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EVALUATION OF ANIMAL MODELS BY COMPARISON WITH HUMAN *DIABETES MELLITUS*: A REVIEW

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ABSTRACT

Animal models are widely used to imitate human diseases to improve the understanding of the pathophysiology of disease and to test treatment interventions. A chronic disease, such as *diabetes mellitus*, is a fast-growing epidemy worldwide connected with obesity, lack of physical exercise, aging and genetics. This review brings an introduction to *diabetes mellitus* and compares individual animal models, mainly rodents, with respect to this disease. The selection of a suitable model is important and essential for the progression of new therapeutic methods of preclinical and clinical studies.

Key words: diabetes; human; animal model; diet

INTRODUCTION

Diabetes mellitus (DM) is a disease of the endocrine system diagnosed by abnormally high blood glucose levels. It is one of the most common and fastest growing diseases in the world, which estimated to affect 693 million adults by 2045. DM has more than 50 % increases since 2017 (Cho *et al*., 2018). According to American Diabetes Association (2015) the most common forms of diabetes are the type 2 diabetes (DMT2), when insulin resistance can lead to hyperglycaemia and the type 1 diabetes (DMT1), when there is an absolute lack of insulin due to the destruction of β-cells in the pancreas. DM is rather a group of metabolic conditions categorized by a hyperglycaemia than a single disease. Recent findings show that DMT2, as the predominant subtype of diabetes, is heterogeneous itself in terms of both mechanisms of action and relationships with health outcomes (American Diabetes Association 2018; Udler *et al*., 2018). The essence of the pathophysiology of DMT2 is that adipose tissue, which is an endocrine organ, can secrete several hormones and cytokines (TNF – α, IL – 6, resistin) that are able to induce chronic inflammatory status and insulin resistance (Millo, 2002). Obesity is connected with increased levels of cytokines, such as TNF – α, IL – 1β or IL – 6 by both immune cells and adipocytes. Such increased secretion induces insulin resistance by multiple mechanisms, including activation of Ser/Thr kinases, decreasing IRS – 1, GLUT4 and PPARγ expression or activation of SOCS3 in adipocytes (Hirosumi *et al*., 2002; Jager *et al*., 2007; Boucher *et al*., 2014). Low levels of adiponectin and leptin resistance are common in obese patients with metabolic syndrome. Leptin is a hormone with orexigenic activity that helps regulate energy balance by inhibiting hunger, while adiponectin is a peptide synthesized by adipocytes that has anti-inflammatory effects (Yadav *et al*., 2011). In such a situation, insulin has no antilipolytic effect, resulting in increased production and secretion of free fatty acids (FFA), which are

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also responsible for the state of insulin resistance. Elevated plasma concentrations of FFA are lipotoxic to β-cells. In addition, cholesterol and triglyceride levels are also elevated, especially the levels of low-density lipoproteins (LDL), which have a negative effect on the cardiovascular system (Boden, 2003). This multifactorial pathophysiology of DMT2 is presented in Figure 1 (Artasensi *et al*., 2020).

Genetic and environmental components are important factors in the heterogenity of diabetes and its complications. When early studies identified differences in the susceptibility to diabetic complications in patients, who appeared to be the same in terms of diabetes control, clinical signs and family management studies were able to show clear and remarkable differences in the incidence of microvascular and macrovascular complications in individuals with both diabetes and complications compared to people with diabetes but without complications (Deckert and Poulsen, 1981; Toumilehto *et al*., 1998). There is still no cure for any type of diabetes, however there is a number of treatments with antidiabetic activity. The most commonly used drugs in the treatment of diabetes include insulin, α-glucosidase inhibitors, amylin analogs, dipeptidyl peptidase-4 inhibitors, incretin mimetics, meglitinides, non-sulfonylureas, sulfonylureas and thiazolidinediones. Despite the wide variety of different types of antidiabetic agents, such agents have side effects that are commonly associated with oral antidiabetic agents, causing serious problems and challenges in the effective management of the disease. Recently, extensive research has focused on finding alternative but effective and safer thermostatic agents to improve diabetic syndrome (Hasan *et al*., 2018). Of the thousands of plants that have shown antidiabetic effects, only a few are characterized by safety, efficacy and a potential antidiabetic agent (Bnouham *et al*., 2005).

DM is a complex disorder and the human or animal physiology is also very complex. Animal models are one of the major tools to progress with establishing an effective model to examine the mechanism of action as well as to explore the beneficial substances within plants. However, the disease itself is very heterogeneous, what leads to many approaches to cause the diabetes as well as the other complications related to diabetes. Thus, a single animal model to examine the efficacy of the treatment is not possible owing to the existence of different types of DM (Vedtofte *et al*., 2010).

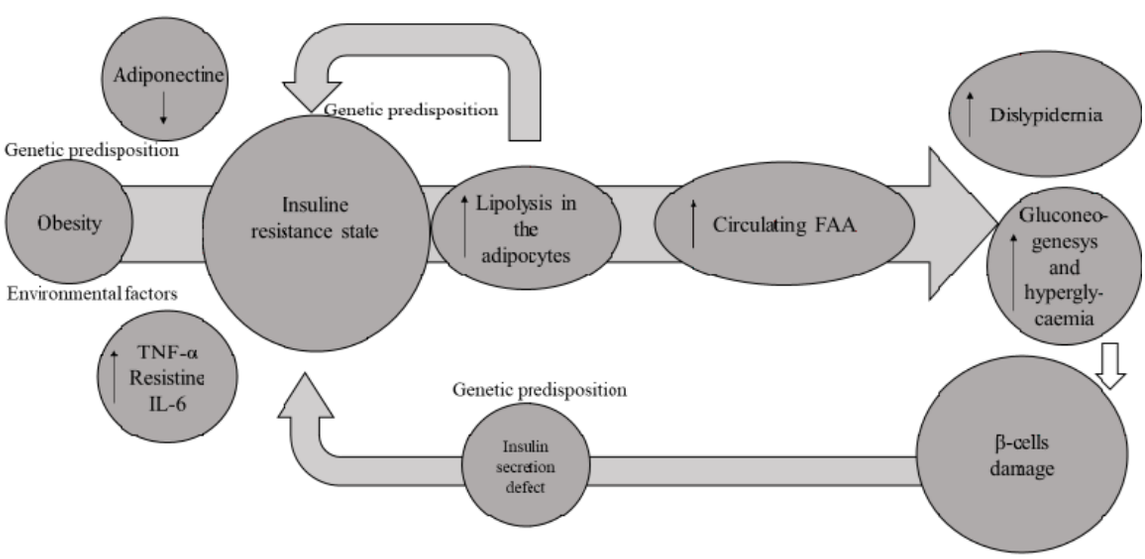

Figure 1. Pathophysiology of *Diabetes mellitus* type 2 (Modified according to Artasensi *et al.*, 2020)

There are several types of animal models for diabetes. Animal models can be divided into spontaneous diabetic animals, diet/nutrition induced diabetics, chemical induced diabetic animals and surgical diabetic animals. Each type of a model has advantages and disadvantages. In addition to *in vivo* methods, *in vitro* methods are also used for diabetes research, which are more cell-specific and less time-consuming, but the human body is a more complex system that cannot be explored by *in vitro* assays alone. Therefore, the efficacy of sample molecules should also be tested through an *in vivo* system to achieve a better understanding. Cell models can be especially recommended for specific mechanism research as well as for primary stage research to determine the exact target molecule or receptor. However, animal models are recommended for further studies on drug development as well as for the evaluation of toxicological profiles (Hasan *et al*., 2018). Animal models may differ in their physiological severity with some models more similar to the development of the disease than others. When selecting a model for DM, it is desirable to use a variety of different animal models to express the diversity observed in human diabetes (King, 2012). For this reason, the aim of this study was to review and compare existing animal models on human physiology with respect to diabetes.

Type 1 diabetes animal models

DMT1 is characterized by an autoimmune destruction of the pancreatic β-cells, leading to lack of insulin production. Animal models of type 1 diabetes has deficiency in insulin production achieved by a variety of different mechanisms like chemical ablation of the β-cells or breeding rodents that spontaneously develop this type of diabetes (King, 2012). Among chemically induced diabetes substances, alloxan (ALX) and streptozotocin (STZ) are the most commonly used. STZ and ALX can be administered through either intraperitoneal, subcutaneous or intravenous ways. The mechanism of action is, that both chemicals are selective cytotoxic agents and consequently destroy the pancreatic β-cells. They are both glucose analogues which are transported to pancreatic β-cells by GLUT2 transporter. STZ causes alkylation of DNA by methylnitrosourea and ALX forms reactive oxygen species (ROS), which lead to the destruction of β-cells (Lenzen, 2008). The destruction of β-cells can cause various complications, such as hyperglycaemia, glycosuria, polyuria, polydipsia, hyperphagia and weight loss (Han and Liu, 2010). Chemically induced diabetes is mostly used when experimenting with drugs or therapies, where the main mechanism of action is lowering glycaemia (Jederstrom *et al*., 2005). Disadvantage of chemically induced diabetes is, that the chemicals can be toxic for other organs of the body and may cause changes in the liver, kidney, lung, intestines, testis or brain. This should be considered when examining effects of drugs on these models (Lee *et al*., 2010).

Surgical induction of diabetes is an another way to cause DMT1 in animals. In addition to chemical induction, surgical removal of the pancreas is an alternative to reduce toxic side effects of chemically induced diabetes but, on the other hand, such an operation requires extensive experience, finances and sufficient sterility of the environment. The limitations of this surgical model outweigh its advantages as large amount of analgesic and antibiotic, post-surgery pancreatic enzyme supplement and risk of animal infection are major drawback of this technique. There have been many attempts to perform partial pancreatic surgery, which is yet to be developed or acclaimed to achieve desired diabetogenic action. Such surgical removal has been reported on animal models of some species, such as rats, dogs or pigs (Weir *et al*., 1983; Wang *et al*., 2009; Vedtofte *et al*., 2010; Müller, 2016).

Other commonly used autoimmune models are the non-obese diabetic (NOD) mouse, the AKITA mouse, the LEW.1AR1/Ztm-iddm rat and the biobreeding (BB) rat (Lenzen *et al*., 2001; Yang and Santamaria, 2006). DMT1 is developed spontaneously or by genetic alteration in these models. Animal models of this type (spontaneous or genetic alteration) have many varieties that more accurately depict the complex nature of diabetes in humans (Ro *et al*., 2010). These models usually have mutations in genes that encode transcription factors important for β-cell identity or protein components of machineries that regulate insulin secretion. A spontaneous mutation in Ins2 in the Akita mouse causes the accumulation of misfolded proinsulin and leads to endoplasmic reticulum stress and ultimately loss of β-cells (Yoshioka *et al*.,

1997; Kleinert *et al*., 2018). DMT1 susceptibility is determined largely by major histocompatibility complex (MHC). Genes encoding MHC class II analog (H2g7 in mice) show the same diabetogenic amino acid substitution (Atkinson and Leiter, 1999). In BB rats, the expression of diabetes requires the presence of at least one MHC class II RTB/Du allele, what is DMT1 susceptibility loci in rats (Chatzigeorgiou *et al*., 2009). Defects in antigen-presenting cell maturation have been noted to produce autoantibodies to insulin, glutamic acid decarboxylase and islet cell antibodies (Pociot and McDermott, 2002). Similar to human beings, ketoacidosis is very severe in the rat models and can not survive without insulin (Chatzigeorgiou *et al*., 2009). However, one of the main disadvantage is, that these models are not very available and post-diabetes maintenance is a major problem to keep the animals healthy (Pravenec, 2010). In addition to predominantly rodent models, pigs, primates or dogs are also used in research of DMT1 (Mellert *et al*., 1998; Fisher *et al*., 2001; He *et al*., 2011). Pancreatectomy in pigs with autotransplantation of the isolated islets reflects islet autotransplantation in humans (Matsumoto, 2011).

Another type of models of DMT1 are models created using a virus (Werf *et al*., 2007). Viruses in animal models are initiating β-cell destruction. The β-cell destruction can be achieved by direct infection of the β-cells or initiation of an autoimmune response against the β-cells (Jun and Yoon, 2003). Viruses used to induce DMT1 include coxsackie B virus, encephalomyocarditis virus and Kilham rat virus (Guberski *et al*., 1991; Shimada and Maruyama, 2004; Jaidane *et al*., 2009). Disadvantage of the virus-induced models is that the outcome is dependent on replication levels of the virus as well as on timing of the infection. Viruses can both induce autoimmunity as well as prevent it depending on the conditions (Herrath *et al*., 2011). Furthemore, it is still unclear to what extent viruses are involved in the pathogenesis of DMT1 in humans (Werf *et al*., 2007).

Type 2 diabetes animal models

DMT2 is a complex metabolic disease in which the pathophysiology is greatly influenced by genetic and environmental factors. Hyperglycaemia occurs as a consequence of the pancreatic islet failure. Failure of the pancreatic islets results in β-cell mass deficiency and increased glucagon secretion (Kahn *et al*., 2014). There are currently many models for DMT2 research, but they must meet three essential criteria for validation. The first criterion is the characteristics of the disease in humans: increased fasting glucose and glucose intolerance. Next criterion

Table 1. Different rodent models of *Diabetes mellitus* type 2 with the signs of metabolic syndrome (Modified according to Panchal and Brown, 2011)

Rodent model	Age (weeks)	Obesity	Hypertension	Dyslipidaemia	Cardiovascular dysfunction	Impaired glucose tolerance	Fatty liver	Kidney dysfunction
ob/ob mice	4	Y	N	N	N	N	N	X
	12	Y	N	N	N	Y	Y	X
	24	Y	N	N	y	Y	Y	X
db/db mice	6	Y	N	N	N	N	N	X
	12-13	Y	N	N	Y	Y	N	X
	20	Y	N	N	Y	Y	Y	X
ZDF rats	12-15	Y	Y	Y	Y	N	N	N
	20	Y	Y	Y	Y	N	Y	N
	31-47	Y	Y	Y	Y	N	Y	Y
Goto-Kakizaki rats	4	N	N	N	N	Y	N	N
	8	N	N	Y	N	Y	Y	N
	20	N	N	Y	Y	Y	Y	N
	60	N	N	Y	Y	Y	Y	Y

Y indicates the presence, N indicates the absence of the signs of metabolic syndrome and X indicates unavailability of the data.

is that the all existing DMT2 treatments in humans are equally effective at reversing DM symptoms in animal models. Finally, they mostly predicted the translation potential of the new therapeutic molecules into results of clinical trials. The most common models are mice and rats due to its small size, high fertility, availability of genetic tools to manipulate its genome and short generation time. Pigs or monkeys, as animal models, can also develop obesity and insulin resistance, however, their postprandial glycemic index often remains quite low as plasma insulin levels increase (Baribault, 2016). The widespread occurrence of metabolic disorders connected with DMT2 in humans means that there is an urgent need to research relevant causes, progression and treatment of the signs. Rodent models reflecting these features differ from each other and it is important to choose the right model for research (Panchal and Brown, 2011). Table 1 shows different rodent models with the signs of metabolic syndrome.

The models of DMT2 are often linked with obesity, which reflects the human condition to DMT2 development. These models may have abnormalities in a single gene or multiple genes related to obesity, glucose intolerance and insulin resistance leading to hyperglycaemia (Kawano *et al*., 1999). Obesity can be achieved through genetic manipulation, naturally occurring mutations or with a high-energy diet (King, 2012). Monogenic obese models include the $Lep^{ob/ob}$ mouse, $Lepr^{db/db}$ mouse and Zucker Diabetic Fatty (ZDF) rat. These rodent models have defect in leptin signalling, what means that leptin induces satiety, because of a lack of functional leptin, which induces hyperphagia and subsequent obesity (Gault *et al*., 2011). Mechanism of the action of leptin and its receptor deficiency is presented in Figure 2 (Panchal and Brown, 2011).

Both $Lep^{ob/ob}$ and $Lepr^{db/db}$ mice are the models designed at the Jackson Laboratory (Hummel *et al*., 1966; Zhang *et al*., 1994). These mice are developing hyperinsulinaemia at around 2 weeks of age and hyperglycaemia starts at 4-8 weeks of age and gradually become obese due to hyperphagia. They have a relative short lifespan (Lindstrom, 2007; Srinivasan and Ramarao, 2007). In addition, $Lep^{ob/ob}$ mice are sterile (Chehab *et al*., 1996).

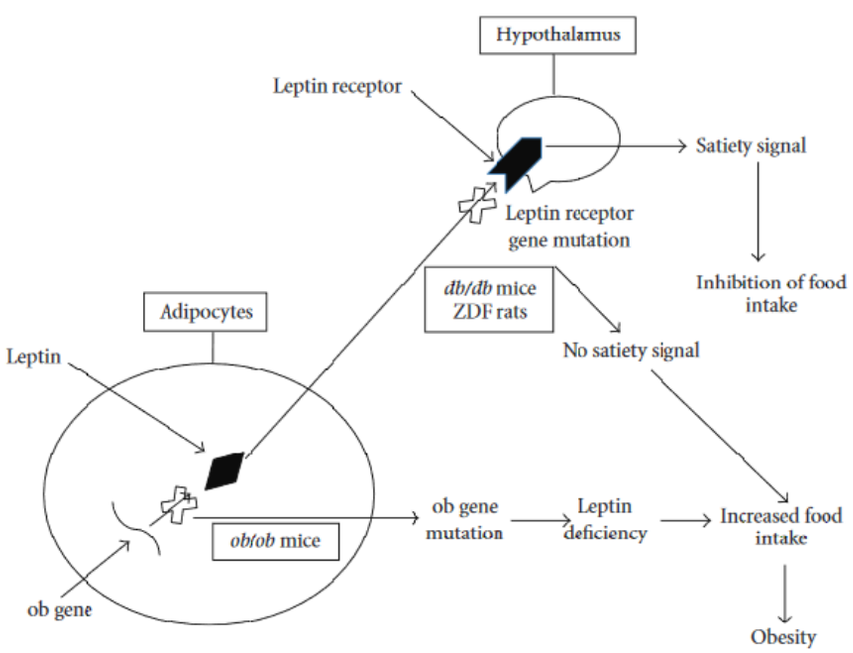

Figure 2. Mechanism of the action of leptin and its receptor deficiency in rodent models (Panchal and Brown, 2011).

Another monogenic obese model, also used in our experiments to describe effects of potential treatments for DMT2 and its complications, is Zucker Diabetic Fatty (ZDF) rat (Capcarova *et al*., 2019; Dupak *et al*., 2020). ZDF rats has origin in the Zucker Fatty (ZF) rats, which were created in 1961 by a crossing between Merck M-strain and Shermanrats. Like previous models, ZF rats have a mutated leptin receptor causing hyperphagia and obesity at around 4 weeks of age. They have also hyperinsulinaemia, hyperlipidaemia, hypertension and impaired glucose tolerance (Srinivasan and Ramarao, 2007). A difference between ZDF and ZF rats is that ZDF rats are less obese, have a higher insulin resistance, show signs of diabetic complications, and diabetes usually develops at 8-10 weeks only in males (Pick *et al*., 1998; Shibata *et al*., 2000). Furthermore, infertility of ZDF males is a problem that is holding back research, which has been addressed by the use of testosterone propionate, which increases the probality of ejaculation and sexual activity. In addition, ZDF rats do not develop hypertension or cardiovascular disease spontaneously (Clark *et al*., 1983; Hemmes and Schoch, 1988). The development of *diabetes* in ZDF rats has become a popular model for preclinical studies of DMT2 due to the fact that these rodents exhibit impaired islet architecture, β-cell degranulation and increased apoptosis of β-cells (Clark *et al*., 1983).

Goto-Kakizaki are spontaneously diabetic, but non-obese rats created by a Japanese group using repetitive breeding of Wistar rats with the poorest glucose tolerance (Goto *et al*., 1976; Yasuda *et al*., 2002). Several distinct genetic lesions exist in Goto-Kakizaki rats, including disorders of β-cell metabolism and function. In combination with chronic hyperglycaemia, inflammation and oxidative stress, disorders of β-cell metabolism and function result in impaired islet architecture and loss of β-cell mass (Portha, 2005; Kleinert *et al*., 2018). They are a lean model of DMT2, which is characterized by glucose intolerance and defective glucose-induced insulin secretion (Ostenson and Efendic, 2007). Studies have confirmed the involvement of macrophages in inducing inflammation around β-cells, leading to altered islet architecture and morphology. Once these islets are distorted, they are unable to secrete insulin in response to glucose, thereby causing β-cell dysfunction. Significantly altered macrophage levels were observed by islet immunohistochemistry with various antibodies such as MHC class II, CD68, CD53 and granulocytes (Homo-Delarche *et al*., 2006). Inflammatory processes of islets in Goto-Kakizaki rats show increased islet expressions of IL – 1β, IL – 6, TNF–α and chemokines including CXCL1/KC, MCP–1 and MIP–1α, that leads to impaired insulin secretion and β-cell dysfunction (Ehses *et al*., 2009). Goto-Kakizaki rats have been used in experiments ranging from β-cell dysfunction in DMT2 to diabetic complications (Okada *et al*., 2010; Giroix *et al*., 2011).

High fat feeding

Diet plays a crucial role in growth as a source of nutrition. An increased caloric intake has been connected with many diet-induced complications including metabolic syndrome, cardiovascular diseases and non-alcoholic fatty liver disease. High fat feeding is used in animal models to reflect these signs and symptoms of human metabolic syndrome associated with DM (Panchal and Brown, 2011). An obesity, hyperinsulinaemia and altered glucose homeostasis are among the most common signs of high fat feeding due to insufficient compensation by the pancreatic islets, which leads to impaired glucose tolerance (Winzell and Ahren, 2004). High fat feeding animal models are one of the best models, because they mimic human situation more accurately than genetic models, since the obesity is induced by environmental manipulation rather than genes (Surwit *et al*., 1995). In the experiments a normal diet (around 26 % protein, 63 % carbohydrate and 11 % fat) is substituted for a high fat diet, which significantly increases the number of calories from fats (about 58 % of the energy obtained from fats). It is necessary to ensure that experimental rodents do not eat less than normal. Studies have shown that they weighed much more than control animals as early as one week after starting a high fat feeding (Winzell and Ahren, 2004). High fat feeding presents the proper etiological, pathological and treatment options, because most patients with DMT2 became ill due to their diets, not to their genetics. From this reason, high fat feeding is the most appropriate disease model. However, creating a suitable protocol for high fat feeding is not easy. High fat feeding models and DMT2 have a complex and overlapping pathophysiology (Figure 3; Heydemann, 2016).

The disadvantage of high fat feeding may be that not all obese animals develop DMT2 or they sometimes become overtly obese and long period is needed since the animals are fed over diet to increase blood glucose level (Hasan *et al*., 2018; Suleiman *et al*., 2020). For studying effects of therapeutics against DMT2 symptoms, high fat feeding model is a valuable choice in preclinical protocols. According to Heydemann (2016) both male and female C57B1/6J mice were used. They were fed starting at 4 weeks to 20 weeks old, using a high fat feed plus high fructose feeding. This was found to be the best option for reflecting the human DMT2. Furthemore, age, exercise and duration of diet should be considered.

Animal models as a subject of antidiabetic research

At present, the goal of diabetic research is to find a cure for DM or at least to find active substances that would alleviate the symptoms of this disease. Preclinical studies use mainly animal models to study antidiabetic agents. The most commonly used models are rodents due to several advantages, such as genomic composition, robust breeding performance, ease of testing, diagnosis, biopsy, autopsy and ethical and economic reasons (Acharjee *et al*., 2013).

Recent studies have focused on the effects of medicinal plants for the prevention and management of DMT2, as it is the most common form of DM. Such plants or their bioactive substances include antioxidant, cardio-protective, anti-inflammatory, anti-microbial, nephro-protective, anti-neoplastic, hepato-protective, immunomodulatory, hypoglycemic and anti-rheumatic effects and, therefore, demonstrated various pharmacological and biological effects on animal models (Kim *et al*., 2017; Pivari *et al*., 2019; Dupak *et al*., 2020). The specific mechanisms of bioactive compounds underlying these effects are still not fully described and understood. Furthermore, the most of antidiabetic effects of *in vivo* studies with natural products have not been verified in clinical studies. Hence, further investigation and application of natural products should be considered (Xu *et al*., 2018). When comparing *in vitro* and *in vivo* methods, *in vitro* are more cell specific and less time consuming than *in vivo*. On the other hand, human organism is a complex system which

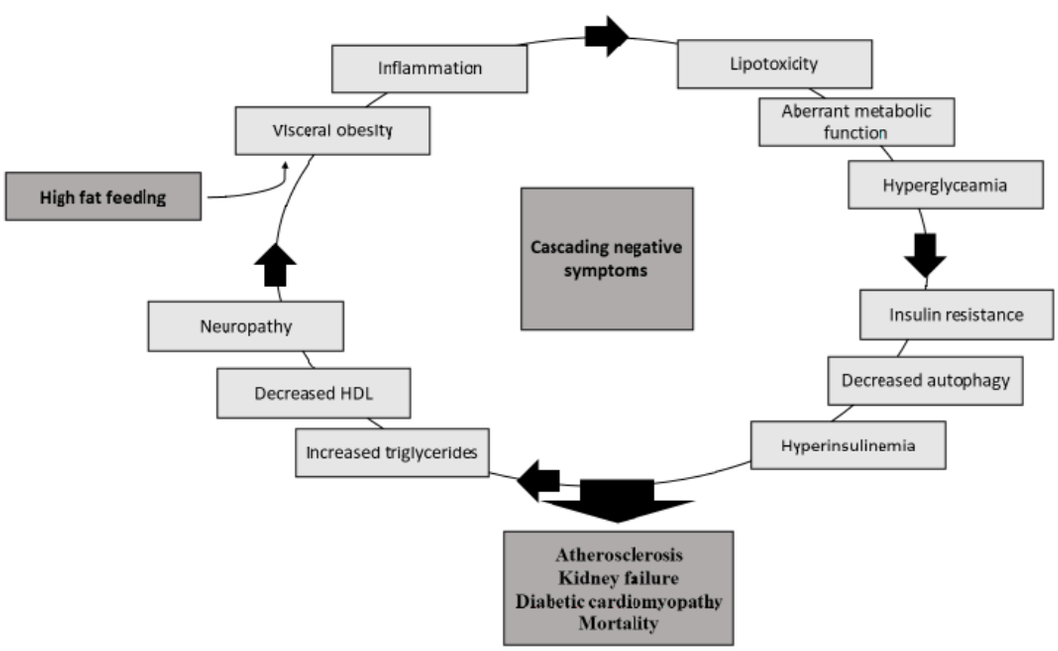

Figure 3. Pathophysiology of type 2 *diabetes mellitus* with high fat feeding impact (Modified according to Heydemann, 2016).

can be better understood by *in vivo* assays. Therefore, *in vitro* studies are recommended for specific research of mechanisms and *in vivo* for developing a drugs or evaluation of toxicological effects (Hasan *et al*., 2018).

CONCLUSION

The prevalence of *diabetes mellitus* is increasing worldwide and animal models play an important role in investigating the pathogenesis of human diabetes and its complications. The aim of this literature-based study was to review and compare the most common animal models reflecting the human *diabetes mellitus*. We conclude that universal animal model for studying diabetes does not exist yet and the choice of a model depends on the purpose of the study. When choosing a right model for either type 1 or 2 diabetes, it is recommended to use different models taking into account the diversity observed in diabetic patients.

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