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Zucker Diabetic Fatty Rats for Research in Diabetes

Marcela Capcarova and Anna Kalafova

Abstract

The rising incidence of diabetes mellitus (DM) worldwide presents a global public health problem. DM is classified into two main groups: type 1 (T1DM) and type 2 (T2DM). T1DM requires insulin treatment. T2DM is complex, heterogeneous, polygenic disease defined primarily by insulin resistance, ongoing hyperglycemia, and β cells' dysfunction. For research in diabetes, an appropriate experimental model reflecting symptoms and complications of human T2DM is required for understanding the pathogenesis, molecular nature, and the possibilities of the treatment. Among the many animal models, rodent models that develop DM spontaneously are frequently used in the studies due to their similarity to the humans and economic effectiveness. This work gives a detailed overview of the literature, covering the characteristic of DM, its symptoms and complications, the description of Zucker diabetic fatty (ZDF) rats as an appropriate model for research in T2DM, and the possibility of the treatment.

Keywords: diabetes, animal model, pancreatic β cells, Zucker diabetic fatty rats, treatment

1. Introduction

1.1 Diabetes mellitus

Diabetes mellitus (DM) is often incident endocrine disorder in many countries [1]. The International Diabetes Federation reported that 6 million people die directly from diabetes every year, and additional 318 million people are suffering with DM. This number is predicted to reach 642 million by 2040 [2] and 693 million by 2045 [3]. DM is a heterogeneous group of chronic disease characterized by a relative or absolute lack of insulin resulting in hyperglycemia [4]. It causes a variety of complications as cardiovascular disease, renal failure, neuropathy, and retinopathy [5]. Chronic hyperglycemia mostly deteriorates the vascular tree and promotes the development of micro- and macrovascular disease [6]. It was reported that hyperglycemia accelerates the development of DM complications through some mechanisms such as increased aldose reductase-related polyol pathway flux, formation of advanced glycation end products (AGEs), increased hexosamine pathway flux, activation of protein kinase C isoforms, and rising generation of reactive oxygen species [7]. Metabolic imbalance in the peripheral nervous system that is activated in the diabetic milieu of hyperglycemia, impaired insulin signaling, and dyslipidemia are the key parameters in the development of diabetic neuropathy [8]. The determining points involve multiple mechanisms of glucose toxicity

including polyol pathway activity, hexosamine pathway, nonenzymatic glycations of proteins, and altered protein kinase C activity [9]. Activation of these pathways can eventually flow into inflammatory and oxidative stress in neurons and adjacent microvascular system [10].

DM is divided into two main forms—type 1 and type 2 [4]. Type 1 diabetes mellitus (T1DM) or insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease and is a result of β cells' death, because a foreign protein is incorporated into islet β cells. In response, lymphocytes attack the foreign protein and unwillingly destroy β cells as collateral damage. It causes an absolute insulin deficiency [11]. It is uncertain what activates the autoimmune response, but some environmental factors as toxins, viral infections, and psychosocial inputs are thought to play a plumbless role [12].

Historically, the usual ratio for T1DM to T2DM has been 1:20. Now it is changing because of expressive increase in the incidence of T2DM in children and young people [11].

1.2 Diabetes mellitus type 2

Type 2 diabetes mellitus (T2DM) or non-insulin-dependent diabetes mellitus (NIDDM) is a syndrome of β cells' dysfunction including relative insulin deficiency associated with insulin resistance [11] and compensatory increases in insulin secretion [13]. It is associated with incorrect sensing of glucose signals by the β cells. T2DM is linked to a stage of insulin resistance. Insulin secreted by the β cells and bound to liver, muscle, and fat cells is subnormally efficacious in carrying out its metabolic action [11]. Generally, T2DM is characterized by the incapability of the pancreatic β cells to secrete appropriate quantities of insulin in order to offset hyperglycemia arising from peripheral insulin resistance and increases hepatic glucose output [14]. It is a multifactorial and complex disorder [13] that is estimated to affect more than 100 million people worldwide [15]. About 80% of all people with diabetes suffer from T2DM [16]. Insulin resistance alone is insufficient to cause diabetes. A progression to overt diabetes required β cells' failure as well [16–18]. Insulin resistance is associated with decrease in insulin receptors in target tissues (muscle, fat, or liver) and insulin receptor kinase activity that causes decrease in glucose transporter 4 (GLUT 4) translocation due to impaired signaling [19]. The onset of T2DM is preceded by an expressive increase in the plasma levels of free fatty acids (FFA) and by sixfold rise in triglyceride (TG) concentration in the pancreatic islets [20]. Chronic exposure to high glucose level and rising FFA concentration is detrimental to β cell function. This situation results in weak glucose-induced insulin secretion and rising level of apoptosis [21].

In spite of the increasing number of T2DM, little is known about the prevention of the disease and its complications at early stages [22]. In this stage insulin-sensitive tissue such as adipose tissue and skeletal muscle become insulin resistant. This causes the development of impaired glucose tolerance, and it can occur over a few years [23].

The pathogenesis of T2DM is complex and is primarily related to gene variation, external and internal environmental factors, abnormal protein modifications, oxidative stress, epigenetic effects, and energy metabolism disorders [24]. It was revealed that also the gut microbiota has been recognized as a key contributor to T2DM, and T2DM is linked to dysbiosis of the intestinal microbiota [25].

1.3 Obesity in type 2 diabetes

Onset and development of T2DM is commonly incurred by several factors, which are combined with lifestyle, obesity, genetic defects, virus infection, and

drugs [16]. Obesity is defined as a pathological excess of body fat that results from a permanent positive energy balance [26]. Persistent positive energy balance is pertinent to increased storage of triglycerides. This expands the adipose depots and increases the proportion of hypertrophied adipocytes [27]. Under condition of obesity, the lipid storage capacity of adipocytes is overcome, resulting in adipocyte-derived fatty acids and cytokines leaking into the circulation [28]. Damaging lipid species accumulates in ectopic tissue causing local inflammation and provides lipotoxicity [29]. Lipotoxicity determines an important link between obesity, insulin resistance, and T2DM. It interprets the harmful cellular effects of chronically increased concentrations of fatty acids and excess lipid accumulation in tissues other than adipose tissue. Excess adiposity is considered to promote the onset and severity of insulin resistance, contributing to emergence and progression of impaired glucose tolerance and T2DM [27].

Obesity-induced insulin resistance accelerates pancreatic islet exhaustion and thus the onset of T2DM [13]. Generally, obesity is a major risk factor for developing T2DM [30]. High-fat diet applied in animal's model that has inclination to DM results in obesity, hyperinsulinemia, and altered glucose homeostasis due to insufficient compensation by the islets [31]. Whereupon it is required in human population suffering T2DM to follow diet regimes and restriction of energy in the food so to maintain glucose concentration in acceptable level. In this case the diet has more considerable impact on diabetic primary complications than genetic predisposition [32]. Genetic disposition to obesity is probably commonly due to the small impingements of a wide selection of genes such as those encoding the beta3adrenoceptor, PPARγ and its co-activator-1, fat mass and obesity-associated gene, and adiponectin and a selection of genes that could potentially influence behavior and hypothalamic hunger-satiety mechanisms [33].

Currently, therapeutic strategies for T2DM are limited. They involve insulin and four main classes of oral antidiabetic agents in order to stimulate pancreatic insulin secretion. However, these agents suffer from generally inadequate efficacy and various adverse effects. So there is the possibility to try new therapeutic agents or treatments, most of them are under preclinical and early clinical stages [34].

2. Zucker diabetic fatty rats

An animal model for biomedical investigation is one in which normative biology, behavior, and pathological process can be studied and in which the phenomenon in one or more respects resembles the same phenomenon in humans [35]. Research in diabetes on humans is not possible or only partially possible. Hence, animal model of DM is very useful and advantageous [36]. Animal disease models are essential tools for studying the pathophysiology of DM enabling therapeutic interventions to be developed [37]. It is true that the present therapeutic approaches to treat DM and obesity, which are saving many lives every day, were invented, validated, and optimized on animal models [38]. When studying T2DM the use of an animal model with a homogenous genetic background is advised [39]. Most of the available models are based on rodents [36]. Rodents are most commonly utilized due to their small size, short generation interval, and easy availability [39] and because of economic consideration [36]. Being mammals, the physiology of rats is similar to humans than nonmammalian species [40]. Nevertheless, nonrodent models of diabetes are needed as a valuable supplement to rodents for both practical and physiological reasons with respect to humans [36]. Many animal models for DM research are obese, reflecting the human condition where obesity is closely related to T2DM development [41]. Animals exhibiting a syndrome of insulin resistance

and T2DM reflecting the human disease involve many species with genetic, nutritional, or experimental causation [36]. There are a lot of rodent models available for the research in T2DM, but some of them may not always be satisfactory to mirror human T2DM due to the large heterogeneity in the latter. There are no fully unified classification criteria for this type of animal model. But, the spontaneous type 2 diabetic rodent models are considered the most outstanding and most useful [42].

According to Srinivasan and Ramarao [36], spontaneous diabetic animal models have special advantages and also disadvantages. The advantages are:

a. The development of T2DM is of spontaneous origin involving genetic factors.

b. Animals develop characteristic features resembling human T2DM.

c. Most of inbred animal model in which the genetic background is homogeneous and environmental factors can be controlled allow genetic dissection of this multifactorial disease easy.

d. Variability of results is minimal and it required smaller sample size.

Among the disadvantages are mainly:

a. Highly inbred, homogenous, and mostly monogenic inheritance and development of diabetes are highly genetically determined unlike heterogeneity in humans.

b. Limited availability and expensive for the diabetes study.

c. Mortality due to ketosis problem is high in the case of animals with brittle pancreas and requires insulin treatment in later stage for survival.

d. Require sophisticated maintenance.

One of the rodent models that reflect human form of T2DM is Zucker diabetic fatty (ZDF) rats. ZDF rats as spontaneous diabetic animal model exhibit both the prediabetic and the end stage observed in human T2DM patients [43]. Spontaneously diabetic animals of T2DM may be acquired from the individuals with one or several genetic mutations transmitted from generation to generation or selected from nondiabetic outbred animals by repeated breeding through several generations. The result is that these animals inherited DM either as single or multigene defects. The metabolic particularities result from single gene defect (monogenic) which is due to dominant gene or recessive gene, or it can be of polygenic origin [36].

ZDF rats come from a colony of outbred Zucker rats in the laboratory of Dr. Walter Shaw at Eli Lilly Research Laboratories in Indianapolis (USA) during the years 1974–1975. In early 1981, some animals with diabetic lineage were designated and redefined. An inbred line of ZDF rats was established in 1985. Development to a genetic model was established in 1991 [44]. The Zucker fatty (ZF) rats carry a spontaneous mutation in the leptin receptor gene (fa) [45]. ZF rats resulted from the simple autosomal recessive (fa) gene on chromosome 5 [36]. This mutation causes hyperphagia, early onset of obesity, and insulin resistance [14] along with increased growth of subcutaneous fat depot [46]. At the age of 4 weeks, ZF rats gain weight more rapidly due to increased growth of subcutaneous fat depot, and

they have a noticeably higher body weight at about the age of 9 weeks [47]. The hyperphagia and obesity in ZF rats are attributed to hypothalamic defect in leptin receptor signaling that is related to mild hyperglycemia, mild glucose intolerance, insulin resistance, hyperlipidemia, and moderate hypertension [46]. ZF rats have impaired glucose tolerance rather than apparent diabetes [42].

Thereafter, a mutation in ZF strain led to a substrain with an evident diabetic phenotype—the Zucker diabetic fatty (ZDF) rats [42]. ZDF rats are less obese than ZF rats having a decrease beta cell mass which resulted in inability to compensate for severe insulin resistance [48]. The ZDF rats were derived by selective inbreeding of hyperglycemic ZF rats [49] within the first months of life due to leptin receptor defect and a genetically reduced insulin promoter activity [17]. ZF rats maintain normoglycemia despite their obese phenotype, hyperlipidemia, and hyperinsulinemia [42].

The ZDF male rats became an experimental model for type 2 diabetes mellitus (T2DM). They have a predictable progression from prediabetic to diabetic state [50]. The ZDF rats carry a genetic defect in β-cell transcription. It is inherited independently of the leptin receptor mutation and insulin resistance [17]. In prediabetic stage of ZDF rats, there is no change in insulin mRNA levels. But, significant reduction (30–70%) of other islet mRNA levels, such as glucokinase, mitochondrial glycerol-3-phosphate dehydrogenase, voltage-dependent Ca^{2+} and K^+ channels, Ca^{2+}-ATPase, and transcription factor islet-1 may be detected [51]. It is known that FFA-induced suppression of insulin output in prediabetic stage of ZDF rats is conveyed by nitric oxide (NO) [52]. ZDF rats start to develop T2DM as early as 10 weeks of age, reaching 100% incidence at around 20 weeks of age [53]. It is possible to shorten prediabetic state and reach the symptoms of T2DM after high-energy diet. But, the animals receiving this diet are in the risky group because the diabetic state with its complications arrives quickly and rats can perish. In our experiment with ZDF rats, high-energy diet caused ketoacidosis that meant two cases of animal death in 7th week after initializing feeding with this caloric diet [54].

Blood glucose concentrations in ZDF rats usually increase from 7 to 10 weeks of age and impaired glucose tolerance at 5–7 weeks of age. At the age of 12 weeks, glucose intolerance becomes more severe than at 5–7 weeks of age [55]. Chronic and increasing hyperglycemia in ZDF rats is related to the loss of insulin and pancreatic duodenal homeobox (PDX-1) mRNAs. The lack of glucose stimulated insulin secretion. The possible prevention of hyperglycemia could block the deficit in insulin amount and PDX-1 gene expression and improve insulin secretion [56].

Male ZDF rats that are homozygous recessive have nonfunctional leptin receptors (fa/fa) and develop hyperlipidemia, obesity, and hyperglycemia. Rats that are homozygous dominant (+/+) or heterozygous (fa/+) are lean with normoglycemia. They are healthy, display no symptoms of diabetes, and are usually used as age-match control rats in the experiments. In young fa/fa rats, insulin resistance appears which extends to a deployed insulin secretory defect that initiates hyperglycemia and inadequate β-cell compensation [17, 49]. The insulin resistance is a result of a mutant leptin receptor that causes obesity [17].

In ZDF rats, there are sex differences for phenotypes of diet-induced insulin resistance and glucose intolerance. The most affected are male individuals [57]. On normal diet, male rats from ZDF strain develop severe hypoinsulinemia and hyperglycemia by 4 months of age. Female individuals maintain normal level of blood glucose and insulin despite advanced obesity [38]. Female ZDF rats with fa/fa genotype become also obese and insulin resistant, but do not progress to hyperglycemia, except when fed a high-fat diet [58, 59]. The female

ZDF rats develop T2DM just on a diabetogenic diet [60]. Thus, male ZDF rats are widely used as animal models for human T2DM and diabetic nephropathy and neuropathy [58, 61]. Hyperglycemia in diabetic ZDF rats was recorded at 2.5 month of age, and then blood glucose increased and reached an average value of 29.5 ± 0.9 mM at the age of 5 months. At the age of 5 months, fatty ZDF rats developed significant symptoms of thermal hypoalgesia indicated by prolonged response latencies in a tail-flick test. With progressing diabetes, the markers of thermal hypoalgesia increased at the age of 7 months and persisted till the 10th month [10]. The ZDF rats undergo a rapid transition between 10 and 15 weeks of age. At 10 weeks of age, they are insulin resistant, hyperlipidemic, and hyperin-sulinemic. But, the high plasma insulin levels are insufficient to control glucose level, and the animals are hyperglycemic. Between 10 and 15 weeks of age, a loss in insulin secretory function occurs which leads to a marked decline in plasma insulin levels along with hyperglycemia [43]. The ability to secrete insulin to compensate peripheral insulin resistance is limited. β cells of ZDF rats are brittle and easily succumb to over-secretion pressure. The primary defect lies not in the ability of β cells to proliferate but rather in an enhanced rate of apoptosis. It shows impaired insulin secretory β-cell response to glucose, while it remains untouched to non-glucose secretogogues like arginine, a phenomenon similar to human T2DM. Downregulation of β-cell GLUT 2 transporters together with impaired insulin synthesis is probably responsible for hyperglycemia in ZDF animals. Decreased glucose transport activity and lowered GLUT 4 levels are present in the skeletal muscle and adipose tissue [34, 36, 48, 62]. Generally, it was reported that in the progression of ZDF rats, the decline of β-cell glucose transporter 2 (GLUT 2) membrane receptors and the incidental loss of muscle glucose transporter 4 (GLUT 4) are responsible for the impaired insulin secretion and subsequent hyperglycemia. The activity of GLUT 4 receptors decreased in adipose tissue and skeletal muscle. This results in reduced β-cell transport ability together with the peripheral insulin resistance [50].

Siwy et al. [63] characterized the strain of ZDF rats as appropriate model for human disease based on urinary peptidomic profiles. In the study the diabetic rats were heavier than lean individuals. Consistent with a diabetic phenotype, ZDF rats were hyperglycemic and dyslipidemic already at early (2 months of age) and more severely at late (8 month of age). Renal function was markedly impaired at 8 months. Proteinuria was present at 2 months and progressively increased at 8 months. At 8 months, ZDF rats' renal histology showed pathological changes, including glomerular sclerosis with thickening of the Bowman capsule and retraction of the tuft, tubular atrophy and dilatation, and hyaline casts. Lean rats did not develop any histopathological changes. Chen and Wang [44] introduced the following pharmacologically related characteristics of ZDF rats: 25–55% reduction of GLUT 4 in the adipose tissue, heart, and skeletal muscle; loss of pancreatic duodenal homeobox gene expression; and free fatty acids and nitric oxide induced suppression of insulin output (**Table 1**).

Obesity of ZDF rats (fa/fa) is caused by hyperphagia [59], and food restriction can counteract or delay development of T2DM [55]. Hyperphagia leads to hyperinsulinemia, which upregulates transcription factors that stimulate lipogenesis. This results in ectopic deposition of triacylglycerol in non-adipocytes, thereby providing fatty acid substrate for pathological non-oxidative metabolism, such as ceramide synthesis [64].

The severity of DM in the adult hyperglycemic ZDF rats is reflected in body weight and food consumption [42]. High-energy diet in animal's models inclined to DM leads to obesity, hyperinsulinemia, and altered glucose metabolism

Main feature	Characteristic	Description
Type of diabetes	T2DM • Characteristic features resembling human type • Associated with obesity • Hyperphagia • Polyuria • Polydipsia • Hyperglycemia • Hyperlipidemia • Hyperinsulinemia • Insulin resistance • Reduction of GLUT 4 in adipose tissue	Development spontaneously involving genetic factors Acceleration of symptoms by high-fat diet Mortality due to ketosis after high-fat diet Brittle pancreas
Genetic feature	Leptin receptors • Defect in leptin signaling • Genetic defect in β cell transcription • Insulin receptor deletion	Homozygous recessive (fa/fa) rats—diabetic homozygous dominant (+/+) and heterozygous (fa/+) remain lean and normoglycemic
Using in research	Mechanism of T2DM Obesity	Minimal variability of results, the possibility to use small size groups
Progression of diabetes	Insulin secretory defects Inadequate β cell compensation	Predictable progression from prediabetic to diabetic state

Table 1.
Major genetic, physiological, and pathophysiological characteristics of ZDF rats.

because of insufficient compensation by the pancreatic islets [31]. In our experiment [54] high-energy diet immediately induced hyperglycemia in ZDF rats, animals developed obesity, and we observed disturbance in some hematology parameters as neutrophils, mean platelet volume (MPV), and platelet count (PLT), which is a possible marker of angiopathy. In the groups of diabetic rats, we observed the significant weight decrease when compared to the control animals. It is probably linked to accelerated switch from prediabetic to diabetic state. It suggests the inability to utilize the calories consumed [42] and the degradation of structural proteins and muscle wasting in diabetic individuals [65]. Hempe et al. [59] found that body weight of ZDF obese rats was higher than the lean rats at the beginning of their study. Later, at around 16 weeks of age, body weight of obese animals started to decline. Lean rats reached the same body weight as obese rats at 25 weeks of age. The decrease of weight in obese diabetic rats on high energy is linked to accelerated switch from prediabetic to diabetic accomplished with associated complications of T2DM. Oyedemi et al. [65] explained this reduction in body weight as degradation of structural proteins and muscle wasting.

Hempe et al. [59] evaluated if nephropathy and neuropathy in ZDF rats are linked to the hyperglycemic state of the rats and are real diabetic late complications or are related to other characteristics of the fa/fa genotype. Good glycemic control may be effective in delaying the neuropathic symptoms in diabetic patients [66].

2.1 Hematological parameters of ZDF rats

In general, diabetic patients have increased values of some hematological parameters as platelet count, mean platelet volume (MPV), and platelet distribution width (PDWc). Platelet activation can result in the generation of vascular disease [67]. Hematological parameters can be altered as a result of infection that occurs during DM [65]. In the human study, the total granulocyte count was increased in diabetic patients. It was confirmed that granulocyte count is associated with T2DM [68]. In our experiment significant increase in granulocyte count was also observed in diabetic ZDF rats in comparison with the control animals [54]. It was also published that increased count of one part of granulocytes (neutrophils) correlated with the rising risk of vascular disease in T1DM [69] with consequences as diabetic angiopathy [70]. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were decreased in the diabetic ZDF rats when compared to the lean control [54]. Similar results are published by Mahmoud [71] in white albino rats with experimentally induced DM and Oyedemi et al. [65] in streptozotocin-induced diabetic Wistar rats. Generally, the decrease of these hematological parameters during the diabetes could be an indicator of abnormal hemoglobin synthesis, failure of blood osmoregulation, and plasma osmolality [72].

Platelets play a critical role in atherogenesis and thrombosis-mediated myocardial ischemia accelerated in diabetic state [73]. They are source of inflammatory mediators [74]. We observed increased values of platelets in diabetic ZDF rats against the lean control [54]. Through inflammatory process during the DM, the platelets are highly activated. Activated platelets presumably support neutrophil activation and recruitment through expressing selectins, inflammatory cytokines, and chemokines [75]. It was proven that platelets and neutrophils regulate and affect each other's functions by platelet-leukocyte contact and releasing soluble effector mediators [76].

The marker of platelet function and activation is hematological parameter—MPV [77]. Increased MPV can be an independent risk factor for arterial thrombotic events such as myocardial infarction and cerebral thromboembolism [78]. Usually diabetic patients have increased MPV values [79] correlated with a large thrombocyte size that are more reactive and aggregable [77] which can upset hemostatic system during the diabetic state [80]. In diabetes the risk of retinopathy onset increases with higher MPV [79, 81]. In our study diabetic ZDF rats had increased MPV values in comparison with the healthy lean control [54]. In diabetic patients there is usually higher value of PDWc—the hematological parameter that presents an indicator of variation in platelets' size and activity [79, 82]. In our previous research [83], we observed that the rise in the secondary symptoms of T2DM complications caused by high-energy diet was accompanied with disturbed hematological parameters. It could be also a potential marker of angiopathy.

3. Other rodent models used in research in diabetes

Rodent animal models for investigation of T1DM are streptozotocin- or alloxan-induced animals, nonobese diabetic (NOD) mouse, and bio-breeding (BB) rat [84]. NOD mice and BB rats are rodent animal model with spontaneous development of T1DM [85]. Rodent models for T2DM include except ZDF rats also Goto-Kakizaki (GK) rats, Otsuka Long-Evans Tokushima Fatty (OLETF) rats,

spontaneously diabetic Tori (SDT) rats, Kuo Kondo (KK) mice, ob/ob+/+ mice, and db/db+/+ mice [84]. GK rats are nonobese Wistar substrain which develops T2DM early [86]. Male OLETS rats suffer from diabetes at 18–25 weeks of age. The symptoms include polyphagia, mild obesity, hypertriglyceridemia, hyperinsulinemia, and impaired glucose tolerance in 16 weeks of age [87]. Tori SDT rat is inbred strain of Sprague-Dawley rat. Male individuals have high glucose levels by 20 weeks, pancreatic islet histopathology, hemorrhage in pancreatic islets, and inflammatory cell infiltration with fibroblasts, prior to diabetes glucose intolerance with hypoinsulinemia [88].

KK mice are a polygenic model of obesity and T2DM. They are characterized by insulin resistance, hyperinsulinemia, and hyperphagia [89]. The ob/ob+/+ mice carry a mutation in the leptin gene, manifested as obesity, hyperglycemia, impaired glucose intolerance, and hyperinsulinemia [90]. The db/db+/+ mice have a leptin receptor mutation, are spontaneously hyperphagic, and suffer from obesity, hyperglycemia, hyperinsulinemia, and insulin resistance within the first month of life [91]. The advantages of ZDF rats in diabetes research are mainly due to the fact that it is a spontaneous model for T2DM research. It shows characteristics such as hyperglycemia, obesity, hyperphagia, polyuria, insulin disorders, and dyslipidemia due to the mutation in the leptin receptor gene and provides an appropriate model for common human T2DM. Moreover, these rats are calm and dispassionate; the handling and manipulation with them is comfortable.

4. Conclusion

The ZDF strain is of increasing preclinical interest due to its pathophysiological similarities to human T2DM [92, 93]. They are generally used in studies of diabetes with obesity and cardiovascular complications because of dyslipidemia background [44]. Defective insulin release in ZDF rats could be partially restored by glucagon-like peptide (GLP-1). The action of GLP-1 therapy is mediated through Ca^{2+}-independent signaling pathway in pancreatic islets [44]. The use of rosiglitazone protected ZDF rats against the loss of β-cell mass through sustaining cell proliferation, and blocking increased β cells' death [94]. Metformin prevented hyperglycemia in ZDF rats aged between 6 and 12 weeks. This compound significantly reduced free fatty acid level and triglycerides. It delayed the onset of DM which is linked to the improvement in β cell functions, on a par with the lipotoxicity hypothesis for adipogenic diabetes [95]. Some experimental interventions provided on ZDF rats are shown in **Table 2**. In general, animal model for DM research is required and needed to uncover and understand the pathophysiology of disease. This is the key to the development of new therapies and treatment [96].

Today, the number of patients suffering from DM is increasing. The most common form of DM is T2DM. It is a genetic disease demonstrating insulin insufficiency. Therefore the research on this disease is deepening and required. Due to its complex, complicated, multifactorial heterogeneous disease resulting from both environmental factors and genetic responsiveness, accurate animal model that can mirror human T2DM symptoms and complication is required. Presently, the spontaneous T2DM rodent model for research in DM and obesity is ZDF rats. This strain shows characteristics such as obesity, hyperglycemia, insulin disorders, and dyslipidemia due to the mutation in the leptin receptor gene and provides an appropriate model for common human T2DM.

Source	Aim of the study	Treatment	Results and conclusion of the study
Tanaka et al. [97]	To assess if the use of antioxidants prevents glucose toxicity and ameliorates the progression of DM	6 weeks of age till 12 weeks of age, antioxidants—N-acetyl-L-cysteine, aminoguanidine, ZDF rats	• The treatment with antioxidants can partially prevent the progressive β cells' dysfunction
Wasan et al. [43]	To examine the effect of organic vanadium compounds	3-week treatment with the insulin-enhancing agent—vanadium compounds, ZDF	• Increase in plasma homocysteine and cysteine level
Hempe et al. [59]	If the complications in the kidney and nerves correspond to human diabetic complications	Food restriction or pioglitazone (peroxisome proliferator-activated receptor gamma - PPARγ agonist) treatment, ZDF rats	• Food restriction delayed (not prevented) the onset of DM for 8–10 weeks and pioglitazone prevented the development of DM • ZDF rat is a good model for diabetic nephropathy, but alterations in nerve functions were not diabetes-related
Siwy et al. [63]	Evaluation of the similarity between ZDF rats and T2DM in humans	24 hours study, ZDF rats 2 month and 8 month of age	• ZDF rats may be more suitable to study the macrovascular branch within the pathophysiologic cascade of diabetic angiopathies, but it is not a good model for microvascular disease
Wang et al. [42]	Determination whether salsalate, a salicylate with anti-inflammatory properties, is effective in mitigating DM progression	Chronic administration of salsalate from 5 weeks of age to 24 weeks of age, ZDF rats	• The therapy is effective in particular animal model; it may only be effective in a subpopulation of humans with the disease
Kim et al. [98]	Investigation of therapeutic effect of resistance training on T2DM	8 weeks of resistance training, ZF and ZDF rats	• Regular resistance training initiated at the onset of DM improved glucose tolerance and GLUT 4 expression
Ďuračka et al. [99]* Tvrdá et al. [100]*	The effect of bee bread on the oxidative profile of testicular tissue and fertility in diabetic rats	3 months therapy with bee bread in the dose of 250 mg/kg/day, ZDF rats	• Significant increase of total antioxidant capacity of in testicular tissue lysate • Bee bread effectively protected proteins against oxidative damage • Bee bread provided substantial protection against testicular oxidative stress
Soltesova Prnova et al. [10]	Observation of the effect of Cemtirestat on symptoms of peripheral diabetic neuropathy	2 months treatment with Cemtirestat in doses 2.5 and 7.5 mg/kg/day, ZDF rats	• Partial inhibition of sorbitol accumulation in red blood cells and the sciatic nerve • Decrease in plasma level of TBARS • Normalization of peripheral neuropathy symptoms
Álvarez-Cilleros et al. [101]	To examine potential antidiabetic properties of cocoa	10 weeks treatment with cocoa-rich diet, ZDF rats	• Improvement in glucose homeostasis and insulin resistance, protection of renal structure and functionality

Source	Aim of the study	Treatment	Results and conclusion of the study
Capcarova et al. [102][*]	To effect of bee bread on DM complications	4 months therapy with bee bread in the dose of 700 mg/kg/day, ZDF rats	• Treatment of hyperglycemia, used as the prevention of DM in young age

[*]*Experiments realized at the Department of Animal Physiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovak Republic.*

Table 2.
The investigation of some therapeutic strategies in DM research using ZDF rats.

In general, in the future research, many novel strategies in treatment of DM will be surveyed, and the use of ZDF rats in these experiments will be worthy to study.

Acknowledgements

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Gavard JA, Lustman PJ, Clouse RE. Prevalence of depression in adults with diabetes: An epidemiological evaluation. Diabetes Care. 1993;**16**(8):1167-1178. DOI: 10.2337/diacare.16.8.1167

[2] Ogurtsova K, Da Rocha Fernandez JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF diabetes atlas: Global estimates for prevalence of diabetes for 2015 and 2040. Diabetes Research and Clinical Practise. 2017;**128**:40-50. DOI: 10.1016/j.diabres.2017.03.024

[3] Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF diabetes atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Research and Clinical Practise. 2018;**138**:271-281. DOI: 10.1016/j.diabres.2018.02.023

[4] King AJF. The use of animal models in diabetes research. British Journal of Pharmacology. 2012;**166**:877-894. DOI: 10.1111/j.1476-5381.2012.01911.x

[5] Pang YL, Hu JW, Liu GL, Lu SY. Comparative medical characteristics of ZDF-T2DM rats during the course of development to late stage disease. Animal Models and Experimental Medicine. 2018;**1**:203-211. DOI: 10.1002/ame2.12030

[6] Nakagawa T, Tanabe K, Croker BP, Johnson RJ, Grant MB, Kosugi T, et al. Endothelial dysfunctions of diabetes. Journal of the American Medical Association. 2004;**292**:2495-2499. DOI: 10.1038/nrneph.2010.152

[7] Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature. 2001;**414**:813-820. DOI: 10.1038/414813a

[8] Feldman EL, Nave KA, Jensen TS, Bennet DLH. New horizons in diabetic neuropathy: Mechanisms, bioenergetics, and pain. Neuron. 2017;**93**(6):1296-1313. DOI: 10.1016/j.neuron.2017.02.005

[9] Tomlinson DR, Gardiner NJ. Glucose neurotoxicity. Nature Reviews. Neuroscience. 2008;**9**(1):36-45. DOI: 10.1038/nrn2294

[10] Soltesova Prnova M, Svik K, Bezek S, Kovacikova L, Karasu C, Stefek M. 3-mercapto-5H-1,2,4-triazino[5,6-b]indole-5-acetic acid (Cemtirestat) alleviates symptoms of peripheral diabetic neuropathy in Zucker diabetic fatty (ZDF) rats: A role of aldose reductase. Neurochemical Research. 2019;**44**:1056-1064. DOI: 10.1007/s11064-019-02736-1

[11] Robertson RP, Harmon JS. Diabetes, glucose toxicity, and oxidative stress: A case of double jeopardy for the pancreatic islet β cell. Free Radical Biology & Medicine. 2006;**41**:177-184. DOI: 10.1016/j.freeradbiomed.2005.04.030

[12] Akerblom HK, Knip M. Putative environmental factors in type 1 diabetes. Diabetes/Metabolism Reviews. 1998;**14**:31-36. DOI: 10.1002/(sici)1099-0895(199803)14:1<31::aid-dmr201>3.3.co;2-1

[13] Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006;**444**:840-846. DOI: 10.1038/nature05482

[14] Garnett KE, Chapman P, Chambers JA, Waddell ID, Boam DSW. Differential gene expression between Zucker fatty rats and Zucker diabetic fatty rats: A potential role for the immediate-early gene Egr-1 in regulation of beta cell proliferation. Journal of Molecular Endocrinology. 2005;**35**:13-25. DOI: 10.1677/jme.1.01792

[15] Gutterman DD. Vascular dysfunction in hyperglycemia: Is

protein kinase C the culprit? Circulation Research. 2002;**90**(1):5-7. DOI: 10.1161/res.90.1.5

[16] Miyake K, Yang W, Hara K, Yasuda K, Horikawa Y, Osawa H, et al. Construction of a prediction model for type 2 diabetes mellitus in the Japanese population based on 11 genes with strong evidence of the association. Journal of Human Genetics. 2009;**54**(4):236-241. DOI: 10.1038/jhg.2009.17

[17] Griffen SC, Wang J, German MS. A genetic defect in beta-cell gene expression segregates independently from the fa locus in the ZDF rat. Diabetes. 2001;**50**:63-68. DOI: 10.2337/diabetes.50.1.63

[18] Polonsky KS, Sturis J, Bell GI. Seminars in medicine of the Beth Israel hospital, Boston. Non-insulin-dependent diabetes mellitus- a genetically programmed failure of the beta cell to compensate for insulin resistance. The New England Journal of Medicine. 1996;**334**:777-783. DOI: 10.1056/NEJM199603213341207

[19] Lencioni C, Lupi R, Del Prato S. Beta-cell failure in type 2 diabetes mellitus. Current Diabetes Record. 2008;**8**:179-184. DOI: 10.1007/s11892-008-0031-0

[20] Lee Y, Hirose H, Zhou YT, Esser V, McGarry JD, Unger RH. Increased lipogenic capacity of the islets of obese rats: A role in the pathogenesis of NIDDM. Diabetes. 1997;**46**:408-413. DOI: 10.2337/diab.46.3.408

[21] Prentki M, Joly E, El-Assad W, Roduit R. Malonyl-CoA signalling, lipid partitioning, and glucolipotoxicity: Role in beta-cell adaptation and failure in the etiology of diabetes. Diabetes. 2002;**51**:S405-S413. DOI: 10.2337/diabetes.51.2007.S405

[22] Gheni G, Yokoi N, Beppu M, Yamaguchi T, Hidaka A, Hoshino Y, et al. Characterization of the prediabetic state in a novel rat model of type 2 diabetes, the ZFDM rat. Journal Diabetes Research. 2015;**2015:**1-8. Article ID 261418. DOI: 10.1155/2015/261418

[23] DeFronzo R. The triumvirate: Beta cell, muscle, liver. A collusion responsible for NIDDM. Diabetes. 1988;**37**:667-685. DOI: 10.2337/diab.37.6.667

[24] Uchigata Y. Pathogenesis, diagnosis, and treatment of type 2 diabetes in adolescence. Nihon Rinsho. 2016;**74**:517-522

[25] Yano JM, Yu K, Donaldson GP, Shastri GG, Ann P, Ma L, et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. Cell. 2015;**161**:264-276. DOI: 10.1016/j.cell.2015.02.047

[26] Hill J, Wyatt HR, Peters JC. Energy balance and obesity. Circulation. 2012;**126**:126-132. DOI: 10.1161/CIRCULATIONAHA.111.087213

[27] Day C, Bailey CJ. Obesity in the pathogenesis of type 2 diabetes. British Journal of Diabetes and Vascular Disease. 2011;**11**(2):55-61. DOI: 10.1177/1474651411407418

[28] Guilherme A, Virbasius JV, Puri V, Czech MP. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. Nature Reviews. Molecular Cell Biology. 2008;**9**:367-377. DOI: 10.1038/nrm2391

[29] Feldstein AE, Nathan W, Werneburg AC, Guicciardi ME, Bronk SF, Rydzewski R, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-α expression via a lysosomal pathways. Hepatology. 2004;**40**:185-194

[30] Lumeng CN, Saltiel AR. Inflammatory links between obesity

and metabolic disease. The Journal of Clinical Investigation. 2011;**121**:2111-2117. DOI: 10.1172/JCI57132

[31] Winzell MS, Ahren B. The high-fat diet-fed mouse: A model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes. 2004;**54**(Suppl 3):S215-S219. DOI: 10.2337/diabetes.53.suppl_3.s215

[32] Izumi Y, Ishibashi G, Nakanishi Y, Kikunaga S. Beneficial effect of 3% milled-rice on blood glucose level and serum lipid concentrations in spontaneously non-insulin-dependent diabetic rats. Journal of Nutritional Science and Vitaminology. 2007;**53**: 400-409. DOI: 10.3177/jnsv.53.400

[33] O'Rahilly S. Human genetics illuminates the paths to metabolic disease. Nature. 2009;**402**:307-314. DOI: 10.1038/nature08532

[34] Bailey CJ. Drugs on the horizon for diabesity. Current Diabetes Reports. 2005;**5**:353-359. DOI: 10.1007/s11892-005-0093-1

[35] Chatzigeorgiou A, Halapas A, Kalafatakis K, Kamper E. The use of animal models in the study of diabetes mellitus. In Vivo. 2009;**23**:245-258

[36] Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: An overview. The Indian Journal of Medical Research. 2007;**125**:451-472

[37] Engel H, Xiong L, Reichenberger MA, Germann G, Roth C, Hirche C. Rodent models of diet-induced type 2 diabetes mellitus: A literature review and selection guide. Diabetes and Metabolic Syndrome: Clinical Research and Reviews. 2019;**13**:195-200. DOI: 10.1016/j.dsx.2018.07.020

[38] Kleinert M, Clemmensen C, Hofmann SM, Moore MC, Renner S, Woods SC, et al. Animals models of obesity and diabetes mellitus. Nature.

2018;**140**:140-184. DOI: 10.1038/nrendo.2017.161

[39] Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM. A new rat model of type 2 diabetes: The fat-fed, streptozotocin-treated rat. Metabolism. 2000;**49**(11):1390-1394. DOI: 10.1053/meta.2000.17721

[40] Rees DA, Alcolado JC. Animal models of diabetes mellitus. Diabetic Medicine. 2005;**22**:359-370. DOI: 10.1111/j.1464-5491.2005.01499.x

[41] Fang JY, Lin CH, Huang TH, Chuang SY. In vivo rodent models of type 2 diabetes and their usefulness for evaluating flavonoid bioactivity. Nutrients. 2019;**11**(3):23pp. DOI: 10.3390/nu11030530

[42] Wang X, DuBois DC, Sukumaran S, Ayyar V, Jusko WJ, Almon RR. Variability in Zucker diabetic fatty rats: Differences in disease progression in hyperglycemic and normoglycemic animals. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy. 2014;**7**:531-541. DOI: 10.2147/DMSO.S69891

[43] Wasan KM, Risovic V, Yuen VG, McNeill JH. Differences in plasma homocysteine levels between Zucker fatty and Zucker diabetic fatty rats following 3 weeks oral administration of organic vanadium compounds. Journal of Trace Elements in Medicine and Biology. 2006;**19**:251-258. DOI: 10.1016/j.jtemb.2005.10.001

[44] Chen D, Wang MW. Development and application of rodent models for type 2 diabetes. Diabetes, Obesity & Metabolism. 2005;**7**:307-317. DOI: 10.1111/j.1463-1326.2004.00392.x

[45] Philips MS, Liu Q, Hammond HA, Dugan V, Hey PJ, Caskey CJ, et al. Leptin receptor missense mutation in the fatty Zucker rat. Nature Genetics. 1996;**13**:18-19. DOI: 10.1038/ng0596-18

[46] Durham HA, Truett GE. Development of insulin resistance and hyperphagia in Zucker fatty rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2006;**210**:652-658. DOI: 10.1152/ajpregu.00428.2004

[47] McCaleb ML, Sredy J. Metabolic abnormalities of the hyperglycemic obese Zucker rat. Metabolism. 1992;**41**(5):522-525. DOI: 10.1016/0026-0495(92)90212-s

[48] Pick A, Clark J, Kubstrup C, Levisetti M, Pugh W, Bonner-Weir S, et al. Role of apoptosis in failure of beta-cell mass compensation for insulin resistance and beta-cell defects in the male Zucker diabetic fatty rat. Diabetes. 1998;**47**:358-364. DOI: 10.2337/diabetes.47.3.358

[49] Etgen GJ, Oldham BA. Profiling of Zucker diabetic fatty rats in their progression to the overt diabetic state. Metabolism. 2000;**49**:684-688. DOI: 10.1016/S0026-0495(00)80049-9

[50] Slieker LJ, Sundell KL, Heath WF, Osborne HE, Blue J, Manetta J, et al. Glucose transporter levels in tissues of spontaneously diabetic Zucker fa/fa (ZDF/drt) and viable yellow mouse (Avy/a). Diabetes. 1992;**41**:187-193. DOI: 10.2337/diab.41.2.187

[51] Tokuyama Y, Sturis J, DePaoli AM, Takeda J, Stoffel M, Tang J, et al. Evolution of beta-cell dysfunction in the male Zucker diabetic fatty rat. Diabetes. 1995;**44**:1447-1457. DOI: 10.2337/diab.44.12.1447

[52] Shimabukuro M, Ohneda M, Lee Y, Unger RH. Role of nitric oxide in obesity-induced beta cell disease. The Journal of Clinical Investigation. 1997;**100**:290-295. DOI: 10.1172/JCI119534

[53] Yokoi N, Hoshino M, Hidaka S, Yoshida E, Beppu M, Hoshikawa R, et al. A novel rat model of type 2 diabetes: The Zucker fatty diabetes mellitus ZFDM rat. Journal Diabetes Research. 2013;**2013**:9. Article ID 103731. DOI: 10.1155/2013/103731

[54] Capcarova M, Kalafova A, Schwarzova M, Soltesova Prnova M, Svik K, Schneidgenova M, et al. The high-energy diet affecting development of diabetes symptoms in Zucker diabetic fatty rats. Biologia. 2018;**73**:659-671. DOI: 10.2478/s11756-018-0076-8

[55] Ohneda M, Inman LR, Unger RH. Caloric restriction in obese pre-diabetic rats prevents beta-cell depletion, loss of beta-cell GLUT 2 and glucose incompetence. Diabetologia. 1995;**38**:173-179. DOI: 10.1007/s001250050267

[56] Harmon JS, Gleason CE, Tanaka Y, Oseid EA, Hunter-Berger KK, Robertson RP. In vivo prevention of hyperglycemia also prevents glucotoxic effects on PDX-1 and insulin gene expression. Diabetes. 1999;**48**:1995-2000. DOI: 10.2337/diabetes.48.10.1995

[57] Nadal-Casellas A, Proenza AM, Llado I, Gianotti M. Sex-dependent differences in rat hepatic lipid accumulation and insulin sensitivity in response to diet-induced obesity. Biochemistry and Cell Biology. 2012;**90**:164-172. DOI: 10.1139/o11-069

[58] Figarolla JL, Loera S, Weng Y, Shanmugam N, Natarajan R, Rahbar S. LR-90 prevents dyslipidaemia and diabetic nephropathy in the Zucker diabetic fatty rat. Diabetologia. 2008;**51**:882-891. DOI: 10.1007/s00125-008-0935-x

[59] Hempe J, Elvert R, Schmidts HL, Kramer W, Herling AW. Appropriateness of the Zucker diabetic fatty rat as a model for diabetic microvascular late complications. Laboratory Animals. 2012;**46**:32-39. DOI: 10.1258/la.2011.010165

[60] Wang Y, Sun G, Sun J, Liu S, Wang J, Xu X, et al. Spontaneous type 2 diabetic rodent models. Journal Diabetes Research. 2013; ID 401723:8. DOI: 10.1155/2013/401723

[61] Brussee V, Guo G, Dong Y, Cheng C, Martinez JA, Smith D, et al. Distal degenerative sensory neuropathy in a long-term type 2 diabetes rat model. Diabetes. 2008;**57**:1664-1673. DOI: 10.2337/db07-1737

[62] Zhang BB, Moller DE. New approaches in the treatment of type 2 diabetes. Current Opinion in Chemical Biology. 2000;**10**:703-710. DOI: 10.1016/s1367-5931(00)00103-4

[63] Siwy J, Zoja C, Klein J, Benigni A, Mullen W, Mayer B, et al. Evaluation of the Zucker diabetic fatty (ZDF) rat as a model for human disease based on urinary peptidomic profiles. PLoS One. 2012;**12**:e51334. DOI: 10.1371/journal.pone.0051334

[64] Unger RH, Orci L. Diseases of liporegulation: New perspective on obesity and related disorders. FASEB Journal. 2001;**15**:312-321. DOI: 10.1096/fj.00-0590

[65] Oyedemi SO, Adewusi EA, Aiyegoro OA, Akinpelu DA. Antidiabetic and haematological effect of aqueous extract of stem bark of Afzelia Africana (Smith) on streptozotocin-induced diabetic Wistar rats. Asian Pacific Journal of Tropical Biomedicine. 2011;**1**(5):353-358. DOI: 10.1016/S2221-1691(11)60079-8

[66] Waldfogel JM, Nesbit SA, Dy SM, Sharma R, Zhang A, Wilson LM, et al. Pharmacotherapy for diabetic peripheral neuropathy pain and quality of life: A systematic review. Neurology. 2017;**88**(20):1958-1967. DOI: 10.1212/wnl.0000000000003882

[67] Curtis TM, Gardiner TA, Stitt AW. Microvascular lesions of diabetic retinopathy: Clues towards understanding pathogenesis? Eye. 2009;**23**:1496-1508. DOI: 10.1038/eye.2009.108

[68] Gkrania-Klotsas E, Ye Z, Cooper AJ, Sharp SJ, Luben R, Biggs ML, et al. Differential white blood cell count and type 2 diabetes: Systematic review and meta-analysis of cross-sectional and prospective studies. PLoS One. 2010;**5**(10):e13405:11 pp. DOI: 10.1371/journal.pone.0013405

[69] Huang J, Xiao Y, Xu A, Zhou Z. Neutrophils in type 1 diabetes. Journal of Diabetes Investigation. 2016;**7**:652-663. DOI: 10.1111/jdi.12469

[70] Piwowar A, Knapik-Kordecka M, Warwas M. Concentration of leukocyte elastase in plasma and polymorphonuclear neutrophil extracts in type 2 diabetes. Clinical Chemistry and Laboratory Medicine. 2000;**38**(12):1257-1261. DOI: 10.1515/cclm.2000.198

[71] Mahmoud A. Hematological alterations in diabetic rats – Role of adipocytokines and effect of citrus flavonoids. EXCLI Journal. 2013;**12**:647-657

[72] Stookey JD, Burg M, Sellmeyer DE, Greenleaf JE, Arieff A, Van Hove L, et al. A proposed method for assessing plasma hypertonicity in vivo. European Journal of Clinical Nutrition. 2006;**61**:143-146. DOI: 10.1038/sj.ejcn.1602481

[73] Kraakman MJ, Lee MK, Al-Sharea A, Dragoljevic D, Barrett TJ, Montenont E, et al. Neutrophil-derived S100 calcium-binding proteins A8/A9 promote reticulated thrombocytosis and atherogenesis in diabetes. The Journal of Clinical Investigation. 2017;**127**(6):2133-2147. DOI: 10.1172/jci92450

[74] Ruggeri ZM. Platelets in atherothrombosis. Nature Medicine. 2002;**8**:1227-1234. DOI: 10.1038/nm1102-1227

[75] Semple JW, Italiano JE Jr, Freedman J. Platelets and the immune continuum. Nature Reviews. Immunology. 2011;**11**:264-274. DOI: 10.1038/nri2956

[76] Li N, Hu H, Lindqvist M. Platelet-leukocyte cross talk in whole blood. Arteriosclerosis, Thrombosis, and Vascular Biology. 2000;**20**:2702-2708. DOI: 10.1161/01.atv.20.12.2702

[77] Park Y, Schoene N, Harris W. Mean platelet volume as an indicator of platelet activation: Methodological issues. Platelets. 2002;**13**(5-6):301-306. DOI: 10.1080/095371002220148332

[78] Khandekar MM, Khurana AS, Deshmukh SD, Kakrani AL, Katdare AD, Inamdar AK. Platelet volume indices in patients with coronary artery disease and acute myocardial infarction: An Indian scenario. Journal of Clinical Pathology. 2006;**59**:146-149. DOI: 10.1136/jcp.2004.025387

[79] Tetikoglu M, Aktas S, Sagdik HM, Yigitoglu ST, Özcura F. Mean platelet volume is associated with diabetic macular edema in patients with type-2 diabetes mellitus. Seminars in Ophthalmology. 2016;**32**(5):651-654. DOI: 10.3109/08820538.2016.1157612

[80] Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. Diabetes Care. 2001;**24**:1476-1485. DOI: 10.2337/diacare.24.8.1476

[81] Citirik M, Beyazyildiz E, Simsek M, Beyazyildiz O, Haznedaroglu IC. MPV may reflect subclinical platelet activation in diabetic patients with and without diabetic retinopathy. Eye. 2015;**29**(3):376-379. DOI: 10.1038/eye.2014.298

[82] Jindal S, Gupta R, Gupta R, Kakkar A, Singh AV, Gupta K, et al. Platelet indices in diabetes mellitus: Indicators of diabetic microvascular complication. Hematology. 2011;**16**:86-89. DOI: 10.1179/102453311x12902908412110

[83] Capcarova M, Schwarzova M, Kalafova A, Soltesova Prnova M, Svik K, Schneidgenova M, et al. Alterations in haematological parameters in diabetic rats. Slovak Journal of Animal Science. 2017;**50**(4):161

[84] Kim JH, Nishina PM, Naggert JK. Genetic models for non insulin dependent diabetes mellitus in rodents. Journal of Basic and Clinical Physiology and Pharmacology. 1998;**9**:325-345. DOI: 10.1515/jbcpp.1998.9.2-4.325

[85] Makino S, Kunimoto K, Munaoko Y, Mizushima Y, Katagiri K, Tochino Y. Breeding of a non-obese diabetic strain of mice. Experimental Animals. 1980;**29**:1-13. DOI: 10.1538/expanim1978.29.1_1

[86] Bedow AA, Samuel VT. Fasting hyperglycemia in the Goto-Kakizaki rat is dependent on corticosterone: A confounding variable in rodent models of type 2 diabetes. Disease Models & Mechanisms. 2012;**5**(5):681-685. DOI: 10.1242/dmm.009035

[87] Kawano K, Hirashima T, Mori S, Saitoh Y, Kurosumi M, Natori T. Spontaneous long-term hyperglycemic rat with diabetic complications, Otsuka Long-Evans Tokushima Fatty (OLETF) strain. Diabetes. 1992;**41**:1422-1428. DOI: 10.2337/diabetes.41.11.1422

[88] Sasase T, Ohta T, Masuyama T, Yokoi N, Kakehashi A, Shinohara M. The spontaneously diabetic Torii rat: An animal model of nonobese type 2 diabetes with severe diabetic complications. Journal Diabetes Research. 2013;**2013**:12. Article ID 976209. DOI: 10.1155/2013/976209

[89] Reddi AS, Camerini-Davalos RA. Hereditary diabetes in the KK mouse: An overview. Advances in Experimental Medicine and Biology. 1988;**246**:7-15. DOI: 10.1007/978-1-4684-5616-5_2

[90] Dubuc PU. The development of obesity, hyperinsulinemia, and hyperglycemia in Ob/Ob mice. Metabolism. 1976;**25**:1567-1574. DOI: 10.1016/0026-0495(76)90109-8

[91] Shariff E. Animal models of non-insulin dependent diabetes. Diabetes & Metabolism. 1992;**8**:179-208. DOI: 10.1002/dmr.5610080302

[92] Jones HB, Nugent D, Jenkins R. Variation in characteristics of islets of Langerhans in insulin-resistant, diabetic and non-diabetic-rat strains. International Journal of Experimental Pathology. 2010;**91**:288-301. DOI: 10.1111/j.1365-2613.2010.00713.x

[93] Kakimoto T, Kimata H, Iwasaki S, Fukunari A, Utsumi H. Automated recognition and quantification of pancreatic islets in Zucker diabetic fatty rats treated with exendin-4. The Journal of Endocrinology. 2013;**216**:13-20. DOI: 10.1530/joe-12-0456

[94] Finegood DT, McArthur MD, Kojwang D. Beta-cell mass dynamics in Zucker diabetic fatty rats. Rosiglitazone prevents the rise in net cell death. Diabetes. 2001;**50**:1021-1029. DOI: 10.2337/diabetes.50.5.1021

[95] Sreenan S, Sturis J, Pugh W, Burant CF, Polonsky KS. Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin or troglitazone. The American Journal of Physiology. 1996;**271**:e742-e747. DOI: 10.1152/ajpendo.1996.271.4.e742

[96] Capcarova M, Soltesova Prnova M, Svik K, Schneidgenova M, Slovak L, Kisska P, et al. Rodent animal model for research in diabetes: A mini review. Slovak Journal of Animal Science. 2018b;**51**(3):138-145

[97] Tanaka Y, Gleason CE, Tran POT, Harmon JS, Robertson RP. Prevention of glucose toxicity in HIT-T15 cells and Zucker diabetic fatty rats by antioxidants. Proceedings of the National Academy of Sciences. 1999;**96**:10857-10862. DOI: 10.1073/pnas.96.19.10857

[98] Kim J, Cho MJ, So B, Kim H, Seong JK, Song W. The preventive effects of 8 weeks of resistance training on glucose tolerance and muscle fiber type composition in Zucker rats. Diabetes and Metabolism Research. 2015;**39**:424-433. DOI: 10.4093/dmj.2015.39.5.424

[99] Ďuračka M, Sukova M, Valentova G, Tokarova K, Kalafova A, Schneidgenova M, et al. Perga (bee bread) improves the oxidative profile of testicular tissue in obese diabetic rats. In: Nutricon 2018. Skopje: Consulting and Training Center KEY; 2018. pp. 169-170

[100] Tvrdá E, Ďuračka M, Sukova M, Valentova G, Tokarova K, Kalafova A, et al. The effect of perga on the testicular oxidative profile of Zucker diabetic fatty rats. In: Nutricon 2018. Skopje: Consulting and Training Center KEY; 2018. pp. 167-168

[101] Álvarez-Cilleros D, López-Oliva E, Goya L, Martín MA, Ramos S. Cocoa intake attenuates renal injury in Zucker diabetic fatty rats by improving glucose homeostasis. Food and Chemical Toxicology. 2019;**127**:101-109. DOI: 10.1016/j.fct.2019.03.002

[102] Capcarova M, Kalafova A, Schwarzova M, Schneidgenova M, Soltesova Prnova M, Svik K, et al. Consumption of bee bread influences glycaemia and development of diabetes in obese spontaneous diabetic rats. Biologia. 2019 in press