

## Animal Models in Type 2 Diabetes Mellitus Research: Pros and Cons

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### ABSTRACT

Worldwide, the prevalence of type 2 diabetes mellitus (T2DM) continues to rise at an alarmingly high rate, constituting one of the leading causes of mortality and morbidity. Research is central to the investigation, creation, and design of new therapeutic approaches for T2DM. For this purpose, not many tests can be conducted on humans; thus, animal models are the only currently available alternative. This article discusses the pros and cons of different animal models used in T2DM research. PubMed, Medline, Science Direct, ADI, and WHO databases were searched through June 2021. Mice and rats are the most widely used models for diabetes studies. Many other animals are also used, such as pigs and non-human primates. Animal models develop diabetes either spontaneously or by using chemical toxins, such as streptozotocin and alloxan, or by surgical or genetic techniques and depict clinical features or related phenotypes of the disease. Although their importance is generally accepted, animal models are criticized for their poor accuracy in predicting human outcomes due to the low rate of translation between preclinical and clinical studies. However, this problem is partly explained by inadequate methodologies and designs in animal trials. It remains to emphasize that animal models add an indispensable value to the basic, clinical, and applied science of T2DM by opening new avenues of research and innovation.

**Keywords:** Type 2 diabetes mellitus, Animal models, Rats, Mice, Pigs, Non-human primates.

### INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder resulting in hyperglycemia. This disorder is mainly associated with defects in insulin secretion, insulin resistance (IR), or both (Goldenberg and Punthakee, 2018; Straub et al., 2019). DM presents a global health challenge, with a

worldwide prevalence that is increasing at an exponential rate (Mayer et al., 2017). There are over four hundred million people worldwide who suffer from DM, and most of them belong to low or middle-income countries (Yaribeygl et al., 2020). This number is expected to reach over 590 million cases by 2035. Furthermore, DM is considered a significant risk factor for death-related diseases, such as cardiovascular and kidney diseases (Rehman and Aksh, 2016).

Among the various types of diabetes observed in humans, the predominant ones include type 1 (T1DM) and type 2 (T2DM). T1DM is an autoimmune disorder resulting from the destruction of pancreatic  $\beta$  cells, while in T2DM, which accounts for >90% of diabetic patients, insulin

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resistance is diminished, which is considered insulin resistance. (Nolan et al., 2011). Several risk factors for developing T2DM have been identified, including genetic predisposition, aging, diet, and many environmental aspects (Ling and Ronn, 2019; Ahmad et al., 2020 a and b; Farah et al., 2020). Besides, the most predominant types of diabetes other types include gestational diabetes, or those arising from defects of the pancreas, such as cystic fibrosis or pancreatitis, or secondary causes that affect glucose metabolism (e.g., Cushing syndrome, hyperthyroidism) (Solis et al., 2018). Furthermore, few other rare diabetic diseases such as maturity-onset diabetes of adults' young or latent autoimmune are not classified as either T1DM or T2DM. This article summarizes the main characteristics of the different animal models used in T2DM research and addresses the various advantages and disadvantages of each model. The appropriate animal model for basic, interventional, and clinical T2DM studies is emphasized.

### LITERATURE SEARCH

A recent literature search was carried out to address the pros and cons of the different animal models used in T2DM studies. The search was limited to recent English publications covering up to June 2021. Relevant articles were primarily identified by an online search of the PubMed, Medline, Science Direct, and WHO databases. Google Scholar and other databases were also searched. The articles included were mainly original experimental, preclinical, clinical, and intervention research. Some review articles were also consulted. For greater search accuracy, the work reference lists were checked for additional publications from major databases.

### INSULIN SECRETION AND GLUCOSE METABOLISM IN TYPE 2 DIABETES

Insulin secretion relies on glucose levels and crosstalk between various insulin-sensitive tissues (Kahn et al., 2014). Enzymatic digestion of carbohydrates post-meal results in the release of glucose into the gastrointestinal

tract. Glucose leaves the Gastric tract and enters the bloodstream via sodium-dependent glucose transporter (GLUT)-3 expressed on intestinal cells. This absorption leads to an increase in blood glucose levels, which in turn is sensed by GLUT-2 receptors expressed on  $\beta$ -cells of the pancreas and hepatocytes. GLUT-2 receptors are highly glucose-dependent, and the enhanced blood glucose levels facilitate the entrance of glucose into  $\beta$ -cells which results in insulin production. Apart from the mechanism mentioned above, the intestinal absorption of glucose results in the secretion of hormones by the gut-derived enteroendocrine cells that enhances insulin release from  $\beta$ -cells. The hormones secreted by the enteroendocrine cells include glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1, which activate  $\beta$ -cells to produce more insulin (Pais et al., 2016).

The secreted insulin binds to receptors expressed on insulin-sensitive tissues such as skeletal muscles, adipose tissue, and other tissues. This binding is a complex process involving enzymes and mediators that open the insulin-dependent transporter GLUT-4 expressed on muscles and adipose tissue. The binding of insulin to the  $\alpha$ -chain of the receptor will result in phosphorylation of the tyrosine residue in the  $\beta$ -chain of the receptor. Multiple signaling events follow the phosphorylation. The signaling results in binding insulin receptor substrates to phosphoinositide 3-kinase result in phosphatidylinositol 3,4,5-trisphosphate. In turn, it is an activator for serine/threonine-protein kinase activation, which facilitates glucose transport via GLUT-4, resulting in its utilization in glycogen, lipid, and protein synthesis (Yaribeygl et al., 2020). Also, insulin reduces glucose levels by inhibiting hepatic gluconeogenesis and glycogen degradation to glucose (Yaribeygl et al., 2020).

Many genes and proteins help mediate the crosstalk between  $\beta$ -cells and insulin-sensitive tissues (McCarthy, 2010; Morris et al., 2012; Kahn et al., 2014). For instance, peroxisomal proliferated activated receptors (PPARs) are

a subfamily of the ligand-dependent nuclear receptors consisting of three isoforms  $\alpha$ ,  $\beta/\delta$ , and  $\gamma$ . These have been linked to a broad spectrum of biological processes, including their lipid and glucose sensors (Grygiel, 2014). Initially, it was thought that PPAR $\gamma$  has insulin-sensitizing properties and functions as a regulator of adipogenesis (Anghel et al., 2007). However, later, it has been found that all PPARs are linked to insulin sensitivity and glucose metabolism in different tissues (Duszka et al., 2020). PPAR can be activated by binding to natural, free fatty acids, eicosanoids, leukotrienes, prostaglandins, and synthetic lipophilic acids such as essential fatty acids.

Such binding activates each isotype of PPAR in different tissues and organs. Of these, PPAR $\gamma$  has been shown to increase glucose metabolism, free fatty acids uptake, and reducing inflammation. Furthermore, the PPAR $\gamma$  gene with single nucleotide polymorphisms variants have been identified in T2DM patients, and many other genes with single nucleotide polymorphisms have been associated with T2DM (Mambiya et al., 2019). Figure 1 shows the possible links between PPAR $\gamma$ , glucose homeostasis, and insulin sensitivity.

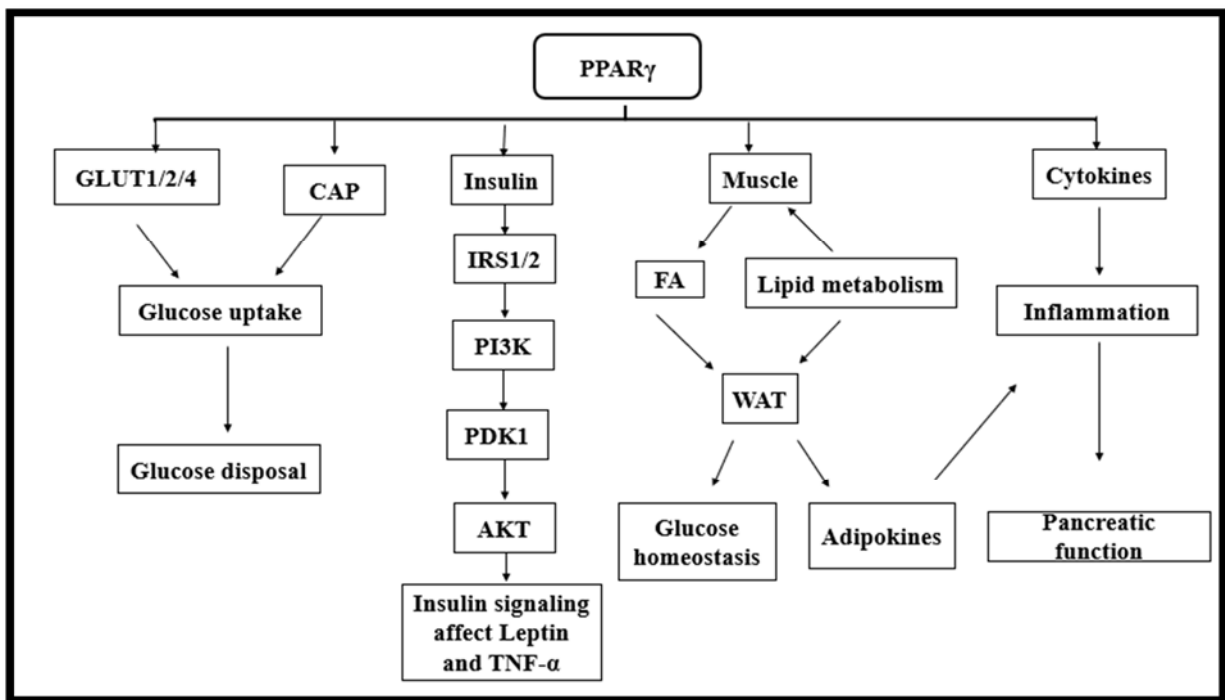


Figure 1. The links between peroxisome proliferator-activated receptor gamma and insulin sensitivity.

Abbreviations: PPAR $\gamma$ : Peroxisome proliferator-activated receptor gamma, GLUT: Glucose transporter; CAP: Cb1-associated protein; IRS: insulin receptor substrate; PI3K: phospho-inositide 3-kinase; PDK1: phosphoinositide-dependent protein kinase-1; AKT: protein kinase B; TNF- $\alpha$ : Tumor necrosis factor alpha; FA: Fatty acid; WAT: White adipose tissue.

Insulin resistance (IR) usually occurs when insulin could not induce an opening to GLUT-4 and cannot reduce plasma glucose levels. IR is associated with age, lifestyle, and epigenetics, and it occurs before the onset of T2DM. Furthermore, substantial evidence exists that T2DM is associated with obesity and fat accumulation in the abdomen (Ajlouni et al., 2020). Such fat goes into lipolysis and release of free fatty acids and influences lipid (Tremmel et al., 2017) and glucose metabolism contributing to obesity-related IR and diabetes (Ingle et al., 2018).

T2DM often results from both environmental and genetic factors. Insulin resistance in T2DM is accompanied by impaired functioning of pancreatic  $\beta$  cells, impaired insulin processing, and loss of pulsatile secretion of insulin. It is also associated with metabolic abnormalities arising from hypertension (Leggio et al., 2018), older age, abdominal obesity, unhealthy lifestyle, dyslipidemia, and glucose intolerance (Ighodaro et al., 2017). Similarly, pathogenesis associated with T2DM is very complex and not completely understood. Many factors such as sedentary lifestyle, smoking, and alcohol intake result in diabetes onset (Kolb and Martin, 2017). It has been shown that practicing exercise and consuming healthy foods enriched with micronutrients such as vitamins and antioxidants could suppress the development of diabetes (Bajaj and Khan, 2012). The diabetic phenotype induced by diet can be rescued by different methods, which include treatment with antibiotics, fecal transplantation (Di Luccia et al., 2015), lipoic acid (Thirunavukkarasu et al., 2004), and Ursodeoxycholic acid (Mahmoud and Elsazhly, 2014). Antioxidants such as resveratrol (extract of red grape) might reverse the diabetic phenotype in rat models. Pioglitazone is an anti-diabetic drug that acts as an insulin sensitizer (either alone or in combination with resveratrol) can reverse the diabetic phenotype and help

normalize glucose levels in DM-induced animals (Mansour et al., 2013).

Neuroendocrine control of insulin secretion has been well-studied. Incretins and bile acids directly or indirectly affect insulin secretion by altering glucose homeostasis (Yehya and Sadhu, 2018). Two of the incretin hormones, namely glucagon-like peptide-1, and glucose-dependent insulinotropic polypeptide, help adequate insulin secretion after food intake. Glucagon-like peptide-1 is degraded by dipeptidyl peptidase-4 and has a half-life of 2 minutes in the blood. Thus, dipeptidyl peptidase-4 inhibitors have become an attractive therapy. In addition, sodium-glucose transporters (SGLT) in the kidney regulate the extent of glucose absorption from urine. Sodium-glucose transporters inhibitors enhance the excretion of glucose, thus helping in stabilizing blood glucose levels (Ighodaro et al., 2017).

Long-term complications of T2DM include damage to brain vessels, depression, foot ulcers, vision damage, nerve and kidney damage. The complex symptoms associated with T2DM make it essential to study the underlying events that develop in T2DM animal models play an essential role in understanding the molecular mechanisms that govern the development of T2DM. Studying the various animal models developing T2DM would help in developing appropriate interventions that could be translated into therapies. Various methods have been successfully employed to develop genetic models for DM in animals, including the use of drugs, genetic screening methods, and dietary methods (Srinivasan and Ramarao, 2007; Ahmad et al., 2020a and b).

#### **ROLE OF ANIMAL MODELS IN UNDERSTANDING TYPE 2 DIABETES**

Replicating the various metabolic abnormalities associated with T2 DM in animal models would help understand the detailed signaling mechanisms involved in

developing the disease in humans. Figure 2 exhibits the most common animal models used in diabetes mellitus studies. Though the exact pathogenic conditions might not be replicated in every animal model, the presence of similar pathological characteristics would help in studying the underlying factors that cause the onset and progression of the disease (Cefalu, 2006). Animal models of T2DM are mainly associated with understanding the role of obesity with diabetes, diet and exercise, and various drugs in the induction of diabetes. Several animal types have been utilized to study T2DM, including mouse, rat, swine, pig, and monkey.

**SPONTANEOUS TYPE 2 DIABETES ANIMAL MODELS**

Compared to various non-mammalian species used for studying diabetes, rodents have physiology closer to humans and thus serve as better models for diabetes research. Although monogenic or single gene-based mutations are usually insufficient to cause diabetes in humans, such observations have been made in rodents.

For instance, leptin is a hormone produced by adipocytes to inhibit hunger, fat storage and for regulating glucose homeostasis (Denroche et al., 2012). However, spontaneous mutations in the leptin gene (lepob/ob) or its receptor in rats developed obesity and hyperglycemia similar to T2DM (Neubauer, 2006). The obese phenotype is observed after two weeks of birth, following which hyperinsulinemia onset is seen. Seven to twelve weeks after that, hyperglycemia is detected along with hyperlipidemia and enhanced hepatic fatty acid synthesis (Drel et al., 2006). Furthermore, although leptin inhibits hunger and fat storage, leptin administration in deficient rats regulated blood glucose more than food intake and body weight (Denroche et al., 2012). Table 1 demonstrates the different rodent models used for obesity induced T2DM.

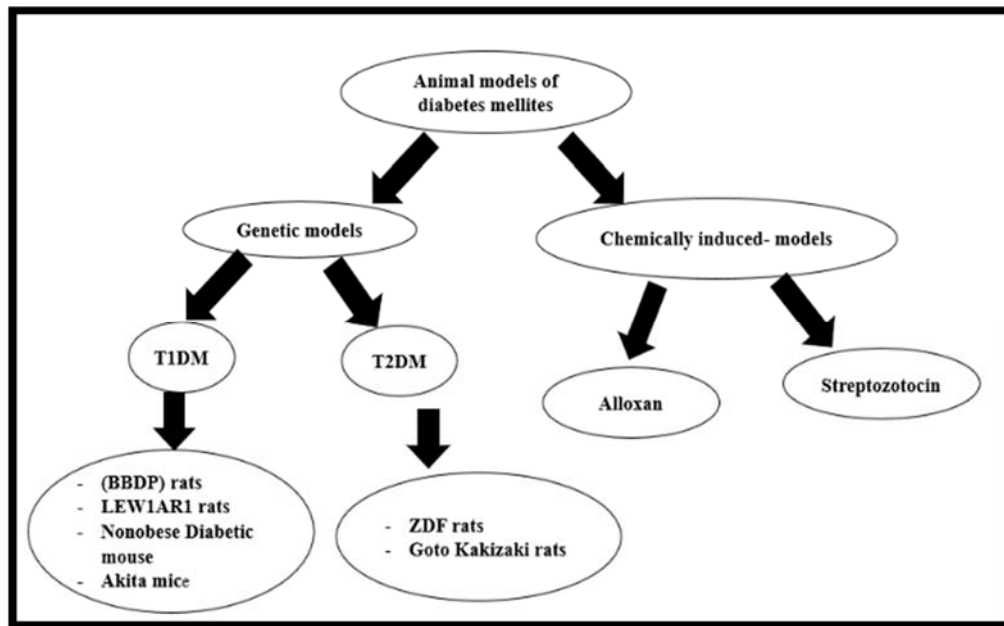


Figure 2. Animal models in diabetes mellitus research

Abbreviations: T1DM: Type 1 diabetes mellitus; T2DM: Type 2 diabetes mellitus; BBDP: Bio-breeding diabetes-prone; LEW1AR1: Lewis rats congenic with defined major histocompatibility complex haplotype; ZDF: Zucker diabetic fatty.

In the strain of mice is the C57BL/KsJ, which has a mutated  $Lepr^{db/db}$ . These mice show rapid obesity by 3-4 weeks, followed by hyperglycemia within 4-8 weeks.

In addition, the leptin receptor mutant in C57BL/KsJ background causes severe diabetes symptoms, and these mice have a short life span (Ramarao et al., 2007).

**Table 1: Various rodent models used for obesity-induced type 2 diabetes mellitus**

<b>Animal strain</b>	<b>Nature of genetic change</b>
<b>ob/ob mouse</b>	<b>Leptin deficient, monogenic</b>
<b>db/db mouse</b>	<b>Leptin receptor-deficient, monogenic</b>
<b>Zucker fatty rat</b>	
<b>Zucker diabetic fatty rat</b>	
<b>Sprague Dawley (SD) rat</b>	
<b>KK (Kuo Kondo mouse)</b>	<b>Polygenic models</b>
<b>KK /Ay mouse</b>	
<b>New Zeland obese mouse</b>	
<b>NSY mouse (Nagoya-Shibata-Yasuda)</b>	
<b>OETF (Otsuka Long Evans Tokushima Fatty) rat</b>	

Similarly, the Zucker fatty rats (ZF) (fa/fa), discovered in 1961. When crossing Sherman rats and Merck-M-strain of rats, inherited a leptin receptor gene mutation. As a result, these rats develop obesity around four weeks of birth and become insulin-resistant (Durham and Truett, 2006). When these rats were fed high-sugar and high-fat diets, hyperglycemia developed. Eight to ten weeks later, rats exhibit classic symptoms of Type 2 DM, such as hyperinsulinemia, hyperglycemia, hyperlipidemia, and hypertension (Pang et al., 2018).

In another rat model, a Sprague Dawley (SD) rat has been developed, an on-obese model that shows hyperglycemia in male rats with decreased levels of insulin production and inflammation of  $\beta$ -cells in 16 weeks of age (Masuyama et al., 2004). When these SD rats

were introduced with a mutated leptin receptor gene, fatty rats, T2DM was developed in both sexes but only in the fa/fa homozygous rats. These rats developed hyperglycemia, with increased plasma insulin and leptin levels and exhibited other features associated with prolonged diabetes such as eye, nerve, kidney, and bone complications (Masuyama et al., 2005)

Goto Kakizaki rats were first developed by Goto and his collaborators in 1973 as a genetic model for Type 2 DM. The Goto Kakizaki rats were selected from Wistar rats that exhibited glucose intolerance over several generations. It is a polygenic model for T2DM. These rat models do not show obesity. However, various features of diabetes, such as hyperglycemia and hyperinsulinemia, are seen by two weeks of age (Portha et al., 2001).

Moreover, rats by 14-21 weeks showed distinct changes in islet cells, such as decrease  $\beta$ -granules with enhanced immature granules. The  $\beta$ -cells complex changes are primarily responsible for the diabetic phenotype, and these rats exhibit excellent diabetic neuropathy (Momose et al., 2006).

Non-human primates such as Rhesus, bonnet, cynomolgus, pigtailed, African green monkeys, and baboons have been reported to develop diabetes. The Non-human primates' development of T2DM and obesity is age-dependent and also showing an identifiable pre-diabetic phase. The pre-diabetic phase involves an initial phase of insulin resistance followed by alteration in insulin levels (Wagner et al., 2006). In monkeys, overt diabetes is associated with changes in pancreatic islet cells, such as amyloidosis, which is also a characteristic feature of human diabetes (Wagner et al., 2006). Furthermore, prolonged phases of increased plasma triglycerides and glucose are observed before the complete appearance of diabetic symptoms. Besides, the development of atherosclerosis is a significant feature with diabetic monkey models. Therefore, these models are immensely helpful in understanding the mechanism that could result in cardiovascular diseases arising due to D2M (Cefalu, 2006)

#### **DRUG-INDUCED ANIMAL MODELS OF DIABETES**

Chemical agents have been extensively utilized for inducing diabetes. These chemical agents target  $\beta$ cells, resulting in their destruction and therefore causing reduced insulin secretion leading to type 1 like DM. Streptozotocin and Alloxan are the most widely used drugs for the induction of diabetes. Both drugs bind GLUT2 receptors and end up inside any cell that expresses such receptors, including  $\beta$ -cells and hepatocytes. Streptozotocin is a nitrosourea that is produced by *Streptomyces achromogenes*. It is a Deoxyribonucleic acid (DNA) alkylating agent that

causes the alkylation and subsequent fragmentation of DNA. Streptozotocin is most widely used in rats for inducing diabetes due to its ability to destroy the  $\beta$ -cells resulting in the onset of DM (Lenzen, 2008). In adult Sprague Dawley (SD) rats, an intravenous dose of 60mg/kg results in swelling of the pancreas and destruction of  $\beta$ -cells in islets of Langerhans. Experimental diabetes is seen 2-4 days post-treatment with streptozotocin (Akbarzadeh et al., 2007). On the other hand, alloxan causes  $\beta$ -cells toxicity by producing reactive oxygen species inside the cells and causes a toxic effect. Besides, Alloxan concentration varies from animal to animal for induction of DM in rats, a dose of 40-200mg/kg either intraperitoneal or intravenous. Results in the development of DM in mice, a dose of 50-200mg/kg by intravenous or intraperitoneal route induces DM (Igodharo et al., 2017). However, a low dose of streptozotocin with and high-fat diet was found to induce T2DM in rats (Reed et al., 2000). In such an approach, a high-fat diet induces higher plasma insulin and triglycerides and insulin resistance. In another streptozotocin-induced T2DM model, nicotinamide, an antioxidant that is known to protect the  $\beta$ -cells from the damage induced by streptozotocin, is administered 15 minutes before streptozotocin treatment in a 3-month-old Sprague Dawley rat protected 40% of pancreatic  $\beta$ -cells and developed stable non-fasting hyperglycemia (Wang and Sadhu, 2018).

However, in pigs, slow infusion of streptozotocin at 130mg/kg induces type-2 DM, which can be treated with metformin. Insulin resistance in pigs is associated with metabolic dysfunction and often shows hyperlipidemia or hyperglycemia (Koopmans and Schuurman, 2015). Furthermore, induction of T2DM in neonatal rats is often achieved by a single dose of streptozotocin at a 100 mg/kg dose via the intraperitoneal route in one-day-old pups. Higher concentrations of 120 mg/kg via the intraperitoneal route induce diabetes in three- to five-day-

old pups. The pups exhibit glucose intolerance and moderate hyperglycemia. The  $\beta$ -cells show similar histopathological features in these rat pups as human diabetic patients, and hence this model serves as a suitable study model (Arulmozhi et al.,2004).

Glucocorticoids have also been used for the induction of diabetes in animal models. Corticosterone and its derivatives enhance food intake resulting in obesity development and subsequent initiation of diabetes. For example, when C57BL/6J mice were treated with 25-100 $\mu$ g/ml of corticosterone in drinking water for five weeks or intraperitoneal 0.1-1mg/kg for five days, these mice developed metabolic syndrome includes glucose intolerance, hyperinsulinemia, insulin resistance, high plasma levels of cholesterol and triglycerides, fat tissue gain, body weight gain and then T2DM (Franson et al., 2014). However, the effects could be reversed by stopping corticosterone administration.

#### **DIET-INDUCED OBESITY AND DIABETES IN EXPERIMENTAL ANIMALS**

Many rodent models are monogenic and show only one of the complex diabetic phenotypes exhibited by humans. However, in polygenic diabetic models, they demonstrate more symptoms that resemble human disease. In the case of polygenic models, the phenotype is environmentally induced. Thus, it is highly comparable to humans. The inbred C57BL/6J mice or C57BL/6N mice are a prototypical example of diet-induced obesity and subsequent glucose intolerance. However, only the C57BL/6N strain develops hyperglycemia and hyperinsulinemia following three weeks on a high-fat diet. Such a model does not usually produce overt hyperglycemia (Kahle et al.,2013; Fang et al.,2018). These differences suggest that genetic variations play an essential role in developing overt hyperglycemia.

Similarly, New Zealand obese mice and Kuo Kondo mice are other polygenic strains that develop

obesity and subsequent T2DM (Fang et al.,2018). In the New Zealand Obese model, mice develop hyperphagia and high leptinemia at 9-12 weeks. When these mice are fed with a high-carbohydrate diet, signaling and histopathological changes occurred in  $\beta$  cells. These changes led to low expression of GLUT2 and loss of protein kinase activation, which resulted in low insulin secretion and the development of hyperglycemia (Jurgens et al.,2007).

Diet plays a vital role in developing obesity, and subsequently, the onset of DM Wistar rats, Sprague-Dawley rats, and C57BL/6J mice all have been utilized to develop effective diet-induced diabetic models. The change of regular chow to a high-energy diet helps in the establishment of diabetes in these samples. A high-fat diet is one of the significant sources for the development of DM Incorporation of 10-45% of fat as a dietary component in Sprague Dawley rats and mice results in increased weight gain and obesity, and diabetic phenotype. To effectively induce type 2 DM in Wistar, Sprague-Dawley rats or mice, a fatty meal consisting of 20- 60% fat should be fed to the animals (Bradley et al., 2017). Vegetable oil, ghee, lard, and plant-derived oils have been successfully utilized to induce T2DM in animal models (Ghibaudi et al.,2002). A high-fat diet suppresses glucose production by insulin in liver cells and also lowers glucose uptake. As a result, the animal model develops hyperinsulinemia, hyperglycemia, and insulin resistance, A high-fat diet in rodents results in increasing glucocorticoid production by altering the pituitary-hypothalamus mediated adrenal functioning. (Geer et al., 2014).

Elevated glucocorticoids result in enhanced triglyceride levels in the plasma due to lower levels of lipoprotein lipase resulting in obesity (Benuck et al., 2020). A high-fructose diet is another routinely used method for the induction of D2M in animals (Table 3). High levels of sucrose consumption result in the



accumulation of fructose in the liver and show obesity and diabetes associated with fructose-induced animal models. Male Sprague-Dawley and Wistar rats treated with 30% and 32% sucrose in drinking water develop diabetic phenotype in 21 and 10 weeks, respectively (Pang et al., 2008). A similar result is obtained when male Sprague-Dawley rats are fed 77% sucrose in the diet for six weeks (Oron-Herman et al., 2008). High levels of fructose in diet either as a monosaccharide or obtained by hydrolysis of high concentrations of sucrose in the digestive tract resulting in its uptake by the liver. It is converted to fructose 1-phosphate and eventually utilized in the central carbon pathways involved in glycolysis, gluconeogenesis, citric acid cycle, and lipogenesis. The excess fructose is converted to lipid in liver cells. This will enhance the

production of free fatty acids and their subsequent accumulation in adipose cells leading to obesity. Constant high levels of fructose result in enhanced free fatty acids in plasma which upon uptake by other tissues results in obesity and subsequent insulin resistance phenotype by the various tissues. Due to insulin resistance and obesity onset, the diabetic phenotype is established due to continuous feeding with a high carbohydrate-rich diet (Benuck et al., 2020). Table 2 shows a summary of some dietary approaches for T2DM induction by fructose-high-fat and fructose-regular chow diets in Sprague-Dawley rats.

**Table 2: Some dietary approaches for type 2 diabetes induction by fructose-high-fat and fructose-regular chow diets in Sprague-Dawley rats.**

Administration route (diet/ drinking water)	Fructose (%)	Induction duration	Reference
Fructose in drinking water	10%	8 weeks	Fang et al., 2018
			Pang et al., 2008
Fructose in drinking water	10%	12 weeks	Mahmoud and Elsazhly, 2014
Fructose in drinking water	20%	8 weeks	Mamikutty et al., 2014
Fructose in drinking water	10%	8 weeks	Sanchez-Lozada. et al., 2007
Fructose	60%		
Corn starch	40%		
Standard diet	60%	8 weeks	Yang et al., 2018
Lard oil	20%		
Egg yolk	10%		
Saccharose/ fructose source	10%		
Standard diet	61%	4-8 weeks	Zhuo et al., 2018
Lard oil	12%		
Egg yolk	2%		
Sucrose/ fructose source	20%		
Milk powder	5%		

### GENETICALLY MODIFIED ANIMAL MODELES FOR TYPE 2 DIABETES

Knockout mice or tissue-specific knockouts have been tested for T2DM. Knockout of the insulin receptor, insulin receptor substrate, GLUT4. Only the insulin receptor substrate and GLUT4 knockouts exhibit enhanced insulin sensitivity.  $\beta$  cells have developed tissue-specific knockouts targeting insulin receptors, mitochondrial transcription factors, and PPAR $\gamma$ . Animals with mitochondrial transcription factors show diabetes upon complete development (Rees, 2005). Knockout mice, in most cases, do not survive till adulthood, and those who survive have phenotype depending on the copy number of integrations in the genome. So, if integration sites are low, then the diabetic phenotype is lost, which poses a significant challenge in the field.

#### CONCLUSIONS

Diabetic phenotype is complex and involves multiple signaling pathways, and its pathophysiology involves

several organs. Therefore, replicating the exact symptoms seen in humans is critical for analyzing and developing the appropriate therapy. In obesity-induced diabetes models, altering the leptin gene or its receptor shows many of the features of human diabetes. However, the major disadvantage with these models is that they are monogenic and do not display all human symptoms. Non-human primate models have most of the symptoms identical to humans, and polygenic models could replicate human disease conditions. However, these models are expensive, and the animals with a long-life span make it challenging to maintain the changes. Table 3 presents a summary of pros and cons of selected animal models used in T2DM studies. Therefore, a combination of genetic and environmental factors such as diet and lifestyle need to be assessed to understand the exact molecular events causing the diabetic phenotype in humans, making these polygenic models more suitable for understanding the human diabetic phenotype.

**Table 3: Summary of pros and cons of animal models used in type-2 diabetes studies.**

Species	Animal profile	advantages	disadvantages
Mouse	Adult, weight 25-30 g Lifespan 1.2-5 years	<ul style="list-style-type: none"> <li>- Spontaneous mutation in insulin gene</li> <li>- Spontaneous diabetes development</li> <li>- Used in subcutaneous and intraperitoneal studies</li> <li>- Study host parasite links</li> <li>- Many immune reagents</li> <li>- Easy breeding</li> <li>- Short gestational period</li> <li>- Small and inexpensive</li> </ul>	<ul style="list-style-type: none"> <li>- Compared to man: different lipid/HDL metabolism, cardiovascular anatomy physiology, and pathology of atherosclerosis</li> <li>- Absence of chronic model</li> <li>- Research requires relatively large numbers of animals</li> <li>- Relatively small size limits frequent blood collection</li> </ul>
Rat	Adult, weight 200-30 g Lifespan 2.5-3 years	<ul style="list-style-type: none"> <li>- Develop early advanced insulin resistance and glucose intolerance</li> <li>- Fed glucose levels: 500 mg/dl by 10-11 weeks</li> </ul>	<ul style="list-style-type: none"> <li>- Relatively small in size</li> <li>- Resistance to periodontitis</li> <li>- Diet differs from humans</li> <li>- Cage specific differences in toxicity testing study for each rat</li> </ul>

		<ul style="list-style-type: none"> <li>- Share similarity with man microbiota composition</li> <li>- Low cost, easily available</li> <li>- Develop diabetes by streptozotocin and alloxan</li> </ul>	<ul style="list-style-type: none"> <li>- Relatively fast dead when Glucose levels up than 650 mg/dL</li> </ul>
<b>Hamster</b>	<p>Adult, weight 80-100 g Lifespan 1.5-2 years</p>	<ul style="list-style-type: none"> <li>- Give good results in type1 diabetes and immunology</li> <li>- Develop insulin resistance and dyslipidemia</li> <li>- Highly susceptible to metabolic diseases</li> </ul>	<ul style="list-style-type: none"> <li>- Relatively expensive</li> <li>- Disease-results problems</li> <li>- Lack of immunological reagents</li> </ul>
<b>Rabbit</b>	<p>Adult, weight 2.2-5 kg Lifespan 4-5 years</p>	<ul style="list-style-type: none"> <li>- Fast hyperglycemia with alloxan</li> <li>- Give good results in biomedical research</li> <li>- Non-aggressive</li> <li>- Low cost to maintain</li> </ul>	<ul style="list-style-type: none"> <li>- High blood N with diabetes</li> <li>- Pancreatic beta cells differ from humans</li> <li>- Literature scarcity for use</li> <li>- Increase intrinsic healing in diabetes due to cell size rise</li> </ul>
<b>Monkey</b>	<p>Adult, weight 10-12 kg Lifespan 15-20 years</p>	<ul style="list-style-type: none"> <li>- Pancreatic beta cells, lipid metabolism, inflammation, obesity, and oxidative stress similar to humans</li> <li>- Anatomical and biological similarity to human</li> </ul>	<ul style="list-style-type: none"> <li>- Ethical clearance</li> <li>- Hard to handle, expensive</li> <li>- Genetic back not similar to human</li> <li>- Males are used in studies, females for breeding</li> </ul>
<b>Pig</b>	<p>Adult, weight 400-500 g Lifespan 4-5 years</p>	<ul style="list-style-type: none"> <li>- Close to man in anatomy, physiology, and disease development</li> <li>- Islet amyloid polypeptide gene similar to humans</li> </ul>	<ul style="list-style-type: none"> <li>- Relatively expensive</li> <li>- Few studies to support use</li> <li>- Rare hyperglycemia</li> </ul>

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#### Conflict of interest

The authors declare that there are no conflicts of interest

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## النماذج الحيوانية في أبحاث مرض السكري من النوع الثاني: الإيجابيات والسلبيات

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### ملخص

في جميع أنحاء العالم، يستمر انتشار مرض السكري من النوع 2 (T2DM) في الارتفاع بمعدل مرتفع بشكل يندرج بالخطر، ويشكل أحد الأسباب الرئيسية للوفيات والمراضة. ويعد البحث أمرًا أساسيًا للتحقيق وإنشاء وتصميم مناهج علاجية جديدة لمرض T2DM. ولهذا الغرض، لا يمكن إجراء العديد من الاختبارات على البشر؛ وبالتالي، فإن النماذج الحيوانية هي البديل الوحيد المتاح حاليًا. وتناقش هذه المقالة إيجابيات وسلبيات النماذج الحيوانية المختلفة المستخدمة في أبحاث مرض T2DM. وتم البحث في قواعد البيانات PubMed و Medline و Science Direct و WHO و ADI حتى حزيران 2021. وتعد الفئران والجرذان النماذج الأكثر استخدامًا في دراسات مرض السكري. وكما يتم استخدام العديد من الحيوانات الأخرى، مثل الخنازير والرئيسيات غير البشرية. ويتم تطوير النماذج الحيوانية لمرض السكري إما تلقائيًا أو باستخدام السموم الكيميائية، مثل الستربتوزوتوسين والألوكسان، أو عن طريق التقنيات الجراحية أو الجينية وتصوير السمات السريرية أو الأنماط الظاهرية ذات الصلة للمرض. وعلى الرغم من أن أهميتها مقبولة بشكل عام، إلا أن النماذج الحيوانية يتم انتقادها لضعف دقتها في التنبؤ بالنتائج البشرية بسبب انخفاض معدل الترجمة بين الدراسات قبل السريرية والسريرية. ومع ذلك، تم تفسير هذه المشكلة جزئيًا من خلال وجود المنهجيات والتصاميم غير الكافية في التجارب على الحيوانات. ويبقى التأكيد على أن النماذج الحيوانية تضيف قيمة لا غنى عنها إلى العلوم الأساسية والسريرية والتطبيقية لمرض T2DM من خلال فتح طرق جديدة للبحث والابتكار.

**الكلمات الدالة:** مرض السكري من النوع الثاني، النماذج الحيوانية، الفئران، الجرذان، الخنازير، الرئيسيات غير البشرية

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