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Review Article

Research Progress on Animal Models of Diabetes Mellitus

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Abstract

Diabetes Mellitus (DM) is a complicated metabolic illness defined by hyperglycemia due to faulty insulin production or activity, and it is one of the world's fastest-growing public health concerns. The construction of a good diabetes mellitus model is crucial for diabetes mellitus pathophysiology research, diabetic mellitus prevention, and the development of effective therapeutic therapy. Diabetes mellitus models are now chemically induced, spontaneous, and genetically created. The models were created using the chemical induction of streptozotocin and alloxan, and the spontaneous type of diabetes mellitus animal models are appropriate for T1DM, T2DM, and related phenotypes, including obesity and insulin resistance. Genetically engineered animal models are much more closely related to human diabetes mellitus, including gene knockout animal models. They can be used to explain the cause of diabetes mellitus in terms of molecular mechanisms and to study the effects of individual genes on insulin secretion and insulin resistance faultlessly. This research provides a detailed description of T1DM and T2DM animal models, illustrating the importance of diabetes mellitus.

Keywords: Diabetes mellitus; Animal models; Model construction

Background

Diabetes Mellitus (DM) is a metabolic condition characterized by chronic hyperglycemia that is one of the most common life-threatening diseases in the world [1]. Diabetes mellitus is classified into three types based on its etiology and clinical features: Type 1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM), and Gestational Diabetes Mellitus (GDM) [2]. T1DM, also known as juvenile diabetes mellitus or insulin-dependent diabetes mellitus, is a type of diabetes that is insulin-dependent [3], is caused by autoimmune death of Beta cells, which results in insulin insufficiency [4], which accounts for 5-10% of diabetes mellitus patients [2]. T2DM, also known as adult diabetes mellitus or non-insulin-dependent diabetes mellitus, is the most frequent form of diabetes mellitus, accounting for 90% of all cases, due to beta cells' failure to properly adjust for insulin resistance, resulting in insulin deficit and hyperglycemia [5]. Their children are also at risk of getting obesity and T2DM at a young age [6]. With the increasing research on diabetes mellitus, clinical and basic research is getting closer and closer. The establishment of an appropriate diabetes mellitus model can improve the mechanism of diabetes mellitus and promote basic research and guide the clinic. In the basic research of diabetes mellitus, the research tools can be divided into cellular models and animal models. Cellular models are utilized in the research of cell function, drug screening, and the interaction of cytokines and receptors. In contrast, animal models are used in studies of diabetes mellitus pathophysiology and efficacy. Animal models, as opposed to cellular models, can completely replicate the beginning and progression of diabetes mellitus and the development of specific treatment medications for diabetes mellitus; As a result, animal models are extensively used in the study of diabetes mellitus pathogenesis. With the development of biomedical technology, animal models of diabetes mellitus have been successfully established in rats, pigs, monkeys, zebra fish, dogs, rabbits, etc. The choice of animal model will be impacted by whether a type 1 diabetes mellitus or type 2 diabetes mellitus model is required, as well as the objective of the study, whether it is to learn more about the pathophysiology, to prevent disease development, or to cure the illness. Therefore, in this review, T1DM animal models and T2DM animal models are discussed systematically to address the above issues respectively.

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Diabetic Mellitus Animal Species

Rodent Diabetes Mellitus Model

Among rodents, rats and mice are the main experimental animals used in diabetes mellitus models, with features such as easy access, low breeding cost, fast reproduction rate, high ethical acceptance, standardized sanitary environment, genetic similarity to human genes of more than 85%, efficient and mature genetic modification techniques, large range of mutants and inbred phenotypes [7]. Rodent models commonly used for T1DM research include alloxan-induced, streptozotocin-induced, Non-Obese Diabetic (NOD) mouse models, and Biologically Bred (BB) rat models. In contrast, rodent models commonly used for T2DM research include Zucker Diabetic Fatty (ZDF) rats, Otsuka Long Evans Tokushima Fatty (OLETF) rats, Kuo Kondo (KK) mice, Goto Kakizaki (GK) rats [8], and to a lesser extent, some other rodent models established by genetic engineering. Genetically engineered rodent models of diabetes mellitus are also available. In conclusion, rodents can be used to establish models of diabetes mellitus and its complications, which can help to understand the pathogenesis, identify therapeutic targets, and develop effective therapeutic drugs [9-11].

Large Mammalian Diabetes Mellitus Model

Large mammals are essential experimental animals in diabetes mellitus models, and the induction of dogs [12], pigs [13], and primates [14] into hyperglycemic diabetes mellitus animal models can improve the relevance to humans and provide more valuable clinical guidance since these animals have a comparable islet structure to humans and a similar portal vein size [9]. A canine model of T2DM, for example, may be produced by combining high-fat food with low dosages of Streptozotocin (STZ) induction. The dog model produced by this technology supports the hyperbolic connection reported in humans between insulin sensitivity and insulin production and fully depicts the coordinated involvement of numerous organs (e.g., pancreas, liver, adipose tissue) to maintain normal blood glucose levels [15]. Feeding high-fat, high-carbohydrate combined with STZ induction can form a porcine model of T2DM, anatomically and metabolically similar to humans and prone to atherosclerosis. All of these factors combine to make it a suitable model for researching the cardiovascular consequences of T2DM [16]. Yuan W et al. reported vital glycated and glycosylated hemoglobin levels with severe glucose tolerance impairments in 11 experimental marmosets following partial pancreatectomy (removing about 70% of pancreatic tissue). They multiplied after STZ infusions (100-160mg/kg). The glucose levels were highly variable, similar to the glycemic instability characteristic of human T1DM patients, with a 73% modeling rate [17]. The difficulty of obtaining spontaneous nonhuman primate diabetes mellitus animals, the high cost of animals and breeding, and the low ethical acceptance of these animals have made them difficult to be widely used.

Non-mammalian Diabetes Mellitus Model

Quantitative and high-throughput analyses can be performed in non-mammals for initial exploration and refinement of basic conceptual analysis of diabetes mellitus, such as zebra fish and drosophila. The pancreatic structure and glucose metabolism control mechanisms of zebrafish are highly homologous to those of humans and share a high 87% similarity with human genes. In addition, zebrafish have the advantages of short sexual maturity, fast reproduction, small size for easy handling, low cost of breeding, and transparent embryos for easy observation of tissue and organ differentiation. Therefore, zebra fish are well suited for studying the mechanism and development of diabetes mellitus. Drosophila and humans have similar biological characteristics. The gene sequencing of drosophila has been completed, making it a great research value in disease development mechanism and gene expression regulation, etc. Moreover, as a model organism, drosophila has the advantages of simple breeding, fast reproduction, and clear genetic background, which provides a more suitable experimental animal for basic research on diabetes mellitus in gene regulation.

Chemically Induced Diabetes Mellitus

Some chemicals can induce experimental animals into diabetes mellitus models, and such chemicals are called diabetogenic factors. The most important and commonly used compounds for inducing experimental diabetes mellitus animal models are alloxan and streptozotocin [18]. Because both chemicals are toxic glucose analogs that accumulate in pancreatic Beta cells via the Glucose Transporter Protein 2 (GLUT2), their doses may fluctuate depending on animal type and manner of delivery [19]. Both alloxan and streptozotocin can induce both T1DM and T2DM; however, these chemicals are most commonly used to induce T1DM because they do not directly induce insulin resistance [20].

Streptozotocin-Induced Diabetes Mellitus

STZ is a broad-spectrum antibiotic with specific toxicity to pancreatic Beta cells because its structure is similar to that of sugar; it can be taken up by GLUT2 on the Beta-cell membrane and reduced to glucose and methyl nitrite urine, which can alkylate Beta cells, leading to Beta-cell death eventually; it can also destroy mitochondrial DNA, which prevents Beta cells from obtaining energy and leads to a decrease in glucose-induced insulin secretion [21]. The STZ induction model can be divided into one high-dose STZ method and several low-dose STZ methods. The introduction of these models is given below.

One High-Dose STZ

This method has a high modeling rate, short cycle time, and easy formation of the T1DM model but a high mortality rate. Barragán-Bonilla MI et al. administered 39 male and 37 female Wistar rats a single injection of STZ (90mg/kg body weight) at birth, followed by 7 weeks of sweetened beverage with 10% sucrose content (SSB; sucrose at 10% or 30%) after weaning, and developed hyperglycemia. After maturity, the individuals developed T2DM-like signs and symptoms, such as hyperglycemia, modest insulin resistance, and hyperactive neuralgia [22]. Wang X et al. established a diabetic peripheral neuropathy model after intraperitoneal administration of STZ (60mg/kg body weight) to SD rats. The established model of diabetic peripheral neuropathy was gavaged with different concentrations of DPDs (Diphenyl diselenide) once a day for 12 weeks, demonstrating that DPDs can be used as a new therapy for diabetic peripheral neuropathy [23].

Multiple Low-dose STZ

It is the method of diabetes mellitus induction by multiple injections of low-dose STZ. The number of injections is mostly 2-7 times, and the time interval of injection is inconsistent and can be adjusted according to the specific experimental environment and the conditions. Wang F et al. administered intraperitoneal injections of 50mg/kg STZ dissolved in 0.1M sodium citrate (pH

4.5) daily to 8-week-old macrophage-specific ubiquitin-binding enzyme E2 (Ubc9) knockout (LyzM-Cre-Ubc9fl/fl, KO) mice [24]. Li L et al. established a T1DM model in healthy male 8-week-old SD rats fed high-fat (10% cooked lard, 20% sucrose, 2.5% cholesterol, 1.0% bile salts, 66.5% normal diet) for four weeks, followed by a 12-hour fast and injected with low-dose STZ (25mg/kg once daily for two days) [25]. Anchi P et al. administered 40mg/kg of STZ to eight male Swiss male mice at 6-8 weeks of age once a day. All eight mice developed T1DM after 5 consecutive days, which helped them study the pharmacokinetics and pharmacodynamics of curcumin [26].

Alloxan-Induced Diabetes Mellitus

Alloxan is a mercaptan reactant, and early studies found that alloxan injections alone can induce diabetes [27], which penetration into Beta cells through Glucose Transporter Protein 4 (GLUT4), and its reduction product, diuretic acid, generates superoxide radicals and hydrogen peroxide. Finally, hydroxyl radicals are generated in iron-catalyzed reactions that damage the DNA of pancreatic Beta cells and eventually lead to the death of pancreatic Beta cells irreversibly, and inhibit the production of glucokinase and thus inhibit Glucose-induced insulin secretion [28]. Alloxan-induced is one of the most effective methods for establishing animal models of experimental diabetes mellitus, and it can be used to induce T1DM and T2DM [29]. Federiuk IF et al. established a T1DM animal model in 41 SD rats by multiple intraperitoneal injections of moderate to high doses of alloxan (100 to 150mg/kg), single intravenous injections of moderate doses of alloxan (100 to 150mg/kg, intravenous injection) and intravenous injections of restricted alloxan doses of 200mg/kg. T1DM animal model, a single high dose (200mg/kg body weight intraperitoneally) was the best molding method, resulting in a 70% incidence of T1DM and 10% mortality [29]. In addition, Sathya A et al. treated SD rats weighing 150g to 200g with overnight fasting. They administered a single intraperitoneal injection of alloxan-hydrate (150mg/kg) prepared in sterile distilled water to induce diabetes mellitus. After injection, these SD rats were allowed to eat, drink and move freely. After 72h, these SD rats with fasting blood glucose levels above 200mg/dl were diagnosed with T2DM (hyperglycemia). They were selected for their study to investigate the health effects of Mucuna pruriens bark extract and empty bean pod extract [30].

Spontaneous Animal Models of T1DM

T1DM is caused by a mix of genetic predisposition and environmental factors, all of which cause an autoimmune illness characterized by the gradual death of pancreatic Beta cells and inadequate insulin output [31]. T1DM is common in children and young adults [32]. Therefore, T1DM is also considered adolescent diabetes mellitus or insulin-deficient diabetes mellitus [3], which is caused by T cells destroying pancreatic Beta cells, leading to insulin insufficiency [33]. Studying animal models of T1DM helps to realize the pathogenesis of T1DM and helps to evaluate new therapies (monotherapies or combination therapies) with therapeutic potential. Animal models of spontaneous T1DM are divided into autoimmune and non-autoimmune. These animal models of spontaneous autoimmune T1DM include the NOD mice, BB rat, LEW.1AR1/-IDDM rat, and KDP rat spontaneous non-autoimmune T1DM models include the Akita mice.

Spontaneous Autoimmune Animal Model of T1DM

The NOD Mouse

The Non-Obese Aiabetic (NOD) mouse, which was created in 1974 at the Shionogi Research Laboratory in Osaka, Japan, is one of the most extensively used models for studying T1DM [34]. Compared to other models used to study autoimmune, NOD mice can simulate spontaneous diabetes similar to humans [35]. In the NOD mouse, 3 or 4-week-old mouse develop pancreatitis and islet infiltration with innate immune cells. The innate immune cells that infiltrate the islets are mostly CD4+ and CD8+ lymphocytes, NK cells, B cells, dendritic cells, macrophages, and neutrophils [36-39]. This pathogenesis is the same as in humans. After roughly 4-6 weeks of age, innate immune cell infiltration in NOD mouse islets attracts CD4+ and CD8+ T cell subsets of the adaptive immune system, resulting in diabetes mellitus in NOD mice [36,37]. Those above innate and adaptive immune cells can infiltrate the islets via immune-mediated or disturbed islet cell initiation, which is required to develop diabetes mellitus. The similarity of T1DM genes in NOD mice and humans, which is critical for understanding the mechanisms and pathways of diabetes mellitus, was revealed [38].

The BB Rat

The most often studied rodent model of T1DM, the BB rat, is a form of autoimmune diabetes mellitus that develops spontaneously. For the first time, rats with this trait were detected in Canadian colonies in 1974. BB rats often acquire diabetes mellitus shortly after juvenile life (prevalence of around 90%), and the prevalence of this spontaneous diabetes mellitus model is optimal; in fact, almost 90% of BB rats develop diabetes mellitus between the ages of 8 and 16 weeks [39], and the prevalence is almost the same in both sexes [40]. The symptoms of diabetes mellitus are very pronounced, characterized by hyperglycemia, hyperinsulinemia, weight loss, and ketonuria [41], and the BB rat strain usually shows severe T-lymphopenia in the circulating blood, especially the lack of ART2-expressing T-lymphocytes [39]. In spontaneous diabetes mellitus BB rats, the normal course of pancreatitis differs from that of NOD mice. This results in a morphology that is comparable to human T1DM and is dominated by Th1-type cells in BB rats [42]. BB rats can reasonably predict the onset of diabetes mellitus and therefore have become a useful model for dissecting the serum factors associated with the early progressive stages of diabetes mellitus, and they have also been shown to be useful for investigating new therapies against the progression of diabetes mellitus.

The LEW.1AR1-IDDM Rat

The Hannover Medical School's Institute of Experimental Animal Science generated the LEW.1AR1-IDDM rat, which is the same species as the Lewis rat and represents a model of spontaneous autoimmune T1DM [43]. As LEW.1AR1-IDDM rats are further inbred, the prevalence can increase from 20% to 60% [44,45], and the prevalence is the same for both male and female rats [45]. The main advantage of using this animal model is that it shows signs of diabetes mellitus in the early stages, with islet cell infiltration occurring approximately one week before hyperglycemia. This one animal model can be employed in diagnostic research, including predicting early T1DM and diabetic mellitus prevention. In addition, the relatively short prediabetes mellitus period can also be used to effectively analyze the different conditions of the immune cell infiltration at various phases [45]. Unlike BB rats and NOD mice, LEW.1AR1-IDDM rats live well after the start of diabetes and do not acquire further autoimmune diabetes problems. As a result of these conditions, the LEW.1AR1-IDDM rat model is ideal for studying diabetic complications [46].

The KDP Rat

The Komeda Diabetes-Prone (KDP) rats are descended from the Long-Evans Tokushima Lean strain, which develops characteristic T1DM symptoms such as polyuria, hyperglycemia, and weight loss after roughly 60 days of selective breeding [47]. Similar to other animal models of T1DM with autoimmunity, the loss of Beta cells is caused by insulitis [48]. However, unlike the pancreas of NOD mice, BB rats, LEW.1AR1-IDDM rats, and T1DM patients, which showed pro-inflammatory cytokines IL-1 and TNF-, and these pro-inflammatory cytokines were expressed in both macrophages and T cells, the pro-inflammatory cytokines expressed by immune cells in the pancreas of KDP rats were primarily IFN- and TNF-, suggesting that the combination of high expression of pro-inflammatory cytokines [49]. In addition, genetic analysis of KDP rats that spontaneously developed T1DM revealed that most of the genetic susceptibility to diabetes mellitus could be explained by 2 major susceptibility loci, MHC on chromosome 20 and IDDM/KDP on chromosome 11 [50]. Thus, it can be seen that KDP rats are one of the best animal models for studying spontaneous autoimmunity in T1DM [51].

Spontaneous Non-autoimmune Model of T1DM

The Akita Mice

The Akita mice were first bred at the Akita City Research Institute in Japan, hence the name Akita mice. This model was developed using C57BL/6NSlc mice in which the Ins2/C96Y gene in Akita mice was mutated owing to a single nucleotide substitution in the Ins2 gene [52]. Ins2/C96Y gene mutations cause aberrant insulin protein folding and toxic damage to pancreatic Beta cells, and a reduction in the capacity of pancreatic Beta cells to release insulin [32], subsequently leading to Endoplasmic Reticulum stress (ER) and ultimately severe T1DM in Akita mice at 3 to 4 weeks of age [43], This might be related to estrogen's protective action [53]. It has been shown that the survival rate of Akita purebred mice is very low without insulin treatment, not exceeding 12 weeks. The C57BL/6-Ins2 Akita mice model offers several benefits over the STZ-induced paradigm. For example, C57BL/6-Ins2+/C96Y mice exhibit greater proteinuria levels and systemic renal pathological alterations and can be developed into a diabetic nephropathy model [54]. Despite this, proteinuria was infrequent in C57BL/6-Ins2+/C96Y mice, and thylakoid enlargement was the only renal pathological alteration discovered [55]. Due to their strong resemblance to other rodent models as well as human clinical traits, the Akita mice have been established to be a viable model of diabetic sympathetic autonomic neuropathy [56].

Spontaneous Animal Models of T2DM

T2DM is primarily caused by insulin resistance [57]. Insulin Resistance (IR) inhibits muscle cells' capacity to absorb and retain glucose and lipids, resulting in higher blood glucose and triglyceride levels [58]. Therefore, the establishment of animal models of T2DM can help researchers understand the pathogenesis of T2DM and develop new drugs with targeted effects. In this regard, this article summarizes the commonly used T2DM models for obese and non-obese animals suffering from symptoms such as hyperglycemia, insulin resistance, and hyperinsulinemia [59], and classifies the T2DM models into obese and non-obese types. The spontaneous T2DM obesity models include $Lepr^{db/db}$ mice, $Lep^{ob/ob}$ mice, KK mice, Zucker fatty rats and Zucker diabetic fatty rats, and the spontaneous T2DM non-obese models include GK rats. These spontaneous animal models exhibit the same clinical symptoms as T2DM patients to some extent, and the pathophysiology of T2DM, obesity, leptin signaling, and the relationship between the three have all been studied using and spontaneous type 2 diabetes obesity models.

Spontaneous Obesity Model of T2DM

The $Lepr^{db/db}$ Mice

The Leprdb/db mice are the most common T2DM model [60], originating from the Jackson laboratory [61], and are an autosomal recessive obesity phenotype caused by a mutation in the leptin receptor [62]. They are characterized by excessive food consumption and insulin overproduction. Within the first month, $Lepr^{db/db}$ mice acquire clinical and metabolic indications of diabetes mellitus, including as obesity, hyperinsulinemia, and hyperglycemia, followed by hyperinsulinemia and hyperglycemia, which peak between 3 and 4 months. After 3-4 weeks of age, $Lepr^{db/db}$ mice develop obesity and hyperinsulinemia. Because hyperinsulinemia appears at two weeks of age, hyperglycemia appears at four to eight weeks, renal function declines at fifteen to eighteen weeks, and their islet cells are sensitive to the toxic response to hyperglycemia, these mice eventually become insulin-dependent to control hyperglycemia and survive [32]. $Lepr^{db/db}$ mice have persistently elevated blood glucose levels, severe islet depletion, and myocardial disease, subsequently leading to death around the age of 10 months [63]. This model is used to study the advanced stages of diabetes mellitus because $Lepr^{db/db}$ mice develop advanced reactive gliosis as well as vascular leakage [64].

The $Lep^{ob/ob}$ Mice

Inheritance of a spontaneous mutation on chromosome 6 in the phenotypic C57BL/6 mice, which was initially detected in a distantly related population at Bar Harbor, Jackson Laboratory, in 1949, resulted in a mouse model of $Lep^{ob/ob}$ with extreme obesity. However, it was not until 1994 that the altered protein was identified as leptin [65]. Thus, infusing leptin into $Lep^{ob/ob}$ mice reduces weight gain, decreases food intake, increases energy expenditure, and improves insulin sensitivity [66]. With hyperinsulinemia, $Lep^{ob/ob}$ mice begin to gain weight at 2 weeks of age and can reach up to 3 times the typical weight of wild-type controls. After 4 weeks, substantial hyperglycemia emerges, and blood glucose concentrations steadily rise, peaking at 3-5 months and then declining as the mice age [67]. In addition, islet cells in $Lep^{ob/ob}$ mice were shed in large numbers due to abnormal insulin release [68,69]. This is manifested by hyperlipidemia, irregular body temperature, reduced physical strength, and infertility [70,71]. Insulin resistance in Lepob/ob mice appears to be caused by decreased insulin binding to receptors, reduced Insulin Receptor (IR) autophosphorylation, and decreased signaling [72]. Owing to the model's extreme obesity, lifelong hyperinsulinemia, and insulin resistance, it can be used to create medications that enhance peripheral insulin sensitivity and lower body weight, such as insulin sensitizers, anti-obesity agents, and other anti-hyperglycemic therapies [63,64,73].

The Zucker Fatty Rat

In 1961, Zucker Fatty (ZF) rats were developed by crossing the Merck M line with Sherman rats [74]. ZF rats are caused by mutations in the leptin receptor and exhibit a lack of appetite [51], become markedly fatty from about 4 weeks, and become severely fatty by week 5 [75], when they weigh almost twice as much as the same litter of lean heterozygous rats [76], and by

30 weeks, plasma insulin levels had returned to normal, which is in stark contrast to the human illness condition [77]. Separately, reduced glucagon secretion was found in ZF rats, reducing resistance to insulin [78]. They had just critical hypertension in addition to modest hyperglycemia [79,80], which has a significant effect on diabetic nephropathy in humans [81]. Furthermore, ZF rats, like $Lep^{ob/ob}$ mice, do not develop overt diabetic mellitus but do suffer from hyperinsulinemia, which begins at 3-4 weeks of age [63], resulting in low glucose tolerance [48]. Thus, ZF rats are considered a model for prediabetes.

The Zucker Diabetic Fatty Rat

Zucker Diabetic Fatty (ZDF) rats are an experimental animal model that reflects human T2DM [82]. Metabolic problems in ZDF rats include hyperinsulinemia, hyperlipidemia, hypertension, and impaired glucose tolerance [63]. Islet morphology differed between ZDF rats and leptin control rats, and these variations were more obvious at 12 weeks of age, with ZDF rats having bigger islets with irregular boundaries [83]. Furthermore, a strong relationship between increased DNA content in islets and serum insulin levels has been shown, suggesting that islet hyperplasia plays a significant role in the development of hyperinsulinemia in ZDF rats [84]. Leptin protects Beta cells from free fatty acid-induced apoptosis in ZDF rats [85]. Long-term exposure to leptin was discovered to trigger beta-cell apoptosis in cultured human islet cells [90]. Still, due to mutations in leptin receptors, significantly higher levels of triglycerides and cholesterol were observed in ZDF rats than in lean rats, which was attributed to lipotoxicity due to excessive skeletal muscle and islet fatty acid metabolism [86,87]. Furthermore, "adipocyte apoptosis" is assumed to be the source of the primary consequences of fatty, insulin resistance, cardiovascular disease, and diabetes mellitus in ZDF rats [87]. These rats also exhibit diabetes consequences [88], and the evolution of diabetes mellitus in leptin receptor-deficient male ZDF rats (ZDF/CrlCrlj) has become an appealing model for experimental T2DM research, since these rats demonstrated islet structural disruption, B-cell degranulation, and increased B-cell mortality [89].

The KK Mouse

The KK mouse has been used extensively in studies of diabetes mellitus associated with obesity [2]. The mice are characterized by moderate obesity, polyphagia, and polyuria. They develop hyperinsulinemia, insulin resistance, islet hypertrophy, and degranulation, as well as hyperinsulinemia and insulin sensitivity in muscle and adipose tissue. This mouse strain also exhibits indications of diabetic nephropathy [90]. KK mice are more prone to acquire T2DM in the context of a high-fat diet and age [91]. Furthermore, because of the increased number and size of islets, hyperinsulinemia compensates for insulin resistance and keeps blood glucose levels within normal limits [90], and there is evidence of Beta-cell compensation [43]. KK mice would greatly help to identify factors associated with obesity-induced diabetes mellitus [84].

Spontaneous Non-obesity Model of T2DM

The GK Rat

Goto Kakizaki (GK) rats are a non-obese model of T2DM [92]. GK rats are selected for high blood glucose levels during glucose tolerance testing on Wistar rats and selectively bred over numerous generations. GK rats acquire glucose intolerance at 2 weeks of age because of a delay in forming islet cells, which produce insulin in response to insulin resistance. Therefore, elevated blood glucose levels were observed in GK rats after glucose feeding at 4 weeks of age [93]. Aside from insulin resistance, GK rats' islets may grow into starfish-like islets, which have a disordered structure and obvious fibrosis separating the endocrine cell chains, giving the islets a starfish appearance. These abnormalities do not occur in adolescent GK rats' pancreas, but they degrade with age [94]. These islet function modifications might be related to cellular differentiation loss produced by long-term mild high blood glucose levels as well as high blood non-esterified lipid acid levels, a phenomenon is deemed to "glycolipid toxicity" [36]. In a nutshell, GK rats are one of the most effective animal models for exploring the link between changes in Beta-cell mass and the development of T2DM and diabetic consequences, particularly diabetic nephropathy [95].

Genetically Engineered Diabetes Mellitus Model

Genetically engineered diabetes mellitus models are animal models in which certain gene fragments that cause diabetes mellitus are modified using transgenic and knockout technologies (Cre/loxP system, CRISP/Cas9 system, and the latest ZFN and TAILEN technologies) to obtain a stable genetic inheritance. Knockout technology can be used to obtain stable genetic models of diabetes mellitus, such as mice [96-98], rats [99], rabbits [100], and pigs [101]. This can explain the cause of diabetes mellitus in terms of molecular mechanisms and investigate the effect of individual genes on insulin secretion and resistance. It has been found that Islet Amyloid Polypeptide (IAPP) can regulate postprandial glucose. Still, its accumulation in vivo can cause islet amyloidosis, leading to islet Beta-cell death and the formation of T2DM [102]. In contrast, in animal models of diabetes mellitus, IAPP is less likely to accumulate in vivo in pigs, which is ideal for studying the mechanism of its toxicity to pancreatic islet cells. The common gene loci associated with diabetes mellitus are Insulin Receptor (IR), insulin receptor substrate (IRS-1, IRS-2, IRS-3), Glucose Transporter Protein-4 (GLUT4), peroxisome proliferator-activated receptor (PPAR-γ), Insulin-like Growth Factor I Receptor (IGF-IR), PAX4 gene, etc.

The IRS-1 Mice/IRS-2 Mice

Since discovering the insulin receptor's tyrosine kinase activity, researchers have been attempting to understand the physiological roles of the IR and its key downstream targets, Insulin Receptor Substrate 1 (IRS-1) and Insulin Receptor Substrate 2 (IRS-2). Kasuga et al. showed the tyrosine kinase activity of IR in 1982 by employing antibodies collected from individuals with type B insulin resistance to specifically immunoprecipitate human lymphocytes and rat liver cancer cells [103]. Kadowaki et al. demonstrated in 1988 that IR ablation causes significant insulin resistance in people and that IR tyrosine kinase activity is essential for insulin activity in humans to keep glycemic control [104]. This allowed molecules downstream of IR to enter, and Mansour et al. investigated the participation of molecules downstream of IR using a homologous recombination approach pioneered by Capecchi in 1988 [105]. Since then, studies using tissue-specific Knockout (KO) mice have confirmed that in obese and T2DM patients, preferential downregulation of IRS-2 and its associated targets resulted in reduced insulin action, correlated with greater insulin action through IRS-1 in different organs with hyperinsulinemia [103]. According to these results, Kubota T et al. proposed a unique notion of "organ-and pathway-specific insulin imbalance" in obesity and T2DM, which incorporates and extends upon this concept of "preferential insulin sensitivity," which arises as insulin signaling via the IRS-2 pathway is typically obstructed. Excess insulin signaling via the IRS-1 route is

maintained in several organs and pathways when insulin signaling via the IRS-2 pathway is normally inhibited [106].

The IRS-1$^{-/-}$Mice/IRS-3$^{-/-}$Mice

IRS-1 and IRS-2 have unique and somewhat overlapping physiological roles in single knockout mice, whereas IRS-3 and IRS-4 have partial involvement in the effects of insulin on growth, development, and glucose homeostasis. Laustsen et al. used a double deletion of IRS-1 and IRS-3 in mice to create an IRS-1/IRS-3/ double knockout animal model, which demonstrated early adipose atrophy as well as severe hyperglycemia, hyperinsulinemia, insulin resistance, glucose intolerance, and islet hyperplasia [107]. IRS-1/IRS-3/double knockout mice, according to Reitman et al., have similar metabolic traits to patients with this condition and other transgenic animal models, including hyperglycemia, hyperinsulinemia, insulin resistance, and glucose intolerance [108]. In conclusion, IRS-1 and IRS-3 have complimentary functions in adipogenesis. The unique animal model of adipose dystrophy diabetes mellitus produced by this double deletion provides a helpful tool for studying diabetes mellitus etiology.

The Syn4 $^{+/-}$ Mice

Yang C et al. created a Syn4$^{+/-}$ animal model by employing homologous recombination to knock out the Syntaxin4 gene in mice. The pure-zygote deletion of the Syntaxin4 gene resulted in early embryonic death in this example, whereas heterozygous knockout mice Syn4$^{+/-}$ showed normal viability with no notable impairment in growth, development, or reproduction. Syn4$^{+/-}$mice, on the other hand, have decreased glucose tolerance, with a 50% reduction in systemic glucose absorption. This shortfall was related to a 50% decrease in skeletal muscle glucose transport as measured by 2-deoxyglucose uptake during the hyperinsulinism-normoglycemic clamp. In addition, insulin-stimulated GLUT4 translocation was considerably decreased in these mice's skeletal muscle. Syn4$^{+/-}$mice, on the other hand, have normal insulin-stimulated glucose uptake and metabolism in adipose tissue and the liver [109]. Yang C et al. Showed that heterozygous Syntaxin4-mutant mice have a 40% loss of Syntaxin4 protein and develop insulin resistance, as demonstrated by considerably worse systemic glucose clearance and glycolysis rates.

The PPAR-γ Full Body Knockout Mice

Peroxisome Proliferator-Activated Receptor (PPAR) is a nuclear receptor super family ligand-inducible transcription factor [110], and PPAR- is extensively expressed in White Adipose Tissue (WAT) and Brown Adipose Tissue (BAT) and is important in adipogenesis, lipid metabolism, and insulin sensitivity [111]. When the expression of PPAR- in human and mouse tissues is compared, the expression patterns are extremely comparable, indicating that PPAR- has a conserved role across species [112]. PPAR- has received a lot of attention in the last two decades as a transcription factor linked to metabolic syndrome. Obesity, insulin resistance, hyperglycemia, hypertension, hypertriglyceridemia, and low serum HDL cholesterol levels are all indications of metabolic syndrome, a worldwide public health problem [113]. PPAR- full-body Knockout (KO) mice demonstrate that PPAR- deletion results in defective trophectoderm and placental vasculature terminal differentiation, culminating in uterine mortality in nulliparous embryos. Lipodystrophy, organomegaly, decreased plasma leptin and lipocalin, insulin resistance, increased Free Fatty Acids (FFA), and hypotension were all observed in these animals. Furthermore, these mice demonstrated a sex-dependent response to rosiglitazone, which stimulated regeneration of particular fat depots and enhanced insulin sensitivity in female mice but not male mice [114].

INS^{C94Y} Pig

Heterozygous Insulin (INS) gene mutations have been identified as the cause of human Persistent Newborn Diabetes Mellitus (PNDM) [115]. Simone Renner et al. employed transgenic technology to create pigs with a mutant porcine INS gene (INSC94Y) that is similar to the human INSC96Y mutation. The mutant gene progeny exhibited considerably higher random blood glucose levels at 24 hours of birth and during subsequent upbringing compared to non-transgenic pig progeny [116]. These diabetic pigs' Peripheral Blood Mononuclear Cells (PBMC) was compared to their non-transgenic wild-type siblings. Giese I-M et al. discovered a 5-fold decrease in the proliferative response of T cells from INSC94Y tg pigs to polyclonal T cell mitogens (PHA), and 2704 proteins were quantified using label-free LC-MS/MS, with significant changes in protein abundance detected in CD4+ T cells from early diabetic pigs. Furthermore, we discovered that PBMC from diabetes INSC94Y tg pigs had substantially greater mitochondrial Oxygen Consumption Rate (OCR) and baseline glycolytic activity, indicating an altered metabolic immune cell profile in diabetic patients [117]. Furthermore, egg maturation and early development of human and porcine embryos are comparable 123. As a result, INSC94Y transgenic pigs provide an excellent model for researching T1DM.

PAX4$^{+/-}$ Rabbit

Transcription factor 4 (PAX4) is a genetically encoded transcription factor that is required for the creation, differentiation, development, and survival of insulin-producing Beta cells throughout mammalian pancreatic development [118]. Additionally, PAX4 is a significant susceptibility gene for diabetes mellitus that has been linked to many different forms of diabetes, including T2DM [119], T1DM [120], Maturity-Onset Diabetes Young type 9 (MODY9) [121], and ketosis-prone diabetes mellitus [122]. The islet structure was disrupted in PAX4 knockout rabbits, resulting in a decrease in the number of insulin-producing Beta cells and an increase in the number of glucagon-producing Alpha cells, as well as persistent hyperglycemia and fetal death (PAX4+/- rabbits died shortly after birth due to severe hyperglycemia, and all died within 4 days) [123]. In PAX4 rabbits, typical DM-related phenotypes such as diabetic nephropathy, hepatopathy, myopathy, and cardiomyopathy were also seen. Furthermore, PAX4+/- rabbits develop diabetes and chronic hyperglycemia due to pancreatic cell malfunction, leading to long-term health issues [124,125]. Therefore, the PAX4 knockout rabbits can serve as a perfect model for studying diabetes mellitus and its complications.

Conclusions

This paper describes various frequently used T1DM and T2DM animal models and model applications for studying the etiology of diabetes mellitus and its consequences. With the rising incidence and complications of diabetes mellitus worldwide, there is an urgent need for a feasible and effective method to study the pathogenesis of diabetes mellitus and its complications, the prevention of diabetic attacks, and the treatment of diabetes mellitus. As previously stated, some animals are biologically similar to humans, and thus many are used to study some factors related to diabetes mellitus. Although these ani-

mals assist in studying and developing new, rational drugs, their limitations also limit the design of new drugs and therapeutic interventions. Therefore, the choice of model is important, and animal models of diabetes mellitus should have the phenotypic characteristics and clinical signs that diabetes mellitus possesses. The autoimmune model is generally preferred for studying T1DM, followed by the model of T2DM, which should have the characteristics of obesity, hyperglycemia, insulin resistance, and Beta-cell defects. Some animal models of spontaneous diabetes mellitus formation and animals with chemically induced diabetes mellitus formation can mimic the phenotype and clinical symptoms of human diabetes mellitus to a certain extent. Due to the limitations of many factors, these two models are not perfectly the pathogenesis of diabetes mellitus can be studied at the molecular level. The genetic engineering of experimental animals can explain the origin of diabetes mellitus at the molecular level and explore the influence of particular genes on insulin production and resistance. Because no one animal model may accurately depict the etiology of any single illness, the animal model used depends on which element of the disease is being studied. To illustrate the impact of prospective therapies, various animal models should ideally be employed.

Author Statements

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Conflicts of Interest

The authors state that they do not have any competing interests.

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Author Contributions

N. N. Z. Responsible for designing. Y. Z.: Responsible for writing manuscripts. N. N. Z.: Responsible for full-text evaluation, guidance, and final approval of the submitted version. All authors read and approved the final manuscript.

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