

RESEARCH ARTICLE

Molecular and Biochemical Comparison of Various Rat Models for Experimental Diabetes Induction

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ABSTRACT

The study aimed to compare three different experimental type 2 diabetes mellitus (T2DM) models performed on rats. 3 equal groups of 54 Wistar albino rats were randomly categorized as follows; first group: The rats treated by high-fat diet and streptozotocin (35 mg/kg, SC), second group: The rats received drinking water containing 20% fructose, and third group: Nicotinamide (110 mg/kg, IP) and streptozotocin (60 mg/kg, SC) were administered. Six animals from each group were euthanized at 6, 10 and 12 weeks to evaluate the diabetic process in the models. Glucose and HbA1c levels tended to be higher in the third model, though overall profiles were comparable to first model group. In the early phases, the first model group's triglyceride levels were statistically greater than those of the other groups, and its insulin levels dropped over time. The GLUT4, IRS1 and PIK3R expressions in skeletal muscle decreased in the first and third model groups. Based on practical considerations, the third model appeared to be more feasible for long-term studies and showed a better reflection of the T2DM phenotype; however, the first model demonstrated more pronounced effects in the short-term. The findings of this research may provide valuable insights for future studies by guiding researchers in selecting the appropriate experimental model for T2DM research in rats and will be a guide for developing new drugs and treatments in the search.

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease marked by elevated blood sugar, decreased insulin production, and/or reduced insulin receptor activity and post-receptor pathways (insulin resistance) (Nelson *et al.*, 2014, Kottaisamy *et al.*, 2021). The development of diabetes in dogs involves genetic factors such as the dog leukocyte antigen gene complex and autoantibodies, whereas in cats, the disease predominantly exhibits characteristics similar to type 2 diabetes, often associated with obesity, insulin resistance, and amyloid deposition in the islets of Langerhans (Hussein *et al.*, 2024; Dik *et al.*, 2025).

Insulin receptor substrate (IRS)-1 translocates to the plasma membrane to interact with insulin after being phosphorylated with tyrosine. Phosphatidylinositol 3-kinase (PI3-K), activated by IRS1, activates Pdk1 and phosphatidylinositol 3,4,5-trisphosphate (PIP3) (Mori *et al.*, 2009; Zhou *et al.*, 2022; Patra *et al.*, 2024). The

translocation of glucose transporter 4 (GLUT4) to the cell membrane, which permits glucose entry, is facilitated by the phosphorylation of serine/threonine kinases (Akt) by activated PI3K, PIP3, and Pdk1 (Mori *et al.*, 2009; Sah *et al.*, 2016; Zhou *et al.*, 2022). Insulin resistance is caused by abnormalities in the tyrosine phosphorylation of the insulin receptor as well as in the activities of GLUT4, PI3 kinase subunits, IRS1, and PIK3R1 (Mori *et al.*, 2009; Nelson *et al.*, 2014, Lee *et al.*, 2022).

Insulin resistance is a significant symptom for T2DM. Therefore, insulin receptor sensitivity reduces in muscles and the liver. Increased gluconeogenesis, glucose release, and impaired glucose oxidation in muscles due to insulin resistance contribute to elevated plasma glucose levels. The glucose and lipids induce oxidative stress, glucotoxicity, insulin resistance, and activation of inflammatory pathways in pancreatic beta cells, disrupting insulin secretion (Rand *et al.* 2013; Nelson *et al.*, 2014; Lee *et al.*, 2022).

Animal models play a crucial role in investigating the etiology of the disease and evaluating the efficacy of novel therapeutic agents and in vivo treatments (AlMasoud *et al.*, 2024). Therefore, diabetic rat models are necessary for the fundamental researches to understand the diabetic background and development of new drugs for T2DM (Al-Awar *et al.*, 2016; Belhan *et al.* 2023; Singh *et al.*, 2024).

Streptozotocin (STZ) selectively accumulates in pancreatic β -cells via GLUT2 transporters, leading to DNA damage, reduced insulin secretion, and apoptosis (Al-Awar *et al.*, 2016) while effective, high doses of STZ may cause systemic toxicity, including kidney damage and oxidative stress (Habib *et al.*, 2024; Yasin *et al.*, 2025; Abdulllah *et al.*, 2024). To better replicate the pathology of T2DM, STZ is often combined with a high-fat diet, which leads to increased circulating free fatty acids (FFAs) and the establishment of the disease model (Magalhães *et al.*, 2019; Guo *et al.* 2022). These FFAs contribute to insulin resistance by impairing insulin signaling pathways, particularly through inhibition of IRS/PI3K/GLUT4 and promoting serine phosphorylation of IRS proteins (Elkanawati *et al.*, 2024).

In the T2DM model, nicotinamide partially protects insulin-secreting cells against STZ, which causes damage to pancreatic beta cells. This approach allows for the creation of a more specific (optimum glucose elevation and insulin reduction) T2DM model with lower doses of STZ (Mokhtare *et al.*, 2018). STZ causes DNA fragmentation through the GLUT2 pathway, and the physiological system repairs DNA by increasing poly (ADP-ribose) polymerase (PARP-1) activity. Nevertheless, high PARP-1 activity causes intracellular NAD⁺ and ATP to be depleted, which causes insulin-secreting cells to necrosis. Nicotinamide protects against this by limiting PARP-1 activity and preventing the loss of NAD⁺ and ATP and consequently protects beta cells. It is emphasized that this model better mimics T2DM disease by partially limiting insulin secretion (Szkudelski, 2012).

Fructose chronically leads to hyperinsulinemia and obesity. However, excessive fructose intake leading to the formation of advanced glycation end products (AGEs) and lipid peroxidation, both of which generate free radicals (Iqbal *et al.*, 2024). Additionally, excessive fructose consumption bypasses insulin-regulated metabolic control and promotes uncontrolled hepatic gluconeogenesis and lipogenesis, primarily through the activation of lipogenic transcription factors such as sterol regulatory element-binding protein 1 (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP). This metabolic dysregulation contributes to hepatic lipid accumulation, insulin resistance, and ultimately the development of obesity and T2DM (Basciano *et al.*, 2005; Zarfeshani *et al.*, 2012). However, fatty diet or fructose models are generally long and costly (Reuter, 2007). In addition, the development of insulin resistance may take a long time in fructose-induced diabetes models (Wilson Islam, 2012).

The research aims to compare the diabetic status in three different experimental T2DM models in rats at different time periods. It is hypothesised that three distinct experimental T2DM models can be used to assess insulin resistance, hyperglycemia, and decreased beta cell function in rats using biochemical and molecular techniques. The

novel aspect of the current research is the determination and comparison of the molecular (particularly on the insulin pathway) and multifaceted biochemical effects of three distinct diabetes models.

MATERIALS AND METHODS

Animals and Diet: Fifty-four female Wistar Albino rats, weighing an average of 220-250 g and aged 8–12 weeks, were housed in cages. Prior to the experiment, measurements of the basal lipid profiles and fasting blood glucose levels were performed along with general health examinations. The Selcuk University Chair of Experimental Medicine Research and Application Center's Ethics Committee approved the study (Approval number: 2022-33).

Experimental T2DM models and experimental groups design: Three groups were randomly selected from among the rats.

1. Model Group (n=18): The rats assigned to the high-fat diet (HFD)+STZ group were initially fed a high-fat diet (Table 1) for two weeks, during which 58% of the total metabolic energy was derived from animal fat. This dietary intervention was employed to induce peripheral insulin resistance. Following the high-fat feeding period, animals received a single subcutaneous injection of STZ at a moderate dose of 35 mg/kg. STZ was freshly prepared prior to each administration using in 0.1 mmol/L cold citrate buffer (pH ~4.5), protected from light, and used within 15 minutes to preserve its stability and biological activity. Subsequently, the animals were deemed to have developed T2DM (fasting blood glucose levels $\geq$ 250 mg/dL) (Dik *et al.*, 2024).

Table 1: Composition of the experimental high-fat diet.

Composition	% Ratio
Vegetable oil	3,00
Animal fat	37,00
Yellow corn	30,50
Casein (Dried)	20,00
Soya Meal (%48)	4,50
Dicalcium Phosphate	1,70
DL-Methionin	0,20
Limestone	1,60
Salt	0,50
Vitamin-Mineral Mix	1,00

2. Model Group (n=18): This T2DM model was established based on fructose by (Incir *et al.*, 2016). The rats in this model consumed drinking water containing 20% fructose throughout the experimental period.

3. Model Group (n=18): For the T2DM model, the rats were fasted the overnight before receiving an intraperitoneal (IP) injection of 110 mg/kg nicotinamide dissolved in 0.9% NaCl (Sayeli and Shenoy, 2021). A subcutaneous injection of streptozotocin (60 mg/kg) prepared in 0.1 mmol/L cold citrate buffer (pH ~4.5) was administered 15 minutes after the preceding injection.

The experiment was conducted for 12 weeks in all groups. The blood samples were collected from the hearts of six rats in each group at the end of the 6th, 10th, and 12th weeks to monitor insulin resistance and other diabetic

markers over time. After a 12-hour fasting period, blood samples were collected, and the animals were subsequently euthanized under thiopental sodium anesthesia (40 mg/kg, IP) using the decapitation method at the experimental.

After euthanasia, the skeletal muscle tissues from the hind limbs of the animals were placed in cyro tubes for molecular analyses and frozen at -80°C. The HbA1c parameter was measured from blood samples collected in EDTA tubes using an HbA1c analyzer (Trinity Biotech, Premier Hb9210, Ireland). Blood samples collected in gel tubes (serum-separated) were centrifuged at 4000 rpm, and the serum was aliquoted into Eppendorf tubes and frozen at -80°C.

Oral glucose tolerance test (OGTT): Before the Oral Glucose Tolerance Test (OGTT) on the last day of the trial, all rats were fasted for twelve hours. Fasting blood glucose levels (0 hours) were measured from the tail vein using glucose test strips (VivaCheck Eco, China). The rats then received a dose of 2 g/kg of glucose dissolved in saline orally. Blood glucose levels were assessed at 30, 60, 90, and 120 minutes following the application of glucose using tail vein samples.

Biochemical and endocrinological analyses: At the time of measurement, frozen serum samples were thawed to room temperature. Biochemical parameters [Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), fasting glucose, blood urea nitrogen (BUN), creatinine, low density lipoprotein (LDL), high density lipoprotein (HDL), cholesterol, triglycerides, total protein, etc.] were assessed using an autoanalyzer (Abbott c8000, Chicago, USA). The insulin levels (Rat Insulin, Cat No: E0707Ra, Bioassay Technology Laboratory, Shanghai, China) were measured using rat-specific commercial ELISA kits with an ELISA reader (Bio-Tek Instruments Inc., MWGt Lambda Scan 200)

A final protein content of 90–100 mg/mL was obtained by homogenizing the rat liver tissues in phosphate-buffered saline (PBS). The glucose-6-phosphatase (Rat Glucose-6-phosphatase, Cat No: E1982Ra, Bioassay Technology Laboratory, Shanghai, China) and hexokinase (Rat Hexokinase, Cat No: E0330Ra, Bioassay Technology Laboratory, Shanghai, China) parameters were examined from liver homogenates using rat-specific commercial ELISA kits.

Molecular analyses: The expression levels of IRS-1, PIK3R1, and GLUT4 genes in muscle tissue were analyzed by the real-time PCR (RT-qPCR) method, to determine insulin resistance-related molecular mechanisms. The primer sequences used for the gene expression analysis of IRS1, GLUT4, PIK3R1, and GAPDH are listed in Table 2.

Initially, 30 mg of muscle tissue was obtained from rats, and homogenized in liquid nitrogen. Total RNA isolations were performed using a commercial RNA extraction kit (RF-1 total RNA isolation kit, Vivantis, USA) following the directions of the kit. RNA quantification (quality, concentration) measurements were carried out with spectrophotometric analysis (NanoDrop, Thermo Fisher Scientific, USA). Isolated RNAs were converted to cDNA using a 2-step cDNA synthesis kit (RTPL12®, Vivantis, Malaysia) according to the supplier's guidelines.

Table 2: Primer Sequences Used for Gene Expression Analysis in Experimental Rat Models of Diabetes

Gene	Primer sequence (5'–3')	Product length (bp)	Reference
IRS1	Forward: GTGGAGTTGAGTTGGGCAGA	227	Zheng et al 2022
	Reverse: CCTGTCCGCATGTCAGCATA		
GLUT4	Forward: CCAGTATGTTGCGGATGCTATG	171	Zheng et al 2022
	Reverse: TGGTTTCAGGCACTCTTAGGA		
PIK3R1	Forward: CGGTCGTAGCTGGATCGAAG	363	Designed by using NCBI primer blast
	Reverse: GTCCGTCAGAGCACAAAGGA		
GAPDH	Forward: ATCAACGGGAAACCCATC	201	Zheng et al 2022
	Reverse: GAAGACGCCAGTAGACTCCA		

Appropriate primers were determined for target genes and the housekeeping gene GAPDH was used for normalization.

The SYBR green qPCR master mix was used for RT-qPCR. The target gene expression levels were interpreted through the calculation of delta Ct (Δ Ct) obtained in real-time PCR.

The Δ Ct value represents a doubling of the DNA amount in each PCR cycle, and the Ct value is inversely proportional to the amount of DNA. Therefore, the high expression levels have low Δ Ct values while a high Δ Ct value indicates low expression levels.

Statistical analyses: Statistical significance of the data was calculated by one-way ANOVA followed by Duncan post-hoc test using the SPSS 25.0 software (SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was considered significant.

RESULTS

The effects of diabetes on the biochemical parameters in rats in three distinct experimental models of T2DM:

The impact of diabetes at 6, 10, and 12 weeks on biochemical parameters in rats with three different experimental models of T2DM were presented in Table 3. At six weeks, the third model group's serum glucose levels were statistically greater than those of the other groups ($P < 0.05$). The glucose levels at 10 and 12 weeks were highest in the 3rd model group, while the 1st model group was statistically similar to the 3rd model group. HbA1c levels in the 3rd model group had the highest levels at three sampling times. However, the HbA1c level in the first model group was statistically comparable to that in the third model group, at six weeks. Additionally, the HbA1c level in the second model group were the lowest at all three sampling times.

The first model group's triglyceride levels were considerably greater than those of the other groups at both 6 and 10 weeks. At six weeks, the first model group's cholesterol levels were greater than those of the other groups ($P < 0.05$), but there was no distinction in the groups' levels at later sampling points. HDL levels were statistically lower in the 2nd and 3rd model groups compared to the 1st model group at 6 weeks ($P < 0.05$), while its levels in the 3rd model group at 10 weeks were lowest, and lowest

in the 2nd model group at 12 weeks. At six weeks, the first model group had the highest LDL levels; however, at ten weeks, there were no differences between the groups. LDL levels were highest in the 2nd model group at 12 weeks.

The second model group's AST levels were statistically greater than those of the other groups at six weeks ($P<0.05$). At 10 weeks, there was no significant difference observed across the groups. However, the third model group's AST level was significantly greater than that of the other groups at 12 weeks ($P<0.05$). The second model group's ALT levels were statistically greater than those of the other groups at six weeks ($P<0.05$). At 10 and 12 weeks, there was no difference between the groups. At six weeks, total protein levels were significantly higher in the first and third model groups than in the second model group ($P<0.05$), and at ten weeks, the levels in the second model group were significantly higher ($P<0.05$) than in the other groups. No significant difference was observed between groups at 12 weeks.

Creatinine levels had no difference between the three model groups at all three sampling times. At each of the three sample times, the third model group's BUN levels were statistically greater than those of the other groups ($P<0.05$).

The effects of diabetes on endocrinological and enzymatic parameters in rats in three distinct experimental models of T2DM: The results of endocrinological and enzymatic parameters in rats with experimentally induced T2DM by three different methods

were presented in Table 4. There was no statistical difference between groups for liver glucose-6-phosphate levels at 6, 10, and 12 weeks. However, liver hexokinase levels were statistically higher in the 3rd model group at 12 weeks compared to other groups. Insulin levels have no difference between groups at 6 and 10 weeks. However, insulin levels were lower in the 1st model group compared to other groups at 12 weeks.

The effects of diabetes on the expressions of GLUT4, IRS1, and PIK3R in rats in three distinct experimental models of T2DM: GLUT4, IRS1, and PIK3R gene expressions in the muscle tissue of rats with experimental T2DM were presented in Figure 1. Although there was no statistical difference between groups for GLUT4, IRS1, and PIK3R1 gene expression at any week, it was clearly determined that the expression levels of these important molecular actors in T2DM development had changed. GLUT4, IRS1, and PIK3R1 expression levels were more downregulated in the 3rd model group compared to the other groups at week 10.

The effects of diabetes on oral glucose tolerance test in rats in three distinct experimental models of T2DM: The OGTT results at the end of the experiment were presented in Table 5. OGTT glucose level in the 2nd model group was statistically lower than the other groups, while its level was statistically higher in the 3rd model group than the other groups at 60, 90 and 120 minutes.

Table 3: Effects of different experimental T2DM models on some biochemical parameters in rats (mean $\pm$ SD)

Time	6 th Week			10 th Week			12 th Week		
Parameters	1.Model Group	2.Model Group	3.Model Group	1.Model Group	2.Model Group	3.Model Group	1.Model Group	2.Model Group	3.Model Group
Glucose(mg/dL)	273.5 $\pm$ 32.7 ^b	113.16 $\pm$ 31.0 ^c	517.7 $\pm$ 132.9 ^a	275.0 $\pm$ 114.9 ^{ab}	113.5 $\pm$ 24.8 ^b	305.8 $\pm$ 195.1 ^a	538.0 $\pm$ 214.3 ^a	57.8 $\pm$ 35.3 ^b	558.3 $\pm$ 35.0 ^a
HbA1c (%)	9.9 $\pm$ 2.7 ^a	5.4 $\pm$ 0.3 ^b	11.5 $\pm$ 1.3 ^a	9.2 $\pm$ 2.3 ^b	4.8 $\pm$ 0.3 ^c	11.8 $\pm$ 2.3 ^a	10.2 $\pm$ 1.3 ^b	5.0 $\pm$ 0.2 ^c	13.5 $\pm$ 0.5 ^a
Triglyceride (mg/dL)	224.2 $\pm$ 76.7 ^a	103.0 $\pm$ 20.3 ^b	80.5 $\pm$ 69.4 ^b	441.3 $\pm$ 253.2 ^a	225.7 $\pm$ 84.7 ^b	137.7 $\pm$ 85.6 ^b	175.8 $\pm$ 99.4 ^a	167.0 $\pm$ 63.4 ^a	243.0 $\pm$ 112.8 ^a
Cholesterol (mg/dL)	96.0 $\pm$ 12.9 ^a	34.5 $\pm$ 9.5 ^b	59.0 $\pm$ 25.7 ^c	106.7 $\pm$ 33.5 ^a	98.2 $\pm$ 11.7 ^a	82.8 $\pm$ 18.7 ^a	61.7 $\pm$ 19.5 ^a	63.6 $\pm$ 23.8 ^a	83.0 $\pm$ 11.4 ^a
HDL (mg/dL)	60.06 $\pm$ 5.0 ^a	12.04 $\pm$ 4.4 ^c	43.7 $\pm$ 7.1 ^b	48.7 $\pm$ 12.4 ^{ab}	54.9 $\pm$ 6.8 ^a	40.1 $\pm$ 10.0 ^b	35.9 $\pm$ 10.9 ^{ab}	28.0 $\pm$ 13.8 ^b	44.5 $\pm$ 7.8 ^a
LDL (mg/dL)	28.0 $\pm$ 4.9 ^a	18.0 $\pm$ 5.1 ^b	22.2 $\pm$ 6.8 ^{ab}	37.2 $\pm$ 11.7 ^a	29.0 $\pm$ 5.3 ^a	25.2 $\pm$ 11.2 ^a	19.8 $\pm$ 2.2 ^b	25.4 $\pm$ 6.5 ^a	22.0 $\pm$ 2.45 ^{ab}
Total Protein	73.2 $\pm$ 4.3 ^a	54 $\pm$ 5.8 ^b	72.27 $\pm$ 2.9 ^a	80.5 $\pm$ 5.8 ^b	97.6 $\pm$ 4.0 ^a	79.1 $\pm$ 17.2 ^b	70.6 $\pm$ 5.2 ^a	107.0 $\pm$ 71.2 ^a	71.0 $\pm$ 7.1 ^a
ALT (U/L)	55.3 $\pm$ 10.4 ^b	357.5 $\pm$ 144.3 ^a	92.6 $\pm$ 54.8 ^b	82.0 $\pm$ 93.6 ^a	71.8 $\pm$ 22.7 ^a	103.0 $\pm$ 54.2 ^a	37.5 $\pm$ 13.1 ^a	44.8 $\pm$ 15.2 ^a	263.0 $\pm$ 316.2 ^a
AST (U/L)	104.3 $\pm$ 17.7 ^b	466.0 $\pm$ 202.0 ^a	192.8 $\pm$ 87.7 ^b	127.2 $\pm$ 70.0 ^a	213.3 $\pm$ 88.5 ^a	180.7 $\pm$ 85.3 ^a	107.0 $\pm$ 104.9 ^b	91.4 $\pm$ 34.4 ^b	428.0 $\pm$ 367.6 ^a
Creatinin (mg/dL)	0.65 $\pm$ 0.11 ^a	0.4 $\pm$ 0.16 ^a	0.78 $\pm$ 0.25 ^a	0.91 $\pm$ 0.15 ^a	0.86 $\pm$ 0.15 ^a	0.73 $\pm$ 0.23 ^a	0.84 $\pm$ 0.1 ^a	0.76 $\pm$ 0.1 ^a	0.76 $\pm$ 0.1 ^a
BUN	32.3 $\pm$ 12.2 ^c	54.3 $\pm$ 19.1 ^{ab}	60.8 $\pm$ 26.0 ^a	23.8 $\pm$ 5.53 ^b	16.3 $\pm$ 1.4 ^b	36.2 $\pm$ 14.6 ^a	21.8 $\pm$ 4.9 ^b	18.40 $\pm$ 6.3 ^b	46.2 $\pm$ 8.9 ^a

^{a, b, c}: Values within line with no common superscripts are significantly different ($P<0.05$). HbA1c (%): Hemoglobin A1c, HDL: High density cholesterol, LDL: Low density cholesterol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, BUN: Blood Urea Nitrogen.

Table 4: Effects of different experimental T2DM models on endocrinologic and oxidative parameters in rats (mean $\pm$ SD)

Time	6 th Week			10 th Week			12 th Week		
Parameters	1.Model Group	2.Model Group	3.Model Group	1.Model Group	2.Model Group	3.Model Group	1.Model Group	2.Model Group	3.Model Group
Liver Glucose 6 phosphate (U/mL)	8.4 $\pm$ 1.5 ^a	9.04 $\pm$ 1 ^a	9.0 $\pm$ 1.2 ^a	6.6 $\pm$ 3.0 ^a	4.6 $\pm$ 3.0 ^a	7.6 $\pm$ 1.6 ^a	6.3 $\pm$ 0.8 ^a	5.5 $\pm$ 2.9 ^a	7.3 $\pm$ 0.8 ^a
Liver Hexokinase (ng/mL)	4.3 $\pm$ 0.6 ^a	3.5 $\pm$ 1.9 ^a	4.1 $\pm$ 0.3 ^a	3.4 $\pm$ 0.6 ^a	3.0 $\pm$ 1.5 ^a	3.5 $\pm$ 0.3 ^a	3.5 $\pm$ 0.2 ^b	3.6 $\pm$ 0.6 ^{ab}	4.4 $\pm$ 1.0 ^a
Serum Insulin (IU/L)	5.84 $\pm$ 1.8 ^a	6.18 $\pm$ 1.7 ^a	4.57 $\pm$ 1.4 ^a	5.57 $\pm$ 0.6 ^a	4.85 $\pm$ 0.7 ^a	5.12 $\pm$ 0.8 ^a	3.69 $\pm$ 0.9 ^b	4.94 $\pm$ 0.7 ^{ab}	5.46 $\pm$ 1.2 ^a

^{a, b, c}: Values within line with no common superscripts are significantly different ($P<0.05$).

Table 5: Effects of different experimental T2DM models on oral glucose tolerance test in rats (mean $\pm$ SD)

Time	12 th Week		
Parameters	1.Model Group	2.Model Group	3.Model Group
OGTT 0. Min	355.5 $\pm$ 24.34 ^a	98.6 $\pm$ 14.79 ^b	414.8 $\pm$ 171.43 ^a
OGTT 30. Min	391.5 $\pm$ 30.38 ^a	111.6 $\pm$ 14.64 ^b	469.4 $\pm$ 189.89 ^a
OGTT 60. Min	452.6 $\pm$ 57.54 ^b	141.2 $\pm$ 29.61 ^c	580.2 $\pm$ 34.21 ^a
OGTT 90. Min	414.0 $\pm$ 35.31 ^b	109.4 $\pm$ 16.52 ^c	568.6 $\pm$ 43.41 ^a
OGTT 120. Min	388.8 $\pm$ 73.20 ^b	104.0 $\pm$ 13.51 ^c	565.6 $\pm$ 76.92 ^a

^{a, b, c}: Values within line with no common superscripts are significantly different ($P<0.05$).

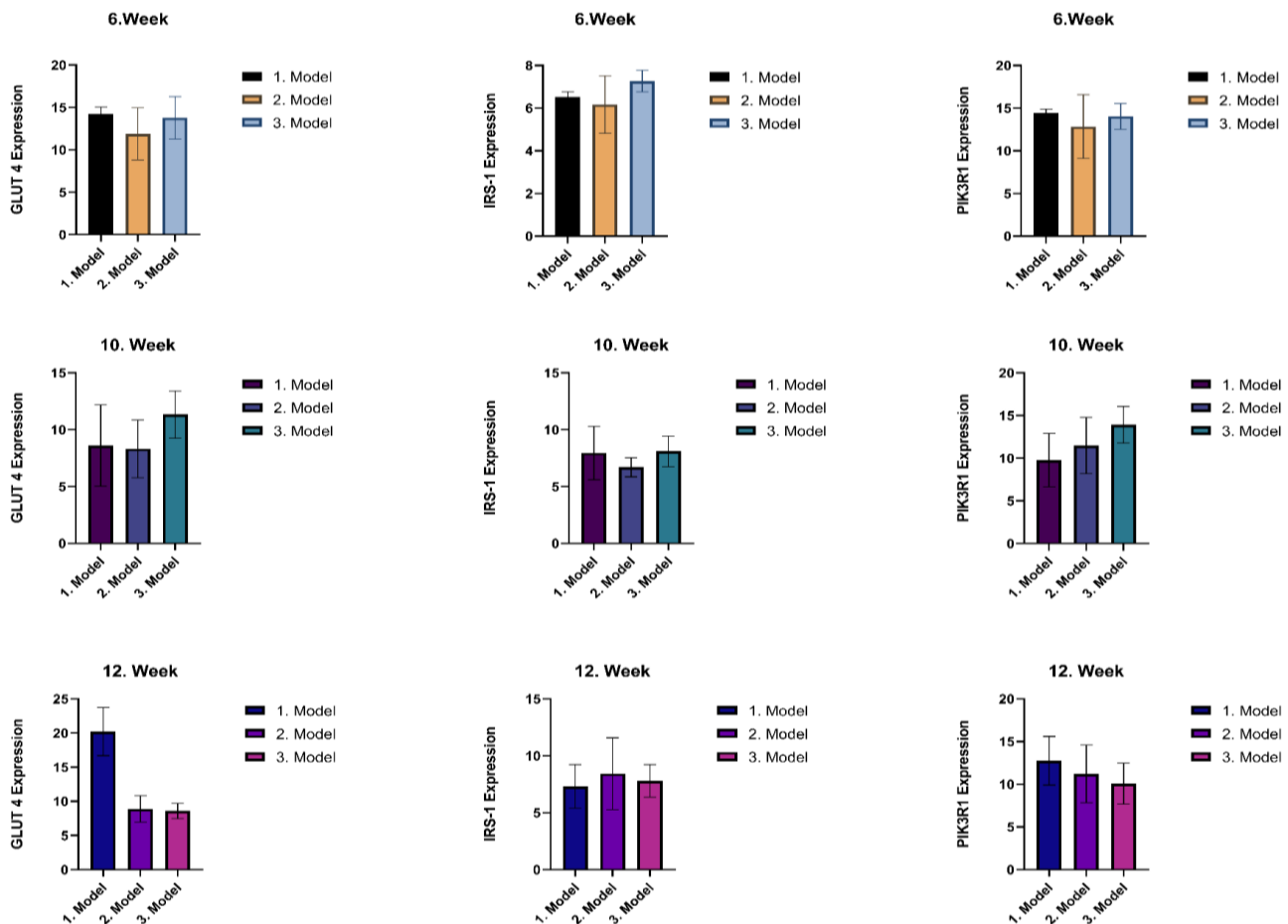


Fig. 1: Relative mRNA expression levels of GLUT4, IRS1, and PIK3R1 in three different experimental models of type 2 diabetes in rats. Gene expression was analyzed by qRT-PCR and presented as relative expression values among the diabetic models. The relative expression levels of target genes were calculated using the $\Delta\Delta C_t$ method and normalized to the expression of the housekeeping gene GAPDH.

DISCUSSION

Animal models of diabetes, particularly in rodents, play a crucial role in understanding the pathophysiological mechanisms underlying disease progression and evaluating novel therapeutic approaches. These models provide valuable insights into metabolic disturbances such as insulin resistance, beta-cell dysfunction, and dyslipidemia, which are also observed in companion animals like dogs and cats, thus enhancing translational relevance in veterinary medicine (Niaz *et al.* 2018; Suleiman *et al.* 2019, Singh *et al.* 2024).

T2DM is a long-term metabolic disorder marked by elevated blood glucose levels, insulin resistance, and/or insufficient synthesis of the insulin hormone (Guo *et al.* 2022; Korkmaz and Dik, 2024). Early treatment is necessary to prevent late complications of diabetes and increased treatment costs. Various models have been developed in rats to determine the pathophysiological features of T2DM and to evaluate new therapeutic agents in the treatment of the disease (Agircan *et al.*, 2024; Al-Awar *et al.*, 2016; Dik *et al.*, 2018). Experimental design of T2DM includes models such as STZ and high-fat diet-induced, fructose-induced, and STZ and nicotinamide-induced diabetic models (Dik *et al.*, 2024; Incir *et al.*, 2016; Sayeli and Shenoy, 2021).

Liver tissues and skeletal muscles of rats developed inflammation, which led to the development of insulin

resistance after feeding rats a high-fat diet for ten weeks. Insulin resistance increased over time during the experimental period that lasted up to 16 weeks with STZ-induced diabetes and high-fat diet intake exhibited significantly higher levels of VLDL, triglycerides, glucose, and HOMA-IR after 30 days. These effects are due to enhanced hepatic glucose synthesis and reduced insulin action on liver and muscle tissues. It has also been stated that OGTT and a high HOMA-IR index indicate insulin resistance. Due to the short research duration, cholesterol levels did not increase significantly. However, the triglyceride level has increased in the research (Magalhães *et al.*, 2019). In a diabetes model induced by 20% fructose added into drinking water, plasma glucose and triglyceride levels elevated, whereas cholesterol levels did not change for 8 weeks (Mamikutty *et al.*, 2014). In another study, a fructose-rich diet suppressed hyperglycemia and prevented the decline in plasma insulin levels. Additionally, both high-fat and fructose-rich diets have been shown to aggravate dyslipidemia by elevating plasma total cholesterol and phospholipid levels, likely due to their differential contributions to endogenous and exogenous lipid accumulation. These metabolic alterations may also contribute to obesity and related complications (Namekawa *et al.* 2017). Glucose, triglyceride, cholesterol, and HOMA-IR levels in diabetic rats induced by STZ together with 10% fructose were statistically higher compared to the diabetic group induced by STZ alone after 11 weeks. Additionally,

the insulin level in diabetic rats induced by STZ along with 10% fructose was lower than in the STZ-alone group. These changes have been caused by insulin resistance and increased lipid concentration (Wilson and Islam, 2012). In a recent study, insulin resistance developed on the 30th day, beta cell functions and serum insulin level decreased, and the glucose level increased in the STZ and nicotinamide-induced T2DM model (Sayeli and Shenoy, 2021). The STZ–nicotinamide model offers a significant advantage over STZ-only models by partially preserving pancreatic β -cell function and insulin secretion, thereby better simulating the early hyperinsulinemic phase followed by gradual insulin decline seen in Type 2 diabetes (Hamid *et al.*, 2025). In experimental animal models of insulin resistance, elevations in blood glucose, triglyceride, and cholesterol levels are commonly observed (Kottaisamy *et al.* 2021). In diabetic rats, insulin resistance reduces intracellular glucose consumption, which in turn decreases liver hexokinase activity. In the present study; the third model group (STZ + nicotinamide) exhibited consistently higher fasting glucose and HbA1c levels compared to the other groups throughout the entire experimental period. However, glucose levels in the first model group were comparable to those in the third group at weeks 10 and 12, and HbA1c levels were only slightly lower than those of the third group. Notably, triglyceride levels in the first model group were higher than those in the other groups during the early phase (weeks 6 and 10). This may be attributed to the high-fat diet, which accelerates adipose tissue accumulation and induces early hyperglycemia and hypertriglyceridemia. Insulin resistance in the first model group may have occurred through this pathway that reduced liver hexokinase activity at week 12 and muscle GLUT4 expression at weeks 6 and 12. Furthermore, β -cell degeneration and hypoinsulinemia in the first model group, may have developed by week 12 in this group.

Disruptions in the function of proteins encoded by IRS1, PI3 kinase, and GLUT4, which play key roles in the post-receptor insulin signaling pathway, contribute to the development of insulin resistance (Patra *et al.* 2024). Increased circulating fatty acids due to a high-fat diet, inhibition of IRS1 and PI3K regulatory subunit 1 expression in muscle, and reduced translocation of GLUT4 protein to the membrane all impair insulin signal transduction (Sundaresan *et al.*, 2016, Rusdiana *etl. al.*, 2023). In the current study, similar disorders occurred in the post-receptor events of insulin in all groups. However, the defects in insulin signaling associated with more pronounced hyperglycemia in the first and third model groups may have exacerbated insulin resistance and further downregulated the expression of IRS1, PIK3R1, and GLUT4 genes in muscle tissue.

Oral glucose tolerance can impair in diabetic models induced by fructose, STZ, a high-fat diet, or other agents (Niaz *et al.*, 2018; Iqbal *et al.*, 2024). In the third model group, hyperglycemia-induced inflammation may have contributed to the development of insulin resistance, which in turn may have resulted in higher blood glucose levels during the 120-minute OGTT analysis compared to the other groups. In this group, insulin resistance may have arisen from decreased glucose oxidation and impaired glycogen synthesis. Moreover, in the first model group, insulin resistance may have been triggered by impaired

insulin signaling and defective glucose transport mechanisms.

BUN levels increase in STZ-nicotinamide-induced diabetic rats due to the stimulation of gluconeogenesis, the release of free glycolytic amino acids deaminated in the liver, and enhanced proteolysis (Balakrishnan *et al.*, 2019). In the present study, proteolysis resulting from more severe diabetes may have occurred in the third model group. The BUN levels may have been higher in the third model group at all three sampling points due to ongoing proteolysis. Furthermore, this process may have limited renal glucose excretion, thereby contributing to the elevated glucose levels observed during OGTT in the third model group.

A high-fructose diet has been shown to cause steatohepatitis and liver damage, leading to elevated ALT and AST levels (Zhou *et al.*, 2023). In the present study, early excessive fructose consumption in the second model group may have transiently induced steatohepatitis and mild hepatic injury, as suggested by elevated ALT and AST levels observed at week 6. However, the subsequent decline in these enzyme levels at later time points may indicate a compensatory hepatic adaptation or resolution of the initial damage.

Various models have been developed in experimental animals for the study of T2DM pathophysiology and the evaluation of potential therapeutic agents. Although no formal cost analysis was performed, based on our observations, we found that first model were more practical, easier to implement, and appeared to better replicate the clinical features of the disease. The STZ and nicotinamide-induced T2DM model effectively replicates early-stage type 2 diabetes progression from the sixth week onward, with sustained effects observed for at least 12 weeks. Both the high-fat diet combined with STZ model and the STZ-nicotinamide model demonstrated comparable diabetic characteristics and metabolic parameters. Nevertheless, if the aim is to develop a T2DM model associated with obesity (i.e., hypertriglyceridemia) and insulin resistance within a relatively short period (less than 10 weeks), the high-fat diet plus STZ model may be a more appropriate choice. In contrast, the 20% fructose model did not adequately replicate T2DM characteristics and led to severe dehydration in the animals.

Finally, the results present a comprehensive comparison of the different experimental T2DM models that will be useful to select the optimal model for experimental T2DM rat models according to the aim of the researcher such as basic background investigation diagnostic or therapeutic purposes.

Although all three models aim to replicate different aspects of type 2 diabetes, their translational relevance may vary depending on the therapeutic strategy being evaluated. While the HFD+STZ model appears more suitable for evaluating insulin-sensitizing agents, the nicotinamide+STZ model may better represent β -cell dysfunction and secretagogue responsiveness. In contrast, the fructose model—despite showing relatively limited metabolic impact in this study—could still serve as a model for investigating early insulin resistance and diet-induced metabolic changes.

Undoubtedly, more detailed research will contribute to current results for future antidiabetic drug trials.

Limitations: This study provides a comprehensive molecular and biochemical comparison of three different rat models used for the induction of type 2 diabetes mellitus. However, several limitations should be acknowledged. First, the sample size at each time point was limited to six animals per group, which—although ethically and practically justified—may have reduced the power to detect subtle intergroup differences. Second, while changes in gene expression were analyzed, the study did not include corresponding protein-level analyses (e.g., Western blotting or immunohistochemistry) or functional assays, which could have provided stronger validation of the molecular findings. Third, the study duration was limited to 12 weeks, and a longer follow-up period might reveal more extensive or progressive metabolic and molecular alterations.

In addition to these methodological considerations, there are intrinsic limitations associated with the models themselves. Chemical models, such as those using STZ, may lack reversibility and thus do not fully replicate the dynamic nature of disease progression or remission observed in type 2 diabetes. Furthermore, inflammatory responses can vary significantly among models, potentially affecting systemic outcomes. Some models, such as the fructose-induced model, may not adequately reflect genetic predisposition, which is a critical factor in the pathogenesis of type 2 diabetes. Finally, STZ-based models are limited in their ability to mimic β -cell regeneration, which restricts their use in studies investigating recovery or regenerative therapies.

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