ALS mouse model during the disease progression. *Pharmacol Res*. 2018;138:25-36. doi:10.1016/j.phrs.2018.09.008
- Han J, Liu YQ. Reduction of islet pyruvate carboxylase activity might be related to the development of type 2 diabetes mellitus in agouti-K mice. *J Endocrinol*. 2010;204(2):143-152. doi:10.1677/JOE-09-0391

33. Rider BJ. Streptozotocin. In: Enna SJ, Bylund DB, eds. *xPharm: The Comprehensive Pharmacology Reference*. Elsevier; 2007:1-4. doi:[10.1016/B978-008055232-3.62679-0](https://doi.org/10.1016/B978-008055232-3.62679-0)
34. Mostafavinia A, Amini A, Ghorishi SK, Pouriran R, Bayat M. The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of type1 diabetes mellitus and mortality rate in rats. *Lab Anim Res*. 2016;32(3):160-165. doi:[10.5625/lar.2016.32.3.160](https://doi.org/10.5625/lar.2016.32.3.160)
35. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. *Indian J Med Res*. 2007;125(3):451-472.
36. White MG, Shaw JAM, Taylor R. Type 2 diabetes: the pathologic basis of reversible  $\beta$ -cell dysfunction. *Diabetes Care*. 2016;39(11):2080-2088. doi:[10.2337/dc16-0619](https://doi.org/10.2337/dc16-0619)
37. Sheshala R, Peh KK, Darwis Y. Preparation, characterization, and in vivo evaluation of insulin-loaded PLA-PEG microspheres for controlled parenteral drug delivery. *Drug Dev Ind Pharm*. 2009;35(11):1364-1374. doi:[10.3109/03639040902939213](https://doi.org/10.3109/03639040902939213)
38. Jansson L, Eizirik DL, Pipeleers DG, Borg LA, Hellerström C, Andersson A. Impairment of glucose-induced insulin secretion in human pancreatic islets transplanted to diabetic nude mice. *J Clin Invest*. 1995;96(2):721-726. doi:[10.1172/JCI118115](https://doi.org/10.1172/JCI118115)
39. Graham ML, Janeczek JL, Kittredge JA, Hering BJ, Schuurman HJ. The streptozotocin-induced diabetic nude mouse model: differences between animals from different sources. *Comp Med*. 2011;61(4):356-360.
40. Halim D, Khalifa K, Awadallah R, El-Hawary Z, El-Dessouky EA. Serum mineral changes in dithizone-induced diabetes before and after insulin treatment. *Z Ernährungswiss*. 1977;16(1):22-26. doi:[10.1007/BF02021207](https://doi.org/10.1007/BF02021207)
41. Penfornis A, Kury-Paulin S. Immunosuppressive drug-induced diabetes. *Diabetes Metab*. 2006;32(5 Pt 2):539-546. doi:[10.1016/s1262-3636\(06\)72809-9](https://doi.org/10.1016/s1262-3636(06)72809-9)
42. Clemetson CA. Ascorbic acid and diabetes mellitus. *Med Hypotheses*. 1976;2(5):193-194. doi:[10.1016/0306-9877\(76\)90037-2](https://doi.org/10.1016/0306-9877(76)90037-2)
43. Kadota I, Midorikawa O. Diabetogenic action of organic reagents: destructive lesions of islets of Langerhans caused by sodium diethylthiocarbamate and potassium ethylxanthate. *J Lab Clin Med*. 1951;38(5):671-688.
44. Dhuria RS, Singh G, Kaur A, Kaur R, Kaur T. Current status and patent prospective of animal models in diabetic research. *Adv Biomed Res*. 2015;4:117. doi:[10.4103/2277-9175.157847](https://doi.org/10.4103/2277-9175.157847)
45. Papich MG. Streptozotocin. In: Papich MG, ed. *Saunders Handbook of Veterinary Drugs*. 4th ed. W.B. Saunders; 2016:742-743. doi:[10.1016/B978-0-323-24485-5.00523-4](https://doi.org/10.1016/B978-0-323-24485-5.00523-4)
46. Wallig MA. Chapter 20: Endocrine system. In: Wallig MA, Haschek WM, Rousseaux CG, Bolon B, eds. *Fundamentals of Toxicologic Pathology*. 3rd ed. Academic Press; 2018:565-624. doi:[10.1016/B978-0-12-809841-7.00020-4](https://doi.org/10.1016/B978-0-12-809841-7.00020-4)
47. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiol Res*. 2001;50(6):537-546.
48. Islam M, Code Q. Streptozotocin is more convenient than Alloxan for the induction of type 2 diabetes. *IJPR*. 2017;7(1):10-7439. doi:[10.7439/ijpr.v7i1.3790](https://doi.org/10.7439/ijpr.v7i1.3790)
49. Saleem Mir M, Maqbool Darzi M, Khalil Baba O, et al. Streptozotocin induced acute clinical effects in rabbits (*Oryctolagus cuniculus*). *Iran J Pathol*. 2015;10(3):206-213.
50. Janapati Y. Optimize diabetes by herbal medicine: a review. *J Adv Med Pharm Sci*. 2015;3(3):98-111. doi:[10.9734/JAMPS/2015/15873](https://doi.org/10.9734/JAMPS/2015/15873)
51. Janapati YK, Junapudi S, Dachani SR. Optimization of diabetes by herbal medicine. *Technol Innov Manag Rev*. 2021;6:1-18. doi:[10.9734/Bpi/Tipr/v6/2277E](https://doi.org/10.9734/Bpi/Tipr/v6/2277E)
52. Chen D, Thayer TC, Wen L, Wong FS. Mouse models of autoimmune diabetes: the nonobese diabetic (NOD) mouse. *Methods Mol Biol*. 2020;2128:87-92. doi:[10.1007/978-1-0716-0385-7\\_6](https://doi.org/10.1007/978-1-0716-0385-7_6)
53. Bortell R, Yang C. The BB rat as a model of human type 1 diabetes. *Methods Mol Biol*. 2012;933:31-44. doi:[10.1007/978-1-62703-068-7\\_3](https://doi.org/10.1007/978-1-62703-068-7_3)
54. Yokoi N, Namae M, Fuse M, et al. Establishment and characterization of the Komeda diabetes-prone rat as a segregating inbred strain. *Exp Anim*. 2003;52(4):295-301. doi:[10.1538/expanim.52.295](https://doi.org/10.1538/expanim.52.295)
55. Weiss H, Bleich A, Hedrich HJ, et al. Genetic analysis of the LEW.1AR1-iddm rat: an animal model for spontaneous diabetes mellitus. *Mamm Genome*. 2005;16(6):432-441. doi:[10.1007/s00335-004-3022-8](https://doi.org/10.1007/s00335-004-3022-8)
56. Conaway HH, Faas FH, Smith SD, Sanders LL. Spontaneous diabetes mellitus in the New Zealand white rabbit: physiologic characteristics. *Metabolism*. 1981;30(1):50-56. doi:[10.1016/0026-0495\(81\)90218-3](https://doi.org/10.1016/0026-0495(81)90218-3)
57. Kramer JW. Animal model of human disease: inherited early-onset, insulin-requiring diabetes mellitus in keeshond dogs. *Am J Pathol*. 1981;105(2):194-196.
58. Gerritsen GC. The Chinese hamster as a model for the study of diabetes mellitus. *Diabetes*. 1982;31(suppl 1):14-23. doi:[10.2337/diab.31.1.S14](https://doi.org/10.2337/diab.31.1.S14)
59. Harwood HJ Jr, Listrani P, Wagner JD. Nonhuman primates and other animal models in diabetes research. *J Diabetes Sci Technol*. 2012;6(3):503-514. doi:[10.1177/193229681200600304](https://doi.org/10.1177/193229681200600304)
60. Hau J. Animal models for human diseases. In: Conn PM, ed. *Sourcebook of Models for Biomedical Research*. Humana Press; 2008:3-8. doi:[10.1007/978-1-59745-285-4\\_1](https://doi.org/10.1007/978-1-59745-285-4_1)
61. Bnouham MM, Ziyat AA, Mekhfi HH, Tahri AA, Legssyer AA. Medicinal plants with potential antidiabetic activity—a review of ten years of herbal medicine research (1990–2000). *Int J Diabetes Metab*. 2006;14(1):1-25. doi:[10.1159/000497588](https://doi.org/10.1159/000497588)
62. Serreze DV, Niens M, Kulik J, Di Lorenzo TP. Bridging mice to men: using HLA transgenic mice to enhance the future prediction and prevention of autoimmune type 1 diabetes in humans. In: Proetzl G, Wiles MV, eds. *Mouse Models for Drug Discovery*. Vol 602. Methods in Molecular Biology. Humana Press; 2010:119-134. doi:[10.1007/978-1-60761-058-8\\_8](https://doi.org/10.1007/978-1-60761-058-8_8)
63. Hasan MDM, Ahmed QU, Mat Soad SZ, Tunna TS. Animal models and natural products to investigate in vivo and in vitro antidiabetic activity. *Biomed Pharmacother*. 2018;101:833-841. doi:[10.1016/j.biopha.2018.02.137](https://doi.org/10.1016/j.biopha.2018.02.137)
64. Müller G. Methods to induce experimental diabetes mellitus. In: Hock FJ, ed. *Drug Discovery and Evaluation: Pharmacological Assays*. Springer International Publishing; 2016:2569-2581. doi:[10.1007/978-3-319-05392-9\\_63](https://doi.org/10.1007/978-3-319-05392-9_63)
65. Britsch S. Transgenic and knock-out animals. *Encyclopedic Reference of Genomics and Proteomics in Molecular Medicine*. Springer; 2006:1900-1903. doi:[10.1007/3-540-29623-9\\_1140](https://doi.org/10.1007/3-540-29623-9_1140)
66. Habener JF, Kemp DM, Thomas MK. Minireview: transcriptional regulation in pancreatic development. *Endocrinology*. 2005;146(3):1025-1034. doi:[10.1210/en.2004-1576](https://doi.org/10.1210/en.2004-1576)
67. Kahn CR. Knockout mice challenge our concepts of glucose homeostasis and the pathogenesis of diabetes. *Exp Diabetes Res*. 2003;4(3):169-182. doi:[10.1155/EDR.2003.169](https://doi.org/10.1155/EDR.2003.169)
68. Wang Q, Jin T. The role of insulin signaling in the development of  $\beta$ -cell dysfunction and diabetes. *Islets*. 2009;1(2):95-101. doi:[10.4161/isl.1.2.9263](https://doi.org/10.4161/isl.1.2.9263)
69. Neubauer N, Kulkarni RN. Molecular approaches to study control of glucose homeostasis. *ILAR J*. 2006;47(3):199-211. doi:[10.1093/ilar.47.3.199](https://doi.org/10.1093/ilar.47.3.199)
70. Accili D, Drago J, Lee EJ, et al. Early neonatal death in mice homozygous for a null allele of the insulin receptor gene. *Nat Genet*. 1996;12(1):106-109. doi:[10.1038/ng0196-106](https://doi.org/10.1038/ng0196-106)
71. Roncero I, Alvarez E, Acosta C, et al. Insulin-receptor substrate-2 (IRS-2) is required for maintaining glucokinase and glucokinase regulatory protein expression in mouse liver. *PLoS ONE*. 2013;8(4):e58797. doi:[10.1371/journal.pone.0058797](https://doi.org/10.1371/journal.pone.0058797)
72. Yan R, Zhang Y, Yang Y, et al. Peroxisome proliferator-activated receptor gene knockout promotes podocyte injury in diabetic mice [retracted in: *Biomed Res Int*. 2022 Nov 23;2022:9764610]. *Biomed Res Int*. 2022;2022:9018379. doi:[10.1155/2022/9018379](https://doi.org/10.1155/2022/9018379)

73. Baker DJ, Atkinson AM, Wilkinson GP, Coope GJ, Charles AD, Leighton B. Characterization of the heterozygous glucokinase knockout mouse as a translational disease model for glucose control in type 2 diabetes. *Br J Pharmacol*. 2014;171(7):1629-1641. doi:10.1111/bph.12498
74. Jun HS, Yoon JW. A new look at viruses in type 1 diabetes. *Diabetes Metab Res Rev*. 2003;19(1):8-31. doi:10.1002/dmrr.337
75. Horwitz MS, Bradley LM, Harbertson J, Krahl T, Lee J, Sarvetnick N. Diabetes induced by Coxsackie virus: initiation by bystander damage and not molecular mimicry. *Nat Med*. 1998;4(7):781-785. doi:10.1038/nm0798-781
76. Yoon JW, Jun HS. Viruses cause type 1 diabetes in animals. *Ann N Y Acad Sci*. 2006;1079:138-146. doi:10.1196/annals.1375.021
77. Alkanani AK, Hara N, Gianani R, Zipris D. Kilham rat virus-induced type 1 diabetes involves beta cell infection and intra-islet JAK-STAT activation prior to insulinitis. *Virology*. 2014;468-470:19-27. doi:10.1016/j.virol.2014.07.041
78. von Herrath MG, Coon B, Wolfe T, Chatenoud L. Nonmitogenic CD3 antibody reverses virally induced (rat insulin promoter-lymphocytic choriomeningitis virus) autoimmune diabetes without impeding viral clearance. *J Immunol*. 2002;168(2):933-941. doi:10.4049/jimmunol.168.2.933
79. Menser MA, Forrest JM, Bransby RD. Rubella infection and diabetes mellitus. *Lancet*. 1978;1(8055):57-60. doi:10.1016/s0140-6736(78)90001-6
80. Craighead JE. Viral diabetes mellitus in man and experimental animals. *Am J Med*. 1981;70(1):127-134. doi:10.1016/0002-9343(81)90419-8
81. von Herrath M, Filippi C, Coppieters K. How viral infections enhance or prevent type 1 diabetes-from mouse to man. *J Med Virol*. 2011;83(9):1672. doi:10.1002/jmv.22063
82. Richardson SJ, Willcox A, Bone AJ, Foulis AK, Morgan NG. The prevalence of enteroviral capsid protein vp1 immunostaining in pancreatic islets in human type 1 diabetes. *Diabetologia*. 2009;52(6):1143-1151. doi:10.1007/s00125-009-1276-0
83. Morel P, Kaufmann DB, Matas AJ, et al. Total pancreatectomy in the pig for islet transplantation. *Tech Altern Transplant*. 1991;52(1):11-15. doi:10.1097/00007890-199107000-00002
84. Zettler S, Renner S, Kemter E, et al. A decade of experience with genetically tailored pig models for diabetes and metabolic research. *Anim Reprod*. 2020;17(3):e20200064. doi:10.1590/1984-3143-AR2020-0064
85. Suriano F, Vieira-Silva S, Falony G, et al. Novel insights into the genetically obese (ob/ob) and diabetic (db/db) mice: two sides of the same coin. *Microbiome*. 2021;9(1):147. doi:10.1186/s40168-021-01097-8
86. Guest PC, Rahmoune H. Characterization of the db/db mouse model of type 2 diabetes. *Methods Mol Biol*. 2019;1916:195-201. doi:10.1007/978-1-4939-8994-2\_18
87. Fu C, Zhang X, Ye F, Yang J. High insulin levels in KK-ay diabetic mice cause increased cortical bone mass and impaired trabecular micro-structure. *Int J Mol Sci*. 2015;16(4):8213-8226. doi:10.3390/ijms16048213
88. Tomino Y. Lessons from the KK-ay mouse, a spontaneous animal model for the treatment of human type 2 diabetic nephropathy. *Nephro-Urol Mon*. 2012;4(3):524-529. doi:10.5812/numonthly.1954
89. Kluge R, Scherneck S, Schürmann A, Joost HG. Pathophysiology and genetics of obesity and diabetes in the New Zealand obese mouse: a model of the human metabolic syndrome. *Methods Mol Biol*. 2012;933:59-73. doi:10.1007/978-1-62703-068-7\_5
90. Leiter EH, Reifsnnyder PC. Differential levels of diabetogenic stress in two new mouse models of obesity and type 2 diabetes. *Diabetes*. 2004;53(suppl\_1):S4-S11. doi:10.2337/diabetes.53.2007.S4
91. Hirayama I, Yi Z, Izumi S, et al. Genetic analysis of obese diabetes in the TSOD mouse. *Diabetes*. 1999;48(5):1183-1191. doi:10.2337/diabetes.48.5.1183
92. Allan MF, Eisen EJ, Pomp D. The M16 mouse: an outbred animal model of early onset polygenic obesity and diabetes. *Obes Res*. 2004;12(9):1397-1407. doi:10.1038/oby.2004.176
93. Otani K, Funada H, Teranishi R, Okada M, Yamawaki H. Cardiovascular characteristics of Zucker fatty diabetes mellitus rats, an animal model for obesity and type 2 diabetes. *Int J Mol Sci*. 2022;23(8):4228. doi:10.3390/ijms23084228
94. Peterson RG, Shaw WN, Neel MA, Little LA, Eichberg J. Zucker diabetic fatty rat as a model for non-insulin-dependent diabetes mellitus. *ILAR J*. 1990;32(3):16-19. doi:10.1093/ilar.32.3.16
95. Shiota M, Printz RL. Diabetes in Zucker diabetic fatty rat. *Methods Mol Biol*. 2012;933:103-123. doi:10.1007/978-1-62703-068-7\_8
96. Greene SF, Johnson PR, Eiffert KC, Greenwood MR, Stern JS. The male obese Wistar diabetic fatty rat is a new model of extreme insulin resistance. *Obes Res*. 1994;2(5):432-443. doi:10.1002/j.1550-8528.1994.tb00090.x
97. Augstein P, Salzsieder E. Morphology of pancreatic islets: a time course of pre-diabetes in Zucker fatty rats. *Methods Mol Biol*. 2009;560:159-189. doi:10.1007/978-1-59745-448-3\_12
98. Frisbee JC. Hypertension-independent microvascular rarefaction in the obese Zucker rat model of the metabolic syndrome. *Microcirculation*. 2005;12(5):383-392. doi:10.1080/10739680590960241
99. Weir GC, Marselli L, Marchetti P, Katsuta H, Jung MH, Bonner-Weir S. Towards better understanding of the contributions of overwork and glucotoxicity to the beta-cell inadequacy of type 2 diabetes. *Diabetes Obes Metab*. 2009;11(suppl 4):82-90. doi:10.1111/j.1463-1326.2009.01113.x
100. Suleiman JB, Mohamed M, Bakar ABA. A systematic review on different models of inducing obesity in animals: advantages and limitations. *J Adv Vet Anim Res*. 2019;7(1):103-114. doi:10.5455/javar.2020.g399
101. Akash M, Rehman K, Chen S. Goto-kakizaki rats: its suitability as non-obese diabetic animal model for spontaneous type 2 diabetes mellitus. *Curr Diabetes Rev*. 2013;9(5):387-396. doi:10.2174/15733998113099990069
102. Cohen AM, Rosenmann E, Rosenthal T. The cohen diabetic (non-insulin-dependent) hypertensive rat model. Description of the model and pathologic findings. *Am J Hypertens*. 1993;6(12):989-995. doi:10.1093/ajh/6.12.989
103. Mathews CE, Bagley R, Leiter EH. ALS/Lt: a new type 2 diabetes mouse model associated with low free radical scavenging potential. *Diabetes*. 2004;53(1):S125-S129.
104. Höppener JW, Jacobs HM, Wierup N, et al. Human islet amyloid polypeptide transgenic mice: in vivo and ex vivo models for the role of hIAPP in type 2 diabetes mellitus. *Exp Diabetes Res*. 2008;2008:697035. doi:10.1155/2008/697035
105. Seal SV, Henry M, Pajot C, et al. A holistic view of the Goto-Kakizaki rat immune system: decreased circulating immune markers in non-obese type 2 diabetes. *Front Immunol*. 2022;13:896179. doi:10.3389/fimmu.2022.896179
106. Weksler-Zangen S, Yagil C, Zangen DH, Ornoy A, Jacob HJ, Yagil Y. The newly inbred cohen diabetic rat: a nonobese normolipidemic genetic model of diet-induced type 2 diabetes expressing sex differences. *Diabetes*. 2001;50(11):2521-2529. doi:10.2337/diabetes.50.11.2521
107. Sasase T, Ohta T, Masuyama T, Yokoi N, Kakehashi A, Shinohara M. The spontaneously diabetic torii rat: an animal model of nonobese type 2 diabetes with severe diabetic complications. *J Diabetes Res*. 2013;2013:976209. doi:10.1155/2013/976209
108. Shinohara M, Oikawa T, Sato K, Kanazawa Y. Glucose intolerance and hyperlipidemia prior to diabetes onset in female spontaneously diabetic Torii (SDT) rats. *Exp Diabetes Res*. 2004;5(4):253-256. doi:10.1080/15438600490898609
109. Yamada K, Hosokawa M, Fujimoto S, et al. The spontaneously diabetic Torii rat with gastroenteropathy. *Diabetes Res Clin Pract*. 2007;75(2):127-134. doi:10.1016/j.diabres.2006.06.034

110. Stott NL, Marino JS. High fat rodent models of type 2 diabetes: from rodent to human. *Nutrients*. 2020;12(12):3650. doi:10.3390/nu12123650
111. Wondmkun YT. Obesity, insulin resistance, and type 2 diabetes: associations and therapeutic implications. *Diabetes Metab Syndr Obes*. 2020;13:3611-3616. doi:10.2147/DMSO.S275898
112. Kaiser N, Cerasi E, Leibowitz G. Diet-induced diabetes in the sand rat (*Psammomys obesus*). In: Joost HG, Al-Hasani H, Schürmann A, eds. *Animal Models in Diabetes Research*. Humana Press; 2012:89-102. doi:10.1007/978-1-62703-068-7\_7
113. Gouaref I, Detaille D, Wiernsperger N, Khan NA, Leverve X, Koceir EA. The desert gerbil *Psammomys obesus* as a model for metformin-sensitive nutritional type 2 diabetes to protect hepatocellular metabolic damage: impact of mitochondrial redox state. *PLoS ONE*. 2017;12(2):e0172053. doi:10.1371/journal.pone.0172053
114. Sahraoui A, Dewachter C, de Medina G, Naeije R, Aouichat Bouguerra S, Dewachter L. Myocardial structural and biological anomalies induced by high fat diet in *Psammomys obesus* gerbils. *PLoS ONE*. 2016;11(2):e0148117. doi:10.1371/journal.pone.0148117
115. Gaire J, Varholick JA, Rana S, et al. Spiny mouse (*Acomys*): an emerging research organism for regenerative medicine with applications beyond the skin. *Npj Regen Med*. 2021;6(1):1. doi:10.1038/s41536-020-00111-1
116. Shafir E, Ziv E, Kalman R. Nutritionally induced diabetes in desert rodents as models of type 2 diabetes: *Acomys cahirinus* (spiny mice) and *Psammomys obesus* (desert gerbil). *ILAR J*. 2006;47(3):212-224. doi:10.1093/ilar.47.3.212
117. Flanagan P. Nile grass rats see the light of day. *Lab Anim*. 2013;42(4):115. doi:10.1038/lablan.190
118. Subramaniam A, Landstrom M, Luu A, Hayes KC. The Nile rat (*Arvicanthis niloticus*) as a superior carbohydrate-sensitive model for type 2 diabetes mellitus (T2DM). *Nutrients*. 2018;10(2):235. doi:10.3390/nu10020235
119. Noda K, Melhorn MI, Zandi S, et al. An animal model of spontaneous metabolic syndrome: Nile grass rat. *FASEB J*. 2010;24(7):2443-2453. doi:10.1096/fj.09-152678
120. Henson MS, O'Brien TD. Feline models of type 2 diabetes mellitus. *ILAR J*. 2006;47(3):234-242. doi:10.1093/ilar.47.3.234
121. Samaha G, Beatty J, Wade CM, Haase B. The Burmese cat as a genetic model of type 2 diabetes in humans [published correction appears in *Anim Genet*. 2020 Feb;51(1):153]. *Anim Genet*. 2019;50(4):319-325. doi:10.1111/age.12799
122. Samaha G, Wade CM, Beatty J, Lyons LA, Fleeman LM, Haase B. Mapping the genetic basis of diabetes mellitus in the Australian Burmese cat (*Felis catus*). *Sci Rep*. 2020;10(1):19194. doi:10.1038/s41598-020-76166-3
123. Ramsey JJ, Laatsch JL, Kemnitz JW. Age and gender differences in body composition, energy expenditure, and glucoregulation of adult rhesus monkeys. *J Med Primatol*. 2000;29(1):11-19. doi:10.1034/j.1600-0684.2000.290102.x
124. Smith AB, Schill JP, Gordillo R, et al. Ceramides are early responders in metabolic syndrome development in rhesus monkeys. *Sci Rep*. 2022;12(1):9960. doi:10.1038/s41598-022-14083-3
125. Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. *Nat Rev Genet*. 2007;8(5):353-367.
126. Oka T, Nishimura Y, Zang L, et al. Diet-induced obesity in zebrafish shares common pathophysiological pathways with mammalian obesity. *BMC Physiol*. 2010;10:21.
127. Teame T, Zhang Z, Ran C, et al. The use of zebrafish (*Danio rerio*) as biomedical models. *Anim Front*. 2019;9(3):68-77.
128. Papatheodorou K, Banach M, Bekiari E, Rizzo M, Edmonds M. Complications of diabetes 2017. *J Diabetes Res*. 2018;2018:3086167.
129. Choi JSY, De Haan JB, Sharma A. Animal models of diabetes-associated vascular diseases: an update on available models and experimental analysis. *Br J Pharmacol*. 2022;179(5):748-769. doi:10.1111/bph.15591
130. Singh R, Farooq SA, Mannan A, et al. Animal models of diabetic microvascular complications: relevance to clinical features. *Biomed Pharmacother*. 2022;145:112305. doi:10.1016/j.biopha.2021.112305
131. Hassan RH. Defect of insulin signal in peripheral tissues: important role of ceramide. *World J Diabetes*. 2014;5(3):244-257. doi:10.4239/wjdv.5.i3.244
132. Ho HTB, Chung SK, Law JWS, et al. Aldose reductase-deficient mice develop nephrogenic diabetes insipidus. *Mol Cell Biol*. 2000;20(16):5840-5846. doi:10.1128/MCB.20.16.5840-5846.2000
133. Jiang X, Yang L, Luo Y. Animal models of diabetic retinopathy. *Curr Eye Res*. 2015;40(8):761-771. doi:10.3109/02713683.2014.964415
134. Thomas AA, Biswas S, Feng B, Chen S, Gonder J, Chakrabarti S. lncRNA H19 prevents endothelial-mesenchymal transition in diabetic retinopathy. *Diabetologia*. 2019;62(3):517-530. doi:10.1007/s00125-018-4797-6
135. Cai X, McGinnis JF. Diabetic retinopathy: animal models, therapies, and perspectives. *J Diabetes Res*. 2016;2016:3789217. doi:10.1155/2016/3789217
136. B Arden G, Sivaprasad S. Hypoxia and oxidative stress in the causation of diabetic retinopathy. *Curr Diabetes Rev*. 2011;7(5):291-304. doi:10.2174/157339911797415620
137. Tang J, Du Y, Petrash JM, Sheibani N, Kern TS. Deletion of aldose reductase from mice inhibits diabetes-induced retinal capillary degeneration and superoxide generation. *PLoS ONE*. 2013;8(4):e62081. doi:10.1371/journal.pone.0062081
138. Feldman E, Sullivan K, Lentz S, Roberts J Jr. Criteria for creating and assessing mouse models of diabetic neuropathy. *Curr Drug Targets*. 2008;9(1):3-13. doi:10.2174/138945008783431763
139. Drel VR, Mashtalir N, Ilnytska O, et al. The leptin-deficient (ob/ob) mouse. *Diabetes*. 2006;55(12):3335-3343. doi:10.2337/db06-0885
140. Kennedy WR, Quick DC, Miyoshi T, Gerritsen GC. Peripheral neuropathy of the diabetic Chinese hamster. *Diabetologia*. 1982;23(5):445-451. doi:10.1007/BF00260960
141. Bhatti R, Sharma S, Singh J, Ishar MPS. Ameliorative effect of *Aegle marmelos* leaf extract on early stage alloxan-induced diabetic cardiomyopathy in rats. *Pharm Biol*. 2011;49(11):1137-1143. doi:10.3109/13880209.2011.572077
142. Lee WS, Kim J. Application of animal models in diabetic cardiomyopathy. *Diabetes Metab J*. 2021;45(2):129-145. doi:10.4093/dmj.2020.0285
143. Jasińska-Stroschein M. The current state of preclinical modeling of human diabetic cardiomyopathy using rodents. *Biomed Pharmacother*. 2023;168:115843. doi:10.1016/j.biopha.2023.115843
144. Rodrigues B, McNeill JH. Cardiac dysfunction in isolated perfused hearts from spontaneously diabetic BB rats. *Can J Physiol Pharmacol*. 1990;68(4):514-518. doi:10.1139/y90-073
145. Lilao-Garzon J, Valverde-Tercedor C, Muñoz-Descalzo S, Brito-Casillas Y, Wägner AM. In vivo and in vitro models of diabetes: a focus on pregnancy. In: Islam MDS, ed. *Diabetes: from Research to Clinical Practice*. Vol. 1307. *Advances in Experimental Medicine and Biology*. Springer International Publishing; 2020:553-576. doi:10.1007/978-94-007-5584-2020\_536
146. Reed MJ, Scribner KA. In-vivo and in-vitro models of type 2 diabetes in pharmaceutical drug discovery. *Diabetes Obes Metab*. 1999;1(2):75-86. doi:10.1046/j.1463-1326.1999.00014.x
147. Sotelo JR, Horie H, Ito S, Benec C, Sango K, Takenaka T. An in vitro model to study diabetic neuropathy. *Neurosci Lett*. 1991;129(1):91-94. doi:10.1016/0304-3940(91)90727-b
148. Verma A, Verma M, Singh A. Animal tissue culture principles and applications. *Anim Biotechnol*. 2020;269-293. doi:10.1016/B978-0-12-811710-1.00012-4
149. Poovitha S, Parani M. In vitro and in vivo  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibiting activities of the protein extracts from two

- varieties of bitter melon (*Momordica charantia* L.). *BMC Complement Altern Med*. 2016;16(S1):185. doi:[10.1186/s12906-016-1085-1](https://doi.org/10.1186/s12906-016-1085-1)
150. Khadayat K, Marasini BP, Gautam H, Ghaju S, Parajuli N. Evaluation of the alpha-amylase inhibitory activity of Nepalese medicinal plants used in the treatment of diabetes mellitus. *Clin Phytosci*. 2020;6(1):34. doi:[10.1186/s40816-020-00179-8](https://doi.org/10.1186/s40816-020-00179-8)
151. Hanson MS, Steffen A, Danobeitia JS, Ludwig B, Fernandez LA. Flow cytometric quantification of glucose-stimulated  $\beta$ -cell metabolic flux can reveal impaired islet functional potency. *Cell Transplant*. 2008;17(12):1337-1347. doi:[10.3727/096368908787648038](https://doi.org/10.3727/096368908787648038)
152. Jones B, Bloom SR, Buenaventura T, Tomas A, Rutter GA. Control of insulin secretion by GLP-1. *Peptides*. 2018;100:75-84. doi:[10.1016/j.peptides.2017.12.013](https://doi.org/10.1016/j.peptides.2017.12.013)
153. Kalwat MA, Wichaidit C, Nava Garcia AY, et al. Insulin promoter-driven Gaussia luciferase-based insulin secretion biosensor assay for discovery of  $\beta$ -cell glucose-sensing pathways. *ACS Sens*. 2016;1(10):1208-1212. doi:[10.1021/acssensors.6b00433](https://doi.org/10.1021/acssensors.6b00433)
154. Nakajima Y, Ohmiya Y. Bioluminescence assays: multicolor luciferase assay, secreted luciferase assay and imaging luciferase assay. *Expert Opin Drug Discovery*. 2010;5(9):835-849. doi:[10.1517/17460441.2010.506213](https://doi.org/10.1517/17460441.2010.506213)
155. Zhang H, Yang F, Qi J, et al. Homoisoflavonoids from the fibrous roots of *Polygonatum odoratum* with glucose uptake-stimulatory activity in 3T3-L1 adipocytes. *J Nat Prod*. 2010;73(4):548-552. doi:[10.1021/np900588q](https://doi.org/10.1021/np900588q)
156. Yamamoto N, Ueda-Wakagi M, Sato T, et al. Measurement of glucose uptake in cultured cells. *Curr Protoc Pharmacol*. 2015;71(1):12-14. doi:[10.1002/0471141755.ph1214s71](https://doi.org/10.1002/0471141755.ph1214s71)
157. Yamamoto N, Ashida H. Evaluation methods for facilitative glucose transport in cells and their applications. *Food Sci Technol Res*. 2012;18(4):493-503. doi:[10.3136/fstr.18.493](https://doi.org/10.3136/fstr.18.493)
158. Vhora N, Naskar U, Hiray A, Kate AS, Jain A. Recent advances in in-vitro assays for type 2 diabetes mellitus: an overview. *Rev Diabet Stud*. 2020;16(1):13-23. doi:[10.1900/RDS.2020.16.13](https://doi.org/10.1900/RDS.2020.16.13)
159. Efrat S, Leiser M, Surana M, Tal M, Fusco-Demane D, Fleischer N. Murine insulinoma cell line with normal glucose-regulated insulin secretion. *Diabetes*. 1993;42(6):901-907. doi:[10.2337/diab.42.6.901](https://doi.org/10.2337/diab.42.6.901)
160. D'Ambra R, Surana M, Efrat S, Starr RG, Fleischer N. Regulation of insulin secretion from beta-cell lines derived from transgenic mice insulinomas resembles that of normal beta-cells. *Endocrinology*. 1990;126(6):2815-2822. doi:[10.1210/endo-126-6-2815](https://doi.org/10.1210/endo-126-6-2815)
161. Scharfmann R, Staels W, Albagli O. The supply chain of human pancreatic  $\beta$  cell lines. *J Clin Invest*. 2019;129(9):3511-3520. doi:[10.1172/JCI129484](https://doi.org/10.1172/JCI129484)
162. Szot GL, Koudria P, Bluestone JA. Murine pancreatic islet isolation. *J Vis Exp*. 2007;7:255. doi:[10.3791/255](https://doi.org/10.3791/255)
163. Corbin KL, West HL, Brodsky S, Whitticar NB, Koch WJ, Nunemaker CS. A practical guide to rodent islet isolation and assessment revisited. *Biol Proced Online*. 2021;23(1):7. doi:[10.1186/s12575-021-00143-x](https://doi.org/10.1186/s12575-021-00143-x)
164. Da Silva XG. The cells of the islets of Langerhans. *J Clin Med*. 2018;7(3):54. doi:[10.3390/jcm7030054](https://doi.org/10.3390/jcm7030054)
165. Balboa D, Barsby T, Lithovius V, et al. Functional, metabolic and transcriptional maturation of human pancreatic islets derived from stem cells. *Nat Biotechnol*. 2022;40(7):1042-1055. doi:[10.1038/s41587-022-01219-z](https://doi.org/10.1038/s41587-022-01219-z)
166. Bani Hamad FR, Rahat N, Shankar K, Tsouklidis N. Efficacy of stem cell application in diabetes mellitus: promising future therapy for diabetes and its complications. *Cureus*. 2021;13(2):e13563. doi:[10.7759/cureus.13563](https://doi.org/10.7759/cureus.13563)
167. Chen S, Du K, Zou C. Current progress in stem cell therapy for type 1 diabetes mellitus. *Stem Cell Res Ther*. 2020;11(1):275. doi:[10.1186/s13287-020-01793-6](https://doi.org/10.1186/s13287-020-01793-6)
168. Zhang X, Ma Z, Song E, Xu T. Islet organoid as a promising model for diabetes. *Protein Cell*. 2022;13(4):239-257. doi:[10.1007/s13238-021-00831-0](https://doi.org/10.1007/s13238-021-00831-0)
169. Dayem AA, Lee SB, Kim K, Lim KM, Jeon TI, Cho SG. Recent advances in organoid culture for insulin production and diabetes therapy: methods and challenges. *BMB Rep*. 2019;52(5):295-303. doi:[10.5483/BMBRep.2019.52.5.089](https://doi.org/10.5483/BMBRep.2019.52.5.089)

**How to cite this article:** Janapati YK, Junapudi S. Progress in experimental models to investigate the in vivo and in vitro antidiabetic activity of drugs. *Anim Models Exp Med*. 2024;7:297-309. doi:[10.1002/ame2.12442](https://doi.org/10.1002/ame2.12442)