Comparison of some diabetic and oxidative status parameters in three different experimental type 2 diabetic rat models

ABSTRACT

The aim of the study was to compare the levels of postprandial glucose, oral glucose tolerance test, and malondialdehyde parameters in 3 different experimental type 2 diabetic models induced rats. In the study, 18 Wistar albino rats were divided into 3 groups. The high-fat diet and streptozotocin (35 mg/kg, SC) were administered to the rats in the first group, water containing 20% fructose was administered to the second group, and nicotinamide (110 mg/kg, IP) and streptozotocin (60 mg/kg, SC) were administered to the third group. Oral glucose tolerance test, postprandial glucose, and malondialdehyde analyzes in 3 different experimental type 2 diabetic rat models were performed and they were euthanized at the 70th days. The postprandial glucose level was higher in the 1st and 3rd model groups than in the 2nd model group, while malondialdehyde level was no difference between the groups. Moreover, the second model group was significantly lower than the other two groups at all times according to oral glucose test results. In conclusion, the results of this research will contribute to researchers choosing the right model and parameters in experimental type 2 diabetic models in rats in the future.

Keywords: Malondialdehyde, oral glucose tolerance test, postprandial glucose

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) characterize hyperglycemia, insulin secretion and/or insulin activity defects (Punthakee et al., 2018). In the early stages, T2DM progresses with insulin resistance, hyperinsulinemia, and hyperglycemia, whereas there is observed loss of beta-cell function leading to inadequate synthesis of insulin hormone and elevation of blood glucose levels in the later stages of the disease (Chatterjee et al., 2017; Khan et al., 2020).

Diabetes mellitus, one of the most important metabolic diseases, leads to the loss of millions of lives worldwide (Khan et al., 2020). The global annual prevalence of T2DM is 6.1%, and it is estimated that the number of diabetic individuals will reach 1.3 billion by the year 2050 (Watkins & Ali, 2023) The diabetic complications such as diabetic nephropathy, neuropathy, retinopathy, adversely affects the quality of life in individuals with diabetes mellitus with glycated hemoglobin (HbA1c) levels between 6-9% (Chatterjee et al., 2017; Quinn, 2002). Diabetes mellitus has also been reported in certain animal species such as cats and dogs, similarly to humans (Kerem et al., 2023; Nelson & Reusch, 2014).

Insulin plays a crucial role in the utilization and storage of energy-providing molecules in tissues such as the adipose tissue, skeletal muscles, and liver as well as in maintaining the balance of energy metabolism. Moreover, insulin provides glucose uptake into cells, inhibits hepatic glucose production by suppressing gluconeogenesis and

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Research Article

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glycogenolysis, while promoting glycogen synthesis (Ruan & Lodish, 2003). Insulin resistance is a significant condition for T2DM, and it is characterized decreased insulin receptor sensitivity in muscles and the liver. Additionally, increased gluconeogenesis in the liver leads to elevated glucose release, while impaired glucose oxidation in muscles contributes to an increase in plasma glucose levels. The increased glucose levels are stored as fat in various tissues, primarily in the liver, and it leads to hepatic steatosis. The increased glucose and lipid levels induces oxidative stress, glucotoxicity, lipotoxicity, insulin resistance, and activation of inflammatory pathways in pancreatic β-cells, ultimately disrupting insulin secretion (DeFronzo et al., 2015; Scheen, 2003).

Insulin resistance and impaired glucose tolerance are present in prediabetic patients. The insulin resistance is one of the main treatment targets, especially in the management of T2DM (Sah et al., 2016; Saini, 2010; Young et al., 2019).

In a chronic disease like diabetes mellitus, it is important to control treatment costs and prevent the development of complications. Animal models have historically critical role in investigating the pathophysiology of the disease and evaluating new therapeutic agents in vivo. It is very important to choose the right experimental diabetic rat model for the research and development of new drugs in the treatment of T2DM (Al-Awar et al., 2016).

Until today, many in vivo studies have been conducted on the pathophysiology and treatment of T2DM. However, it is important to choose the right animal model for the research results to be reflected in the clinic. In order to choose the right animal model, the reliability, repeatability and costs of the model should be carefully analyzed (Frode & Medeiros, 2008).

The majority of T2DM models performed in experimental animals are models induced by streptozotocin (STZ). STZ tends to accumulate in pancreatic beta cells via glucose transporter 2 (GLUT2) and it causes cytotoxicity and reduces insulin secretion. The DNA alkylating activity of the methyl-nitrosourea part of STZ creates toxic effects and causes DNA fragmentation. Therefore, STZ-only models are more similar to type 1 diabetes mellitus models (Al-Awar et al., 2016). In addition, the method may cause undesirable effects such as kidney damage, oxidative stress in various organs, inflammation and endothelial dysfunction. It has been emphasized that STZ should be administered together with a high-fat diet in order to achieve symptoms similar to clinical T2DM (hyperglycemia, insulin resistance, altered lipid profile and hyperglycemia) (Ergel & Ertuğrul, 2022; Magalhães et al., 2019).

A more specific (proportional glucose increase and insulin decrease) T2DM model can be created in the diabetes model induced by the application of both STZ and nicotinamide. While STZ causes DNA fragmentation via GLUT2, poly (ADP-ribose) polymerase (PARP-1) activity increases to repair DNA. PARP-1 activity is limited and depletion of NAD+ and ATP in cells is prevented with the protective effect of nicotinamide. In this model, administration of nicotinamide before STZ prevents complete damage to β cells. Thus, hyperglycemia occurs while the insulin level decreases slightly (Szkudelski, 2012). Fructose causes chronic hyperinsulinemia and obesity. T2DM develops as a result of developing obesity and oxidative stress. However, this pattern can take weeks to induce (Basciano et al., 2005).

In the current study, it was aimed to reveal oral glucose test analysis, malondialdehyde (MDA) and post prandial glucose levels at the 10th week in 3 different experimental T2DM models in rats.

MATERIALS AND METHODS

Experimental design

In this study, 18 Wistar Albino male rats (8-12 weeks) were divided 3 groups. Experimental T2DM diabetes was induced in these rats in 3 different ways.

1. Model Group (n=6): In this model, animals were fed with a high-fat diet (58% of metabolic energy from animal fat) for two weeks and then low-dose STZ (STZ, 35 mg/kg, sc) was administered. Animals with a fasting blood glucose level ≥ 250 mg/dL were considered to have T2DM (Soetikno et al., 2020; Srinivasan et al., 2005).
2. Model Group (n=6): In this model, animals were given drinking water containing 20% fructose throughout the experiment (Incir et al., 2016).
3. Model Group (n=6): In this model, animals were injected with nicotinamide (i.p.) at a dose of 110 mg/kg and 15 minutes after this injection, STZ injection (s.c.) was administered at a dose of 60 mg/kg (Sayeli & Shenoy, 2021).

The blood samples were taken from the heart under thiopental Na anesthesia (40 mg/kg, ip) at the end of the 10th week, and the serum were frozen at -80°C until analysis.

Oral glucose tolerance test (OGTT)

The animals were fasted for 12 hours and fasting glucose (0th hour) was detected with glucose test strips (VivaCheck Eco, China) for OGTT analysis on the last day of the experiment. Subsequently, glucose at a dose of 2 g/kg was administered to all rats by oral gavage. Then, blood samples were taken from the tail vein at the 30th, 60 th, 90th and 120th minutes and blood glucose values were determined with glucose test strips.

Biochemical analyzes

Post prandial glucose was measured from frozen serum samples using by autoanalyzer (Abbott c8000, Chicago, USA). MDA (Rat Malondialdehyde Cat No: E0156Ra, Bioassay Technology Laboratory, Shangai, China) was analyzed on an ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200) via a rat-specific commercial ELISA kit.

Statistical analysis

The data were statistically analyzed using the SPSS 25.0 program (SPSS, Inc, Chicago, IL, USA). They were analyzed using one-way analysis of variance (ANOVA) and post hoc Duncan test. P<0.05 value was considered statistically significant.

RESULTS

The postprandial glucose levels and MDA levels in rats in which T2DM was induced with three different experimental models are presented in Figure 1 and Figure 2, respectively. Although the MDA level decreased in group 3, there was no statistical change between the groups. While the post prandial serum glucose level was highest in the third model group, the first model group was statistically similar to the third model group. The glucose level in the second group did not exceed the limit for diabetes in rats (250 mg/dL).

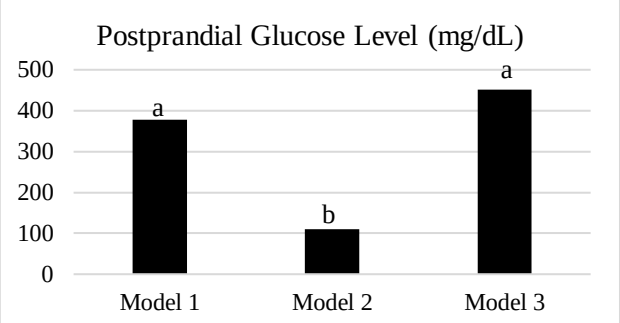

Figure 1. The postprandial glucose level in three different experimental T2DM models in rats.

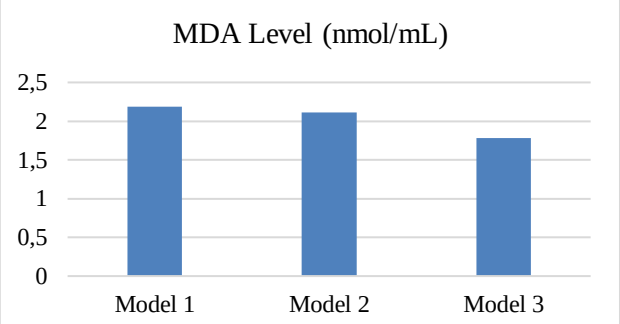

Figure 2. The MDA levels in three different experimental T2DM models in rats.

The OGTT results at the end of the experiment are presented in Table 1. The glucose level in OGTT was statistically lower in the second model group than in the other groups at all sampling times.

Table 1. Effects of three different experimental T2DM models on oral glucose tolerance test in rats (mean ± SD).

Sampling Time/Groups	1.Model Group	2.Model Group	3.Model Group
OGTT 0. min	275.0±114.9[a]	113.5±24.8[a]	305.8±195.1[a]
OGTT 30. min	447.8±127.9[a]	141.0±8.2[b]	398.8±136.3[a]
OGTT 60. min	418.8±127.0[a]	147.0±16.4[b]	452.8±131.4[a]
OGTT 90. min	430.0±116.1[a]	153.3±56.6[b]	436.3±128.9[a]
OGTT 120. min	409.5±168.5[a]	119.8±3.71[b]	380.7±144.4[a]

[a,b] : Values within line with no common superscripts are significantly different(P <0.05).

DISCUSSION

T2DM is a chronic metabolic disease characterized by high glucose levels. Early diagnosis of the pathophysiological changes in diabetes is important for the treatment of the disease and costs (Al-Awar et al., 2016; Chatterjee et al., 2017; Khan et al., 2020). Diabetes models induced in various ways are needed to evaluate the disease diagnostically and to determine the treatment effectiveness of various substances (Al-Awar et al., 2016; Incir et al., 2016; Sayeli & Shenoy, 2021; Soetikno et al., 2020; Srinivasan et al., 2005).

The inflammation and insulin resistance developed in rats fed a high-fat diet for 10 weeks (Lee et al., 2011). The glucose levels increased after 30 days in rats in which an experimental diabetes model was induced with high-fat diet and STZ. In addition, glucose levels after OGTT analysis and high HOMA-IR index showed that insulin resistance developed (Magalhães et al., 2019). In the 20% fructose-induced diabetes model, glucose level increased after 8 weeks (Mamikutty et al., 2014). In the T2DM model induced by STZ and nicotinamide, insulin resistance developed and glucose level increased on the 30th day (Sayeli & Shenoy, 2021). The hyperglycemia and oxidative stress caused renal and liver degeneration after 45 days in rats induced by STZ (65 mg/kg) and nicotinamide (110 mg/kg) application (Murugan & Pari, 2006). High glucose is reabsorbed into the bloodstream by the kidneys and contribute to hyperglycemia, which continues in a vicious cycle. As a result, this condition further contributes to abnormal glucose homeostasis, causing insulin resistance (DeFronzo et al., 2012). When blood glucose concentration does not return to baseline levels within 60 minutes after glucose challenge, insulin secretion has reduced, increasing the risk of insulin resistance and T2DM (Abdul-Ghani et al., 2010).

In the current study, post prandial glucose and glucose levels after OGTT analysis were higher in the STZ-high fat diet and STZ-nicotinamide groups (Models 1 and 3 group) during the experiment, compared to other models. In these groups, hyperglycemia may have occurred due to β cell damage caused by STZ leading to decreased insulin secretion. However, the experimental period may be short since fructose may increase glucose by causing insulin resistance. It can be speculated that model 1 and model 3 are more realistic for future short-term T2DM models and that the possibility of insulin resistance may be higher in these models according to the post prandial glucose and OGTT results.

Previous studies have shown that MDA increases due to the development of T2DM (Ibuki et al., 2020). Additionally, MDA levels have been shown to increase significantly as a complication of T2DM (Lu et al., 2010). Lipid peroxidation (MDA), which occurs due to hyperglycemia, can be inhibited by antioxidant enzymes in living organisms. However, the

levels of both free radicals and antioxidant enzymes increase with the development of diabetic complications in the later stages of the disease (Ahmed et al., 2006). In the current study, MDA level may have increased in all three T2DM model groups due to diabetic complications and no difference was observed between the models.

CONCLUSION

Researcher are carried out on different models on experimental animals for various complications and treatments of T2DM disease. However, it is very important for researchers that these experimental models should cheap, easy to create, and reflect the real symptoms of the disease. In the current study, the model in which STZ and nicotinamide combined model and the high-fat diet and STZ combined model were generally similar. However, the model created with 20% fructose did not reflect T2DM very well. As a result, current research results will be guiding for the experimental T2DM rat model that researchers will choose based on postprandial glucose and oral glucose tolerance testing. However, new experimental designs are required in which more parameters are analyzed, and longer-term studies are conducted.

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Conflict of interest: The authors declare no conflicts of interest.

Ethical statement or informed consent: The study procedure was approved by Selcuk University Veterinary Faculty Ethics Committee (Ethical approval number 2024/083).

Author contributions: BD: Design of the project, creation of the model and preparation of the text by making statistical evaluations. OT: Carrying out the treatment protocol, performing analyzes and contributing to the writing of the text by evaluating the analysis results. TMP: Carrying out the treatment protocol, performing analyzes and contributing to the writing of the text by evaluating the analysis results.

Availability of data and materials: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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