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Review Article

Animal models for type 2 diabetes: A review

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ABSTRACT

Diabetes mellitus is a potentially morbid condition with high prevalence worldwide thus the disease constitutes a major health concern. The search for compounds with novel properties to deal with the disease condition is still in progress. This makes the use of experimental models for the disease imperative. Appropriate experimental models are considered as an essential tool for understanding the pathogenesis, complications, and genetic influences that increase the risks of type 2 diabetes. In recent years, large numbers of new genetically modified animal models including transgenic, generalized knock-out and tissue-specific knockout mice have been engineered for the study of diabetes. In this review our efforts have been devoted to gather the animal models of type 2 diabetes with reference to their characteristic features, underlying causes or mechanisms, advantages and disadvantages to the investigators in diabetes research.

Key words: Animal models, Diabetes, mechanisms, Transgenic, knock-out

INTRODUCTION

The increasing worldwide incidence of diabetes mellitus in adults constitutes a global public health burden. It is predicted that by 2030, India, China and the United States will have the largest number of people with diabetes.[1] Diabetes mellitus is categorized as a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Over the years, several animal models have been developed for studying diabetes mellitus or testing anti-diabetic agents. These models include chemical, surgical (pancreatectomy) and genetic manipulations in several animal species to induce diabetes mellitus. The diabetogenic drugs used include: Alloxan monohydrate, streptozotocin with or without nicotinamide, ferric nitrilotriacetate, ditizona and anti-insulin serum. The selections of inappropriate animal models have been identified as one of the common problem associated with ethno botanical researches. [2]The aim of the present review is to gather together the various experimental models developed for studying diabetes.

Animal models of type 2 diabetes and their classification:

Category	Obese model	Non-obese model
Surgical diabetic animals	VMH lessoned dietary obese diabetic rat	Partial pancreatectomized animals. *e.g.* dog, primate, pig & Rats
Chemically induced Diabetic animals	GTB treated obese mice	Low dose alloxan or Streptozotocin treated adult rats, mice, *etc*.
Diet/nutrition induced diabetic animals	Sand rat, C57/BL 6J mouse Spiny mouse	----------

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Spontaneous or genetically derived diabetic rat	*ob/ob* mouse, *db/db* mouse, KK mouse, NZO mouse, KK/Ay mouse, M16 mouse, TSOD mouse. Jucker fatty rat, JCR/LA-cp rat, OLETF rat	GK rat, Cohen diabetic rat, Torri Rat Non obese C57BL/6(Akita) mutant mouse, ALS/Lt mouse
Transgenic knock-out diabetic animals	Uncoupling protein(UCP1)knock-out mouse,β_3 receptor knock-out mouse	Transgenic mice involves genes of insulin, insulin receptor and its component of downstream insulin signaling e.g. IRS-1,IRS-2,GLUT-4,PTP-1B and others

VMH, ventromedial hypothalamus; GTG, gold thioglucose; KK, Kuo Kondo; NZO, New Zealand obese; ; KK/Ay, yellow KK obese ;TSOD, Tsumara Suzuki obese diabetes; JCR, James C Russel; ZDF, Zucker diabetic fatty; SHR/N-cp, spontaneously hypertensive rat/NIH-corpulent; Goto-Kakizaki; OLETF, Otuska Long Evans Tokushima fatty; IRS, insulin receptor substrate; GLUT-, glucose transporter; PTP, Phosphotyrosine phosphates;

***In vivo* animal models of diabetes mellitus:**

Surgical type 2 diabetic models: Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals such as dogs. Surgical method consists of complete or partial pancreatectomy in animals used for the induction of type 1 or type 2 diabetes, respectively. Historically, the diabetic dog model discovered by Oskar Minkowski through surgical complete pancreatectomy has been considered to be the first animal model of diabetes and is rarely now used for the investigation. [3] Partial pancreatectomy in animals performed as 70 or 90 per cent (usually 90%) dissection of pancreas has been reported in various animal species mostly in dogs, pigs, rabbit and also rats [4-6]. It does not cause severe form of diabetes and is characterized by moderate hyperglycaemia with neither reduction in body weight nor reduction in plasma insulin levels.

Chemically induced Diabetic models:

Gold thioglucose induced diabetes: Gold thioglucose is diabetogenic compound, which is induced hyperphagia and severe obesity induced Type -2 diabetes. It is derivative of sugar glucose. Gold thiglucose is precipitated with methanol and re-crystallized with water and methanol. Gold thioglucose developed obesity induces diabetes in genetically normal mouse strains. Gold thioglucose treated DBA/2 (Dilute Brown Non- Agouti), C57BLKs, and BDF1 mice gained weight rapidly and significantly increase non fasting plasma glucose level within 8-12 weeks. These mice showed impaired insulin secretion, mainly in early phase after glucose load and reduced insulin content in pancreatic islets [7].

Alloxan (ALX) induced diabetes: Alloxan is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3[8]. Because of its low stability, relatively very shorter half-life (less than 1 min) and acidic nature of solution, intravenous route of administration of alloxan is preferred. Alloxan causes diabetes in many rodent and non rodent animals and is most preferably used incase of rabbit because of the relative ineffectiveness of streptozotocin (STZ) in rabbits for induction of diabetes and development of well characterized diabetic complications. However, guinea pig has been reported to be resistant to the action of alloxan due to certain unclear mechanisms [8-10]. Alloxan exert its diabetogenic action when it is administered parenterally: intravenously, intra-peritoneally or sub-cutaneously. The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status. The range of the diabetogenic dose of alloxan is quite narrow and even light overdosing may be generally toxic and may cause the loss of many animals. This loss is likely to stem from kidney tubular cell necrotic toxicity, in particular when too high doses of alloxan are administered [11].The most frequently used intravenous dose of alloxan in rats is 65mg/kg, but when it is administered intraperitonially (i.p.) or subcutaneously its effective dose must be higher[12].For instance, an intra-peritoneal dose below 150 mg/kg may be insufficient for inducing diabetes in this animal species[13].In mice, doses vary among 100–200 mg/kg by intravenous route (i.v.) [14].Alloxan acts by selectively destroying the pancreatic beta islets leading to insulin deficiency, hyper- glycaemia and ketosis[8]. Alloxan is disadvantageous as the percentage incidence of diabetes is quite variable and is not proportionately related to increasing doses of alloxan. Further, the incidence of ketosis and resulting mortality is high. The reversal of hyperglycemia due to pancreatic regeneration is early and common in case of Alloxan treated animals. Because of these limitations, Alloxan is now almost replaced by STZ for induction of diabetes in laboratory animals [9].

Streptozotocin (STZ) induced diabetes: Streptozotocin is a deoxy-s [((methyl-nitrosoamino) carbonyl)- amino]-D gluco pyranose molecule that produces a selective toxic effect on β cells and induces diabetes mellitus in most laboratory animals[15-16].Streptozotocin enters the pancreatic β-cell via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA). Furthermore, STZ induces activation of poly adenosine diphosphate ribosylation and nitric oxide release. As a result of STZ action, pancreatic β-cells are destroyed by necrosis [17]. Although the exact mechanism of its toxicity is still a matter of debate, one proposed site of action of STZ is at nuclear DNA. During the decomposition of STZ, highly reactive carbonium ions are formed, which cause alkylation of DNA bases and also STZ may damage the β cell membrane and break the DNA strand which leads to the activation of poly (ADP-ribose) synthetase and NAD depletion, which ultimately leads to cell death .[16, 18, 19]

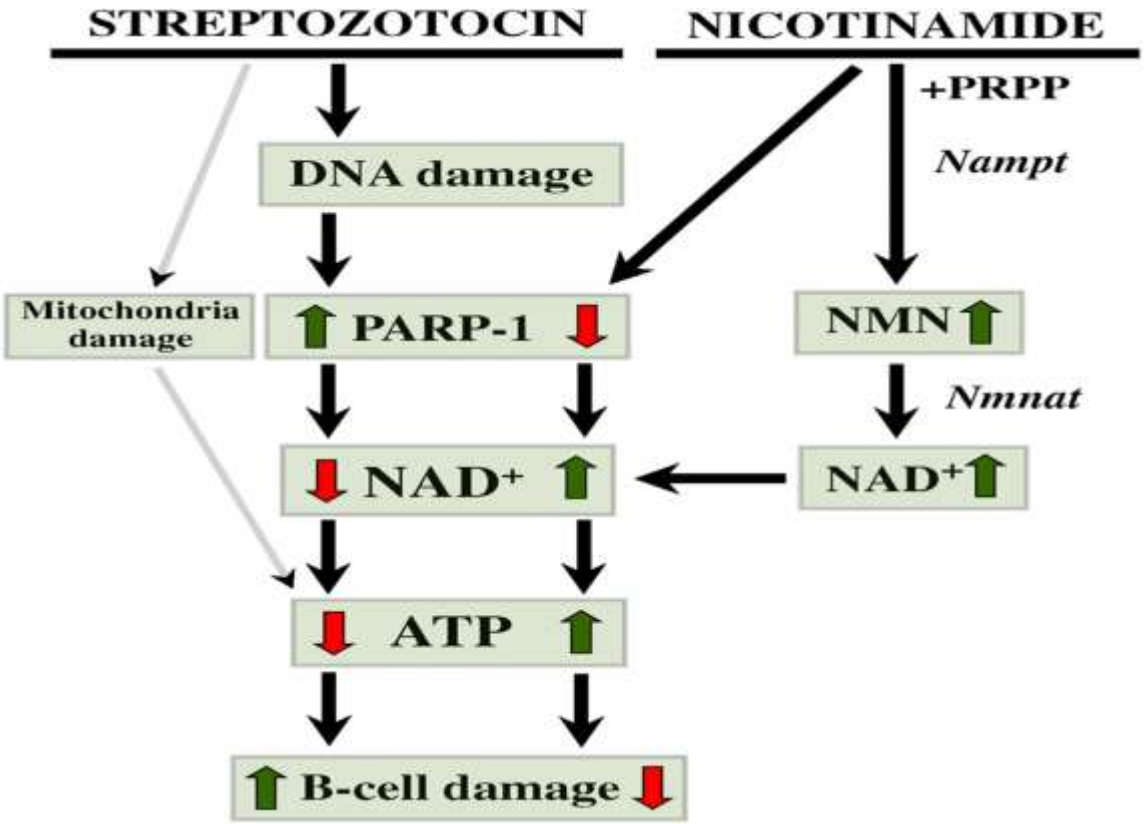

Fig 1: Suggested mechanism of STZ induced Diabetes

Diet-induced type 2 diabetic atherosclerosis mouse models:

SAND RAT: Sand rat (*Psammomys obesus*) is a model of nutritionally induced Type 2 diabetes mellitus. Sand rat remains normal in its natural habitat but develop obesity and diabetes in captivity when fed on standard laboratory chow (high energy diet) instead of its usual low energy vegetable diet (mainly of *Atriplex*) [20, 21]. Also primary insulin resistance is a species characterization of *Psammomys*. However, the potential to become diabetic decreases with age. In *Psammomys* of ages 1–12 months, maintained on a low-energy diet from weaning and transferred at different ages to a high-energy diet, the sensitivity to the development of diabetes mellitus increases from weaning to a peak of about 5 months of age and decreases thereafter [22].

***C57BL/6J mouse*:** This is a type 2 diabetic model by simply feeding high fat feed to non obese; non diabetic C57BL/6J mouse strain was initially developed in Japan. It is characterized by marked obesity, hyperinsulinaemia, insulin resistance and glucose intolerance [23]. These mice are demonstrated to develop peripheral leptin resistance. This animal model represents both genetic and environmental risk factors. Further, its usefulness for drug testing has been reported in the literature as these mice treated with orally active inhibitor of dipeptidyl peptidase-IV are shown to

have normalized glucose tolerance in association with augmented insulin secretion [24].

Spiny mouse: *(Acomys calirinus)*, is a small rodent generally living in semi desert areas of eastern Mediterranean, in fact they are low insulin secretors. However, when they are placed in captivity on high energy rodent lab chow, they gain weight and exhibit marked pancreatic beta cell hyperplasia, hypertrophy and increased pancreatic insulin content in comparison to other animal models exist, the plasma insulin response to glucose as well as to other secretogogues is impaired suggesting a impairment in hormone release mechanisms[25]. They are reported to accumulate insulin in beta cells, which may disintegrate and produce insulin-deficiency. These animals develop frank hyperglycemia with glucosuria leading to fatal ketosis [20, 26].

Spontaneous or genetically derived diabetes model: *Ob/ob mouse models*: *Ob/ob* mouse (obese mouse) (now relabeled as *Lep* ob) is inherited as (monogenic) autosomal recessive mutation on chromosome 6(obese) in C57BL/6J mouse strain. *Ob/ob* and *db/db* mice have identical phenotypes, each weighing three times more than normal mice (even when fed the same diet) and exhibiting a fivefold increase in body fat content. The mutation in *ob/ob* mice are now identified in leptin gene, which encodes for leptin. Leptin, a 16 kDa protein expressed predominantly in adipose tissue of normal mice, is missing from these mice homozygous for the mutation 'obese'. Leptin functions as a hormone keeping the brain apprised about the amount of body fat and activating centers involved in regulating feed intake and energy expenditure. Hence the major deficiency of satiety factor leptin in these mice significantly alters feeding behaviour, metabolism, and endocrine function, resulting in hyperphagia, decreased energy expenditure and obesity. [27]

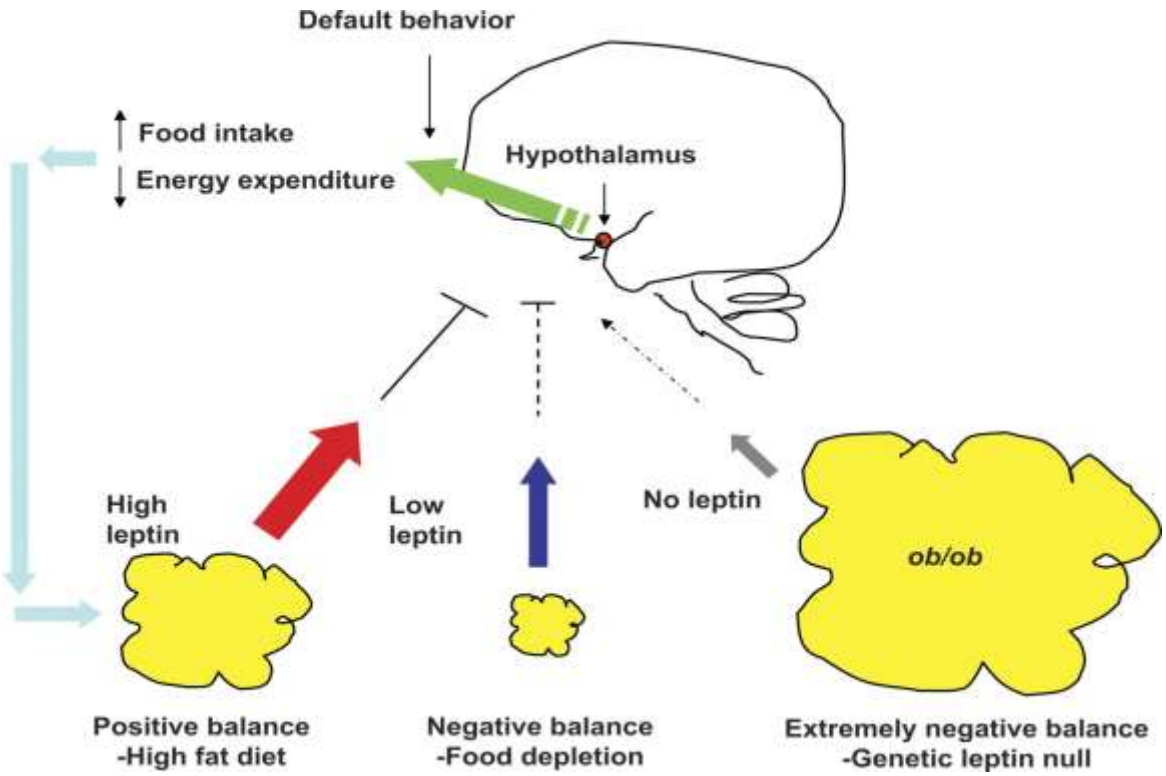

Fig: 2 Leptin and the regulation of body weight in *ob/ob* mouse

***KK mouse*:** KK (Kuo Kondo) mouse also named as Japanese KK mouse is polygenic model of obesity and type 2 diabetes [25, 28]. These animals are hyperphagic, hyperinsulinaemic, insulin resistant and show moderate obesity by two months of age, which attains maximum at four-five months. Insulin resistance precedes the onset of obesity. The increase in pancreatic insulin content is associated with increase in number and size of pancreatic islets but histologically degranulation of beta cells and hypertrophy of islets are found[26,29].

NZO MOUSE: New Zealand obese (NZO) mice present a syndrome of morbid obesity, insulin resistance, hypertension, and hypercholesterolemia which resembles the human metabolic syndrome. As a consequence of the syndrome, male NZO mice develop type 2 like diabetes characterized by marked hyperglycaemia and hyperinsulinemia at earlier age (8–12 weeks), and later on by low serum insulin levels associated with beta-cell destruction. The syndrome has a polygenic basis, and outcross progeny of the strain as well as generation of subcongenic lines has previously been used for identification of genes associated with adiposity, hypercholesterolemia, and hyperglycaemia[30].

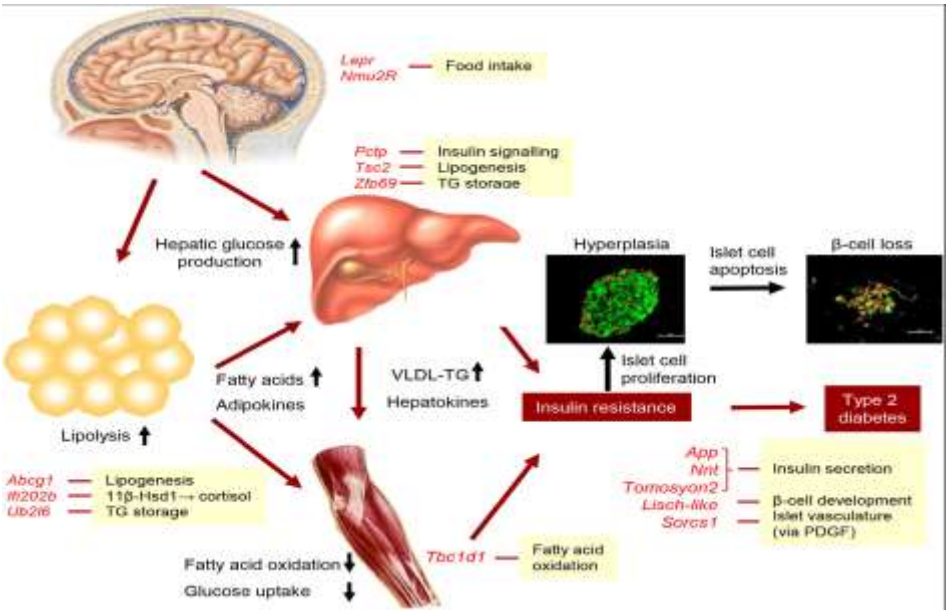

Fig. 3: Pathophysiology of insulin resistance and type 2 diabetes with Beta-cell failure and site of action of candidate genes [30].

***KK/Ay mouse*:** KK/Ay mouse (also known as Yellow KK obese mouse) carries both lethal yellow obese (Ay) and diabetic gene unlike KK mouse where it carries only diabetic gene. KK/Ay mouse is heterozygous which shows severe obesity, hyperglycaemia, hyperinsulinaemia and glucose intolerance by 8 wk of age. Hyperphagia and obesity in young is more pronounced in males than in females [31].

Zucker fatty Rats: Male ZDF rats develop a phenotype of obesity, insulin resistance, and eventually, hyperglycemia due to a leptin receptor mutation, resulting in a phenotype very similar to humans with T2DM. In these rodents, glucose intolerance usually develops by age 8 weeks, followed by overt hyperglycemia by age 10 to 12 weeks. In the pre-diabetic phase, ZDF rats maintain normoglycemia by a compensatory increase in beta cell function resulting in hyperinsulinemia. This compensatory adaptation begins to fail as animals enter the diabetic phase, defined by a dramatic increase in beta cell apoptosis and a corresponding decrease in beta cell mass. In obese pre-diabetic humans, there is a similar period of adaptive beta cell expansion followed by a marked reduction in beta cell mass as the disease progresses [32].

JCR: LA-cp rat: The JCR: LA-cp rat is a unique small-rodent model possessing the major elements of the metabolic syndrome such as insulin resistance/obesity/hyper-triglyceridemia seen in humans including end stage cardiovascular disease. If homozygous for the autosomal recessive *cp* gene (cp/cp), the rats are obese and become insulin resistant, hyper-insulinemic, and hyper-triglyceridemic. If heterozygous (cp/+) or homozygous normal (+/+), the rats are lean and metabolically normal. Male cp/cp rats are atherosclerosis prone, developing advanced intimal lesions from the age of 3 months and ischemic lesions of the heart in later life. The rats also

exhibit a marked vasculopathy, with hyper proliferative and hyper plastic vascular smooth muscle cells and both hyper contractility in response to nor-epinephrine (NE) and impaired nitric oxide-mediated vascular relaxation [33].

OLETF rat: OLETF rat referred as Otsuka Long-Evans Tokushima Fatty is a spontaneously diabetic rat with various symptoms such as polyuria, polydipsia and mild obesity. The characteristic features of this animal includes late onset hyperglycemia(after 18 weeks of age),a chronic diseased state, increased urinary protein excretion at about 30 weeks of age, higher glomerular filtration rate(GFR),increased kidney weight and glomerular hypertrophy etc. Evidences suggest that defects in the beta cell proliferation *per se* is responsible for the development of diabetes in OLETF rats since 70 per cent pancreatectomized animals exhibit sustained hyperglycemia due to poor capacity of pancreatic islet regeneration after surgery and the treatment with nicotinamide (NAD) corrects hyperglycemia by increasing beta cell proliferation[34]. In the recent years, OLETF rat model has been extremely used in pharmacological research while testing for a number of anti-diabetic and antihypertensive drugs [35-37].

Non-obese model:

***GK rat*:** The GK (Goto-Kakizaki) rat, a polygenic model of type 2 diabetes. This is characterized by non-obesity, moderate but stable fasting hyperglycemia, hypoinsulinaemia, normol ipidaemia, impaired glucose tolerance that appears at 2 wk of age in all animals and an early onset of diabetic complications. In the adult GK rats, total pancreatic beta cell mass is decreased by60% along with same degree of decrease in pancreatic insulin stores [31].

Cohen diabetic rat: The Cohen diabetic rat is a genetically derived experimental model of type 2(diet induced) diabetes that possess many similar features of human diseases. Its distinctive feature is that, it expresses genetic susceptibility (sensitivity and resistance) to a carbohydrate-rich diet, a central feature of type 2 diabetes. Recently, the Cohen rat strain was newly inbred and metabolic phenotypes of this rebred colony of CDs (Cohen diabetic sensitive) and CDr (Cohen460 Indian diabetic resistant) rats and their genetic makeup render the Cohen diabetic rat a useful experimental model that is highly suitable for studying the interaction between nutritional-metabolic environmental factors and genetic susceptibility for the development of type2 diabetes and also distinctively useful for investigating the effect of sex on the expression of the diabetic phenotype[31,38].

CONCLUSION

Some of the important animal models described in this review share similar characteristic features of type 2 diabetes. None of the known single species is exactly equivalent to human diabetes, but each model act as essential tool for investigating genetic, endocrine, metabolic, morphologic changes and underlying various different mechanisms that could also operate during the evaluation of type 2diabetes in humans. Among the animal models for type 2 diabetes some animal models are better suited to screen particular class of anti-diabetic compounds. Hence, care must be taken in interpretation and extrapolation of the results obtained from these animal models to humans.

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