NEU PRESS

SELÇUK TIP DERGİSİ
SELCUK MEDICAL JOURNAL

DOI:http:/dx.doi.org/10.30733/std.2025.01837
Selcuk Med J 2025;41(2): 99-109

OPEN DERLEME / REVIEW

Animal Models of Diabetes and Complications for Studying Disease Mechanisms

Diyabet ve Komplikasyonlarının Hastalık Mekanizmalarını İncelemek İçin Hayvan Modelleri

Asli San Dagli Gul[1], Okan Arihan[1]

[1]Hacettepe University, Faculty of Medicine, Department of Physiology, Ankara, Türkiye

ÖZET

Bu derleme, diyabetin patofizyolojisini ve komplikasyonlarını anlamak için hayvan modellerinin kullanımını ve etkinliğini incelemeyi amaçlamaktadır. Bu derleme, hayvan modellerinde diyabet kaynaklı komplikasyonları inceleyen güncel literatürün analizini içermektedir. Sadece İngilizce makaleler dahil edilmiştir ve çoğunluğu 2018 sonrası yayımlanmıştır. Alloksan ve Streptozotosin gibi kimyasal modellerin etkili bir şekilde diyabet oluşturabildiği, ancak toksisite ve sistemik yan etkilere yol açabileceği görülmektedir. Alloksan, β-hücre toksisitesi yoluyla Tip 1 diyabeti tetiklerken, STZ hem Tip 1 hem de Tip 2 diyabeti modellemek için tercih edilmektedir. Otoimmün diyabeti simüle eden NOD fareleri veya obezite kaynaklı diyabet geliştiren db/db fareleri gibi genetik modeller, hastalığın genetik yönlerini incelemek için avantajlıdır ancak yüksek maliyet ve karmaşıklık gibi dezavantajlar taşımaktadır. Cerrahi yaklaşımlar, insülin sekresyonu ve pankreas fonksiyonlarını değerlendirmede önemli bilgiler sunarken, bu yöntemlerin invaziv olması ve fizyolojik olarak bazı farklılıklara yol açması sınırlayıcı bir faktördür. Modellerin çoğu, diyabete bağlı oksidatif stres ve inflamasyonun, nefropati, retinopati, nöropati ve kardiyovasküler hastalıklar gibi komplikasyonlara yol açtığını göstermektedir. Özellikle diyabetik böbrek hastalığında podosit hasarı, proteinüri ve glomerüler filtrasyon değişiklikleri gözlemlenirken, diyabetik retinopati modelinde vasküler değişiklikler ve görme kaybı tespit edilmiştir. Diyabetik nöropati, duyusal ve motor fonksiyon kayıplarına yol açarken, kardiyovasküler komplikasyonlar damar sertliği, hipertansiyon ve kalp yetmezliği ile ilişkilendirilmiştir. Diyabetin ilerleyişi ve komplikasyonlarını değerlendirebilmek için model seçimi büyük önem taşımaktadır. Ancak her modelin kendine özgü avantajları ve sınırlamaları vardır. Kimyasal ajanlar hızlı ve düşük maliyetli bir seçenek sunarken, genetik modeller daha fizyolojik ancak maliyetli ve teknik olarak karmaşıktır. Bu derleme, diyabet araştırmalarında en uygun hayvan modelinin belirlenmesine rehberlik edebilir ve yeni terapötik stratejilerin geliştirilmesine katkı sağlayabilir.

Anahtar Kelimeler: Diyabet, Komplikasyonlar, Hayvan Modeli, STZ, Alloksan

ABSTRACT

This review aims to examine the use and effectiveness of animal models in understanding the pathophysiology and complications of diabetes. This review includes an analysis of recent literature investigating diabetes-related complications in animal models. Only English-language articles were included, with the majority published after 2018. Chemical models such as Alloxan and Streptozotocin (STZ) effectively induce diabetes; however, they may cause toxicity and systemic side effects. While Alloxan triggers Type 1 diabetes through β-cell toxicity, STZ is preferred for modeling both Type 1 and Type 2 diabetes. Genetic models, such as NOD mice simulating autoimmune diabetes or db/db mice developing obesity-induced diabetes, provide advantages in studying the genetic aspects of the disease. However, these models have drawbacks, including high costs and complexity. Surgical approaches offer valuable insights into insulin secretion and pancreatic function, but their invasive nature and potential physiological differences pose limitations. Most models demonstrate that oxidative stress and inflammation associated with diabetes lead to complications such as nephropathy, retinopathy, neuropathy, and cardiovascular diseases. Specifically, diabetic nephropathy is characterized by podocyte damage, proteinuria, and changes in glomerular filtration, while diabetic retinopathy models show vascular alterations and vision loss. Diabetic neuropathy results in sensory and motor function loss, whereas cardiovascular complications are linked to arterial stiffness, hypertension, and heart failure. Selecting the appropriate model is crucial for evaluating diabetes progression and its complications. However, each model has its unique advantages and limitations. Chemical agents offer a fast and cost-effective approach, while genetic models provide a more physiologically relevant but expensive and technically complex alternative. This review may guide researchers in selecting the most suitable animal model for diabetes studies and contribute to the development of new therapeutic strategies.

Keywords: Diabetes, Complications, Animal Model, STZ, Alloxan

Geliş Tarihi/Received: 21 February/Şubat 2025 **Kabul Tarihi/Accepted:** 23 June/Haziran 2025 **Yayın Tarihi/Published Online:** 27 June/Haziran 2025

INTRODUCTION

Diabetes, a chronic disease that is widely prevalent worldwide, can lead to serious health problems due to disruptions in the regulation of blood sugar in the body. Diabetes can manifest in different types based on the disorders in the body's blood sugar regulation. Type 1 diabetes develops as a result of the immune system attacking the beta cells of the pancreas, which produce insulin. On the other hand, type 2 diabetes represents a condition where the effective use of insulin is impaired. Understanding these types of the disease and developing effective treatment methods is crucial, and for this purpose, experimental diabetes models play a vital role. In the scientific research, animal models are frequently employed to gain deeper insights into the pathophysiology and treatment of diabetes. Through these models, valuable

Sorumlu Yazar/Corresponding Author: Asli San Dagli Gul, Hacettepe University, Faculty of Medicine, Department of Physiology, Ankara, Türkiye
e-mail: aslisandagligul@hacettepe.edu.tr

Atıf yapmak için/ Cite this article as: Dagli Gul AS, Arihan O. Animal Models of Diabetes and Complications for Studying Disease Mechanisms. Selcuk Med J 2025;41(2): 99-109

Disclosure: *The author has no financial interest in any of the products, devices, or drugs mentioned in this article. The research was not sponsored by an outside organization. Author has agreed to allow full access to the primary data and to allow the journal to review the data if requested.*

"**This article is licensed under a** Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)"

information is obtained regarding the interaction of genetic and some environmental factors in diabetes, the progression of the disease, and potential treatment strategies. This review discusses how experimental diabetes models are established in animals, how these models are evaluated in diabetes research, which models are more suitable for specific experiments, and their effects on complications.

MATERIALS AND METHODS

This review examines the investigation of complications induced by diabetes in animal models through a review of the scientific literature. Only English articles have been included in the review, with the majority of the articles being from after 2018.

What is Diabetes Mellitus?

Diabetes is a chronic disease characterized by the impairment of the body's ability to regulate blood sugar levels. Essentially, it arises when the pancreas is unable to generate sufficient insulin or when the body is ineffective in utilizing the insulin it produces. Diabetes can lead to various complications in the long term, such as neuropathy, retinopathy, nephropathy, cardiomyopathy, vascular damage, and others (1). In 2019, the World Health Organization updated the classification of diabetes. According to this classification, Type 1 Diabetes is considered as an autoimmune disease which affects beta cells of the pancreas. This condition leads to almost no insulin production. Type 2 Diabetes is a condition where insulin resistance develops, meaning the body's cells cannot effectively utilize insulin, and over time, there is a reduction in insulin production from the Langerhans islets. Gestational Diabetes is a type of diabetes that arises during pregnancy or is first detected during pregnancy, typically temporary, but sometimes can persist postpartum (2). In addition to these, there are various special types of diabetes that include genetic or other types of diabetes based on reasons such as infection, medications, and chemicals.

Pathophysiology of Diabetes

We encounter different molecules in the pathophysiology of diabetes such as insulin. Insulin is a polypeptide hormone produced as preproinsulin by the beta cells of the Langerhans islets in the pancreas. Its primary functions include increasing glucose uptake into cells, facilitating the storage of glucose as glycogen and fat, and regulating protein synthesis. This hormone initiates mechanisms by binding to receptors on cell membranes, making it easier for glucose to enter cells. This process is crucial for maintaining glucose metabolism and energy homeostasis.

Understanding the mechanism of intracellular insulin secretion and the cellular factors that regulate it is crucial in diabetes treatment strategies. Insulin release from beta cells of the pancreas' Langerhans islets occurs after a complex process. In beta cells, enzymes such as glucose transporters (GLUT) and glucokinase, which allow glucose to enter the cell, are present. When post-digestion blood glucose levels rise, and glucose levels inside beta cells increase, ATP production increases as a result of glucose metabolism. With the increase in ATP/ADP in the cytosol of beta cells, ATP-sensitive potassium channels (KATP) close. This causes cell depolarization, the opening of voltage-sensitive calcium channels, and, consequently, the entry of calcium into the cell. In addition, cyclic AMP further increases intracellular calcium levels by reducing the intake of Ca++ into intracellular organelles and modulating second messenger molecules independently or dependently on protein kinase A (3). Calcium is essential for the exocytosis process of insulin vesicles, allowing insulin to be released from the cell into the bloodstream (4).

The regulation of insulin secretion is associated with various factors, including cellular, hormonal, and neural, in addition to plasma glucose. Among cellular factors, gastrointestinal hormones (e.g., glucagon-like peptide-1), neurotransmitters, and intermediates in the glucose metabolic pathway play a role. For example, glucagon-like peptide-1 (GLP-1), which reaches beta cells through endocrine and paracrine pathways, can increase insulin production (5). Neural regulations have a significant impact, with the activity of the autonomic nervous system playing a major role. Sympathetic nervous system activity can regulate insulin secretion through neural stimulation or alpha and beta adrenergic receptors on the surface of beta cells (6). Various signaling pathways in beta cells that modulate intracellular calcium levels and second messenger molecules also influence insulin release (7).

After insulin is secreted, it reaches target cells through the circulation. Insulin receptors are specialized protein structures located on the surface of cells with tyrosine kinase enzyme activity (8). Insulin receptors are made up of four subunits, resulting from the repetition of two different types called alpha (α) and beta (β). The alpha subunits of the receptor assist in insulin binding and the insertion of the receptor into the cell surface, while the beta subunits transmit the insulin signal, initiating cellular responses (9). The binding of insulin results in the dimerization of the receptor's alpha subunits. This dimerization triggers the activation of intracellular signaling pathways by increasing the interaction of the beta subunits, leading to the opening of voltage-sensitive calcium channels. These signaling pathways regulate intracellular signal transduction and direct the effects of insulin in target tissues. In particular, signaling pathways such as the MAPK (mitogen-activated protein kinase) and the PI3K (phosphoinositide 3-kinase) pathways regulate a significant portion of insulin cellular effects (10).

Diabetic Complications

When predisposing factors for diabetes progress from prediabetes to diabetes, individuals face many complications of these pathophysiological conditions. Diabetes can lead to various complications due to long-term high blood sugar levels. These complications are generally classified as microvascular (related to small blood vessels) and macrovascular (related to large blood vessels) complications. Diabetes can lead to microvascular complications such as nephropathy, retinopathy, neuropathy and macrovascular complications such as diabetic foot syndrome, cardiovascular diseases and thrombotic events.

Retinopathy is one of the most common complications

of diabetes. Prolonged high blood glucose levels can lead to proliferative retinopathy or non-proliferative retinopathy, eventually resulting in vision loss. Diabetic nephropathy results from damage to the filtration system of the kidneys due to chronic uncontrolled glucose metabolism. Impaired kidney function can lead to an increased risk of kidney failure, proteinuria, and the loss of blood pressure control (11). Neuropathy often presents symmetrically in the extremities, resembling gloves and socks, in diabetic patients. It can also affect nerves responsible for autonomic functions in the digestive, cardiovascular, and urinary system (12). Cardiovascular diseases may develop due to impaired glucose metabolism in diabetes, due to the formation of Advanced Glycation End Products (AGES) and oxidative damage (13). Diabetic foot ulcers are observed in approximately 30% of diabetic patients. Poor circulation and sensory nerve damage make wound healing difficult and increase the risk of infection (14). Thrombotic events occur due to reasons such as reduced anti-thrombotic activity, platelet reactivation, increased concentration and activity of coagulation factors (15). These complications highlight the importance of effective diabetes management so we made a particular effort to focus on modelling of diabetic complications in animal experiments in this article.

Diabetes Models in Experimental Animals

Due to the complex pathophysiology and treatment requirements of diabetes, understanding this disease better and developing effective treatment strategies is of great importance. Before clinical research, it is necessary to conduct cell culture studies and test with experimental diabetes models in animals. Experimental diabetes models aim to mimic certain aspects of diabetes types such as Type 1 and 2 in a laboratory setting. These models are used as tools to understand how various factors contribute to the development of diabetes, investigate the effects of drug candidate chemicals or plant extracts in diabetes, and study complications. In diabetes research, experimental models created using genetic methods, chemical compounds, or dietary manipulations are quite common.

Various models are applied to create Type 1 and Type 2 diabetes in mice and rats. The main categories for triggering diabetes include chemical methods (Alloxan and STZ), spontaneous autoimmune and genetic methods (16). Surgical methods can also be added to these methods. One of the oldest and simplest ways to induce experimental diabetes in animals is the partial or complete removal of the pancreas (17). Towards the end of the 19th century, physicians discovered the association between diabetes and the pancreas and began research to understand the role of this organ. Although open abdominal surgery is generally preferred, laparoscopic methods have also been tested recently (18). Complete removal of the pancreas or Langerhans is compatible with Type 1 diabetes since it eliminates insulin production. In contrast, partial removal can be adapted to a Type 2 model (19). It is worth noting that rats and mice have significant anatomical and physiological differences in their pancreas compared to humans (20). The head of the rodent pancreas is located in the duodenal region and is scattered within the mesentery. The body part extends to the spleen, and the tail ends at the hilum of the spleen (21).

In addition to this difference, there are adverse effects of total pancreatectomy. Total pancreatectomy eliminates not only endocrine but also exocrine cells, resulting in a more severe condition than the true diabetic syndrome. Furthermore, this method destroys not only beta cells but also other critical cells that secrete hormones such as alpha, delta, pp, and epsilon cells (22). Since the aim of partial pancreatectomy is to remove over 90% of the pancreas, the disadvantages of this method are similar to total pancreatectomy (23). Despite the ease of this surgical method, its undesired side effects have led researchers towards practices where diabetes can be modeled more easily without causing significant harm to animals. Looking more closely at these methods, it can be seen that the most common applications are chemical or toxin applications. Although within this model framework, the ditizone model (24), ferric nitrilotriacetate injection (25), insulin antibody model (26), and diet modification with high-fat or high-glucose diets can be used to model conditions similar to type 2 diabetes in animals, the two most commonly used chemical substances are Alloxan and Streptozotocin (STZ) (27).

The advantages, disadvantages, mechanisms of action and complications of experimental diabetes models are given in table 1.

Alloxan

Alloxan is one of the molecules frequently used in modeling experimental diabetes in animals. It is a hydrophilic derivative of pyrimidine that is similar to glucose. Due to its resemblance to glucose, it can easily enter pancreatic beta cells and liver cells through the GLUT2 transporters (28). Its chemical structure contains five carbonyl groups, allowing it to react with thiol groups in cells. It inhibits the function of the thiol-based enzyme glucokinase, which acts as a glucose sensor in beta cells by forming disulfide bonds (29). Furthermore, it increases intracellular ROS production, leading to DNA damage and consequently, beta cell apoptosis (30). The increased production of hydroxyl radicals inside the beta cells is related to ascorbic acid, and this effect is pronounced in the mitochondria (31). A study investigating the effects of age-related alloxan administration in Wistar albino rats reported that the best induction of diabetes was observed in rats aged 7-9 weeks (32). Mostafavinia and colleagues reported that subcutaneous experiments with different doses of alloxan resulted in the most desirable outcome for Type 1 diabetes induction at a dose of 120mg/kg (33). However, there are limitations to the use of alloxan. It not only reduces glucokinase activity in beta cells but also in liver cells, which has led to the incompatibility of this model with human diabetes (34).

Streptozotocin

Streptozotocin is a broad-spectrum antibiotic that was initially isolated from *Streptomyces achromogenes* in the 1960s and was later reported to have diabetogenic effects (35, 36). In those years, it was used as a chemotherapeutic agent for

Table 1. Comparison of Common Experimental Diabetes Models and Complications

Model Type	Induction Method	Mechanism	Main Type of DM	Advantages	Disadvantages	Common Complications
Alloxan	Chemical	GLUT2-mediated entry, ROS production	Type 1	Simple, inexpensive, rapid induction	Affects liver cells, short half-life, inconsistent	Kidney damage, Retinopathy, Neuropathy
Streptozotocin (STZ)	Chemical	GLUT2-mediated entry, DNA alkylation, ROS	Type 1 & Type 2 (with high-fat diet)	More stable than Alloxan, high specificity for β-cells	Causes severe β-cell destruction, can induce early death	Kidney damage, Retinopathy, Neuropathy
Pancreatectomy	Surgical	Partial/total removal of pancreas	Type 1 or Type 2 (depending on extent)	Accurately mimics insulin deficiency	Invasive, affects both endocrine and exocrine function	Severe pancreatic damage, multi-organ dysfunction
NOD Mouse	Genetic	Spontaneous autoimmune diabetes	Type 1	Autoimmune resemblance to human T1DM	High cost, variability in diabetes onset	Retinopathy, Neuropathy
db/db Mouse	Genetic	Leptin receptor mutation, leading to obesity	Type 2	Obesity and insulin resistance model	Does not perfectly mimic human T2DM	Nephropathy, Cardiomyopathy
High-Fat Diet + STZ	Combined	Diet-induced insulin resistance, STZ-induced β-cell destruction	Type 2	Mimics human T2DM better than genetic models	Requires precise dosing, variability in response	Nephropathy, Retinopathy
Non-Human Primates	Genetic Surgical Chemical	Multiple pathways	Type 1 & Type 2	Close physiological resemblance to humans	High cost, ethical concerns	Kidney damage, Retinopathy, Neuropathy

metastatic pancreatic cancers. In fact, after a trial with 52 patients, reductions in tumor size were observed, but it was reported that five patients died due to organ damage (37). STZ is not suitable for oral administration because it is affected by stomach acid, so parenteral administration is preferred. It remains in the bloodstream at high levels for 15 minutes after injection and is then excreted through the kidneys and bile ducts (38). Similar to alloxan, STZ is a hydrophilic agent with a structure resembling glucose. STZ's specific effect on pancreatic beta cells is explained by its entry into the cells through GLUT2 receptors on the surface of pancreatic beta cells. In its chemical formula, it contains a nitrosourea group similar to adenosine, leading to DNA methylation in beta cells. Furthermore, it increases nitric oxide production and free radical formation, causing cell death (39). Surviving beta cells continue their existence with oxidative stress and mitochondrial dysfunction (40). Beta cell damage and hence insulin deficiency are typical features of type 1 diabetes, which is why STZ is considered more suitable for type 1 diabetes models. However, it is often added to models for type 2 diabetes, such as high-fat diets (41) or combined with agents like nicotinamide (42) to create these models. The nicotinamide model is based on the experiment conducted by Junod and colleagues in 1969 (43). In this combined model, approximately 60% loss of function is observed in pancreatic islets (44). The STZ-NA protocol's induction of hyperglycemia, reduction of insulin receptors in skeletal muscle, partial reversibility with metformin, development of a dyslipidemic profile, and especially histopathological changes in the liver indicate that it is a suitable model for T2DM (45).

In addition to the chemical and functional differences between alloxan and STZ, there are also differences in stability. Alloxan starts to degrade at approximately 1.5 minutes at 37°C and pH 7.4, whereas STZ can remain stable for up to about one hour at pH 7.4 and 37°C (38). Acidic environments extend the stability periods for both substances.

Dosages of STZ Application

The dosages of STZ can vary depending on the species, gender, age, and the purpose of the experiments with animals. Additionally, both repeated low-dose applications and single high-dose models are used. It is anticipated that diabetes created with multiple low doses leads to beta cell dysfunction through an inflammatory process rather than beta cell destruction, which is considered to be closer to reality (44). In mice, STZ dosages typically range from 100 to 200 mg/kg through intraperitoneal injection for creating type 1 diabetes. Dosages ranging from 40 to 60 mg/kg are used in combination with different models for type 2 diabetes (46). For generating type 1 diabetes in rats through intraperitoneal injection, dosages typically range from 40 to 65 mg/kg, although lower dosages can be used to model insulin resistance or type 2 diabetes. In a study conducted to determine the optimal dosage for inducing diabetes in rats with a single intraperitoneal dose, dosages of 30, 35, 40, and 50 mg/kg were compared. According to the results, it was reported that the likelihood of diabetes occurring was 0.764 with a dosage of 40 mg/kg, despite a low mortality rate (33). The development of diabetes begins in the days following STZ injection. In many protocols, the formation of a diabetes profile is accepted to occur with the increase in fasting blood glucose levels measured at 72 hours. Reference values for plasma glucose vary, but 200 mg/dL and above is commonly considered the lower limit. Hyperglycemia levels are often categorized as stage 1 for 200-450 mg/dL and stage 2 for 451 and above (47).

Untoward effects due to the use of STZ

Although it is widely used in rodent diabetes models, some researchers argue that it is not ideal for experimental diabetes models. Wszola et al. reported that since a single dose of STZ in small rodents caused more than 90% beta cell destruction in the pancreatic islets of Langerhans, it was not a suitable diabetes model for transplantation studies (48).

In the STZ diabetes model, different results are obtained depending on the age of the animal. WangFischer et al investigated age-related effects. In their study, it was observed that acute deaths within 1 week after STZ injection were 3% in rats aged 6-11 weeks, 83% in rats aged 12-17 weeks, and 91% in rats older than 18 weeks (49).

Finally, although there are fewer deaths in the STZ-induced diabetes model compared to alloxan, the resulting diabetes is longer-lasting and irreversible. STZ also shows greater selectivity to beta cells than alloxan (17).

Genetic Models

It is possible to create genetic diabetes models by modifying or silencing specific genes. Some common genetic type 1 diabetes models include: NOD Mouse Model (Non-Obese Diabetic): This model have a tendency to develop a disease similar to autoimmune type 1 diabetes.

Rat Insulin Promoter-LAK Mouse Model: This model involves the addition of a toxin gene (LAK) that halts insulin production. db/db Mouse Model: This model includes mutations in the leptin receptor, leading to obesity and diabetes,

Akt-Insulin Resistance Mouse Model: Mutations are made in the Akt gene, disrupting insulin signaling, Lipodystrophy Mouse Models: These models involve a lack or dysfunction of adipose tissue (16).

Different Experimental Animal Models

Another category to consider is the use of various animal species. Different animal models offer various advantages and disadvantages, providing different opportunities. Among these models: Non-Human Primate Models: These models have the closest physiological resemblance to humans but are often challenging to use due to cost and ethical concerns. Dog and Pig Models: These species pose similar problems in terms of cost and long-life cycles.

Non-Mammalian Models: Non-mammalian models may not be preferred due to their diverse physiological features, despite offering opportunities in terms of life cycle and cost. Rodent Models: Rodent models are the most commonly used models. Although pancreatic islet structures may not closely resemble those of humans, their cost-effectiveness, short life cycles, and demonstrated validity make them a top choice (50). Each of these animal models has its unique advantages and limitations, and the choice of model depends on specific research goals, budget, and ethical considerations. The ultimate goal with these models and species is to induce and study tissue and organ damage such as diabetic cardiomyopathy, nephropathy, neuropathy, and retinopathy, with the aim of advancing our scientific knowledge on the subject (16).

Effect of animal species and age on experimental success in different models In order to evaluate the validity and clinical implications of the data obtained in diabetes models, the age, sex, species and physiological characteristics of the animals used and the induction method of the model are of great importance. For example, while animal age affects the response to insulin secretion in streptozotocin application, alloxan sensitivity may also vary among species. In addition, environmental factors (e.g. diet, housing conditions and stress level) directly affect metabolic responses. Therefore, it is important for researchers to evaluate these variables comparatively when choosing a model. A comparative table summarizing some basic variables related to age and species in the most commonly used diabetes models is presented in Table.2.

Understanding pathophysiology of diabetic complications for model selection Diabetes is a multisystem disorder that leads to serious organ damage in the long term. Chronic hyperglycemia triggers oxidative stress, inflammation and glycation processes at the cellular level, forming the basis for both microvascular (retinopathy, nephropathy, neuropathy) and macrovascular (coronary artery disease, peripheral artery disease) complications. Hyperglycemia increases the production of reactive oxygen species (ROS) within the cell, leading to mitochondrial dysfunction (51). This triggers cellular damage and apoptosis processes. At the same time, the formation of advanced glycation end products (AGEs) disrupts the function of protein and lipid structures; AGE-RAGE (receptor for advanced glycation end products) interaction activates proinflammatory signaling pathways (52). Protein

Table 2. Effect of animal species and age on model success in the most commonly used experimental diabetes models.

Model	Animal Type	Age (weeks)	Administration Method	Age-Related Effects
STZ (Type 1 DM)	Rat, Mouse	4–6 wks (juvenile): High sensitivity 8–12 wks (young adult): Stable glucose response	i.p./i.v. single dose (45–65 mg/kg)	Severe β-cell destruction in 4–6 wk animals, but mortality may increase. 8–12 wk animals provide a more stable model.
Alloxan (Type 1 DM)	Rabbit, Rat	6–8 wks: Maximum oxidative stress response	i.v. or i.p. injection	Juvenile animals have lower antioxidant defense, increasing sensitivity to alloxan.
HFD + STZ (Type 2 DM)	Mouse, Rat	HFD started at 4 wks; STZ at 8–12 wks (30–35 mg/kg)	Diet + low-dose STZ	Starting HFD at 4 wks facilitates insulin resistance development; low-dose STZ in adults causes partial β-cell damage.

kinase C (PKC) activation contributes to processes such as endothelial dysfunction, increased vascular permeability, and angiogenesis (53). Activation of the polyol pathway causes osmotic stress within the cell, causing damage especially, to nerve and kidney cells (54). The hexosamine pathway also tries to process excess glucose through an alternative pathway, affecting transcription factors and increasing fibrosis and inflammation mechanisms (55).

In microvascular complications, these processes manifest themselves with pathologies specific to each organ systems. In retinopathy, thickening of the retinal capillaries, pericyte loss and neovascularization develop (56). Whereas in nephropathy, glomerular basement membrane thickening, mesangial expansion and podocyte damage are seen. In neuropathy, slowing of nerve conduction, axonal degeneration and microvascular perfusion disorders are prominent (57). Macrovascular complications are characterized by the acceleration of the atherosclerosis process (58). The combination of hyperglycemia, dyslipidemia, low-grade chronic inflammation and endothelial dysfunction results in deterioration of the arterial wall structure and accelerates plaque formation (59). Modeling these multifaceted pathophysiological processes is important to more accurately investigate complication-specific targets in experimental systems.

Experimental model selection according to complication

Understanding the mechanisms underlying complications such as nephropathy, retinopathy, neuropathy, and cardiovascular dysfunction is critical for disease management and development of treatment strategies. However, not every experimental diabetes model can accurately reflect the development of every complication. For example, the most commonly used method for diabetic nephropathy models is the application of manipulations that increase renal stress, such as puromycin aminonucleoside (PAN) or unilateral nephrectomy, in addition to STZ-induced hyperglycemia (60). In diabetic retinopathy studies, retinal microvascular changes are observed as a result of long-term hyperglycemia induced by STZ, while this process can be accelerated by agents that increase oxidative stress. For diabetic neuropathy, chronic STZ models or db/db mice with leptin receptor mutation are preferred in terms of slowing of peripheral nerve conduction, nerve fiber loss and development of thermal/mechanical hyperalgesia (61). Cardiovascular complications are studied through models that reveal both metabolic syndrome and cardiac dysfunction as a result of STZ administration combined with a high-fat diet (62). Each complication model shows variable sensitivity depending on the duration of hyperglycemia, age and species of the animal, and meticulous standardization of protocols is of great importance for translational validity. Therefore, suitable models for each complication are discussed separately below.

Studying Diabetes-Induced Complications in Animal Models

Kidney Damage Models

Diabetes affects various tissues, including the kidneys, due to the microvascular damage it causes. In an effort to prevent this damage, different natural substances are being tested in many research studies. One of these substances is quercetin. The effects of quercetin usage in animal models studying kidney damage have been evaluated in a meta-analysis. As inflammation and oxidative stress are known to increase under diabetes, quercetin's anti-inflammatory and antioxidant properties can be predicted as protective (63).

In addition to the approach of trying to prevent damage by providing natural or synthetic substances directly, there is a research approach that targets molecular pathways. Sirtuin-1 (SIRT-1) can be given as an example in this context. Overexpression of SIRT-1 has been reported in structures such as podocytes and renal tubular cells in animals with diabetes. The protective effects of SIRT-1 in diabetic nephropathy are among the research topics in the molecular field (64). Wenshen Jianpi Recipe (WSJPR), widely used in traditional Chinese medicine, is considered and used as beneficial for diabetic nephropathy. Cao and colleagues used this recipe in a diabetes model induced by STZ (60 mg/kg i.p.) in rats. WSJPR given at different doses for 8 weeks reduced urinary total protein, albumin, and urea nitrogen and led to improvements in glomerular hypertrophy and mesangial expansion. Additionally, the expression of nephrin and podocin mRNA was increased. The researchers suggested that WSJPR is beneficial in diabetes-induced kidney damage and could be considered

as an approach for the treatment of diabetic nephropathy (65).

Another study, based on traditional Chinese medicine, tested the Jiedu Tonlguo Baoshen formula (JTBF) for its protective effects against proteinuria and kidney damage induced by diabetes. In a rat diabetes model induced by a high-fat diet + STZ, blood and urine samples were provided with an automatic analysis device. JTBF was found to reduce 24-hour urinary protein excretion and increase the expression of podocin, nephrin, and WT-1 in podocytes. This suggests that podocyte damage was reduced with JTBF. Additionally, it was found that this formula changed the expression of proteins related to autophagy in podocytes and affected signaling pathways through proteins such as Akt and mTOR (66).

Retinopathy

One of the most significant complications of diabetes is diabetic retinopathy (DR). DR is one of the leading causes of non-trauma-related blindness worldwide. This condition can affect patients on a scale ranging from a decline in visual quality to total blindness, impacting millions of people. While rodents are most commonly used in modeling this pathological condition, other organisms like dogs and zebrafish can also be preferred. Each model has its advantages, and for directly modeling the pathophysiological development in humans, one model alone may not suffice (67). Animal models are crucial for understanding the pathogenesis of DR, providing insights into both proliferative and non-proliferative DR. Different DR models have been developed to examine these aspects. In animal models, these conditions can be induced by selecting genetically suitable animals or performing appropriate applications to trigger the disease (68).

In a study conducted on Wistar albino rats, animals that developed diabetes after a 55 mg/kg i.p. STZ application were subjected to experiments. Electroretinography, as well as Evans blue and dextran fluorescence retinal angiography, were performed at 1, 3, 6, and 9 months after this administration. Significant changes were observed in electroangiography in the diabetic groups. Furthermore, the observation of conditions such as vascularization, ischemic changes, increased vascular permeability, and vitreous neovascularization in diabetic rats suggests that this model may be a good one for modeling the pathology of diabetic retinopathy in humans and testing treatment options (69).

In another study related to DR, the effect of melatonin on VEGF, IL-6, TNF-alpha, and parameters related to apoptosis in rats was investigated. In a diabetes model triggered by STZ (60 mg/kg i.p.), melatonin was administered at a dose of 10 mg/kg for 20 days. The results showed that melatonin administration reduced the expression of VEGF, cytokines, and apoptosis. The authors evaluated this result as an indication that melatonin has the potential to improve adverse conditions related to DR (70).

Neuropathy

One of the significant long-term complications that diabetes can cause is neuropathy. This condition is the subject of various research studies that suggest that it can be attributed to not only the effects of elevated glucose but also different pathways involving insulin receptors on peripheral nerves and dyslipidemia (71). While different animal models successfully model various aspects of neuropathy observed in humans, they may not be sufficient in other aspects. For example, diabetes in cats is good for modeling advanced diabetic neuropathy in humans, but rodent models do not provide reliable results to reflect functional impairments observed in early stages in humans. However, it's worth noting that the use of STZ in inducing diabetes itself can be problematic as it has direct neurotoxic effects (72).

Depression

Similar to diabetes, depression is a disease that significantly affects the quality of life and should be taken into consideration. The increased prevalence of depression in individuals with diabetes necessitates the elucidation of the mechanisms linking these two conditions. In a model induced by STZ and accompanied by a high-fat diet, after 12 weeks, depression-like behaviors were observed in diabetic animals, as evidenced by their performance in a challenging swimming test. Researchers attributed these observations to the increased levels of cytokines such as IL-6 and TNF-alpha. The increase in these pro-inflammatory molecules, which is associated with the underlying inflammation in many diseases, appears to be linked to induced diabetes. In this model, researchers found that daily agmatine administration (10-20 mg/kg) reduced depression-like behaviors and inflammation markers examined in brain tissue (73).

Anxiety

Anxiety, like depression, is a topic frequently researched in experimental animals. Increases in anxiety-like behaviors, evaluated in setups such as the light-dark box, open field test, and elevated plus maze test, are used to assess whether animals develop anxiety following a pathology or administration. In STZ induced diabetes in rats, anxiety-like behaviors were increased in the elevated plus maze test, while melatonin reduced these behaviors (74).

Mice with diabetes induced by a single dose of STZ were treated with fluoxetine, a serotonin reuptake inhibitor. It was observed that anxiety-like behaviors decreased in different behavioral tests (elevated plus maze, open field, dark and light transition, Y maze). Additionally, it was noted that fluoxetine reduced the increased astrocyte activation associated with STZ. One possible reason for this effect is suggested to be the reduction of myelin basic protein loss in oligodendrocytes due to diabetes with fluoxetine (75).

Memory and Learning

Memory problems and Alzheimer's disease, which are among the most important health issues related to aging, significantly impact the quality of life and pose significant financial challenges in terms of caregiving. The higher prevalence of Alzheimer's disease in individuals with diabetes suggests that diabetes increases susceptibility to Alzheimer's disease in older individuals. There is growing evidence of similarities in the pathophysiology of both diseases in terms of cognitive impairment. Cognitive impairments triggered by diabetes also constitute a significant topic in behavioral

studies involving animals (76).

Accumulation of amyloid-beta (Aβ) and cerebrovascular inflammation, which are important changes in Alzheimer's disease, have been studied in an Alzheimer's mouse model. Researchers crossed transgenic Alzheimer's mice (APP23) with two different diabetic mice strains (ob/ob and NSY mice). The changes in metabolism and brain pathology provided insights into the role of vascular changes and insulin signaling function in the cognitive impairments observed in Alzheimer's disease (77).

The relationship between the brain and the gut is a research topic that has attracted increasing attention in recent years. In another study related to Alzheimer's disease, *Akkermansia muciniphila* (Akk) from the gut microbiota was administered via gavage to APP/PS1 mice for six months. The results showed that this intervention reduced diabetes-related parameters such as fasting blood sugar, improved intestinal barrier function, enhanced cognitive function as demonstrated in Y-maze tests, and reduced brain AB 40-42 levels. This approach represents an original way to address diabetes through microbiota intervention, beyond the direct application of a chemical substance, active ingredient, or extract (78).

Oxidative Stress

Oxidative stress is an important component in the pathogenesis of many diseases, is also important in diabetes. The effects of exercise or changes in diet towards healthier directions on reducing oxidative stress have been investigated in various studies. In a study that examined the effects of swimming exercise in C57BL/6 mice in which type 2 diabetes was induced, it was shown that diabetes increased oxidative stress in mice by elevating MDA and GSSG levels and that swimming exercise had a protective effect against this stress (79).

The nicotinamide-STZ model, which is a less common method of inducing diabetes, it was reported that crocin, one of the active ingredients of the saffron plant (*Crocus sativus*), restored the disrupted liver oxidant-antioxidant balance related to diabetes in rats and restored Total Antioxidant Capacity. Crocin also exhibited a similar protective effect in the kidneys (80).

Inflammation is often observed alongside oxidative stress, and it is also among the components of diabetes. In response to diabetes, proinflammatory cytokines such as IL-1B and IL-16 increase in tissues. One of these tissues is the brain tissue. Irisin molecules have been observed to reduce neuroinflammation in the mentioned mice and improve cognitive function based on behavioral test results (81).

Coagulation Disorders

In a study conducted to determine the potential protective effects of melatonin on hemostatic parameters in rats with diabetes induced by streptozotocin (40 mg/kg), 32 adult male healthy Wistar Albino rats were divided into four groups. After achieving the desired blood sugar levels for diabetes, melatonin (50 mg/kg i.p.) was administered for 8 weeks. Diabetic rats showed significantly increased platelet counts and fibrinogen levels. The administration of melatonin to diabetic rats partially improved these values, as well as PT and INR levels, indicating an improvement in the procoagulant state caused by diabetes (82).

Another study examining complications related to diabetes-induced coagulation used a leaf aqueous extract of *Terminalia catappa* (400 and 800 mg/kg - 28 days) in rats where diabetes was induced with STZ and a high-fat diet. The results suggested that the plant extract increased coagulation and bleeding time in diabetic rats and, due to its anticoagulant properties, it could be beneficial in reducing hematological problems related to diabetes (83).

Diabetic Foot Ulcer Model

Diabetes, when combined with neuropathic conditions, can lead to tissue damage ranging from ulcers to tissue necrosis. Innovative approaches such as 3D skin models, angiogenesis models, and skin bioprinting are being explored in research on this topic. However, traditional animal models are still being used (84).

Different parameters of this topic, from the development of tissue damage to the healing process, are also tested in animal studies. While rodents are more commonly used in these studies, larger animals like pigs can also be subjects of research. Although pigs have some model advantages in terms of nutrition and physiology, parameters such as cost, skin structure, and the long duration required for healing often lead to the preference for rats. Zucker Diabetic Sprague-Dawley rats are recommended models for investigating diabetic ulcers (85).

CONCLUSION

This review summarizes the pathophysiology of Diabetes Mellitus (DM), treatment strategies, and the link between experimental DM models and diabetic complications. Choosing appropriate animal models is essential for studying complications, as Type 1 DM results from autoimmune beta cell destruction, while Type 2 DM involves insulin resistance and beta cell dysfunction. Models must align with human disease features, though species differences limit full replication. Rodents are widely used due to cost and accessibility, while rabbits and primates serve specific roles. Diabetes can be induced by genetic manipulation, surgery, diets, or chemicals like STZ and Alloxan. NOD mice and chemically induced models are common for Type 1 DM; ob/ob mice and high-fat diets are used for Type 2 DM.

This review emphasizes complications such as nephropathy, retinopathy, neuropathy, and cognitive deficits. Natural compounds have shown benefits in reducing kidney damage, oxidative stress, and inflammation. Psychological and cognitive impairments are also addressed, with some treatments improving memory and anxiety. Antioxidants and anti-inflammatory agents appear effective in mitigating complications.

In conclusion, selecting the right animal model is critical for understanding DM and developing targeted, effective treatments to enhance patient outcomes.

Conflict of interest: *The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.*

Financial conflict of interest: *Author declares that he did not receive any financial support in this study.*

Address correspondence to: *Asli San Dagli Gul, Hacettepe University, Faculty of Medicine, Department of Physiology, Ankara, Türkiye*
e-mail: *aslisandagligul@hacettepe.edu.tr*

REFERENCES

1. Schleicher E, Gerdes C, Petersmann A, et al. Definition, classification, and diagnosis of diabetes mellitus. Exp Clin Endocrinol Diabetes. 2022;130(S 01):S1-S8. doi: 10.1055/a-1624-2897
2. Sweeting A, Wong J, Murphy HR, et al. A clinical update on gestational diabetes mellitus. Endocr Rev. 2022;43(5):763-93. doi: 10.1210/endrev/bnac003
3. Stožer A, Paradiž Leitgeb E, Pohorec V, et al. The role of cAMP beta cell stimulus–secretion and intercellular coupling. Cells. 2021;10(7):1658. doi: 10.3390/cells10071658
4. Mears D. Regulation of insulin secretion in islets of Langerhans by Ca^{2+} channels. J Membr Biol. 2004;200:57-66. doi: 10.1007/s00232-004-0692-9
5. de Souza AH, Tang J, Yadev AK, et al. Intra-islet GLP-1, but not CCK, is necessary for β-cell function in mouse and human islets. Sci Rep. 2020;10(1):2823. 3. doi: 10.1038/s41598-020-59799-2
6. Robertson RP. Glucagon and Insulin Overview: An Odd Couple's History and Physiology. J Endocrinol. 2023; pJOE-22. doi: 10.1530/JOE-22-0224
7. Huang W, Wu T, Xie C, et al. Sensing Intra-and Extra-Cellular Ca^{2+} in the Islet of Langerhans. Adv Funct Mater. 2022;32(3):2106020. doi: 10.1002/adfm.202106020
8. Rygiel KA, Elkins JM. Recent advances in the structural biology of tyrosine kinases. Curr Opin Struct Biol. 2023;82:102665. doi: 10.1016/j.sbi.2023.102665
9. Lin J, Selicharová I, Mitrová K, et al. Targeting the insulin receptor with hormone and peptide dimers. J Pept Sci. 2023;29(4):e3461. doi: 10.1002/psc.3461
10. Hall C, Yu H, Choi E. Insulin receptor endocytosis in the pathophysiology of insulin resistance. Exp Mol Med. 2020;52(6):911-20. doi: 10.1038/s12276-020-0456-3
11. Sulaiman MK. Diabetic nephropathy: recent advances in pathophysiology and challenges in dietary management. Diabetol Metab Syndr. 2019;11:1-5. doi: 10.1186/s13098-019-0403-4
12. Jensen TS, Karlsson P, Gylfadottir SS, et al. Painful and non-painful diabetic neuropathy, diagnostic challenges and implications for future management. Brain. 2021;144(6):1632-45. doi: 10.1093/brain/awab079
13. Yang P, Feng J, Peng Q, et al. Advanced glycation end products: potential mechanism and therapeutic target in cardiovascular complications under diabetes. Oxid Med Cell Longev. 2019;2019:9570616. doi: 10.1155/2019/9570616
14. Chang M, Nguyen TT. Strategy for treatment of infected diabetic foot ulcers. Acc Chem Res. 2021;54(5):1080-93. doi: 10.1021/acs.accounts.0c00864
15. Schneider DJ. Diabetes and thrombosis. In: Diabetes and cardiovascular disease. Springer; 2023:99-127. doi: 10.1007/978-3-031-13177-6_5
16. Kottaisamy CPD, Raj DS, Prasanth Kumar V, et al. Experimental animal models for diabetes and its related complications—a review. Lab Anim Res. 2021;37(1):1-14. doi: 10.1186/s42826-021-00101-4
17. Rees D, Alcolado J. Animal models of diabetes mellitus. Diabet Med. 2005;22(4):359-70. doi: 10.1111/j.1464-5491.2005.01499.x
18. Eulálio JMR, Ferreira ML, Silva PC, et al. Laparoscopic Pancreatectomy in Rats: The Development of an Experimental Model. J Invest Surg. 2022;35(4):776-82. doi: 10.1080/08941939.2021.1946220
19. Masiello P. Animal models of type 2 diabetes with reduced pancreatic β-cell mass. The international journal of biochemistry & cell biology. 2006;38(5-6):873-93. doi: 10.1016/j.biocel.2005.09.007
20. Case RM. Is the rat pancreas an appropriate model of the human pancreas? Pancreatology. 2006;6(3):180-90. doi: 10.1159/000091849
21. Tsuchitani M, Sato J, Kokoshima H. A comparison of the anatomical structure of the pancreas in experimental animals. J Toxicol Pathol. 2016;29(3):147-154. doi: 10.1293/tox.2016-0016
22. King AJ. The use of animal models in diabetes research. Br J Pharmacol. 2012;166(3):877-94. doi: 10.1111/j.1476-5381.2012.01911.x
23. Qamar F, Sultana S, Sharma M. Animal models for induction of diabetes and its complications. J Diabetes Metab Disord. 2023:1-8. doi: 10.1007/s40200-023-01277-3
24. Algul S, Ozcelik O. Comprehensive review of animal models in diabetes research using chemical agents. Lab Anim. 2025; p. 00236772241296199. doi: 10.1177/00236772241296199
25. Zhao ZS, Khan S, O'Brien PJ. The prevention of ferric nitrilotriacetate-induced nephro-and hepatotoxicity by methylenedioxybenzene antioxidants. Chem. Biol. Interact. 1997; 108(1-2): p. 107-18. doi: 10.1016/S0009-2797(97)00103-8.
26. Wright PH. The production of experimental diabetes by means of insulin antibodies. Am J Med. 1961 Dec; 31:892-900. doi: 10.1016/0002-9343(61)90031-6.
27. Maqbool M, Dar MA, Gani I, Mir SA. Animal models in diabetes mellitus: an overview. J Drug Deliv Ther. 2019;9(1-s):472-5. doi: 10.22270/jddt.v9i1-s.2351
28. Lenzen S. The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia. 2008;51(2):216-26. doi: 10.1007/s00125-007-0886-7
29. Tiedge M, Richter T, Lenzen S. Importance of cysteine residues for the stability and catalytic activity of human pancreatic beta cell glucokinase. Arch Biochem Biophys. 2000;375(2):251-60. doi: 10.1006/abbi.1999.1666
30. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50(6):537- doi: 10.33549/physiolres.933247
31. Katoh M, Sakurai K, Fujimoto Y. Alloxan radical-induced generation of reactive oxygen species in the reaction system of alloxan with ascorbate. Yakugaku Zasshi: J Pharm Soc Jpn. 2002;122(10):831-9. doi: 10.1248/yakushi.122.831
32. Aba PE, Edeh MN. Age Susceptibility of Wistar Rats to Alloxan-Induced Diabetes: A Paradox. Not Sci Biol. 2019;11(2). doi: 10.15835/nsb11210438
33. Mostafavinia A, Amini A, Ghorishi SK, et al. The effects of dosage and the routes of administrations of streptozotocin and alloxan on induction rate of type 1 diabetes mellitus and mortality rate in rats. Lab Anim Res. 2016;32:160-5. doi: 10.5625/lar.2016.32.3.160
34. Zhang M, Lv X-Y, Li J, et al. The characterization of high-fat diet

and multiple low-dose streptozotocin induced type 2 diabetes rat model. J Diabetes Res. 2008:704045. doi: 10.1155/2008/704045
35. Singaram S, Lawrence RS et al. Studies on the biosynthesıs of the antibıotic streptozotocin (streptozocin) by streptomyces achromogenes var. streptozoticus. J. Antibiot. 1979;32(4):379-385. doi: 10.7164/antibiotics.32.379
36. Siedlecka D, Micał W. Streptozotocin - an antibiotic used to induce diabetes on experimental animals. J educ helath sport. 2020;10(9):906-909. doi.org/10.12775/JEHS.2020.10.09.110
37. Broder LE, Carter SK. Pancreatic islet cell carcinoma: II. Results of therapy with streptozotocin in 52 patients. Ann Intern Med. 1973;79(1):108-118. doi: 10.7326/0003-4819-79-1-108
38. Ghasemi A, Jeddi S. Streptozotocin as a tool for induction of rat models of diabetes: A practical guide. EXCLI Journal. 2023;22:274. doi: 10.17179/excli2022-5720
39. McNeill JH. Experimental models of diabetes. Routledge; 2018. ISBN: 0-8493-1667-7
40. Wu J, Yan L-J. Streptozotocin-induced type 1 diabetes in rodents as a model for studying mitochondrial mechanisms of diabetic β cell glucotoxicity. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2015:181-188. doi: 10.2147/DMSO.S82272
41. Ghasemi A, Norouzirad R. Type 2 diabetes: an updated overview. Critical Reviews™ in Oncogenesis. 2019;24(3). doi: 10.1615/CritRevOncog.2019030976
42. Yan L-J. The nicotinamide/streptozotocin rodent model of type 2 diabetes: Renal pathophysiology and redox imbalance features. Biomolecules. 2022;12(9):1225. doi: 10.3390/biom12091225
43. Junod A, Lambert AE, Stauffacher W, et al. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. The Journal of Clinical Investigation. 1969;48(11):2129-2139. doi: 10.1172/JCI106180
44. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Current Protocols. 2021;1(4):e78. doi: 10.1002/cpz1.78
45. Elamin N, Fadlalla I, Omer S, et al. Histopathological alteration in STZ-nicotinamide diabetic rats, a complication of diabetes or a toxicity of STZ. Int J Diabetes Clin Res. 2018;5(3):1-8. doi: 10.23937/2377-3634/1410091
46. Gilbert ER, Fu Z, Liu D. Development of a nongenetic mouse model of type 2 diabetes. J Diabetes Res. 2011;2011(1):416254. doi: 10.1155/2011/416254
47. Qinna NA, Badwan AA. Impact of streptozotocin on altering normal glucose homeostasis during insulin testing in diabetic rats compared to normoglycemic rats. Drug Des Devel Ther. 2015:2515-2525. doi: 10.2147/DDDT.S79885
48. Wszola M, Klak M, Kosowska A, et al. Streptozotocin-induced diabetes in a mouse model (BALB/c) is not an effective model for research on transplantation procedures in the treatment of type 1 diabetes. Biomedicines. 2021;9(12):1790. doi: 10.3390/biomedicines9121790
49. Wang-Fischer Y, Garyantes T. Improving the reliability and utility of streptozotocin-induced rat diabetic model. J Diabetes Res. 2018;2018(1):8054073. doi: 10.1155/2018/8054073
50. Kleinert M, Clemmensen C, Hofmann SM, et al. Animal models of obesity and diabetes mellitus. Nat Rev Endocrinol. 2018;14(3):140-162. doi: 10.1038/nrendo.2017.161
51. González P, Lozano P, Ros G, et al. Hyperglycemia and oxidative stress: An integral, updated and critical overview of their metabolic interconnections. Int J Mol Sci. 2023;24(11):9352. doi: 10.3390/ijms24119352
52. Vianello E, Beltrami AP, Aleksova A, et al. The advanced glycation end-products (AGE)–receptor for AGE system (RAGE): An inflammatory pathway linking obesity and cardiovascular diseases. Int J Mol Sci. 2025;26(8):3707. doi: 10.3390/ijms26083707
53. Xiao Q, Wang D, Li D, et al. Protein kinase C: A potential therapeutic target for endothelial dysfunction in diabetes. J Diabetes Complications. 2023;37(9):108565. doi: 10.1016/j.jdiacomp.2023.108565
54. Tigchelaar C, van Zuylen ML, Hulst AH, et al. Elevated cerebrospinal fluid glucose levels and diabetes mellitus are associated with activation of the neurotoxic polyol pathway. Diabetologia. 2022;65(6):1098-1107. doi: 10.1007/s00125-022-05693-7
55. Dissanayake WC, Oh JK, Sorrenson B, et al. Glucose regulates expression of pro-inflammatory genes, IL-1β and IL-12, through a mechanism involving hexosamine biosynthesis pathway-dependent regulation of α-E catenin. Biosci Rep. 2021;41(7):BSR20211066. doi: 10.1042/BSR20211066
56. Dong An, Tan B, Yu DY, et al. Differentiating microaneurysm pathophysiology in diabetic retinopathy through objective analysis of capillary nonperfusion, inflammation, and pericytes. Diabetes. 2022;71(4):733-746. doi: 10.2337/db21-0737
57. Dahlin LB. The dynamics of nerve degeneration and regeneration in a healthy milieu and in diabetes. Int J Mol Sci. 2023;24(20):15241. doi: 10.3390/ijms242015241
58. Zakir M, Ahuja N, Surksha M, et al. Cardiovascular complications of diabetes: From microvascular to macrovascular pathways. Cureus. 2023;15(9):e45835. doi: 10.7759/cureus.45835
59. Maruhashi T, Higashi Y. Pathophysiological association between diabetes mellitus and endothelial dysfunction. Antioxidants (Basel). 2021;10(8):1306. doi: 10.3390/antiox10081306
60. Ren Q, Yu S, Zeng H, et al. The role of PTEN in puromycin aminonucleoside-induced podocyte injury. Int J Med Sci. 2022;19(9):1451-1459. doi: 10.7150/ijms.72988
61. Ye LX, Huang HH, Zhang SH, et al. Lu JS, Cao DX, Wu DD, Chi PW, Hong LH, Wu MX, Xu Y, Yu CX. Streptozotocin-induced hyperglycemia affects the pharmacokinetics of koumine and its anti-allodynic action in a rat model of diabetic neuropathic pain. Front Pharmacol. 2021;12:640318. doi: 10.3389/fphar.2021.640318
62 Prandi FR, Evangelista I, Sergi D, et al. Mechanisms of cardiac dysfunction in diabetic cardiomyopathy: molecular abnormalities and phenotypical variants. Heart Fail Rev. 2023;28(2):597-606. doi: 10.1007/s10741-021-10200-y
63. Hu T, Yue J, Tang Q, et al. The effect of quercetin on diabetic nephropathy (DN): A systematic review and meta-analysis of animal studies. Food Funct. 2022;13(9):4789-4803. doi: 10.1039/D1FO03958J
64. Ji J, Tao P, Wang Q, et al. SIRT1: mechanism and protective effect in diabetic nephropathy. Endocr Metab Immune Disord Drug Targets. 2021;21(5):835-842. doi: 10.2174/1871530320666201029143606
65. Cao X, Wei R, Zhou J, et al. Wenshen Jianpi recipe, a blended traditional Chinese medicine, ameliorates proteinuria and renal injury in a rat model of diabetic nephropathy. BMC Complement Altern Med. 2019;19(1):1-9. doi: 10.1186/s12906-019-2598-1
66. Jin D, Liu F, Yu M, et al. Jiedu Tongluo Baoshen formula enhances podocyte autophagy and reduces proteinuria in diabetic kidney disease by inhibiting PI3K/Akt/mTOR signaling pathway. J Ethnopharmacol. 2022;293:115246. doi: 10.1016/j.jep.2022.115246
67. Olivares AM, Althoff K, Chen GF, et al. Animal models of diabetic retinopathy. Curr Diab Rep. 2017;17:1-17. doi: I 10.1007/s11892-017-0913-0

68. Quiroz J, Yazdanyar A. Animal models of diabetic retinopathy. Ann Transl Med. 2021;9(15):1272. doi: 10.21037/atm-20-6737
69. Naderi A, Zahed R, Aghajanpour L, et al. Longterm features of diabetic retinopathy in streptozotocin-induced diabetic Wistar rats. Exp Eye Res. 2019;184:213-20. doi: 10.1016/j.exer.2019.04.025
70. de Melo IMF, Ferreira CGM, da Silva Souza EHL, et al. Melatonin regulates the expression of inflammatory cytokines, VEGF, and apoptosis in diabetic retinopathy in rats. Chem Biol Interact. 2020;327:109183. doi: 10.1016/j.cbi.2020.109183
71. Calcutt NA. Diabetic neuropathy and neuropathic pain: a (con) fusion of pathogenic mechanisms? Pain. 2020;161(Suppl 1):S65. doi: 10.1097/j.pain.0000000000001922
72. Jin HY, Moon S-S, Calcutt NA. Lost in translation? Measuring diabetic neuropathy in humans and animals. Diabetes Metab J. 2021;45(1):27-42. doi: 10.4093/dmj.2021.0034
73. Kale M, Nimje N, Aglawe MM, Umekar M, Taksande B, Kotagale N. Agmatine modulates anxiety and depression-like behaviour in diabetic insulin-resistant rats. Brain Res. 2020;1747:147045. doi: 10.1016/j.brainres.2020.147045
74. Ergenc M, Ozacmak HS, Turan I, et al. Melatonin reverses depressive and anxiety-like behaviours induced by diabetes: involvement of oxidative stress, AGE, RAGE, and S100B levels in the hippocampus and prefrontal cortex of rats. Arch Physiol Biochem. 2022;128(2):402-10. doi: 10.1080/13813455.2019.1684954
75. Yuan P, Zhang J, Li L, Song Z. Fluoxetine attenuated anxiety-like behaviors in streptozotocin-induced diabetic mice by mitigating inflammation. Mediators Inflamm. 2019;2019(1):4315038. doi: 10.1155/2019/4315038
76. Kimura N. Diabetes mellitus induces Alzheimer's disease pathology: histopathological evidence from animal models. Int J Mol Sci. 2016;17(4):503. doi: 10.3390/ijms17040503
77. Takeda S, Sato N, Uchio-Yamada K, et al. Diabetes-accelerated memory dysfunction via cerebrovascular inflammation and Aβ deposition in an Alzheimer mouse model with diabetes. Proc Natl Acad Sci. 2010;107(15):7036-7041. doi: 10.1073/pnas.1000645107
78. Ou Z, Deng L, Lu Z, et al. Protective effects of Akkermansia muciniphila on cognitive deficits and amyloid pathology in a mouse model of Alzheimer's disease. Nutr Diabetes. 2020;10(1):12. doi: 10.1038/s41387-020-0115-8
79. Matinfar P, Peeri M, Azarbayjani MA. Swimming exercise attenuates anxiety-like behavior by reducing brain oxidative stress in type 2 diabetic mice. Physiol Behav. 2021;237:113449. doi: 10.1016/j.physbeh.2021.113449
80. Margaritis I, Angelopoulou K, Lavrentiadou S, et al. Effect of crocin on antioxidant gene expression, fibrinolytic parameters, redox status, and blood biochemistry in nicotinamide-streptozotocin-induced diabetic rats. J Biol Res Thessalon. 2020;27:1-15. doi: 10.1186/s40709-020-00114-5
81. Wang K, Song F, Xu K, et al. Irisin attenuates neuroinflammation and prevents the memory and cognitive deterioration in streptozotocin-induced diabetic mice. Mediators Inflamm. 2019;2019(1):1567179. doi: 10.1155/2019/1567179
82. Keskin E, Uluisik D. The effect of melatonin on some coagulation parameters in streptozotocin-induced diabetic rats. Kocatepe Vet J. 2019;12(2):130-4. doi: 10.30607/kvj.511340
83. Iheagwam FN, Garuba PA, Ogunlana OO, Chinedu SN. Counteractive role of Terminalia catappa leaf extract on hematological and coagulation disturbance in Type 2 diabetic rats. Veterinary World. 2023;16(8):1593. doi: 10.14202/vetworld.2023.1593-9
84. Phang SJ, Arumugam B, Kuppusamy UR, et al. A review of diabetic wound models—Novel insights into diabetic foot ulcer. J Tissue Eng Regen Med. 2021;15(12):1051-1068. doi: 10.1002/term.3246
85. Rai V, Moellmer R, Agrawal DK. Clinically relevant experimental rodent models of diabetic foot ulcer. Mol Cell Biochem. 2022;477(4):1239-47. doi: 10.1007/s11010-022-04372-w