Research Article

Development of a Type 2 Diabetes Mellitus Model in Rats with Administration of High-Fat Diet and Streptozotocin

Akhilesh Mishra[1], Vandana Roy[1], Ajay Kodiyatar[1], Megh Sing[1], B.C Koner[1], Niket Rai[1]

1. Maulana Azad Medical College (MAMC), New Delhi, India

Background: Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by chronic hyperglycemia, insulin resistance, and β-cell dysfunction. Despite various efforts, the development of a stable and reproducible T2DM model remains a challenge for fundamental and clinical research due to variability in model methodologies and outcomes.

Objective: This study aimed to optimize an induced rat model of T2DM characterized by insulin resistance and β-cell degeneration using a combination of a high-fat diet (HFD) and low-dose streptozotocin (STZ)@ 25mg/kg injections.

Methods: Male Sprague-Dawley rats were divided into two groups. The normal control (NC) group was fed standard chow for four weeks and received vehicle injections, while the diabetic control (DC) group was fed a high-fat diet for four weeks and administered two intraperitoneal injections of STZ (25 mg/kg) at a five-day interval. Rats with fasting blood glucose levels ≥250 mg/dL were classified as diabetic. Key parameters—including body weight, fasting blood glucose, serum cholesterol, serum creatinine, and HbA1c—were measured.

Results: Rats subjected to HFD feeding followed by STZ injections exhibited significant increases in fasting blood glucose, total serum cholesterol, serum creatinine, and HbA1c levels compared to normal controls ($p < 0.05$), confirming the successful induction of a diabetic state. The model demonstrated features of insulin resistance, hyperglycemia, dyslipidemia, and early renal alterations, replicating key aspects of human T2DM pathophysiology.

Conclusion: A combination of high-fat diet and low-dose STZ administration successfully developed a robust and reproducible rat model of T2DM characterized by insulin resistance and β-cell destruction.

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This improved experimental model provides a valuable platform for future research into the mechanisms and therapeutic interventions of T2DM.

Correspondence: papers@team.qeios.com — Qeios will forward to the authors

Introduction

Diabetes mellitus (DM) is a common metabolic illness characterized by hyperglycemia, which results from a complicated interplay between genetic predisposition and environmental variables, leading to a variety of problems. Diabetes is caused by a variety of factors, the most common of which are hypo-insulinemia (low insulin production), insulin resistance, which impairs glucose consumption, and increased gluconeogenesis.[1]

According to the most recent figures as of 2024, over 540 million people worldwide have diabetes, with approximately 240 million remaining undiagnosed. Type 2 diabetes, which constitutes 90% of all cases of diabetes, was earlier considered to be a disease of the affluent “Western” countries, has now spread globally, and has become a major cause of disability and death affecting even younger age groups.[2]

Streptozotocin (STZ) is a chemical agent commonly used to generate DM in experimental animals by destroying animal pancreatic beta cells, resulting in hyperglycemia.[3]

Multiple low doses of streptozotocin (STZ) have been shown to decrease mortality compared to a single high dose in experimental models of type 2 diabetes mellitus (T2DM).[4] This approach involves administering STZ in smaller doses over a period, which induces moderate and gradual pancreatic beta-cell damage, mimicking the progressive nature of T2DM seen in humans.[5] By contrast, a single high dose of STZ can lead to severe and abrupt beta-cell destruction, potentially causing more acute metabolic disturbances and higher mortality rates in animal studies.[6] Therefore, using multiple low doses of STZ is preferred in research settings to better simulate the chronicity and progression of T2DM and to facilitate longer-term studies of interventions and treatments.[6]

In the induced model, high-fat diet (HFD) induction simulates the development of insulin resistance and hyperglycemia caused by diet in a straightforward manner.[7] However, feeding takes a long time and necessitates frequent monitoring of blood glucose and insulin levels. Chemical induction can cause hyperglycemia, particularly by destroying β-cells.[7] However, the intervention dosage is difficult to

control and can result in T1DM and mortality. It is also ineffective for inducing insulin resistance in animals.[7] Currently, HFD combined with streptozotocin (STZ) is one of the most prevalent techniques of establishing T2DM in animal models, and it can readily manifest as diabetic mellitus.[7]

The HFD/STZ rat model was used to induce type 2 diabetes mellitus (T2DM), following a previously published method. In that study, a high-fat diet was combined with multiple low doses of streptozotocin (STZ) administered at 30 mg/kg weekly, which proved effective for establishing a stable model of T2DM.[6] In our study, we initially used 30 mg/kg STZ administered twice, but such doses have been associated with increased mortality in T2DM rat models. Therefore, we modified the protocol to administer two intraperitoneal injections of STZ at a lower dose (25 mg/kg), spaced five days apart. This modified HFD-STZ protocol provides a more suitable method for developing T2DM in animal models without causing mortality.

Materials and Methods

Animals

The study was conducted at the Central Animal Facility and Department of Pharmacology, Maulana Azad Medical College (MAMC), New Delhi, following approval from the Institutional Animal Ethics Committee (IAEC) (Ref. No. IAEC/MAMC/CAF/2023/03). Male Sprague Dawley rats (6-8 weeks old, 150 ± 20 g) were obtained from the same facility. The rats were housed in groups of three per cage, using standard-sized cages under standard laboratory conditions. These conditions included a temperature of 25 ± 5°C, a humidity of 55 ± 10%, and a 12-hour light and dark cycle. Throughout the study, all animals were provided with their respective diets: a normal pellet diet for the normal control group and a high-fat diet (HFD) for the Diabetic control group. Water was provided ad libitum.

Diet and Chemicals

- Streptozotocin (STZ), a chemical powder supplied in a 500 mg vial, was obtained from Sisco-Research Laboratories Pvt. Ltd. (Mumbai, India).
- Citric acid and sodium citrate were obtained from Sigma-Aldrich Company (St. Louis, MO, USA).
- The Eppendorf centrifuge 5702 was obtained from Sigma-Aldrich Company (St. Louis, MO, USA).
- A semi-auto analyzer was obtained from Rapid Diagnostic Pvt. Ltd. (New Delhi, India).
- Electronic weighting machine: Wensar Weighing Scales Ltd. (New Delhi, India).

- Glucose 300-ml kit, cholesterol 100-ml kit, creatinine 100-ml kit, and HbA1c with calibrator 40-ml kit were obtained from KEE Diagnostic Pvt. Ltd. (New Delhi, India)
- High Fat Diet (HFD), composed of 60% fat, 20% protein, and 20% carbohydrates, was obtained from KaryomePvt. Ltd. (Mysuru, India)

Composition and Formulation for High Fat Diet

Ingredient	g/Kg	Cal.	%Cal
Corn Starch (Carbohydrate part)	150	600	10.00
Sucrose	100	400	6.67
Cellulose	50	200	3.33
Carbohydrate	300	1200	**20.00**
Casein	200	800	13.33
Corn Starch (Protein part)	50	200	3.33
Min. Mix (AIN93G Min mix)	35	140	2.31
Vit Mix (AIN93G Vit mix)	10	40	1.03
L- Cysteine	3	12	0
Choline bitartarate	2	8	0
Protein	300	1200	**20.00**
Lard	400	3600	60.00
Fat	400	3600	**60.00**
TOTAL	1000	6000	100.00

Experimental Design

Rats were randomly divided into 2 groups (n=6/group):

1. **Normal Control (NC)** - Normal saline (10 ml/kg)
2. **Diabetic Control (DC)** - HFD + STZ

Induction of Type 2 Diabetes Mellitus (T2DM)

The HFD/STZ rat model for T2DM induction was based on a high-fat diet and low-dose STZ injections. Rats were fed a high-fat diet ad libitum for 21 days to induce insulin resistance.

After 21 days, rats were given two intraperitoneal injections of STZ at a dosage of 25 mg/kg body weight in citrate buffer (0.1 mM, PH = 4.5), with a 5-day gap between each injection. Three days following the second STZ injection, 0.4-0.5 ml of blood was taken from the retro-orbital sinus route in microcentrifuge tubes for outcomes analysis.

Blood Collection and Biochemical Parameters

The rats were anesthetized with ketamine (80 mg/kg) and xylazine (100 mg/kg) in combination. The rat was placed on the operation table, and with a capillary tube (both ends open) 75 mm in diameter, the blood was collected from the retro-orbital sinus route (0.4-0.5 ml) in microcentrifuge tubes. Blood samples were centrifuged at 4000 g for 15 min at 4 °C to obtain the plasma, which were stored at -80 °C until analysis with the Semi-Auto Biochemistry Analyzer.

Observation And Results

Outcomes	Groups	
	Normal Control (NC)	Diabetic Control (DC) - HFD + STZ
Weight (gm)	163.16 ± 9.95	302.83 ± 56.02*
FBG (mg/dl)	101.16 ± 12.78	476.00 ± 80.88*
Cholesterol (g/L)	89.50 ± 9.13	351.6 ± 75.99*
Creatinine (mg/dl)	0.27 ± 0.07	2.05 ± 0.47*
Hb1Ac (g/L)	3.71 ± 0.15	10.55 ± 0.57*
HOMA-IR (mg/dl × µU/ml)	14.85 ± 0.60	8.46 ± 0.82*

Table 1. Induction of diabetes

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*Values are mean ± SEM, n= 6 in each group*P<0.05, compared to normal control group (NC)*

Inference: -

All groups began with similar weights, as shown by the lack of statistically significant differences in baseline weights (p-values > 0.05). After HFD feeding, diabetic rats gained much more weight than the NC group.

Diabetes induction resulted in considerably higher glucose levels in the DC group compared to the NC group ($p<0.05$), but there was significant difference between the two groups. Rats with fasting blood glucose levels more than 250 mg/dL were classed as having T2DM.

All comparisons have p-values of 0.001 ($p<0.05$), demonstrating significant differences in total serum cholesterol, creatinine, and HbA1c levels between NC and DC groups.

Mention that there was no mortality in rats during this process of developing T2DM model. Rats ere active and eating normally during the process indication that no acute complicatins leading to any severe morbidity developed during the process.

Discussion

The present study successfully established a rat model of type 2 diabetes mellitus (T2DM) using a combination of a high-fat diet (HFD) and multiple low-dose STZ (25 mg/kg) injections. This approach effectively replicated key metabolic features of human T2DM, including insulin resistance, hyperglycemia, β-cell dysfunction, and altered lipid and renal profiles.[8][9]

Streptozotocin (STZ), a naturally occurring nitrosourea molecule, is highly toxic to pancreatic β-cells. STZ reaches β-cells via the highly expressed GLUT2 glucose transporter.[10] Once within, STZ alkylates DNA and produces strand breaks. This stimulates poly(ADP-ribose) polymerase (PARP), which depletes cellular NAD+ and ATP levels, ultimately leading to β cell necrosis or apoptosis. Furthermore, STZ produces reactive oxygen species (ROS) and nitric oxide (NO), which impair mitochondrial and cellular function. β-cell loss causes insulin insufficiency and hyperglycemia, similar to diabetes mellitus.[11]

The effective dosage of STZ varies according to species, age, body weight, and experimental objectives. In adult rats, a single large dosage of STZ (45-65 mg/kg body weight) is routinely used to cause type 1 diabetes. To replicate type 2 diabetes, a modest dosage of STZ (e.g., 25- 35 mg/kg) is commonly combined

with a high-fat diet (HFD) to simulate insulin resistance and partial β-cell malfunction.[12] It is crucial to titrate the dosage carefully, as greater doses might result in significant mortality or severe toxicity.[9]

A high-fat diet (HFD) can lead to type 2 diabetes by increasing insulin resistance and β-cell malfunction. The key mechanisms are fat buildup in peripheral organs, which causes lipotoxicity, and decreased insulin signaling, notably by serine phosphorylation of the insulin receptor substrate (IRS).[13] HFD causes low-grade chronic inflammation by releasing pro inflammatory cytokines such TNF-α and IL-6, which can impede insulin function. Excessive fatty acid oxidation, mitochondrial overload, and ER stress all contribute to β-cell destruction. Chronic metabolic stress causes β-cell fatigue and failure, resulting in persistent hyperglycemia.[14]

Rats fed with HFD for 21 days followed by STZ administration exhibited significant increases in fasting blood glucose, total cholesterol, serum creatinine, and HbA1c levels compared to normal controls, indicating the successful induction of a diabetic state. The observed hyperglycemia and elevated HbA1c levels reflect impaired glucose metabolism and chronic glycemic imbalance, while increased cholesterol and creatinine levels suggest dyslipidemia and early renal alterations, respectively both of which are common in human T2DM.

High-fat diets usually cause animals to gain weight. Because fats have a higher calorie density than proteins or carbohydrates, animals that consume a high-fat diet tend to ingest more calories overall, which can result in a rise in body weight if the additional calories are not burned off via exercise.[15]

Combining HFD with low-dose STZ is a well-established method for inducing a pathophysiological state in rodents that closely resembles human type 2 diabetes. This model incorporates both insulin resistance from dietary fats and partial β-cell dysfunction from STZ, making it highly suitable for studying disease mechanisms and testing therapeutic agents.[9][10]

Conclusion

This study successfully developed a reliable and reproducible rat model of type 2 diabetes mellitus (T2DM) using a combination of a high-fat diet (HFD) and multiple low-dose STZ (25 mg/kg) injections. The model closely mimics the dual pathophysiological features of human T2DM, including insulin resistance and partial β-cell dysfunction. Significant alterations in metabolic parameters such as increased fasting blood glucose, serum cholesterol, creatinine, and HbA1c confirm the effective induction

of the diabetic state. The HFD-STZ model provides a valuable experimental tool for investigating the mechanisms of T2DM and evaluating potential therapeutic agents in preclinical settings.

Statements and Declarations

Author Contributions

Akhilesh Mishra was responsible for the conceptualization, hypothesis of the study, data collection, analysis, and manuscript drafting. Vandana Roy provided supervision and contributed to the revision of the manuscript. Ajay Kodiyatar supported the study methodology, benchtop procedures and manuscript preparation. Megh Singh assisted with the statistical analysis and data interpretation. BC Koner reviewed and finalized the manuscript prior to submission. Niket Rai supervised the overall research direction and provided oversight throughout the study.

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Declarations

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