Chapter 17

Development of Improved Animal Models for the Study of Diabetes

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Additional information is available at the end of the chapter

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1. Introduction

Medical research based on animal model is rightly considered a "necessary evil", being a "modus vivendi" in all research activities for more than 2,000 years. It is admitted that the major breakthroughs in medicine such as blood circulation, respiration physiology, the hormonal system used for research purpose different species of animals. In the last 150 years animals used in medical experiments brought huge benefits to humanity by providing crucial responses to the most intriguing questions about prevention and treatment of some devastating diseases. Furthermore, diseases as cancer, AIDS, malaria, tuberculosis, influenza, Alzheimer's disease and diabetes mellitus were approached by creating specific animal models with respect to pathogenesis, genetic insights and treatment. Despite to all these achievements, over the years a lot of people or organizations were and still are reluctant to animal research because this brings intolerable suffer and pain. All of those mentioned emphasized that animal models are not the only scientific methods to achieve important and reliable results. Consecutively, it was constantly sustained that animal research should be abandoned at once and further efforts should be invested in creating alternative methods. For preventing barbarity against animals which was rightly condemned in the past, new concepts were necessary to be enforced. Thus, "animal rights" (animals are granted to live a life free from abuse and exploitation which also includes prevention of use an animal for scientific research) and "animal welfare" (for the animals used in research this implies assessment of breeding, transport, housing, nutrition, disease prevention and treatment, handling and, where necessary, euthanasia) were two of the most invoked [1].

Laboratory animal welfare was first defined in *The Principles of Humane Experimental Technique* written by William Russell and Rex Burch. The essence of this work refers to the *three Rs (3Rs)*:

- *refinement:* decrease in the incidence of the severity of inhumane procedures applied to those animals used for research purpose;
- *reduction*: reduction in the number of animals used to obtain information of given amount and precision;
- *replacement*: the substitution of conscious living animals with insentient materials.

Nevertheless, the 3Rs were the subject of dispute between animal research supporters and those who are against animal experimentation. Animal welfare was consistently improved by implementing of the 3Rs, but some important issues were created in some area of medical research. For instance, validation of the alternative methods which replaces the animals, reliable results based on statistical analysis when a smaller number of animals are used or refinement of the methods for induce less pain and suffering (e.g. administration of analgesics after surgical procedures) were the most debated in the last forty years. The scientific world is still preoccupied by further implementing of the 3Rs [2, 3]. In USA, National Institute of Health stopped financing almost all new projects which use chimpanzees as the closest human's related animal model [4]. This species become nonessential due to alternative research tools and methods, this being one of the last benefits of Russell's and Burch's 3Rs.

2. Experimentally induced hyperglycemia

Hyperglycemia is one of the most important signs of diabetes mellitus, both surgical removal of the pancreas and administration of β-cell toxins being equally used. The first method has been used for the first time in a canine model designed by Oskar Minkowski and Josef von Mering. Partial or total surgical removal of the pancreas was followed by the most "popular" clinical sign of diabetes: glucosuria, body weight loss despite voracious appetite and intake of nourishing food, polyuria, polydipsia and ketonuria [5, 6]. This experiment was followed by another historical breakthrough accomplished by Frederick Banting and Charles Best. These two scientists performed a ligation of pancreas ducts to induce atrophy of exocrine acinar component and thereby to obtain a less contaminated extract of pancreatic islets. This extract succeeded to determine a substantial prolongation of life in dogs with pancreatectomy and also to save the life of a diabetic boy [7].

It is well known that the beginnings of the research in diabetes aimed as animal model the dogs and the rabbits. Later, the scientists preferred to conduct experiments in smaller animals, these being easier to manipulate and involve smaller expenses. Thus, rats and mice were subjected for pancreatectomy. This surgical procedure is challenging because of the particular anatomy of the pancreas and pancreatic ducts in this species. The rat pancreas is spread on a large anatomic area, being divided in three parts (biliary, duodenal and gastrosplenic portions). The duct system is quite polymorphic and represented by numerous independent pancreatic ducts which drain secretion from each corresponding part. The results of pancreatectomy in rat were not always followed by the rapid onset of the diabetes and do not reflect entirely the diabetes in humans, these being speculated by those who consider that larger species are more appropriate for diabetes study [8, 9].

Toxins as streptozotocin [10], alloxan [11], vacor [12], dithizone [13], and 8-hydroxyquinolone [14] were used as non surgical methods. Each toxins aim to induce various destruction of β-cells and produce diabetes and subsequent complications.

Both surgical removal of the pancreas and toxin induced diabetes are valuable methods used for studying the consequence of hyperglycemia and the onset of diabetes complications such as diabetic microangiopathy and macroangiopathy, retinopathy, neuropathy, and cardiomiopathy. Cardiomiopathy, as a complication of streptozotocin induced diabetes was revealed by gravimetric assessments and morphometry. Diabetic rats present hypertrophy of left ventricle, revealed by increased values of ventricular ratio, comparing with control group. Same groups exhibited significant increasing of heart weight/body weight ratio and liver weight/body weight ratio, comparing with control group [15]. Considering that cardiac hypertrophy is the result of potential interstitial fibrosis, thickening of arteriolar media, endothelial cells and basement membrane changes, morphometry of arteriolar media of heart arterioles and cellular density of media were assessed. Arteriolar media/diameter of arteriolar lumen was significantly bigger in rats with streptozotocin induced diabetes, this being the result of fibrosis in arteriolar media [16].

Islet cell transplantation and its consequence is one of the current research targets, being conducted on either surgical removal of the pancreas and toxin induced diabetes. Successful transplantation was achieved for the first time in 1966 in patients with diabetic nephropathy subjected for simultaneous pancreas and kidney transplantation [17]. Despite to consistent benefits of this therapeutic management, the lack of donors, the acquired chronic immunosupression, postoperative complications and graft rejection have to be considered. Thus, islet transplantation era began with two experiments in rodents previously rendered diabetic by the methods described above [17-19]. The methods of transplantation became more refined correlated with and requested by all the shortcomings resulted by immunosupression and graft rejection. Therefore, pancreatic islets graft may be transplanted as alginate or alginate-polylysine immunoisolated microcapsules [20-22], which are implanted in various anatomic sites (subcutaneously, into the splenic parenchyma, under renal capsule, into the peritoneum, into the portal vein for further colonization in the liver) [17-19, 23]. Unfortunately, the lifetime of transplanted islets is shortened by the deleterious immune reaction of the host. Without microcapsule protection and immunosuppressive treatment, islets transplanted into the liver are immediately surrounded by thrombi placed into the vessels of the surrounding tissue. Allogeneic islets from liver and spleen present lymphocytic infiltrations in 2 days after transplantation and are destroyed rapidly by the host [24].

Diabetic rodents are frequently used in research concerning pharmaceutical compounds aimed to lower the level of glycemia in diabetic persons. New formulas are previously tested on diabetic rodents in order to estimate efficacy, and potential toxic effect on the patients.

3. Experimentally induced glycosuria

Phlorizin is an organic compound, member of chalcone class, extracted for the first time from the bark of the apple tree. The compound was also isolated from roots bark, shoots,

leaves and fruits, proving that phlorizin is usually ingested by humans. It was observed that ingestion of more than 1g of phlorizin is followed by glycosuria. Knowing that diabetes mellitus expresses urinary symptoms such as glucosuria and polyuria, an important correlation has been made between these symptoms and the effect of phlorizin. Chronic administration in dog was followed by glucosuria, polyuria and weight loss, creating this way an obvious resemblance between human spontaneous diabetes mellitus and phlorizin effect [25]. Diabetic rats treated with phlorizin express values of glycemia almost equal with normal parameter. This model was used to clarify the implication of hyperglycemia in the progression of islet lesions. The results proved that chronic hyperglycemia might have no effect of islets histopathological changes [26].

4. Chemically induced insulin dependent diabetes mellitus – animal models

Considering that insulin dependent diabetes mellitus (IDDM) features the immune-mediated destruction of β-cells and subsequent insulinopaenia, animal models which reproduce damage of pancreatic islets have been created. For this purpose, streptozotocin and alloxan induced diabetes mellitus were considered the handiest manners to create this condition, although naturally, β-cells become dysfunctional after a long period without evident clinical signs. Streptozotocin and alloxan are diabetogenic chemicals, both being framed in the group of glucose analogues. The onset of β-cells destruction is induced via different mechanisms. Alloxan was the first used as a toxic agent against β-cells, its ability being to generate both reactive oxygen species (ROS) and inhibition of glucose mediated insulin secretion through glucokinase blockage. During the destructive process, β-cells express reversible transformation of cytoplasmic organelles (cytoplasmic vacuolization, dilation of rough endoplasmic reticulum, reduced Golgi apparatus, scattered insulin content secretory granules and swollen mitochondria) finalizing with irreversible damaging of DNA (TUNEL positive staining of β-cells nuclei) [11]. Streptozotocin has antibiotic and chemotherapeutic properties, being isolated from *Streptomyces achromogenes*. The main action of streptozotocin is focused on β-cells DNA via alkylation process. Finally, DNA methylation results into the fragmentation and ultimately generates cell death [11, 14, 27]. Streptozotocin diabetes mellitus can be induced via a single large dose or multiple low doses administration. It is possible that the first option to induce diabetes because of direct toxic effect of streptozotocin, while, low doses repeatedly administrated may exert blockage of insulin secretion [14].

Other diabetogenic compounds were used in experimental models such as dithizone [28]. Administration of this chelator in rabbit has a particular effect expressed as initial hyperglycemia after 2 hours, followed by normoglycemia in 8 hours and finalized by permanent hyperglycemia due to degranulation of β-cells [29].

5. Spontaneous IDDM based on animal models

The non-obese diabetic (NOD) mouse (table 1) is a spontaneous IDDM animal model. This was spontaneously obtained in one of two sublines derived from CTS mice (Immune Deficiency

of Cataract Shionogi). The diabetic line was established after six generations of breeding [30]. About 20% of males and 80% of females develop type 1 diabetes mellitus around 30 weeks of age in particular environment (the incidence of diabetes is higher in colonies maintained in relatively germ-free conditions). The lesions of Langerhans islets are expressed as insulitis, the onset of insulinopaenia being recorded in 12-week-old females. Polyuria, polydipsia, hyperglycemia, glucosuria and hypercholesterolemia are the main clinical signs [30, 31]. Daily administration of insulin improves consistently the body weight and life span, although the mice can survive for weeks without insulin supplement. It is noteworthy that the low level of insulin in NOD mice is correlated with increase secretion of glucagon in treatead and non-treated with insulin individuals. Thus, is concluded that insulin deficiency and glucagon hypersecretion might have an important role in the development and clinical progress of diabetes in NOD mice [32]. Pinealectomy in newborn mice is followed by a more rapid onset of diabetes in female and supplementary melatonin administration protects the animals. The results are somehow intriguing, knowing that melatonin induces increase of insulin autoantibodies [33]. NOD mice are prone to develop autoimmune inflammations, especially those with anti-diabetogenic MHC haplotype and programmed death cell deficiency (sialadenitis of submandibular gland, thyroiditis, gastritis, vasculitis of renal arteries, neuritis) [34-36]. The most important studies which have been run on NOD mice targeted gene implication, MHC genes class II having an important role. Also, knowing that NOD mouse develop cell immune mediated diabetes, many of the experiments aim to picture the immunological status which is responsible for the onset of diabetes [37, 38]. It is important to bear in mind that diabetes in NOD mice is not only the result of cell mediated immunity but also of humoral factors as GAD and IgM [39].

Akita mouse (Ins2^{Akita}) was obtained from a spontaneous point mutation in a female of C57BL/6 line. This mutation disrupts normal synthesis of insulin via incapacity to produce and secrete mature insulin. Clinical signs of diabetes are clearly expressed in male, comparing with female. Heterozygous mutant mice present hyperglycemia, hypoinsulinemia, polydipsia and polyuria. The mice are lean and do not present insulitis. Pancreatic islets exhibit decreased density of β-cells and decreased density of secretory granules in the existing β-cells, increase amount of endoplasmic reticulum and swollen mitochondria [40]. Progressive diabetic retinopathy begins around 12 weeks of age after the onset of hyperglycemia and is consistent with increased vascular permeability, morphological abnormalities of astrocytes and microglia, apoptosis and thinning of inner layer of the retina [41]. Heterozygous Ins2^{Akita} are suited for allogeneic and xenogeneic islet transplantation, because it provides a biological status free of unwanted toxic effect of streptozotocin and alloxan and without β-cell autoimmunity [42].

BB (bio breeding) rat also known as BBDP (bio-breeding diabetes prone) rat is an inbred laboratory rodent which spontaneously develops IDDM. The animals between 2 and 4 months of age develop spontaneous hyperglycemia, different degrees of mononuclear infiltration of the pancreatic islets or total loss of β-cells, insulinopaenia and ketogenesis [43-45]. The overt diabetes can be reversed in 36% of diabetic rats when BB/Worchester (BB/W) are treated with rabbit anitiserum to rat lymphocytes. These results highlight that diabetes mellitus in BB rats is a cell-mediated autoimmune disease [46]. Destruction of β-cells is performed

Rodents	**Mouse**	Non-obese diabetic (NOD) mouse Akita mouse
	Rat	BB/BBDP Long Evans Tokushima Lean (LETL) Komeda Diabetes Prone (KDP)
	Rabbit	New Zealand White Rabbit
	Hamster	Chinese hamster (*Cricetulus griseus*)
Dog		Keeshond dog

Table 1. Animal models for insulin dependent diabetes mellitus

by a cohort of immune cells such as T and B-lymphocytes, macrophages and natural killer cells [38, 47]. The BB/Worchester diabetic rats may develop lymphocytic thyroiditis in individuals between 8 and 10 months of age [48]. The onset of diabetes in BB rats is attributable to many genes, the most important being those which trigger the age of the onset of diabetes, diabetes susceptibility, severity of islet infiltration with inflammatory cells and islet atrophy [49].

As an overview of either differences or resemblances between NOD mouse, BB rats and human IDDM data are presented in table 2 [50].

Characteristics	**Human**	**NOD mice**	**BB rats**
Genetic predisposition (MHC class II)	yes	yes	yes
Genetic control	polygenic	polygenic	polygenic
Haemopoietic stem cell transfer	yes	yes [50]	yes [50]
Lymphocytic insulitis (with T-lymphocytes)	yes	yes	yes
Lymphocytic infiltrates in other organs	sometimes	yes	yes
Humoral reactivity to β-cells	yes	yes [39]	no
Diabetic ketoacidosis (without treatment)	yes	mild	yes
Detection of retroviral antigens expressed in beta cells	no	yes [51]	no
Sex predisposition	no	yes	no

Table 2. Comparative overview in human, NOD mouse and BB IDDM

Long Evans Tokushima Lean (LETL). An outbred colony of Long-Evans rats developed spontaneously remarkable signs attributable to diabetes (polyuria, polyphagia, and polydipsia). This line has been maintained since 1983 in Tokushima Research Institute (Otsuka Pharmaceutical, Yokushima, Japan) and generated another line (Long Evans Tokushima Lean - LETL). LETL rats present no sex predilection concerning the onset of the disease or severity, sudden onset of the diabetes expressed as hyperglycemia, polyuria, polydipsia and weight loss, lymphocytic insulitis at 120-220 days of age followed by the destruction of β-cells, normal levels of T-lymphocytes, lymphocytic infiltration of salivary and lacrimal glands [52, 53].

Komeda Diabetes Prone (KDP) rat is a substrain of LETL, all the individuals presenting moderate to mild insulitis around 220 days of age. The onset of diabetes is 70% at 120 days and 82% within 220 days. This strain present a major IDDM susceptibility gene named

Iddm/kdp1 placed on chromosome 11. Homozigous alleles at this locus are strongly linked with the capacity to develop moderate or severe insulitis [54-56]

New Zealand White Rabbit developed spontaneous diabetes mellitus for the first time in a female in 1969. By inbreeding this female and her offspring, a diabetic line was obtained. The overt diabetes was diagnosed in 19% of animals aged between 1 and 3 years. The diabetic animals present fasting hyperglycemia, hypoinsulinemia and absent ketoacidosis [57]. The lesions of β-cells are expressed as cytoplasmic hypergranulation, this being different comparing with previous animal models featured by insulitis, islet atrophy, degranulation of β-cells. It was postulated that the lesion is the consequence of a secretion defect. In addition, diabetic rabbits present mineral deposits in kidney, particularly in basement membrane of the tubules and Bowman capsules and into the lining cells of proximal convoluted tubules [58, 59].

Certain lines of *Keeshond dog* may develop inherited IDDM expressed as overt diabetes around 2-6 months of age. The dogs have low level of insulin as a consequence of β-cells aplasia. In addition, glucagon secretion is also depressed. The dogs can survive 2-4 months without insulin supplement. Concurrent lesions such as cataracts, skin infections and poor bodily growth are observed. The incidence of diabetes is higher in females. The fertility in diabetic individuals is very low, non-diabetic dogs being used to obtain diabetic offspring. An autosomal recessive disorder is consistent with the onset of diabetes. Keeshond dogs are suitable for studying long term complications of diabetes [60, 61].

Chinese hamster (Cricetulus griseus) has become the subject of research in diabetes mellitus as an animal model since 1959 [62]. The incidence of diabetes in Chinese hamster sublines is more than 85%. At the time of birth, the pups are prediabetic. The overt diabetes range from mild to severe and it is characterized by polyphagia, hyperglycemia, severe polyuria, glucosuria and elevated gluconeogenesis. β-cells present degranulation and hydropic degeneration [63-65]. Other morphologic changes occur in kidneys (glomerulosclerosis), brain (vascular lesions expressed as duplication and thickening of the basement membrane, degeneration in either dendrites or axons, focal demyelination and synaptic degeneration) [66], exocrine pancreas (pancreatic adenoma and adenocarcinoma) [67], teeth (periodontal disease) [68], and macroangiopathy of the thoracic aorta [69]. Genetic defects are responsible for the onset of diabetes, four autosomal recessive genes being involved [70]. Chinese hamster with IDDM have an impaired humoral antibody response similar to that developed in human diabetes, which makes it suitable for research concerning the consequence of diabetes mellitus induced by impaired immune response [71], as well as for diabetic nephropathy [72].

6. Animal models of non-insulin dependent diabetes mellitus (NIDDM)

NIDDM is generated by the failure of β-cells to adapt to a more challenging conditions created by insulin resistance, this being induced by over-nutrition and lack of physical exercises. Mechanisms as oxidative stress, islet amyloidosis, glucotoxicity and lipotoxicity were associated with inappropriate secretory behavior of β-cells. Autoimmune attack and islet inflammation considered previously as a hallmark for IDDM, is now associated with

NIDDM. This concept is sustained by the fact that all mentioned mechanisms may initiate inflammation or are initiated by the inflammation. One of the reasons is that human pancreatic islets release IL-1β as response to glucotoxicity. The inflamation is somehow blocked in the initial stages for allowing β-cell regeneration. The more necrosis and apoptosis become obvious, the more infiltration with inflammatory cells (e.g. macrophages) are seen in pancreatic islets [73, 74].

Creation of animal models of NIDDM needs to meet the heterogeneous background which features human condition. Roughly, the animals have to express insulin resistance, impaired insulin secretion in the condition of fasting or post-challenge hyperglycemia. On the other hand the existent animal models present as dominant at least one characteristic: some animals are insulin resistant, other express mainly glucose intolerance as a part of obesity, others express NIDDM because of a particular sensitivity to dietary components. The animal models used for research in NIDDM present an important diversity, although mice and rats are constantly preferred (table 2).

Rodents	Mouse	Obese	ob/ob mouse db/db mouse KK mouse NZO mouse NONcNZO10 mouse NSY mouse TH mouse TSOD mouse M16 mouse CBA/ca mouse	Gene mutation
		Diet induced	C57/BL 6J mouse	Diet-gene interaction
	Rat	Obese	ZDF rat Wistar fatty rat OLETF rat SHR/NIH-cp	Gene mutation
		Non-obese	GK rat Torii rat	Gene mutation
		Diet induced	Cohen diabetic rat Israeli sand rat Nile rat	Diet-gene interaction
Pig [75]		-	Yucatan minipig Göttingen minipigs Sinclair minipigs Yorkshire and Yorkshire crossed strains Chinese Guizhou minipig Ossabaw minipigs Familial hypercholesterolemic pigs Low-birth-weight pigs	Cardiovascular complications
Cat [76]		-	Shorthaired males	Islet amyloidosis
Monkey [77]		-	Non human primates	Islet amyloidosis

Table 3. Animal models for NIDDM

Ob/Ob mouse was created in Jackson Laboratories in 1949 and resulted from mutation on both obese (ob) genes [78]. The main characteristic of this mutant is the uncontrolled appetite which results rapidly in the onset of obesity and NIDDM around 11 weeks of age. Polyphagia in ob/ob mouse is generated by ob genes mutations which also encode leptine. This hormone is synthesized by adipose tissue and has an important role in appetite downregulation and regulation of body weight. Leptin is absent in obese mice, the treatment with this monomer lowers consistently the food intake and body weight and also improve up to normal the plasma levels of glucose and insulin [79]. Persistent mild hyperglycemia is linked with 60% enlargement of pancreatic islets and subsequent hyperinsulinemia comparing with lean mice. Interestingly, β-cell from obese mice secretes insulin at a lower threshold of glucose that lean mice [80]. High level of plasma insulin may result from metabolic alteration of β-cells that leads to insulin overproduction or is the consequence of the heterogeneity in glucose sensitivity of these cells. Increased concentration of glucose is followed by recruitment of new β-cells with increased glucose sensitivity [81]. Infertility is a current feature in obese mouse, this being supported by fatty degeneration of the ovaries, follicular atresia, damaged mitochondria and apoptosis of the ovocytes [82]. Many studies have been run in ob/ob mice such as amelioration of insulin resistance [83], hypoglycemic effects of some polysaccharids [84, 85] and complication of NIDDM as diabetic cardiomiopathy [86] and peripheral neuropathy [87].

Db/db mouse is a diabetic mutant mouse created in Dunn Nutritional Laboratory, Cambridge, United Kingdom in 1966. Particularly, this mutant expresses a mutation on db gene which encodes the leptin receptor [88]. Thus, leptin signaling in the hypothalamus is absent leading to persistent high levels of both insulin and leptin. The mouse becomes obese around 4-6 weeks of age and develops progressively high levels of plasma insulin and glucose. All characteristic clinical signs are recorded: polyuria, polydipsia, polyphagia, proteinuria, and glucosuria. One of the most intriguing aspects is that the mice of some strains maintain hyperinsulinemia despite severe depletion of β-cells. This can be attributed to stem cells differentiation from pancreatic ducts. Body weight and insulin levels begin to decrease in association with β-cell degeneration when the mouse reaches 5-6 months of age. The cause of death remains unclear, although the mice present ketonuria, hematuria and gastrointestinal hemorrhages in terminal stage [89]. Db/db mouse has a long history in comparative research to human diabetes. Thus, human dietary habits were reproduced in db/db mouse. High lipid and cholesterol reach diet induce dyslipidemia and create similarities with the patients with type 2 diabetes mellitus [90]. Furthermore, diabetic nephropathy in db/db mice is consistent with some features encountered in human diabetic nephropathy such as renal hypertrophy, glomeruli enlargement, albuminuria, and mesangial matrix expansion [91].

KK mouse history began in 1957, this line being derived from numerous strains of Japanese native mice. Later, after many inbreeding procedures, Nakamura obtained KK mouse strain, which was a polygenic model, spontaneously diabetic and named after the region where the strain was founded (Kasukabe in Saitama prefecture) [92]. The KK mice become obese once with the onset of adulthood and develop insulin resistance, subsequent hyperinsulinemia and β-cell hyperplasia. Particularly, KK mice present a chemical diabetic stage preceded by

prediabetes stage accompanied by renal, neurological and retinal complications [93]. The severity of diabetes is strongly correlated with environmental factors such as diet, food intake and social isolation of the animals, the chemical diabetic state being replaced with overt diabetes [94, 95]. Diabetes and obesity in KK mouse has a moderate expression. Introduction of A^y allele creates a new line (KK- A^y) which present enhanced pathophysiological characteristics especially for glucose intolerance [96].

New Zealand obese (NZO) mouse is a polygenic animal model which is prone to express obesity, insulin resistance, glucose intolerance and also autoimmunity featured by perturbation of splenic lymphocyte function and IgM antibodies to insulin receptor. These characteristics are concomitant with poor breeding performance due to ovarian degeneration [97] and diabetic nephropathy expressed as glomerular proliferation, mesangial deposits, mild thickening of basement membrane, glomerular eosinophilic nodules and glomerulosclerosis [98-100]. There are research which concluded that the obesity develops independently to dietary content, the onset of diabetes being recorded earlier in mice fed with carbohydrates and fat reach diet [101]. Other studies emphasized that obesity in NZO individuals is the results of hyperphagia and low energy expenditure due to insufficient physical activity [102].

NONcNZO10 mouse is a recombinant congenic new strain of NIDDM developed by introgressing 5 genomic intervals containg NZO/H1Lt (NZO) diabetogenic quantitative trait loci onto non-obese non-diabetic (NON/Lt or NON) genetic background [103]. Particularly, these mice do not express polyphagia, morbid obesity, poor fertility and variable frequency of hyperglycemia as their parental NZO males do. NONcNZO10 males are normophagic, moderately obese and exhibit normal fertility. NONcNZO10 males become hyperglycemic in 12-20 weeks of age and present atrophy of pancreatic islets and hepatic lipidosis. The resemblance between NONcNZO10 mice and human obesity/diabetes syndrome in higher than ob/ob and db/db mice because of lack of hyperphagia, normal levels of leptin and leptin signaling, normal thermoregulation and lack of hypercorticism [104].

Nagoya-Shibata-Yasuda (NSY) mouse is a spontaneous model of NIDDM, having the same ancestor with NOD mouse (Jcl ICR line). Surprisingly, three major loci contributing to susceptibility to NIDDM in the NSY mouse presented overlapping with the region where susceptibility genes for IDDM have been mapped in NOD mouse. It was postulated that some responsible genes for the onset of diabetes come from the same ancestor genes which express IDDM phenotype in NOD mice and NIDDM in NSY mice [105]. Age and sex related onset of diabetes is the most prominent characteristic for NSY mice. Males develop diabetes at 48 weeks of age as mild obesity and mild hyperinsulinemia. The impaired insulin secretion via glucose challenge is observed after 24 weeks of age. There were no morphological changes in pancreatic islets in NSY mice at any age, these findings suggesting that defective secretion of β-cells may be one of the causes in NIDDM in the NSY mouse. Fasting hyperinsulinemia may contribute to the pathogenesis of diabetes in NSY mouse, insulin sensitivity being under genetic control of *Nidd2nsy* and *Nidd3nsy* genes. Genetic analysis of NSY identified a specific gene mutation of *Tcf2* responsible for encoding hepatocyte nuclear factor 1β (HNF-1β) and implicated in MODY pathogenesis [106, 107]. Spontaneous amyloidosis was reported in old

individuals, deposits being remarked mainly in kidneys, but also in the different segments of digestive system, lung, heart and adrenal glands [108]. NSY mice as well as ob/ob mice prove recently to be the source of creating of new animal models for simultaneous development and research of Allzheimer's disease and NIDDM [109].

TallyHo (TH) mouse is a relatively new NIDDM animal model, reported for the first time in 2001. The mice present obesity, hyperinsulinemia, hyperlipidemia and male-limited hyperglycemia, insulin resistance and glucose intolerance. It has been postulated that female diabetes resistance is the consequence of estrogens which enhance hepatic insulin sensitivity [110]. The genome wide scan proves polygenic involvement and also additional gene-gene interactions to express hyperglycemic phenotype [111]. Comparing with ob/ob and db/db mice, which present severe obesity attributable to leptin synthesis and leptin receptor deficiencies respectively, TH has normal levels of this hormone and also intact leptin signaling. Carbohydrates and fat reach diet enhance the levels of leptin and also the other specific features of NIDDM [112]. The treatment with leptin results in decreased glucose-stimulated insulin secretion, which demonstrate that letin plays an important role in initiation of glucose intolerance in TH mice [113]. Both males and females develop early moderate hyperplasia and hypertrophy of pancreatic islets, but only the males continue these lesions with β-cell degranulation, discrete vacuolization, different degrees of islet atrophy and fibrosis [114]. Vascular dysfunction occurs in TH mice, expressed mainly in aorta, carotid arteries and cerebral arterioles as a consequence of PGH2/TxA2 receptor activation and cytochrome p450 products and oxidative stress and elevated activity of Rho kinase, respectively [115, 116].

Tsumura Suzuki obese diabetic (TSOD) mouse resulted from inbreeding procedure of ddY strain. The diabetic line includes only moderate obese males with polyphagia, polydipsia, glucosuria, hyperglycemia, hyperinsulinemia, and hyperlipidemia. Pancreatic islets exhibit hypertrophy and hyperplasia, without any signs of insulitis or islet fibrosis [117]. Diabetic nephropathy is consistent with thickened basement membrane of the glomeruli and increased mesangial area. Peripheral neuropathy involves both sensitive and motor nerves and expresses high resemblance with human counterpart. The most prominent lesions of the nerve are decreased density of nervous fibers due to endoneurial fibrosis, degeneration of myelin sheath, intralamellar edema and remyelination, total destruction of lamellar structure associated with macrophage invasion around and into the myelin sheath [118]. Insulin resistance in TSOD mouse is probably induced, at least partially, by a decreased GLUT 4 translocation by insulin in skeletal muscles and adipose tissue [119].

M16 mouse is a new obese animal model created in Institute of Cancer Research, London, UK. Both male and female express early onset of a moderate obesity due to hyperphagia and have high levels of insulin, leptin and cholesterol. The diabetic phenotype of M16 permits research of obesity/diabetes syndrome with early onset as it recorded in human population as a current tendency [120].

CBA/Ca mouse diabetes is recorded only in 10-20% of males. The incidence can be enhanced by inbreeding. Hyperphagia, obesity, hyperglycemia, glucose intolerance, hyperinsulinemia, hypertriglyceridemia occur around 12-16 week of age. Pancreatic islets are hypertrophied,

with increased insulin content that persist up to 48 weeks of age. The islet degeneration as a prove of β-cell exhaustion does not appear in this mouse [121, 122].

Zucker diabetic fatty rats (ZDF rats) resulted from inbred as well as outbred lines of rats, which maintain hyperglycemia and glucose intolerance featuring NIDDM. The scientist Lois M. Zucker and Theodore F. Zucher created in 1961 a line of rats which express a gene responsible for the onset of obesity (fatty gene/fa). Usually these rats are not hyperglycemic and present leptine-receptor deficiency, although both male and female express some parameters attributable to insulin resistance. The original colony began spontaneously to present hyperglycemia and glucose intolerance in some bucks and does. These individuals were the founders of the Zucker diabetic fatty rats. ZDF rats develop hyperglycemia with concomitant β-cell death. Compensatory proliferation is maintained as long as plasma glucose levels remain moderate [123-125]. Subsequent exhaustion of β-cells is followed by an increased rate of apoptosis [126]. Lipotoxicity is also considered as a potential cause of β-cell population reduction. Thus, elevated lipogenesis prior to, or in association with hyperglycemia results in excessive accumulation of fatty acid into the β-cell cytoplasm [127]. ZDF rats are frequently used in comparative studies with non-diabetic fatty rats and lean ZDF.

Besides the ZDF rats, other strains have been created, all receiving fa-gene from Zucker rats. *Wistar fatty rats* (fa/fa) resulted from mating of Zucker with Wistar-Kyoto individuals. The rats from this line are obese, and present hyperlypidemia, hyperinsulinemia and insulin resistance. Wistar fatty rats are prone to develop hypertrophy of pancreatic islets and degranulation of β-cells. The symptoms of diabetes have been observed only in males [125].

Otsuka Long-Evans Tokushima fatty (OLETF) rat resulted from an outbred colony of Long-Evans rats which spontaneously develop polyuria, polydipsia and mild obesity. The onset of hyperglycemia occurs in male and relatively late comparing with other lines (after 18 weeks of age). Particularly, OLETF rats present a specific diabetogenic gene associated with X-chromosome Implication of testosterone is considered to have an important influence in the onset of diabetes in male. This feature is sustained also by the administration of estrogen in castrated males which suppress or delay diabetes. The lesions of pancreatic islets begin with discrete lymphocytic infiltration, followed by the second stage expressed as islet hyperplasia with or without fibrosis in or around the islets and final stage represented by islet atrophy [128, 129]. OLETF are prone to develop diabetic nephropathy, some features of this complication comparable with human diabetes being recorded (diffuse glomerulosclerosis, thickening of basement membrane, PAS-positive deposits in the mesangium or capillaries). Mesangial lesions might express some nodular aspect similar but not identical with specific Kiemmelstiel-Wilson lesions [130].

Spontaneously Hypertensive rat/National Institute of Health-cp (SHR/NIH-cp) was created in Bethesda Maryland USA and associates obesity, NIDDM and hypertension. This rat presents a homozygous genotype for corpulent gene (cp/cp). The males are early hyperphagic and become obese and express hyperglycemia, impaired glucose tolerance, hyperinsulinemia, insulin resistance, high plasma levels of cholesterol and triglycerides, hyperleptinemia and mild essential hypertension [131].

JCR/LA-cp rat (James C. Russel/LA-cp rat) was reported in 1984 as a homozygous genotype for cp gene which develops hyperphagia, obesity, insulin resistance, hyperinsulinemia, glucose intolerance, hyperlipidemia and leptin receptor deficiency. Obese males also manifest cardiovascular lesions such as atherosclerosis and myocardial lesions. Hyperinsulinemia is caused by β-cell hyperplasia followed by islet hypertrophy and fibrosis [132]. Pharmacological researches use this animal model to determine the effectiveness of anti-obesity compounds and also to evaluate long-term benefit to prevent atherosclerosis [133-135]

Goto Kikazaki rat (*GK*) is one of the polygenic non-obese models of NIDDM which exhibit high resemblances with human condition, especially on hormonal, metabolic and vascular disorders. The line was founded in Japan (Tohoku University in 1975) based on selective repeated inbreeding of non-diabetic Wistar-Kyoto rats with minor glucose intolerance. Diabetes became overt and stable after 30 generations. Despite minor differences between subcolonies of GK, common characteristics were noticed such as decreased β-cell mass, moderate and stable hyperglycemia in adults, hepatic and peripheric insulin resistance and polyuria. Defective function and morphology of pancreatic islets was recorded since embryonic and fetal period featured by reduction of β-cell mass and insulin levels [136-138]. The complications of diabetes in GK rats refer to nephropathy (significant enhancement of kidney weight, glomerular volume, basement membrane thickness, mesangial fraction and total mesangial volume) [139], peripheral neuropathy [140, 141], diabetic osteopathy (trabecular osteopaenia) [142] and diabetic retinopathy (reduction of retinal blood flow, pericytes ghosts, acellular capillaries, increased production of vascular endothelial growth factor) [143, 144].

Spontaneously Diabetic Torii (SDT) rat has been developed from Sprague-Dawley rats in 1997 in Research Laboratories of Torii Pharmaceutical, Ohnodai, Chiba, Japan. This rat is particularly characterized by non-obese, sex related onset of NIDDM with insulin hyposecretion and severe diabetic retinopathy. The males develop glucosuria around 20 weeks of age. All males are diabetic by 40 weeks, while only 33% of female rats present diabetes even by 65 weeks of age. Glucose intolerance is noticed in 16-week-old individuals and continues with the onset of hyperglycemia, hypoinsulinemia, long-term survival without insulin treatment and hypertriglyceridemia. Fibrosis of pancreatic islets and ocular lesions such as hypermature cataract, hemorrhages in anterior chamber, tractional retinal detachment and subsequent retinal fibrovascular proliferation are the most important histopathological findings in SDT rats [145, 146]. The attempt to clarify the genetic basis of diabetes in SDT rats succeeded to identify seven quantitative trait loci which affect the levels of plasma glucose and one for body weight. One of them (*Dmsdt1*) have particular involvement in islet inflammation and fibrosis. It was suspected that this gene might also have implication in retinal lesions [147].

Cohen diabetic rat (CD) is a particular experimental model for study in NIDDM, which make it distinctive comparing with the other models presented. Diet-induced diabetes correlated with a genetic sensitivity is truly considered the most prominent feature of this rat, although it is still unclear which of the dietary components are responsible for the onset of the diabetes. It was observed that CD rats become overtly diabetic when their diet has a high-sucrose low

copper content. In addition with diet profile, a sex predilection can be observed: male record a lower growth rate and more severe glucose intolerance than female. CD rats fed with diabetogenic diet do not express obesity or hyperlipidemia [148]. Other studies concluded that high-casein low copper diet is responsible for the onset of the diabetes. This result is based on genetic analysis, a gene (*Ica1*) being associated with diabetes and bovine casein. Routine histopathological investigation reveals intact pancreas islets and replacement of exocrine acini with adipose tissue. Degeneration of exocrine pancreas remains intact when diabetogenic diet is replaced with a regular one [149]. CD rats develop early hyperinsulinemia and insulin resistance, followed by the exhaustion of β-cells and hypoinsulinemia. The most common complications are diabetic retinopathy and nephropathy [150, 151].

The Israeli sand rat (Psammomys obesus) is a terrestrial mammal, being mostly found in the desert area of North Africa and Middle East. The sand rat is another experimental model for diet induced NIDDM. The high resemblance with human condition derives from distribution of adipose tissue into the subcutaneous and visceral compartments. This animal readily becomes obese when the diet from the natural habitat is replaced with usual laboratory rat chow. It was suspected but not proved yet that some components from the natural diet might have hypoglycemic effect. Thus, the juice from *Atriplex halimus* (saltbush which has low energy, high water and electrolyte content and represents the basis of the food intake), as well as water extract and dialysate induce a significant decrease of glucose in diabetic sand rat [152]. The development of obesity is accompanied by hyperglycemia, hyperinsulinemia, decreased insulin sensitivity in adipose tissue and liver, and glucosuria, [153, 154]. Comparing with normoglycemic individuals, pancreatic β-cell volume begin to decrease in the obese and diabetic sand rats, as well as GLUT 2 glucose transporter on the cellular membrane and glucokinase in the cytoplasm of β-cells [155, 156]. Progressive loss of β-cells due to cell death is accompanied by hypoinsulinemia and persistent hyperglycemia, generating an irreversible diabetic state in sand rat. Proinflammatory cytokines such as IL-1β are not involved in producing deleterious effect on β-cells [157]. However, initiation of inflammation in sand rat NIDDM seems to be induced by other pathogenic pathways. For instance, a gene named Tanis (the Hebrew word for fasting) and expressed as hepatic receptor for serum amyloid A (SAA) is regulated by glucose and become dysfunctional when diabetes occur. Knowing that SAA and other acute-phase protein received special attention because their implication in cardiovascular disease, Tanis gene may provide answers for questions about the link between diabetes, inflammation and cardiovascular disease [158].

Nile rat (Arvicanthis niloticus) is a recently reported diet-induced model which expresses the features of both Metabolic Syndrome and NIDDM. Nile rats fed with current lab diet present characteristic signs as excessive abdominal adipose tissue, hyperglycemia, hyperinsulinemia, impaired peripheral insulin sensitivity, dyslipidemia (high level of cholesterol and triglycerides), microalbuminuria, and hypertension. Sex predilection was observed in males, which present segregation in two groups: early-onset diabetes and late-onset diabetes. Dietary modulation (high-fat diet) induce the early onset, as well as more accumulation of body fat [159].

7. Transgenic and knockout models used for research in diabetes mellitus

The specific techniques of molecular biology had a valuable contribution for the study of diabetes mellitus. As it was mentioned before, diabetes mellitus involves a considerable heterogeneity given by the multifactorial genetic and environmental conditions. Thus, interpretation of the results in a particular experiment is challenged by this complicated background. For this particular reason, the scientists have felt the need to create transgenic animal model which provide good conditions for studying the effect or implication of a specific gene and corresponding product according to physiological and environmental conditions. The most important outcomes of the transgenic animals are knowledge about gene regulation and development, pathogenesis of diabetes and new approaches in the therapy of this disease.

Transgenic animals, particularly mice, result from two basic techniques of genetic engineering. The first aims to transfer a gene (a new genetic material presented as a foreign DNA construct containing a regulatory region and a coding region for a protein), into the pronucleus of a fertilized ovocyte. After the gene inoculation, the modified ovocytes are transferred in the uterus of a foster mother for further development. After the birth, the pups are genetically scanned to verify whether the new genetic material was incorporated into the host genome. The animals which manifest the transgene are bred and the pups are also analyzed for the same DNA construct. Positive offspring of the second generation are further bred to establish a transgenic line for studying a particular transgenic phenotype. This revolutionary technique has both advantages and disadvantages. The major advantage is that the method enables to obtain transgenic animals with minimal cost and in a short time. The disadvantages are generated by the hazardous integration of the DNA construct in the genome of the host. The locus of integration, as well as the number of copies is unpredictable. Transgene phenotype expression is limited to use for studying a specific protein or RNA. Therefore, this protein will be overexpressed in the transgenic animal. If the target of experimentation is to reduce the expression of a protein, a RNA antisense transgene is used. It is noteworthy that this technique is also disadvantageous because of unpredictable complications and misinterpretation of the results [160].

The second method used for obtaining genetically engineered mice is focused on deleting a specific endogenous gene or gene fragment (knockout) and replacing with an exogenous DNA which present homologous sequences with the endogenous DNA fragment (homologous recombination). The engineered DNA fragment (a vector which is designed to produce a disruption in the target gene) is inoculated in an embryonic stem cell culture. The positive targeted cells are inoculated in a mouse embryo, which will be finally transferred into the uterus of a foster mother. If the experiment is successful, this embryonic stem cells will participate to generate germ cells and finally organs, all having the new recombinant gene [161].

Double transgenic mice can be obtained by maiting. Thus, the offspring of transgenic mice expressing the hemagglutinin of influenza virus under the insulin promoter and transgenic

mice expressing T-lymphocytes with receptor for immunodominant epitope of the same virus present typical features for IDDM. The mice are hyperglycemic, hypoinsulinemic, present lymphocytic insulitis, glucosuria and poor bodily growth, features which are consistent with IDDM. The mortality is up to 90% at 3 months of age [162]. This line (TCR-HA Ins-HA) has consistent improvement glucose levels when treated with potato buds lectin [163, 164].

8. Conclusions

The experimentation in diabetes mellitus has known a long history, as well as a continuous and diverse development. Banting and Best as discoverers of insulin and Minkowski as the scientist who create the first experimental model of diabetes mellitus are truly recognized as the pioneers of the research in this area. Although the diversity of animal models created in the last fifty years is somehow overwhelming, their classification and usefulness follows the pathogenesis, corresponding lesions and subsequent complications recorded in human diabetes mellitus. The scientific literature describes many animal models of IDDM, NIDDM and secondary diabetes, although mice and rats are constantly used regardless the purpose of the research. It is easily noticed that the most famous research centers and laboratories developed their own experimental models and also provided genetic material for the creation of other colonies. Considering that hyperglycemia and glucosuria are two of the most important clinical signs of diabetes, some basic substances which induce these signs are described. Thus, Streptozotocin, Alloxan, Vacor, 8-hydroxyquinolone, Dithizone are usually used in experimentation which reproduce hyperglycemia, while phlorizin is recognized as a vegetal component which is responsible for glucosuria. The animal models of spontaneous diabetes mellitus are consistently represented by rodents, although other species as dog, cat, pig and primates are recommended. The research in NIDDM is sustained by experimental models divided in three major categories: obese, non-obese and diet-induced models. Molecular biology techniques have an important contribution in creation of transgenic animals for research the depth of the pathogenesis of diabetes mellitus.

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9. References

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