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Review

The Use of Animal Models in the Study of Diabetes Mellitus

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Abstract. *Animal models have enormously contributed to the study of diabetes mellitus, a metabolic disease with abnormal glucose homeostasis, due to some defect in the secretion or the action of insulin. They give researchers the opportunity to control in vivo the genetic and environmental factors that may influence the development of the disease and establishment of its complications, and thus gain new information about its handling and treatment in humans. Most experiments are carried out on rodents, even though other species with human-like biological characteristics are also used. Animal models develop diabetes either spontaneously or by using chemical, surgical, genetic or other techniques, and depict many clinical features or related phenotypes of the disease. In this review, an overview of the most commonly used animal models of diabetes are provided, highlighting the advantages and limitations of each model, and discussing their usefulness and contribution in the field of diabetes research.*

"An animal model for biomedical research is one in which normative biology or behaviour can be studied, or in which a spontaneous or induced pathological process can be investigated, and in which the phenomenon in one or more respects resembles the same phenomenon in humans or other species of animals". According to this definition of the American National Research Council Committee on Animal Models for Research and Aging, animal models used in biomedical research can be classified into five groups: a) Spontaneous models in which diseases or conditions occur spontaneously in animals as in humans, b) experimentally and c) genetically modified models in which diseases or conditions are induced chemically/surgically or by genetic manipulation, respectively; d) negative models, including animals resistant to a particular condition or disease and e) orphan models, including animal models with disease unknown to human counterparts (1). In the past century, in an effort to minimize the number of animals used in research, Russell and Burch (2) proposed that the use of animals must follow the three "Rs": Replacement, substituting animals with non-animal (alternative) models; Reduction, reducing the numbers of animals used in research; and Refinement, following the best quality care that can be provided to the animal. The 4th "R", that of Responsibility, was added by Ronald Bank (3, 4).

Diabetes mellitus became known as a disease of pancreatic insufficiency or failure since scientists (Minkowski in the 1880s and later Banting and Best in the 1920s) modelled this condition in dogs by removing part of or the entire pancreas.

Both type 1 and type 2 diabetes mellitus are multifactorial diseases in which a very complex genetic background interacts with environmental factors contributing to the disease development (5). Type 1 diabetes (commonly known as juvenile-onset diabetes) represents about 10% of all cases of diabetes mellitus (6) and is characterised by an autoimmune destruction of the pancreatic beta cells by effector lymphocytes which leads to the loss of insulin production and the prospective hyperglycaemia (7). At least 20 genes of the major histocompatibility complex (MHC) are implicated in type 1 diabetes (8), which is characterized by the presense of autoantibodies against proteins such as insulin, glutamic acid decarboxylase (GAD) and the tyrosine phosphatase ICA512 (also known as IA-2) (9). Type 2 diabetes (commonly known as adult-onset diabetes) represents about 90% of all cases (6) and has nowadays taken on epidemic proportions in Western society (10). This complex and heterogenous disease arises as a combination of insulin resistance and inadequate functional beta cell mass (11), while lifestyle factors like obesity, poor diet and lack of exercise, in association with heredity play a significant role in the risk of developing type 2 diabetes (12, 13).

Although there is much debate about the true value of using animal models in the study of diabetes (14, 15) and

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the ability of implementing an animal-derived therapeutic protocol into clinical use (16), it must be admitted that experimental models are essential tools for understanding the molecular basis, the pathogenesis of complications and the utility of therapeutic agents in a multifactorial disease such as Diabetes Mellitus (17).

This review aims to summarize the essential up-to-date information on diabetic animal models and present a critical evaluation of the utility of three subcategories of animals: a) type 1 diabetes models, b) type 2 diabetes models and c) models not categorized by type.

Type I Diabetes (T1DM) Models

T1DM, a multifactorial autoimmune disease involving genetic and environmental factors, is hallmarked by T-cell and macrophages-mediated destruction of pancreatic β-cells, resulting in irreversible insulin deficiency. Diabetic ketoacidosis, a T1DM immediate consequence, can be fatal without treatment, while the long-term vascular T1DM complications affecting several organs and tissues can significantly affect life expectancy. There is no doubt that T1DM susceptibility is MHC-dependent and MHC genes account for approximately 50% of the total contribution to the disease. However, although to date studies corroborate that both HLA-DR and HLA-DQ genes are important in determining disease risk, the effects of individual alleles may be modified by the haplotypes on which they are carried (18). Besides, immunological, genetic and molecular pathways' differences in the establishment of autoimmune diabetes between animal models indicate that human T1DM can be probably generated from more than one loss of tolerance pathways.

Commonly used models of spontaneous diabetes type I. Five animal models of spontaneous diabetes are mainly preferred for stydying autoimmune diabetes: the NOD mouse, the diabetes-prone BB rat, the LETL rat, the KDP rat and the LEW-iddm rat. NOD mouse and BB rat are by far the most widely used.

The NOD mouse is the most favoured by researchers animal model in the study of autoimmune diabetes. It was developed about 30 years ago in Japan, by inbreeding of a strain called Jcl:ICR, which was used to develop CTS mouse, an inbred cataract-prone strain (19). Insulitis in NOD mice is initiated at the age of 4-5 weeks (much earlier compared to humans) and has many differences from human insulitis, since it begins with lymphocytes surrounding of the islet perimeter and continues with an infiltration of the whole islet by an unusually large number of leukocytes (mainly $CD4^+$ and $CD8^+$ T-cells). Finally, after a period of subclinical β-cell destruction, overt diabetes is usually presented, when more than 90% of the pancreatic β-cells are destroyed (about at the age of 24-30 weeks), with females having a larger trend (90%) to develop diabetes compared to males (50-60%). These frequencies are achievable only in an SPF (specific pathogen-free) environment, because NOD mice are easily prone to protective immunomodulation by a wide spectrum of pathogens. Although, in NOD mice, the typical clinical symptoms (hyperglycaemia, glycosuria, polydipsia and polyuria) are present as in humans, the mice have larger resistance to ketoacidosis development, can remain alive about 2-4 weeks after the disease establishment without insulin administration and if diabetes is not finally treated, death results from dehydration, rather than ketoacidosis (20-22).

NOD mice develop autoantibodies to insulin, GAD and IA-2, as happens in humans, but also typically develop other autoimmune manifestations, such as Sjögren's syndrome and thyroiditis (4, 15). Many genes in the NOD mouse are linked with susceptibility to T1DM and, like in humans, the MHC alleles play an important role in this process. In some cases, genes such as the A^{g7}, or loci such as the CTLA4 seem to be particularly homologous in humans and mice. Besides, both in humans and NOD mice, many MHC alleles need to be accompanied by the presence of other non-MHC genes, in order to express their diabetogenic action (20, 23).

The NOD mouse has also been used as the initial animal for transgenic approaches. For example, the transgenic expression of a T-cell receptor, specific for native β-cells autoantigens, the introduction of further MHC molecules into the NOD genotype or the selective expression of several cytokines in the pancreatic tissue could serve as ways for studying mechanisms, which accelerate or prevent the development of autoimmune diabetes (8, 22).

The Diabetes-prone BB rat, the most widely used rat model for studying autoimmune diabetes, was developed in the 1970s from a colony of outbred Wistar rats in Canada (in the Bio-Breeding Laboratories) (24). Like NOD mouse, the BB rat develops T-cell dependent autoimmune diabetes, which is also characterised by islet auto-antibodies, as well GAD antibodies. However, in contrast with the NOD mouse, the phenomenon of insulitis has many similarities with humans, begins 2-3 weeks before the clinical initiation of the disease, does not start with peri-insulitis and Th1-lymphocytes predominate in the procedure (20, 25). At about the age of 8-16 weeks, the BB rat becomes hyperglycaemic and insulinopaenia, polyuria and polydipsia have already evolved. Though, unlike NOD mouse, ketoacidosis is very severe in the BBrat and as in humans, lethal if not treated with insulin (20, 26).

Spontaneous diabetes in BB rat strains is accompanied by T-cell lymphopenia, where $CD8^+$ T-cells are missing and $CD4^+$ T-cells greatly reduced. In addition, rats lack T-cells that express ART2, an enzyme with nicotinamide adenine nucleotide glycohydrolase activity. However, the adoptive transfer of ART2+ cells into BB diabetic rats prevents

diabetes development (27, 28). As in all rats, the appearance of autoimmune diabetes in BB rat requires at least one MHC-associated gene and especially one class II allele, called RT1 B/Du haplotype (RT1 is designated the rat's MHC) (29). Furthermore, the BB-rat is susceptible to subclinical thyroiditis and sialitis (30). In general, although the existence of lymphopenia calls into question the acceptability of BB rat as a model for human diabetes, it is regarded as a preferable small animal model for studying islet transplantation tolerance induction.

The Long Evans Tokushima Lean (LETL) rat is the first discovered rat model that spontaneously develops an autoimmune destruction of the islet β-cells and rapid frank diabetes at a rate of 20%, without however being lymphopenic (31). LETL rats' substrain that was finally established as the one that develops the disease at a very good rate (70-80% both in males and females) is the KDP rat. The latter is also "non-lymphopenic", shares the RT1 B/D^u haplotype with the BB rat, is characterized by severe insulitis at the age of 120-220 days, and exhibits lymphocyte infiltration in thyroid and kidney (25, 32). Additionally, a non-MHC gene called Cblb (Casitas B-lineage lymphoma b), which codes an ubiquitin ligase important for CD28 co-stimulation during T cell activation, is the major susceptibility gene for type 1 diabetes in KDP rat, but it has no linkage with diabetes in humans (33). Although KDP rat has a similar ability of developing the disease in both sexes and thus seems to be a great model of the human disease, only few studies have been based on this animal and are mainly genotype studies (34).

The LEW.1AR1/Ztm-iddm rat is a new model, which originated from the congenic LEW.1AR1 strain through a spontaneous mutation and as all rat models expresses the RT1 B/Du class II allele. The LEW-iddm is not lymphopenic, develops pancreatic damages similar to BB and KDP rats and frank diabetes is evolved at the age of 60 days. Diabetes appearance rate is about 70% in both sexes, is not lethal without insulin administration and is characterized by low blood insulin, hyperglycaemia, glycosuria and ketonuria (35, 36). Autoantibodies against GAD or IA-2 are not increased in this model and immune cells that infiltrate the pancreas, do not attack to thyroid, parotid or other glands like in other models (35, 37).

Other models of T1DM. Except from the aforementioned typical models of disease representation, scientists develop and use also many other models for studying T1DM. Apart from the case of the genetically modified NOD mouse that has already been mentioned, transgenic and knock-out models of genes that belong to the human immune system (HLA molecules, TCRs, CD4 or CD8 *etc*) have been implemented for studying autoimmunity or transplantation efforts and are known as "humanised models". Moreover, viruses (*e.g.* the encephalomyelitis virus variant D) have already been used to induce insulin dependent diabetes (not autoimmune) in wild-type rodents (22). Besides, viruses are probably thought to play a significant role in human T1DM initiation and this approach is experimentally supported by the fact that Kilham-Rat Virus was the cause of the disease's development in a strain of BB-rat that resists diabetes (BB-DR rat) (38).

Table I. *Animal models of type 1 diabetes (T1DM).*

Animal models of type 1 diabetes	
Commonly used	Other models (rarely used)
NOD mouse	New Zealand white rabbit
diabetes-prone BB rat	Keeshond dog
LETL rat	Chinese hamster
KDP rat	Macaca nemestrina/fascilularis/nigra
LEW-iddm rat	Papio hamadryas

Streptozotocin or other diabetogenic agents (*e.g.* alloxan) with β-cell toxicity abilities have also been used for producing chemically induced T1DM models when administered in a large dose or in repeated low doses for several days (30-40 $mg.kg^{-1}$). Streptozotocin (STZ) and alloxan (AX) accumulate the pancreatic β-cells via the GLUT2 glucose transporter and destroy them through reactive oxygen species and free radicals mechanisms. More specifically, STZ is a nitrosourea related antibiotic and antineoplastic drug, which is produced by Streptomyces achromogenes and due to its alkylating properties, causes alkylation and thus fragmentation of DNA, modifies biological macromolecules and finally destroys β-cells, causing insulin-dependent like diabetes. However, although STZ and AX models result in hyperglycaemia and insulinopaenia, they do not bear strong autoimmune features (22, 26, 39, 40). Eventually, we should not disregard that rabbits, canines, hamsters and non-human primates (especially for transplantation efforts) have also been used in the field of type 1 diabetes research (Table I). In these models, diabetes is usually surgically or chemically induced (41), due to the absence of spontaneity in diabetes development.

Tables I and II synopsize the main T1DM models and the characteristics of the most widely used.

Evaluation of T1DM models' use. Similarities between the aetiopathogenesis of autoimmune diabetes in humans, mice and rats, in which the genes contributing susceptibility to the disease are already expressed at the haemopoietic stem cell level, indicate that animal models are valuable tools for studying T1DM. Besides, even significant differences

Table II. *Characteristics of the most widely used T1DM models.*

	Human	NOD mouse	BB rat	KDP	LEW-iddm
Age of disease presentation	Adolescence	24-30 weeks	8-16 weeks	3-4 months	2-3 months
Disease incidence	?	Females≈ 90% Males≈50-60%	50-80%	70-80%	70%
Ketoacidosis without treatment	Heavy	Mild	Heavy	Heavy	Heavy
Autoantibodies	Insulin, GAD, ICA, ICSA, BSA, CPH, EC, IA-2, IAA	Insulin, GAD, ICA	ICA	unknown	ICA
Insulitis	Destructive T-cells driven	Destructive T-cells driven begins with periinsulitis	Destructive T-cells driven	Destructive T-cells driven	Destructive T-cells driven
Insulin is required immediately after onset	yes	no	yes	yes	no
Other immune/ autoimmune disorders	Thyroiditis, celiac disease, vitiligo, pernicious anemia, polyendocrine syndromes	Thyroiditis, Sjögren's Syndrome, sialitis	Lymphopenia, Subclinical Thyroiditis and sialitis	Sjögren's Syndrome, lymphocyte infiltration in thyroid and kidney	thyroid, parotid or other glands not infiltrated
MHC associated genes	HLA-DQ and DR (susceptibility modified by the haplotype)	Unique I-Ag7	at least RT1 B/D^u haplotype	at least RT1 B/D^u haplotype	at least RT1 B/D^u haplotype
Non-MHC associated genes	CTLA-4, INS (insulin gene promoter region), LYP/PEP tyrosine phosphatase, probably IL-2 and CD25	β-2 microglobulin, CTLA-4, LYP/PEP tyrosine phosphatase, probably IL-2 and CD25	Possibly CTLA-4 and IAN-4 (immune accosiated nucleotide-4)	unknown	Cblb
Enviromental influence on disease appearance	probable	yes	yes	unknown	unknown
Disease alienable by bone marrow	yes	yes	yes	unknown	unknown

between the various rodent models, give researchers the opportunity to understand the potential heterogeneity (mainly in genotypes) underlying this complex disease in humans (42).

Animal models can give us valuable information about molecular pathways that contribute to the induction of T1DM in humans. For example defects in antigen presenting cells' maturation have been reported not only in NOD mice but also in humans and might serve as the beginning of multiple β-cell autoantigen-specific therapies (16). Moreover, methods and strategies to measure anti-insulin auto-antibodies, to detect antigen-specific T-cells or to prevent diabetes using peptide vaccines or other therapies (*e.g.* dendritic cell based immunomodulation) are primarily applied in models, which also serve as reminders of the potential dangers that could exist in human trials, as in the case of the peptide vaccine-induced anaphylaxis (43, 44). Furthermore stem cell research for the production of insulin secretory cells or studies including pancreas or islet transplantation are in any case initiated from animals (45, 46). The use of oral insulin was firstly implemented in the NOD mouse and continued by the Diabetes Prevention Trial (DPT) in subjects with elevated insulin autoantibodies, providing the preliminary data that oral insulin may delay disease progression in individuals expressing high levels of insulin autoantibodies (47). Neogenesis, another aspect of the functional b-cell mass reestablishment, was firstly observed and is now under investigation in animal models. The induction of islet neogenesis was associated with a newly identified protein, the Islet Neogenesis-Associated

Protein (INGAP) that was member of the Reg family of proteins (48). Beyond that, the emergence of T1DM may be influenced by environmental factors (viruses, peptides or others), since even monozygotic twins develop both the disease only at an average rate of 50% (49, 50). Animal models are the only way to start searching for these factors.

Taken together, animal models promote T1DM research into three main research areas. Firstly, through animal models, scientists can investigate immune mechanisms and responses at locations such as the islets or the pancreatic draining lymph nodes, which cannot be accessed in humans. Besides, human studies in the field of disease pathogenesis are only limited to peripheral blood leukocytes or proteins or to results from cultured cells. Secondly, targeted knock-out studies or specific lymphocyte populations' deletion investigations, which are implemented for studying the molecular ways by which immune cells migrate into the pancreatic tissue and destroy the β-cells, can only be realised when using animal models. Last but not least, experiments with animals are essential for the investigation of T1DM complications, the selection of the best drug candidates (either for aetiological or for complications' treatment) and the possible dose ranges and protocols that should be supplied (42, 51, 52). Nevertheless, even when the results from a mouse- or rat-based therapy are equally satisfactory and allow for trials in humans, there must still be in doubt, since there are indeed a plethora of differences between animal models and human disease and each model reflects only few aspects of the disease. Therefore, several therapies such as the subcutaneous injections of insulin at the beginning of weaning that were successful both in NOD mice and BB rats, did not work in humans. However, differences in the extent by which diabetogenic T-cell responses had developed at the time insulin injections were initiated in experimental rodents and the fact that, when weight normalized, the injected insulin doses that inhibited T1DM development in NOD mice were approximately ten times higher than those in humans could excuse the failure of the respective therapy protocol in humans (16).

Generally, T1DM in humans may be the result of a loss of tolerance pathway that cannot be demonstrated by animal models and thus experimental results from models are not relevant to the human disease (53). However, the pathophysiological differences between models and humans are so many, that someone could easily claim that animals are finally useless in T1DM research. First of all, major incompatibilities in both innate and adaptive immunity and also in important immune pathways such as the Th1/Th2 differentiation or the Ag-presenting function of endothelial cells confirm this argument. Furthermore, the phenomenon of insulitis is quite different in humans and animals (especially in NOD mice) and only in BB rats has some similarities and is mainly characterized by the few leucocytes that are detected in the inflamed islets (14). Likewise, although insulin is a major immune target in both humans and animals during the development of diabetes, antibodies against GAD65 and IA-2 are predictors of susceptibility to T1DM only in humans (54). Another controversial issue is the presence of other immune and autoimmune abnormalities such as lymphopenia, thyroiditis and sialitis, which are characteristic in diabetic rodents but not in humans (14, 25). In addition, the fact that the NOD mouse is used as the best known representation of the human disease and thus is the most commonly used, it does not give scientists an objective view of the various aspects of the disease, since this single approach is unlike that in the human condition. In particular, except for the fact that the BB rat displays human insulitis much better than the NOD mouse, the latter exhibits in literature an extreme facility in disease prevention, which is either incompatible with respective results in humans or is often ethically questionable and financially unattainable (*e.g.* insulin administering at the beginning of weaning as described above). Last but not least, many advocate that genetically modified animals (transgenic or knock-outs) or animals with chemically induced diabetes such as STZ are not analogous to any clinical human condition (14).

Whatever is the case, each type of animal model advances the understanding on T1DM step by step and the challenge will always be to combine and translate these findings from the animal models to human disease. Besides, without animals, Banting and Best would never have been able to show that the clinical characteristics of diabetes (*e.g.* hyperglycemia, ketonemia, ketonuria, tissue wasting, coma and death) could be reversed when an extract of the pancreas was given to the pancreatectomised dog and would never have discovered insulin.

Type II Diabetes (T2DM) Models

T2DM is a very complex metabolic disorder in which genetic background and environmental factors (*e.g.* obesity, age *etc.*) both interact and contribute to the establishment of the disease. It is mainly characterised by peripheral insulin resistance, hyperinsulinemia and finally β-cell dysfunction (with or without decrease of β-cell mass) and thus insulin levels and glycemic control vary, depending on the stage of the disease and the ability of hyperinsulinemia to compensate the high glucose levels. However, a large proportion of T2DM patients are not obese and characterised by disproportionally reduced insulin secretion and less insulin resistance than obese phenotype. Due to the variety of genetic impact, environmental factors and complications, T2DM can manifest itself in multiple clinical and pathophysiological conditions and patients are diversified in such a way that the disease in each individual constitutes finally a unique pathophysiological phenomenon. Similarly, animal models of

T2DM can only depict few of the phenotypes that are prevalent in humans, but in each case in models the genetic and environmental factors that predispose them to the disease can be controlled. However, since obesity is the major environmental factor predisposing to T2DM (although 2/3 of obese subjects do not become diabetics), the ability of an animal model to develop firstly obesity, so as to finally develop diabetes, is one of the most important criteria for selecting a model.

Commonly used models of spontaneous diabetes type II. As with T1DM, rodents are the most thoroughly used animals to mimic human T2DM, although other animals such as felines, swine and primates have also been used as T2DM models. Except for the general advantages of using rodents as disease models (*e.g.* small size, easily and economically available, ability of using many animals at the same time), especially the diabetic rodents category includes a variety of models that can spontaneously develop diabetes similar to human T2DM, based either on a monogenic or on a polygenic background.

a) Obese rodent models of spontaneous T2DM. The ob/ob mouse, db/db mouse and Zucker fa/fa rat are the most characteristic examples of T2DM models with monogenic background. These diabetic models develop obesity due to mutations in leptin gene (ob/ob) or leptin receptors (db/db and fa/fa), which may finally lead to the emergence of diabetes. The ob/ob (currently named as Lep^{ob}) genotype has been observed in the C57BL/6J mouse strain and this model is characterised by hyperphagia and low energy expense and thus becomes obese approximately at the age of 4 weeks. The ob/ob mouse is characterised by mild hyperglycaemia due to compensatory hyperisulinemia, which is observed at the age of 3-4 weeks together with hyperphagia, obesity and insulin resistance. However, diabetes becomes very severe and lethal when the ob/ob genotype is expressed on the C57BL/KS strain (55). On the other hand, the db/db mouse also becomes hyperphagic, obese (about at the age of 4 weeks), hyperinsulinemic (about at the age of 2 weeks) and insulin resistant, but later (4-8 weeks) develops hyperglycaemia, due to β-cell failure (56) and does not live longer than 8-10 months. The Zucker (fa/fa) fatty (obese) rat develops the same pathophysiological characteristics with the db/db mouse and is mainly used as a model of human obesity accompanied with hyperlipidaemia and hypertension (55, 57). However, selective inbreeding of fa/fa rat for hyperglycaemia gave birth to the Zucker diabetic fatty rat strain (ZDF), which develops severe diabetes (only in males) at about 8 weeks after birth, due to enhanced apoptosis of β-cells, which are not able to compensate the insulin resistance, as in the fa/fa rat, and becomes insulinopenic at about 14 weeks of age (58).

On the other hand, the KK mouse, the NZO mouse, the OLETF rat and the NSY mouse are the major heralds of the category of obesity-induced diabetes models with polygenic background. The KK (Kuo Kondo) rat comes from the Japanese KK mouse, a strain inbred for large body size (59). Hyperphagia, hyperinsulinaemia and insulin resistance are main features of the KK mouse, which becomes gradually obese from the age of 2 months to the age of 4-5 months, although a decrease in food intake can decrease both obesity and hyperglycaemia. Besides, the hyperinsulinemia, owing to number and size increase of the pancreatic islets, compensates the insulin resistance and keeps blood glucose at mild levels (60, 61). Many lines and colonies have been bred since the development of the KK mouse in 1967. The most prevalent is the KK/A^y mouse which carries the lethal yellow obese gene (A^y). Although the homozygous for A^y animal dies almost before implantation, the heterozygous KK/A^y mouse becomes severely obese, hyperglycaemic and hyperinsulinemic at about the age of 8 weeks (55, 62). KK and KK/A^y mouse are regarded as suitable models for exploring the mechanisms of obesity-induced T2DM, as well as for studying new antidiabetic drugs (62, 63).

The New Zealand obese (NZO) mouse is a model of polygenic obesity, which sharply gains weight during the 2 first months of age, but diabetes frequencies finally differ among the NZO substrains. Although glucose and insulin characteristics are almost similar to those of the KK mouse, the NZO mouse develops hepatic insulin resistance from an early age and progressively represents hyperleptinemia and simultaneously leptin resistance, responsible probably for the hyperphagia (55, 64). Glucose levels and insulin resistance are increased in an age-dependent way and blood glucose reaches the level of 300-400 mg/dL at the age of 20-24 weeks. Furthermore, females seem to be more resistant to diabetes development among several NZO colonies and also males evolve hypertension when fed a high-fat diet (12, 65). Although NZO mouse is a rarely preferred model, new recombinant congenic strains that have been developed by entering NZO loci into other strain genomes [*e.g.* the Nonobese Nondiabetic mouse (NON/Lt)] have attracted a lot of researchers' interest for studying "diabesity" and its treatment (12, 66).

The OLETF rat and the NSY mouse also develop obesity-induced diabetes, although, in contrast with KK and NZO mouse, are mildly and not severely obese. The OLETF (Otsuka Long Evans Tokushima Fatty) rat comes from an outbred colony of Long-Evans rats and males (which are more susceptible to developing the disease) evolve diabetes at about the age of 18-25 weeks. Animals are characterised by polyphagia, high levels of insulin, triglycerides and cholesterol and age increasing hyperglycaemia (67). Many loci on several chromosomes seem to be involved in the disease and regarding metabolism genes, researchers have observed lack of cholecystokinin-A receptors and decreased

content of GLUT-4 in muscles (17). OLETF rat has been widely used for studying and testing antidiabetic (*e.g.* metformin, pioglitazone) or hypertension cure drugs (*e.g.* cilnidipine) (17, 68, 69).

Likewise, the NSY mouse is another polygenic model, which develops diabetes in a sex-dependent manner (almost all males evolve diabetes but only about 30% of females) and the severity of the disease is proportionate to the age of the animal. NSY mouse comes from the Jc1: ICR mouse, which is also the parental strain of the NOD mouse (model of T1DM), but was inbred for glucose intolerance. As mentioned, the NSY mouse is characterised by mild (and not severe) obesity with visceral fat accumulation, accompanied by impaired insulin secretion (firstly observed at about 24 weeks of age) and moderate insulin resistance (70). However, a high fat diet or sucrose administration quickens the development of diabetes. This model is very useful for studying the age-dependent damages and phenotypes of T2DM, as well as the possible genetic correlations between T1DM and T2DM (due to the common origin of NSY mouse and NOD mouse) (5).

b) Non-obese rodent models of T2DM. Clinical experience demonstrates that T2DM can also exist in the absence of an obese phenotype. Thus, the development of non-obese models is essential for studying this condition of the disease. The GK (Goto-Kakizaki) rat is a polygenic non-obese model of T2DM, developed through selective inbreeding of mildly glucose intolerant Wistar rats over many generations (71). This model is characterized by insulin resistance, normolipidaemia and impaired insulin secretion, due to the fact that neonatal GK rats have reduced islets mass (probably owing to defective prenatal β-cell proliferation allied by abnormal apoptosis). Besides, three genetic loci have been correlated with impaired insulin secretion and glucotoxicity is regarded as a mechanism of secondary loss of β-cell differentiation. Thus, adult GK rats show finally a 60% decrease in their total pancreatic β-cell mass. However, blood glucose is elevated only after the 3-4 first weeks of animal's age and generally, during its lifetime, fasting glucose remains mild and stable and rises only after challenge with glucose (72, 73). GK rat is a very useful model for studying the mechanisms of diabetes complications (*e.g.* renal, retinal and peripheral nerves lesions), although the very early β-cell destruction remains a limitation for depicting T2DM.

The non-obese mutant C57BL/6 (Akita) mouse comes from the C57BL/6 colony in Akita (Japan) and contains a spontaneous mutation in the INS2 gene, which is the mouse homologue of human preproinsulin gene (74). This model is characterised by polydipsia, polyuria, progressive hypo-insulinaemia and finally hyperglycaemia at an age of 3-4 weeks. The reduction in insulin secretion is due to a gradual decrease in β-cell mass without the presence of insulitis. This non-obese model, which corresponds well to the administration of exogenous insulin, has been mainly used for transplantation studies (75).

Many other rodent models (obese and non-obese) have been used for studying type 2 diabetes mellitus and are mentioned and categorized in Table III.

c) Non-rodent models of spontaneous T2DM. Feline, swine and non-human primate models have also been implemented to depict spontaneous T2DM.

Felines and especially the domestic cat is a very useful model of T2DM, due to its similarities to the human condition. First of all, the domestic cat shares the same living environment with humans and thus is exposed to the same risk factors, such as obesity and low physical activity. Furthermore, except for the fact that the majority of diabetic cats (about 80%) harbour T2DM like diabetes, the initiation of the disease in cats takes place between 9 and 13 years of age, which is analogous to middle age or older and corresponds to the age of diabetes onset in humans. T2DM in felines is also characterised by insulin resistance and progressively decreased insulin secretion, owing to an approximately 50% loss of β-cell mass in adult diabetic cats. Besides, the latter lesion is due to the development of islet amyloid deposits, detected also in more than 90% of T2DM human patients. Moreover, cats suffer from diabetic complications (*e.g.* peripheral neuropathy and retinopathy) consistent with those appeared in humans. The abovementioned characteristics of diabetes in cats make them an appropriate model for studying the pathophysiological mechanisms of T2DM establishment (6, 76, 77).

Swine are similar to humans regarding cardiovascular anatomy and function, metabolism, lipoprotein profile, size, tendency to obesity, pancreas morphology, gastrointestinal structure and function, and thus are regarded as very suitable models for testing new drugs (*e.g.* statins) or devices (*e.g.* stents). Swine models have been used for many conditions and diseases, including both T1DM and T2DM, although in these cases are mainly useful for the determination of mechanisms that mediate cardiovascular complications of diabetes mellitus (78). Besides, among the several swine strains, only few develop spontaneous T2DM (*e.g.* female Yucatan minipigs) and others need a high fat diet background to become firstly obese and afterwards to gain some characteristics of T2DM (*e.g.* the Gottingen minipigs) (65). Finally, the use of swine models is considered very beneficial in specific studies of complications of chemically induced diabetes mellitus (usually by STZ), specially for cardiovascular, renal or retinal damages (6).

Last but not least, T2DM can be spontaneously established in many primate species such as cynomolgus, rhesus, bonnet, macaques, baboons and others. As in humans, T2DM in non-human primates is developed in an age-dependent way, is influenced by obesity and characterised by insulin resistance,

Table III. *Animal models of Type II diabetes*.

Type of animal models	Obese	Non-obese
Spontaneous or genetically derived models	*ob*/*ob* mouse *db*/*db* mouse Zucker (fa/fa) fatty rat KK (Kuo Kondo) mouse KK/A^y (yellow KK obese) mouse NZO (New Zealand obese) mouse NONcNZO10 mouse OLETF (Otsuka Long Evans Tokushima fatty) rat ZDF (Zucker diabetic fatty) rat JCR/LA-cp (James C Russel/LA corpulent) rat M16 mouse SHR/N-cp (spontaneously hypertensive rat/NIH-corpulent) rat TSOD (Tsumara Suzuki obese diabetes) mouse Obese rhesus monkey female Yucatan minipigs	Cohen diabetic rat GK (Goto-Kakizaki) rat Torri rat Non-obese C57BL/6 (Akita) mutant mouse ALS(alloxan sensitive)/Lt mouse
Diet/nutrition induced models	Israeli Sand rat (Psammomys obesus) Spiny mouse (Acomys calirinus) C57/BL 6J mouse Ctenomis talarum (Tucotuco) Gottingen minipigs	--
Chemically induced	GTG (goldthioglucose) treated obese mice	ALX or STZ adult models Neonatal STZ rat
Surgically induced	VMH (ventromedial hypothalamus) lesioned dietary obese rat	Partial pancreatectomized animals
Genetically modified animals (transgenic/knockout)	β_3 receptor knockout mouse Uncoupling protein (UCP1) knock-out mouse	Transgenic or knockout animals (mainly mice) of genes implicated in insulin resistanse (*e.g. IRS-1, IRS-2, GLUT-4*), lipid and glucose metabolism (*e.g. PPARs*) and insulin secretion (*GLUT-2, Glukokinase, IGF-1R*) human islet amyloid polypeptide (hIAPP) transgenic rodents

hyperinsulinaemia and progressive hyperglycaemia, due to gradually decreased insulin secretion, owing to islet amyloid lesions. Furthermore, these animals develop dyslipidaemia, increased inflammation status, ketoacidosis (in severe, not treated diabetes) and generally the evolution of the disease depends on the same factors as in humans (energy intake, physical activity, *etc*.). Additionaly, in females, pregnancy, menopause or sex hormone treatments can affect insulin resistance and influence the possibility of T2DM development (6, 65, 79). Similarly to swine models, primates have also been used for depicting chemically-induced diabetes and are generally valuable tools for pharmacological studies (*e.g.* for agonists for the PPAR family) or studies on the mechanisms of diabetic complications, such as atherosclerosis. However, limitations such as their cost and their lifespan reduce their usefulness as models of human diabetes (65, 79).

Other models of T2DM. T2DM is not only depicted through spontaneous models of the disease, but also through induced models. This category of non-spontaneous T2DM animal models includes the diet affected diabetic models, the chemically or surgically T2DM induced models and the transgenic models related to this type of disease.

The Israeli sand rat (*Psammomys obesus*), the most widely used diet-induced diabetic animal model, becomes obese and diabetic when changing its natural vegetarian diet with laboratory chow, which is a high energy diet (80). Then, the rat develops hyperphagia, obesity, hyperinsulinaemia, glucose intolerance, increased hepatic glucose production and muscle insulin resistance and this hyperglycaemic state of the animal is characterized by increased circulating proinsulin due to high demand for insulin secretion (6, 55, 81, 82). However, this increased insulin demand is followed by a progressive loss of

β-cells mass owing to increased apoptosis of the cells, which is driven by a glucose-toxicity mechanism (83). Among the several genes that have been found in *Psammomys obesus* and affect obesity and diabetes, the "Tanis", is a liver protein of 188 amino acids, which is upregulated after fasting in the liver of diabetic P.obesus (but not in non-diabetic controls) and shows direct correlation with the progress of T2DM, inflammation and cardiovascular disease (84). This polygenic rat model of T2DM is mainly used for studying the interaction between obesity and diabetes, the effects of diet and exercise and for pharmacological research (*e.g.* for testing protein tyrosine phosphatase inhibitors and glucogan like peptide-1 (GLP-1) analogues) (55, 81).

Except for *Psammomys obesus*, the C57BL/6J mouse and the *Acomys calirinus* (spiny mouse) are also very useful diet induced models among rodent models. Rhesus monkey (*Macaca mullata*), the Gottingen minipigs and the female Ossabaw pigs are some other examples of non-rodent diet-induced models, which under atherogenic diet develop characteristics of metabolic syndrome and coronary artery disease (55, 82, 85).

Furthermore, although diabetogenic agents such as alloxan and streptozotocin are mainly used for T1DM induction, they have also been used for the development of T2DM like diabetes in animals. In rats, when the administration of STZ follows a primary administration of NAD (15 minutes previous), produces a T2DM model at a rate of 75-80% , which develops mild and stable hyperglycaemia without changes in plasma insulin (86). A congener non-rodent model was developed using a protocol like the abovementioned in Gottingen pig (87). Moreover, STZ or alloxan injection, neonatally or immediately after birth in rats (following different dose and time protocol for each agent) produces T2DM in the adult age of animals (88, 89). However, the development of diabetes in these animals is accompanied by a decrease in β-cell mass and thus in insulin secretion. Therefore, the combination of STZ administration with a genetically insulin resistant background (*e.g.* in ZFR model) or under a high fat or high fructose diet produces models that develop overt hyperglycaemia in the presence of normal blood insulin and, hence, are regarded as more appropriate for T2DM studies (55, 90). Likewise, goldthioglucose causes necrotic damage to the cells of ventromedial hypothalamus and provokes the development of hyperphagia, which finally results in the establishment of obesity-related diabetes in mice (91). As already mentioned, other non-rodent animals (*e.g.* pigs or primates) have also been used as chemically-induced diabetic models. However, cats resist the diabetogenic effect of STZ or alloxan and develop diabetes only when this administration is combined with partial pancreatectomy protocols (76). Otherwise, the combination of partial pancreatectomy followed by growth hormone and dexamethasone administration produces an insulin resistant model in cats (92).

Additionally, partial removal of the pancreas (up to 90%) has been implemented in many animals (rats, dogs, pigs, rabbits), so as to induce T2DM. However most of these models develop moderate hyperglycaemia without frank changes in body weight or blood insulin levels. Therefore, nowadays, pancreatectomy protocols are usually combined with the administration of diabetogenic substances in order to develop T2DM (as already described in cats). Moreover, due to the pancreas regeneration abilities observed in human and many animal models, these surgically induced diabetic animals are usually utilized for transplantation or regeneration factors' studies (*e.g.* PDX-1, IDX-1, IGF-1) (55, 93, 94).

Moreover, due to the complexity and the polygenic basis of T2DM, many transgenic models have been developed to study the genes that may contribute to the development and pathogenesis of the disease and thus many review articles are available on this issue (95-98). Concisely, the role of genes implicated in insulin resistance (*e.g. IRS-1, IRS-2, GLUT-4*), lipid and glucose metabolism (*e.g. PPARs* and *C/EBP-a*) and insulin secretion (*GLUT-2*, Glukokinase, *IGF-1R*) is better understood through transgenic or knock-out approaches. Besides, researchers today have the opportunity to develop tissue-specific knock-outs of the abovementioned genes, so as to estimate the impact of these specific tissues in the whole phenotype of the disease. The Cre/loxP system is usually implemented for this procedure. Cre is a bacteriophage recombinase enzyme that recognizes specific sequences of DNA (LoxP sites), which can be placed through genetic engineering techniques next to tissue specific promoters. Thereby, the enzyme is able to remove the gene or exon of interest in a tissue specific manner (26, 95-97). Furthermore, the human islet amyloid polypeptide (hIAPP, a 37-amino acid protein cosecreted with insulin by β-cells) transgenic rodents are also prominent models for studying the effect of pancreatic amyloidosis on β-cells mass and thus on the pathogenesis and development of T2DM (99, 100). Last but not least, models that have genetic modifications that affect fatty acid metabolism pathways (*e.g.* Acyl-diacylglycerol transferase, Acetyl-CoA carboxylase, acyl-CoA dehydrogenase deficient mice *etc.*) when combined with low or high fat diet and with or without physical activity are remarkable tools for understanding the "nutrigenomics" of insulin resitance and T2DM (10).

Evaluation of T2DM models' use. T2DM, an heterogeneous metabolic disorder resulted from defects in one or more diverse molecular pathways, affects >5% of population in Western countries. Monogenic forms are usually linked with insulin secretion defects, while the most common multifactorial forms are linked with insulin resistance and obesity. The heterogeneous nature of T2DM and the fact that its worldwide prevalence is expected to double within the next two decades, makes any new information from animal

models always positively acceptable, even in the case in which the animal model does not seem to appropriately represent the human condition. Besides, the availability of a plethora of models for T2DM, gives researchers the opportunity to study a specific phenomenon, such as the effect of a drug, into different models and thus to make results as applicable as possible to human disease.

Generally, animal models and especially rodents, have already offered valuable information in many sectors of T2DM research. First of all, they have proved essential for studying the different features or phenotypes of the disease (*e.g.* hyperglycaemia, hyperinsulinaemia, insulin resistance, obesity *etc.*), the underlying pathophysiological mechanisms and their correlation with other factors (mainly environmental such as diet or physical activity) that can efficiently contribute to the initiation of the disease. Relatively, animal models nowadays have a stellar role in studies aimed at unravelling nutrient-gene interactions, with the help of nutrigenomics and nutrigenetics, such as the presence, relation and interaction between obesity and diabetes (6, 10, 12, 17, 55, 82, 83). Moreover, experiments which include animal models are very helpful for studying the aetiopathogenesis of T2DM complications (*e.g.* atherosclerosis, nephropathy) (51, 101) and are afterwards necessary for the selection and testing of new drugs such as thiazolidinediones (PPARγ agonists *e.g.* pioglitazone, rosiglitazone) (62, 66), dimethyl amiloride and others (102). Thirdly, genetically modified models (transgenic and knock-out animals) have provided useful knowledge about the role of specific molecules and proteins on glucose and fatty acid metabolism (10, 96, 97) or the mechanisms of the preservation or reduction of the β-cells mass during T2DM (83). Last but not least, due to the fact that T2DM is an age-related disease (and thus known as adults' or elderly' diabetes) is usually accompanied with other diseases such as heart ischemia or Alzheimer. Even these disease combinations are today investigated through animal models (103, 104).

It is true that rodent models of T2DM, which are the most widely used, share many similarities with the diabetic condition observed in humans, such as the fact that phenotype in these animals also depends on genetic background, sex and age of the animal (98). Additionally, they give us the opportunity to study the molecular mechanisms that lead to diabetes and follow all the stages of disease from its onset and development, to the beginning of the disease's complications. Furthermore, they provide the ease of genetic manipulation, a relatively short breeding span and access to physiological and invasive testing. However, there are some limitations in their use. Firstly, in most models (including rodents), diabetes appears as a consequence of the inability to increase β-cells mass in response to obesity-induced insulin resistance. Furthermore, animals (except monkeys and cats) usually develop diabetes without displaying the same islet pathology as in humans (islet amyloidosis) (6). Moreover, many questions have yet to be answered about animal models and their relevance to the human condition. These questions concern specific physiological or pathophysiological mechanisms in humans (*e.g.* the fact that the human brain needs about 20% of the energy of the resting metabolic rate compared with 3% in rodents, the role of menopause in human diabetic complications and generally the role of gender in human diabetes *etc.*) or other factors (*e.g.* the psychosocial stress that contributes to insulin resistance and T2DM development in humans) which we do not know if (or to what extent) they can be depicted by animal models (65, 105). Additionally, apart from limitations such as cost or availability, which also exist in all disease models, time is also a limitation when it comes to T2DM, due to the fact that (as in humans) disease progression or the development of its complications in animals (especially in primates) need frequently long periods to appear.

In general, although no single model of T2DM encompasses all of the disease's characteristics, they represent several of the pathophysiological conditions seen in humans and thus remain valuable if not irreplaceable for T2DM research. However, especially in the case of diabetes development studies, the selection of the most appropriate animal model comprises a serious part of the whole strategy for gaining new knowledge (and not wrong conclusions) about T2DM.

Models not Categorized by Type (1 or 2)

Diabetic animal models cannot always be categorized into T1DM or T2DM and many of them are used for depicting and studying common features of diabetes' types, such as hyperglycaemia effects or diabetic complications.

Such an example is the chemically and surgically induced diabetic models. As already described, diabetogenic agents such as streptozotocin, alloxan, goldthioglucose or dithizone can induce many subtypes and features of T1DM or T2DM, with the two first being the most widely used. However, streptozotocin is more preferred than alloxan, due to some advantages of it over alloxan such as longer half-life time and hyperglycaemia duration and better established diabetic complications with lower possibility of ketosis or mortality incidents. Moreover, alloxan is ineffective in guineapigs and, relatively, streptozotocin in rabbits. Though, most of the times, these models are not chosen for their ability to represent the one type of diabetes or the other, but only for their usefulness in studying the mechanisms of diabetic complications (*e.g.* diabetic nephropathy or retinopathy), or as a tool for testing new drugs (*e.g.* PPARγ agonists), physiological responces (*e.g.* of the growth hormone axis) or current regeneration or transplantation methods. Besides,

even if such a model is supposed to depict one specific type of diabetes, we cannot be sure about its actual homology with this type of diabetes (26, 106-110).

Genetically modified animals (transgenic and knock-outs represent another category of models that cannot always and preclusively be categorized into T1DM or T2DM models, due to the fact that they do not depict a whole diabetic condition organism, but only the role of one or more specific genes under a physiological background. For example, although transgenic mice deficient in factors involved in pancreas development (*e.g. Pdx-1*) or in β-cell growth and/or survival (*e.g. IRS-2, PKB*, cyclin D2, Cyclin dependent protein kinase-4) are usually implemented for T2DM studies, they do not actually represent models of specific diabetes type, but are very useful in providing information about the pathogenic mechanisms of β-cell mass reduction (83). Likewise, transgenic or monogenic mutations models regarding genes such as Apolipoprotein E, Endothelial Nitric Oxide Synthase or *GLUT1* provide a better understanding of the factors that exacerbate diabetic nephropathy and partially atherosclerosis (109, 111).

Furthermore, we must not exclude from diabetic models, those that are useful for depicting specific non-type 1 or 2 diabetes conditions such as the maturity onset diabetes of the young (MODY) or gestational diabetes. MODY is owed to mutations in genes coding for transcription factors or glucose-sensing proteins and thus mutations in hepatocyte nuclear factor-4a (*HNF-4a*), glucokinase gene, *HNF-1a*, insulin promoter factor-1/*PDX-1, HNF-1b* and NeuroD are present in MODY1, MODY2, MODY3, MODY4, MODY5 and MODY6, respectively. Animal models for MODY (usually for the very common form MODY2, which is caused by mutations in the glukokinase gene) are nowadays developed through large-scale mutagenesis projects that employ the chemical mutagen N-ethyl-N-nitrosourea (ENU) (112, 113). On the other hand, animal models of gestational diabetes are generally established using STZ protocols or through diet-induced obesity in pregnancy (114, 115).

Last but not least, as already mentioned, many animal models, such as partial pancreatectomy models or low-dose STZ models are used in the field of β-cells regeneration research. Similarly, the Insulinoma-bearing New England Deaconess Hospital (NEDH) rat and the IFN-g transgenic mice are also two single models of diabetes mellitus that are used almost exclusively for pancreatic regeneration studies. In the first model, animals become hypoglygemic and hyperinsulinemic, due to transplantation of a small insulinoma tumor and the following atrophy of β-cells. However, the removal of the tumor results in hypoinsulinemia and hyperglycaemia, which is followed by the regeneration of pancreatic β-cells and the increase of insulin levels. In the second model, the *IFN-g* gene is linked to human insulin promoter and mice undergo pancreatic inflammation and progressive loss of islets at an age of about 6-8 weeks (93).

Conclusion

By and large, animal models of diabetes mellitus are regarded as very useful tools for studying the pathophysiology and the clinical aspects of the disease and are always used as the first step for investigating a prospective new therapy. Although they have many differences from the human condition and are usually characterised by many limitations (animal size, availability, cost, *etc.*), investigators continue to rely on animal models due to the fact that they can be readily tested, biopsied and autopsied, their genetic and environmental background is already known and generally they serve studies that could not otherwise be accomplished in humans. Therefore, the continuing effort for inventing new models has always positive critics and animal models will continue to have a major and meaningful place in diabetes research. However, all researchers should always keep in mind the ethical limits in the use of animal models for their experiments, utilize animals only when they are indispensable for a study and avoid causing them pain, distress, suffering and lasting harm.

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