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Research Article

Investigation of gender bias in streptozotocin and high fat diet induced mouse model of diabetes mellitus

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Abstract

Five hundred thirty seven million adults suffer from diabetes among which 17.7 million more men than women have the disease, on the other hand among 1.9 billion overweight adults 11% of men and 15% of women are obese, hence, gender is a significant variable in diabetes and obesity research. A prior survey indicated that only 11.2% of preclinical animal studies included both sexes, with 20.7% of studies involving females and 66% involving males, resulting in a male-to-female ratio of 3.2:1, reflecting a significant male gender bias in research. This sex bias leads to generalizations of findings for males and females without adequate justification. In this study Induction of STZ-induced Type I Diabetes and HFD-induced & Type II Diabetes was done in both male and female mice to investigate the presence of any gender bias. The body weight and blood glucose of the mice were routinely monitored. The metabolic changes associated with these models were measured, by monitoring insulin sensitivity via the Oral Glucose Tolerance Test (OGTT). Liver function tests (LFT) and lipid profile tests are conducted to evaluate liver health and lipid metabolism, which are often impaired in diabetic states. Additionally, hematological assessments, including total cell count and differential cell (DC) count was conducted to evaluate any immune or inflammatory responses associated with the induction of the disease diabetes. Finally, histological analysis of tissues, particularly pancreatic and liver tissues, provides insights into the structural changes and potential damage induced by diabetes in these organs focusing from gender specific way. The findings reveal that male and female mice exhibit differential responses to STZ-induced Type I Diabetes and HFD-induced Type II Diabetes in majority of the metabolic, hematological and histological parameters including blood glucose, body weight, OGTT, LFT, highlighting the importance of considering gender as a significant variable.

Increasing evidences suggests, rather than over-reliance on male-only animal model, balancing both sexes in the preclinical studies and drug discovery study is important as gender specific personalized dosage regimen and gender specific therapeutic intervention might be an emerging option for better management of the disease.

Keywords: Diabetes, Gender bias, High Fat Diet (HFD), Preclinical mice model, Streptozotocin (STZ)

Highlights

- The problem of over-reliance on male only animal in preclinical and drug discovery research
- Exploration of the gender bias in commonly used animal models of Type I and Type II diabetes
- Blood glucose levels were significantly higher in diabetic male mice in comparison to females
- Most disease parameters showed significant differences between male & females mice

INTRODUCTION

Metabolic diseases are disorders that interfere with the body's ability to process and distribute essential nutrients like carbohydrates, proteins, and fats, disrupting normal metabolic functions and the cellular process of converting food into energy. Diabetes is a long-term metabolic condition characterized by elevated blood glucose levels, which occur when the pancreas either fails to produce enough insulin or when the body cannot properly use the insulin it produces. Over time, this can result in significant damage to organs such as the heart, blood vessels, eyes, kidneys, and nerves.

According to international diabetes federation 537 million adults (10.5% of all adults in age group 20-79 years) suffers from diabetes the data also indicate sex differences in global adult population. Gender differences are also observed in diabetes prevalence, as the number of men suffering from the disease is 17.7 million more than women. On the other hand, among 1.9 billion overweight adults 11% of male population and 15% of female population are obese, hence gender is a significant variable in diabetes and obesity research. Previous survey reports that 11.2% of preclinical animal research was done in both sexes, only 20.7% in females, and 66% in males indicating a

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male bias of 3.2:1. This sex bias leads to generalizations of findings for males and females without adequate justification (Buoncervello *et al*., 2017; Plevkova *et al*., 2020; Merone *et al*., 2022).

Diabetes mellitus can be broadly categorized into two types, diabetes type I (also known as, juvenile or insulin-dependent), characterized by insufficient production of Insulin, whereas type 2 diabetes (formerly called non-insulin-dependent, or adult-onset) results from the body's ineffective use of insulin (Ilonen *et al*., 2019). More than 95% of people with diabetes have type 2 diabetes (Galicia-Garcia *et al*., 2020). One of the leading factors that predisposes an individual towards diabetes type 2 is obesity (Wen *et al*., 2022).

A broad-spectrum antibiotic Streptozotocin (STZ), isolated from *Streptomyces acromogenes* and is widely used to induct diabetes in preclinical experimental animals (Wu *et al*., 2008; Deeds *et al*., 2011; Furman, 2015; Goyal *et al*., 2016; Ghasemi *et al*., 2023). Structurally STZ is a Glucosamine nitrosourea compound and it resembles 2-deoxy D- glucose hence up taken by GLUT2 transporter of the pancreatic beta cells. Intracellular accumulation of STZ occurs in the beta cells specifically as other cells use GLUT4 transporter for uptake of glucose. STZ within the cell causes diabetogenic action by various pathways like DNA methylation by formation of (DAM), increased NADPH levels and activation of PKC pathway, upregulation of reactive oxygen species production, AGE (advanced glycation end products) accumulation and even cytokine production resulting in cytotoxicity. Death of beta cells of the pancreas results in insufficient insulin supply as beta cells are the sole producer of insulin in pancreas. Lack of insulin causes excess circulatory glucose concentration in the blood hence resulting in pathogenesis of diabetes mellitus. Prolonged excess glucose in peripheral circulation results in formation of advance glycation end products and reactive oxygen species that is responsible for further diabetes associated secondary complications like nephropathy retinopathy neuropathy and cardiomyopathy (Ilonen *et al*., 2019).

High fat diet (HFD), apart from higher calorific value have low satiating effect hence resulting in over consumption of food thereby altering energy balance which paves the way to alteration of metabolic hormones like insulin, leptin. Increased body weight, increased adipose tissue, hepatic steatosis, alteration of lipid metabolism are some of the immediate effects of a high fat diet (Heydemann, 2016). Increased presence of fats in the diet also increases permeability of gut there by increasing the chances of breaching of the gut barrier by pathogenic bacteria and alteration of the gut microbiome which is now an area of focus for modern research (Hariri *et al*., 2010; Wang *et al*., 2012; Carpenter *et al*., 2013; Avtanski *et al*., 2019).

MATERIALS AND METHODS

Mice: Six-week-old both male and female Swiss albino mice, weighing 20 - 25 g, were used in this experiment. All the experimental animals were kept in temperature-controlled environment at 12 hrs light and day cycle along with fresh food and water.

The mice were divided into six groups, with three animals (n = 3) in each group. These included control male and control female groups, which served as untreated healthy controls. Two groups, referred to as STZ male and STZ female, were induced with Type I Diabetes using Streptozotocin (STZ). The remaining two groups, HFD male and HFD female, were subjected to a high-fat diet (HFD) to induce Type II Diabetes.

Induction of the disease: Male mice in Type I Diabetes groups were intraperitoneally injected with two 150 mg/kg dose of freshly prepared Streptozotocin (SRL), in 0.1 M Citrate buffer pH 4.5 (SRL), on day 0 and day 5. For female mice 300 mg/kg dose was used for induction of type I diabetes (Furman, 2015, Goyal *et al*., 2016, Ghasemi *et al*., 2023). Fig. 1 describes the

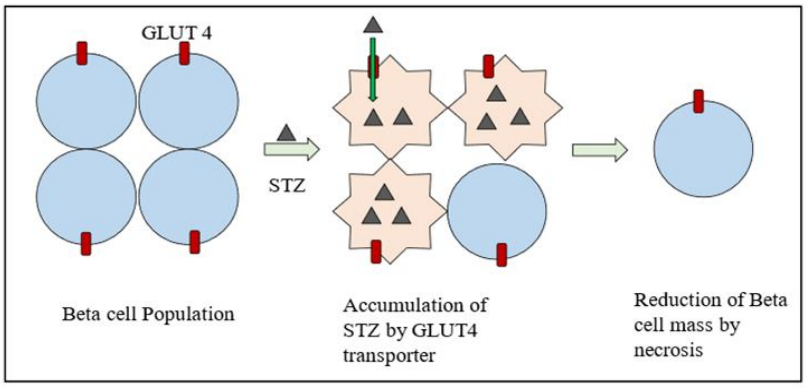


Fig. 1. Mechanism of Action of STZ induced diabetic model, STZ is up taken by GLUT 4 transporter which is selectively present in beta cells of the pancreas, accumulation of STZ results in necrosis of beta cells hence insulin production decreases resulting in increased peripheral blood glucose concentration mimicking the pathophysiology of diabetes mellitus.

mechanism of action of STZ induced diabetic model, while Fig. 2 describes the detailed dosage regimen and induction of the disease.

Obesity was induced in the HFD group by modelling diet constituting more than 60% fat. The HFD group were fed a control diet constituting 10% fat for the first 7 days of acclimatization. The models were fed a high cariogenic diet of which 60% constitutes fat for the next 28 days of the experiment (Hariri *et al*., 2010; Wang *et al*., 2012; Heydemann, 2016). The nutrient composition of the experimental diets reveals significant differences in macronutrient distribution, which are critical for evaluating their metabolic impact. The control diet provides 4.03 kcal/g, with a major proportion of energy derived from carbohydrates (60%), followed by proteins (30%) and a minor contribution from fats (10%). The primary sources of these macronutrients are Atta (carbohydrate), Sattu (protein), and Lard (fat), reflecting a relatively balanced dietary profile suitable for maintaining normal metabolic function (Table 1).

In contrast, the high-fat diet (HFD) is formulated to deliver 5.87 kcal/g, thus constituting a hypercaloric intake aimed at promoting obesity-induced diabetes. This diet shows a marked shift in macronutrient distribution, with fats contributing 60% of the total energy, while carbohydrates and proteins each contribute 20%. The increase in fat content, primarily from Lard, underscores the diet's obesogenic potential and its relevance for modelling metabolic disorders such as insulin resistance and type 2 diabetes (Table 2). Fig. 3 depicts the mechanism by which a high-fat diet

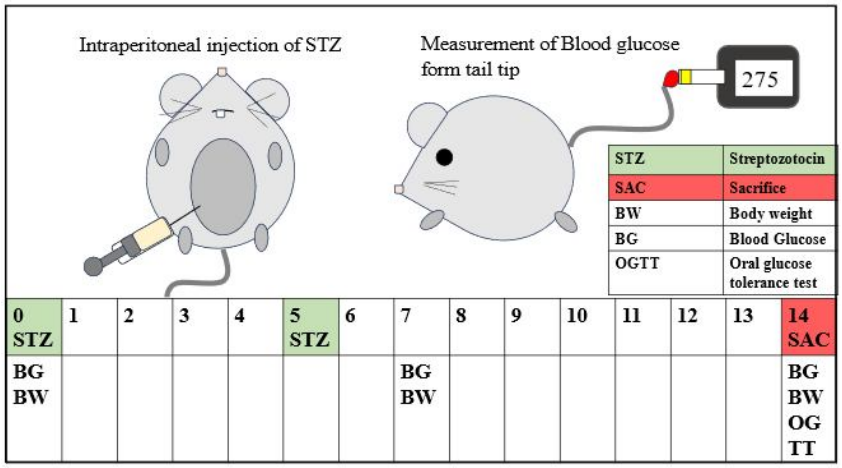


Fig. 2. Induction of disease, Streptozotocin is dissolved in 0.1 M Citrate buffer at pH 4.5 and injected intraperitoneally in the mouse. STZ administration was done on day0, and day5. Blood glucose was measured by glucometer from a drop of blood taken from the tail tip

Table 1. Nutrient distribution of control dietary intake 1g of high-fat diet constituted 4.03 calories

Control diet	Ingredients	Proportions/kg	% of kg/cal
Carbohydrate	Atta	662.425 g	60
Protein	Sattu	293.081 g	30
Fat	Lard	44.274 g	10

Table 2. Nutrient distribution of high-fat diet dietary intake 0.1 g of high-fat diet constituted 5.87 calories making it a high calorigenic meal for the high-fat diet group targeted for the induction of obesity induced diabetes.

Control diet	Ingredients	Proportions/kg	% of kg/cal
Carbohydrate	Atta	286.047 g	20
Protein	Sattu	324.28 g	20
Fat	Lard	390.047 g	60

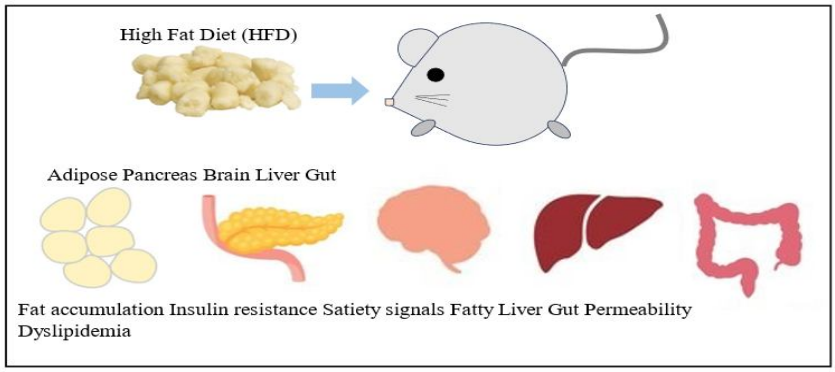
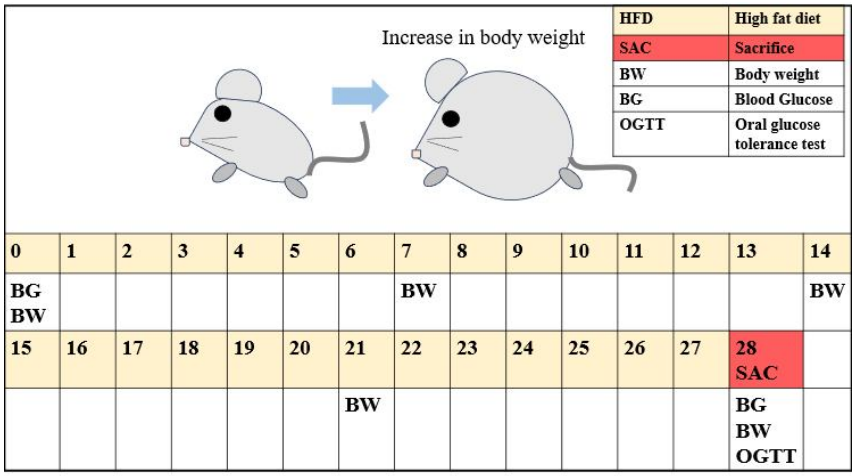


Fig. 3. Mechanism of Action of HFD induced diabetic model, Intake of high fat diet impairs satiety signals resulting in body fat accumulation, altered lipid metabolism, NFLAD and insulin resistance that in increased peripheral blood glucose concentration mimicking the pathophysiology of diabetes mellitus type II



0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
BG BW							BW							BW
15	16	17	18	19	20	21	22	23	24	25	26	27	28 SAC	
						BW							BG BW OGTT	

Fig. 4. Induction of disease, high-fat diet, 1 g of constituted 5.87 calories were fed to mice daily. Body weight (BW) was measured on day 0, 7, 14, 21, 28; Blood glucose (BG) was measured on day 0, 28, from a drop of blood taken from the tail tip

(HFD) induces type II diabetes and Fig. 4 describes the detailed dosage regimen and induction of the disease.

Measurement of metabolic parameters: Blood glucose, OGTT and body weight- Blood glucose was measured from the tail tip by Accu-check glucose monitor machine. Mouse was gently restrained and the tail is wiped with alcohol, a small insertion was made in the distal tip of the tail with a sterilized needle followed by milking of the tail. A drop of blood is then placed in the testing strip of the glucometer to measure the blood glucose amount. Blood glucose was measured on day 0, day 7 and day 14 for type I diabetes mice model, on day 0 and day 28 of high fat diet induced type II diabetes model (Wang *et al*., 2012). The OGTT was done to monitor the peripheral disposal of orally administered glucose. OGTT was performed by fasting the animals for 5-6 hours prior to the test. Blood glucose of all the groups were measured at 0 mins, followed by oral administration of 40 µL dextrose (50% dextrose solution) at a dose of 1g/kg. Blood glucose was measured at an interval of 0, 15, 30, 60 and 120 minutes (Wang *et al*., 2012). Body weight was measured on a digital weighing scale. Measurement of body weight was done on day 0, day7, day14 for diabetic mice model (Avtanski *et al*., 2019).

Sacrifice and experimental tissue collection: Experimental animals were sacrificed by over dose of Thiopental sodium and samples were collected following tissues were collected. By myocardial puncture, peripheral blood (PB) was taken, and it was then gathered in K2-EDTA tubes (BD Biosciences, USA). On a microscope slide, a smear was made for differential cell counts (Mitra and Ray Banerjee, 2023). For Serum extraction from peripheral blood, PB was left to stand for at least 30 minutes in tubes (without EDTA to allow for coagulation). After that, it was centrifuged at 10,000 rpm at 4°C for 10 minutes, with the supernatant being collected in new tubes. For later use, the serum was kept refrigerated at -80°C (Mitra and Ray Banerjee, 2023). The femurs and tibias of the mice were collected and bone marrows were flushed out by pbs buffer and collected in properly labelled centrifuge tube (Ghosh *et al*., 2018). The organs were carefully dissected from each mouse from all the groups. Pancreas and liver were collected in 10% formalin for histological processing. Pancreas and adipose were also collected in DMEM media for flow cytometry preparation (Ghosh *et al*., 2018).

Liver function test (LFT): Blood was collected during sacrifice in EDTA vials and sent for liver function test (SGPT and SGOT). The liver function test was performed by the process of UV without PS5 in the laboratory of Medicity Specialty Laboratory Pvt. Ltd (Jamal Gilani *et al*., 2021).

Lipid profile test: Blood was collected during sacrifice in EDTA vials and sent for lipid profile test. Lipid profile test was done by the process of CHOD-PAP, GPO-PAP in the laboratory of Medicity Specialty Laboratory Pvt. Ltd (Jamal Gilani *et al*., 2021).

Cell count: After digestion of the organs using 0.1% collagenase and subsequent centrifugation, the resulting cell pellets were resuspended in PBSG buffer. Total cell count (TC) was performed by staining the samples with Trypan blue dye in a 1:1 ratio (Himedia, India), and the cells were counted using a hemocytometer under 10X magnification to assess cell viability, following the method described by Ghosh *et al*. (2018). For the differential cell count (DC), peripheral blood smears were prepared, fixed with methanol (SRL, India), and stained with Giemsa stain (SRL, India). These smears were then examined under

40X magnification using a light microscope, as described by Ghosh *et al*. (2018).

Histology: For 72 hours, tissues were preserved at room temperature using 10% formalin. The initial step in dehydrating tissues was to subject them to a series of ethanol concentration increases, starting at 50% and working up to 100% in graded steps. After that, the tissues were fixed in paraffin, treated with xylene, and divided into 5 µm slices using a rotating microtome. Under a light microscope (Dewinter Fluorex LED) with magnifications of 4X, 10X, and 40X, the stained slices were examined (Avtanski *et al*., 2019).

RESULTS

Body weight: After 2 weeks of follow-up, both STZ male and STZ female mice gradually lost body weight. The body weight decrease was 17.5% STZ male from day-0 to day-14 when compared with control male counterpart while there was 16.6% decrease in body weight in STZ female from day-0 to day-14 compared to control females. As shown in Fig. 5, body weight significantly decreased in STZ-induced type I diabetic mice (A), while HFD-induced type II diabetic mice (B) showed a marked increase in body weight ($p<0.05$ vs. control; #$p<0.05$ vs. male diabetic mice). The decrease in body weight were 5.2% more in STZ males than STZ female. After 4 weeks while feeding ad libitum on high fat diet containing 60% calories from fat, slight increase in body was seen in HFD male while there was a slight decrease in body weight of HFD female compared to control female mice though both these changes were statistically insignificant.

Blood glucose: Blood glucose was measured from the tail vein on day 0, day 7 and day 14 for STZ models and day 0 and day 28 for HFD models. STZ mice with diabetes displayed wide swings in blood glucose levels, ranging from 250 mg/dL to 500 mg/dL, characteristic of type I diabetes while the blood glucose swings in HFD induced type II diabetes were around 100 mg/dL to 250 mg/dL. The increase in day 14th blood glucose were 69.7% & 51.9% in STZ male & STZ female respectively when compared to controls. 35.5% & 24.3% increase in day-28th blood glucose levels in HFD male and HFD female respectively when compared to controls. The increase in STZ male and HFD male blood glucose levels were 25.5% & 25.2% more in comparison to the females respectively. As depicted in Fig. 6, blood glucose levels significantly increased in both STZ-induced type I diabetic mice (A) and HFD-induced type II diabetic mice (B) ($p<0.05$ vs. control; #$p<0.05$ vs. male diabetic mice).

OGTT: The increase in area under the curve in oral glucose tolerance test were 57% & 46.7% for STZ male and STZ female respectively, the increase in area under the curve in STZ male mice are 18% more compared to females (0.34-fold increase in males). On the other in high fat diet induced diabetic mice the increase in area under the curve for OGTT are 46% & 36% for HFD male and HFD female when compared to control group

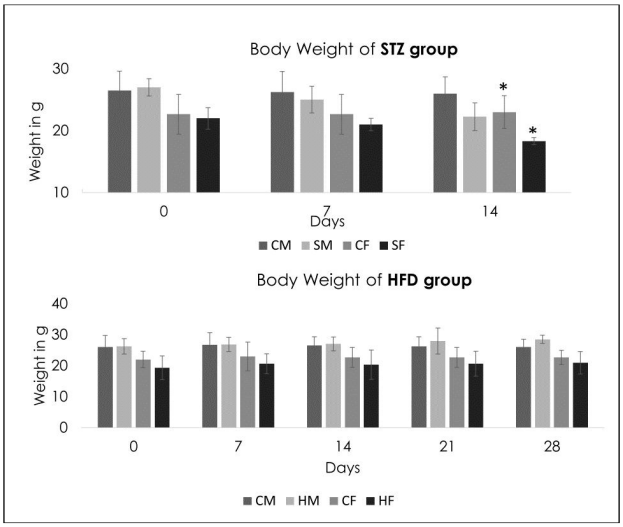


Fig. 5. Body weight changes in (A) STZ induced type I diabetes mice. (B) HFD induced type II *p < 0.05, compared to control #p<0.05, compared to male diseases diabetes mice

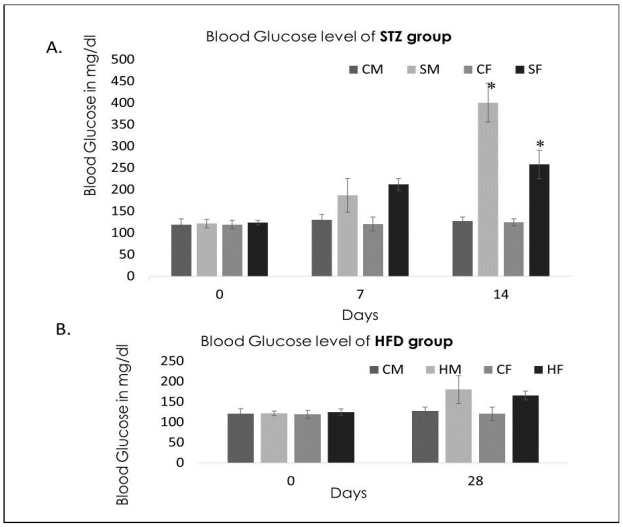


Fig. 6. Increase in blood glucose (A) STZ induced type I diabetes mice. (B) HFD induced type II *p<0.05, compared to control #p<0.05, compared to male diseases diabetes mice

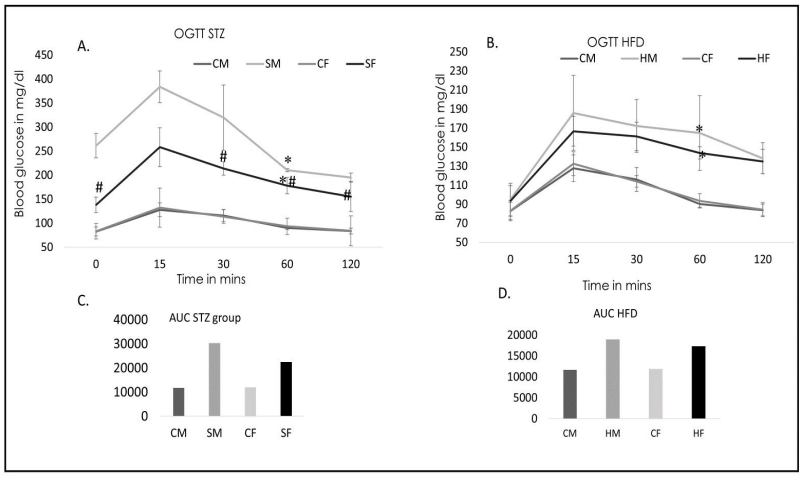


Fig. 7. Oral glucose tolerance test and area under the curve (A) STZ induced type I diabetes mice. (B) HFD induced type II *p<0.05, compared to control #p<0.05, compared to male diseases diabetes mice

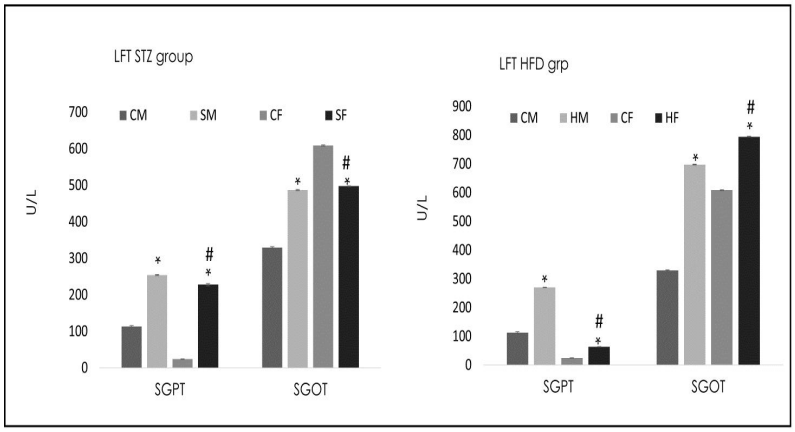


Fig. 8. Liver function test (LFT), Serum glutamic-pyruvic transaminase (SGPT), Serum glutamic-oxaloacetic transaminase (SGOT) (A) STZ induced type I diabetes mice. (B) HFD induced type II *p<0.05, compared to control #p<0.05, compared to male diseases diabetes mice

and the increase in are under the curve were 0.09-fold when compared between HFD male and HFD female. As illustrated in Fig. 7, oral glucose tolerance and area under the curve (AUC) were significantly impaired in both STZ-induced type I (A) and HFD-induced type II (B) diabetic mice ($p<0.05$ vs. control; #$p<0.05$ vs. male diabetic mice).

Liver function test: The SGPT and SGOT levels of STZ male increased by 55.5% and 32% respectively. Whereas the SGPT and SGOT levels of STZ female increased by 89% and 22% respectively when compared to control mice. The SGOT and SGPT levels of HFD male increased by 58.2% and 58.7% respectively similarly the SGOT and SGPT levels of HFD female increased by 61% and 23.3% respectively when compared to control mice. Fig. 8 shows SGPT and SGOT levels in diabetic mice. In both STZ-induced type I (A) and HFD-induced type II (B) models, enzyme levels were significantly elevated compared to controls (*$p<0.05$). Female diabetic mice also showed significant differences from males (#$p < 0.05$), indicating sex-related liver function changes.

Lipid profile test: There were 33% and 18% increase in cholesterol in STZ induced type I diabetic male and female respectively and 37% and 9% increase in the same parameter in HFD male and female respectively when compared with their gender matched control. Triglyceride levels also showed an increase of 45% in STZ male and 26% in STZ female, 14% increase of triglyceride were also observed in HFD male while the triglyceride levels were 6.7% lower in HGD females compared to control females. High density lipoprotein (HDL) levels increased in both male and female diseased groups with a rise of 18%, 20%, 34.8%, 12% in STZ male, STZ female, HFD male and HFD female respectively. In both the diseased group male showed larger increase in low density lipoprotein or LDL levels. There was 54% increase in LDL in STZ male compared to an 8% rise in the diseased female, similarly 59% and 12.82% rise in LDL levels in HFD male and HFD females respectively. Very low-density lipoprotein or VLDL levels showed a increase of 45% and 26% increase in diseased male and female of STZ group while a 14% increase in HFD males and a 6.7% decrease in VLDL of HFD females were observed. On of the major differences based on gender were observed in the LDL:HDL ratio as for STZ male and HFD male the ratio increased by 43.8% and 35% respectively while for both the females the ratio

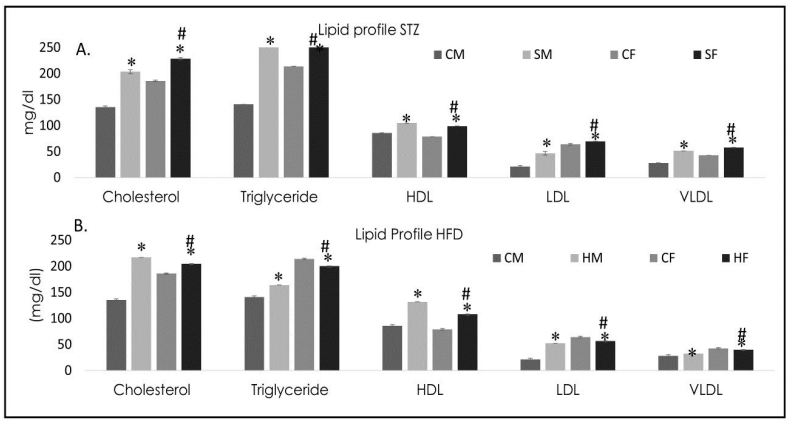


Fig. 9. Lipid profile (A) STZ induced type I diabetes mice. (B) HFD induced type II *p<0.05, compared to control #p< 0.05, compared to male diseases diabetes mice

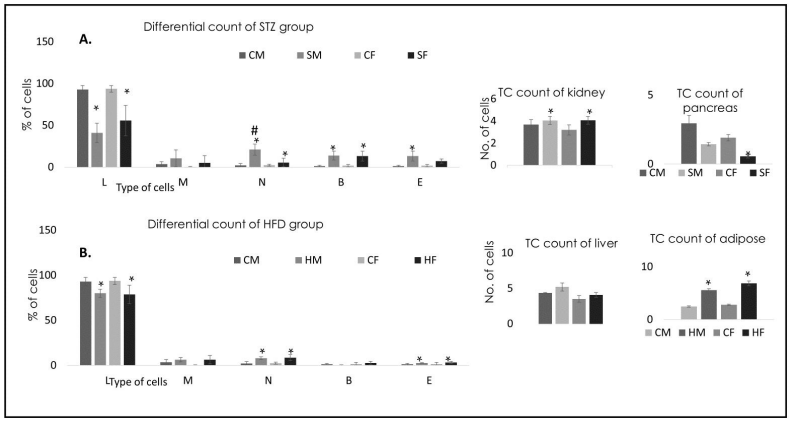


Fig. 10. Differential cell count (DC) and Total cell count (TC) (A) STZ induced type I diabetes mice. (B) HFD induced type II *p<0.05, compared to control #p<0.05, compared to male diseases diabetes mice

decreased by 15.3% and 56% respectively. Fig. 9 presents the lipid profile of diabetic mice. In both STZ-induced type I (A) and HFD-induced type II (B) models, lipid levels were significantly altered compared to controls (*p<0.05). Female diabetic mice showed significant differences from males (#p<0.05), indicating sex-based variations in lipid metabolism.

Cell count: In differential cell count 9.08-, 9.71- and 9.20-fold increase of neutrophil, basophil and eosinophil was observed in STZ male and 6.75- and 3.18-fold increase of basophil and eosinophil in STZ female when compared with gender-based control mice. Similarly, 1.06- & 0.86-fold increase of neutrophil & eosinophil and 2.88- & 2.43-fold increase HFD male & HFD female respectively, when compared with their gender matched controls. In total cell count sign 0.09- & 0.21-fold increase of kidney cell in STZ males & STZ females respectively, 1.05- & 2.42-fold decrease of pancreatic cell in STZ male & STZ female respectively. In high fat diet induced diabetes type II model 0.19-fold & 0.15-fold increase of liver cell in HFD male & HFD female respectively though the increase were statistically insignificant. Significant 1.2- & 1.4-fold increase of adipose cell in HFD male & HFD female respectively were observed. Fig. 10 shows total cell count (TC) and differential cell count (DC) in diabetic mice. Both STZ-induced type I (A) and HFD-induced type II (B) groups showed significant changes compared to controls (*p<0.05). Differences between female and male diabetic mice were also significant (#p<0.05), suggesting sex-related immune cell alterations.

Histology: In STZ induced mice pancreas histology shows atrophy in Islets of Langerhans along with swollen acinar cells, vacuoles and mononucleated cell infiltration. Histology score was 0.368-fold higher in diseased males compared to diseased females. In diseased liver tissue of STZ and HFD diabetes model, congestion of central vein portal vein, hepatic artery along with mononuclear cell infiltration Mallory body formation and hepatocyte with ballooning degradation is observed. According to liver histology- Brunt System for Grading and Staging of Steatohepatitis HFD male showed a significant 0.352-fold increase in histological score compared to HFD female. Fig. 11 and 12 present histological analyses of the pancreas and liver, respectively, in diabetic mice. In both STZ-induced type I (A) and HFD-induced type II (B) models, significant tissue alterations were observed in comparison to controls (*p<0.05). Additionally, female diabetic mice exhibited distinct histopathological changes compared to males (#p<0.05), indicating sex-specific effects on pancreatic and liver structure.

DISCUSSION

The investigation on the gender bias of streptozotocin induced mice model of type I diabetes mellitus shed light on an important aspect of preclinical studies and highlight the need for considering gender as a significant variable in experimental design. The findings demonstrate that male and female mice may

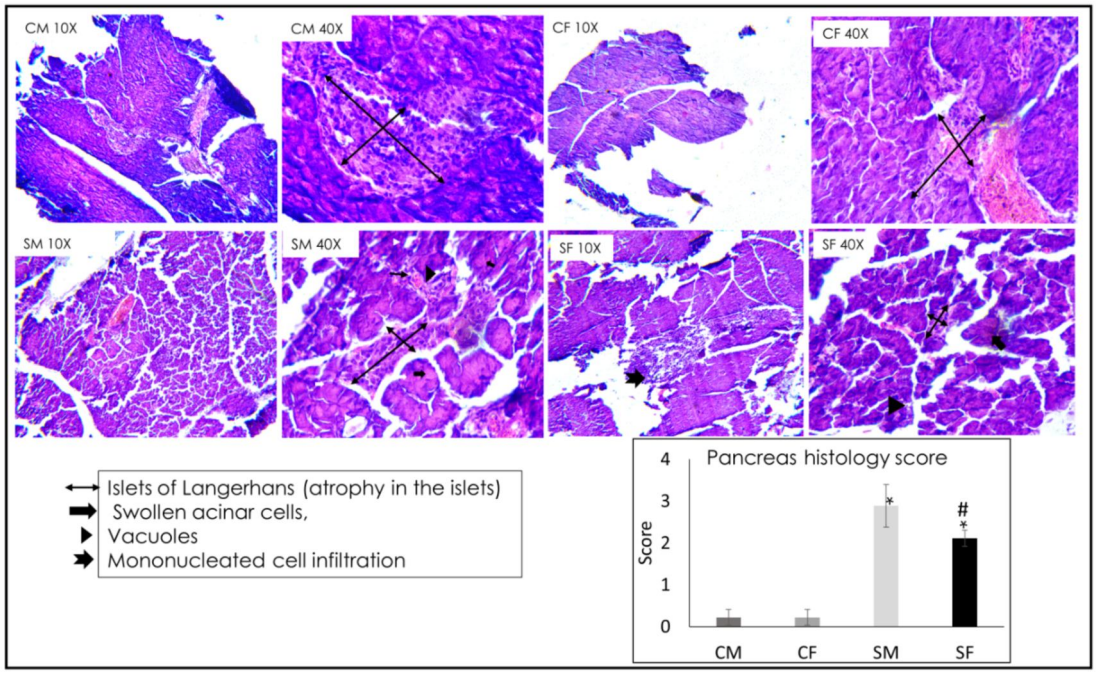


Fig. 11. Histology on Pancreas (A) STZ induced type I diabetes mice. (B) HFD induced type II *p < 0.05, compared to control #p < 0.05, compared to male diseases diabetes mice

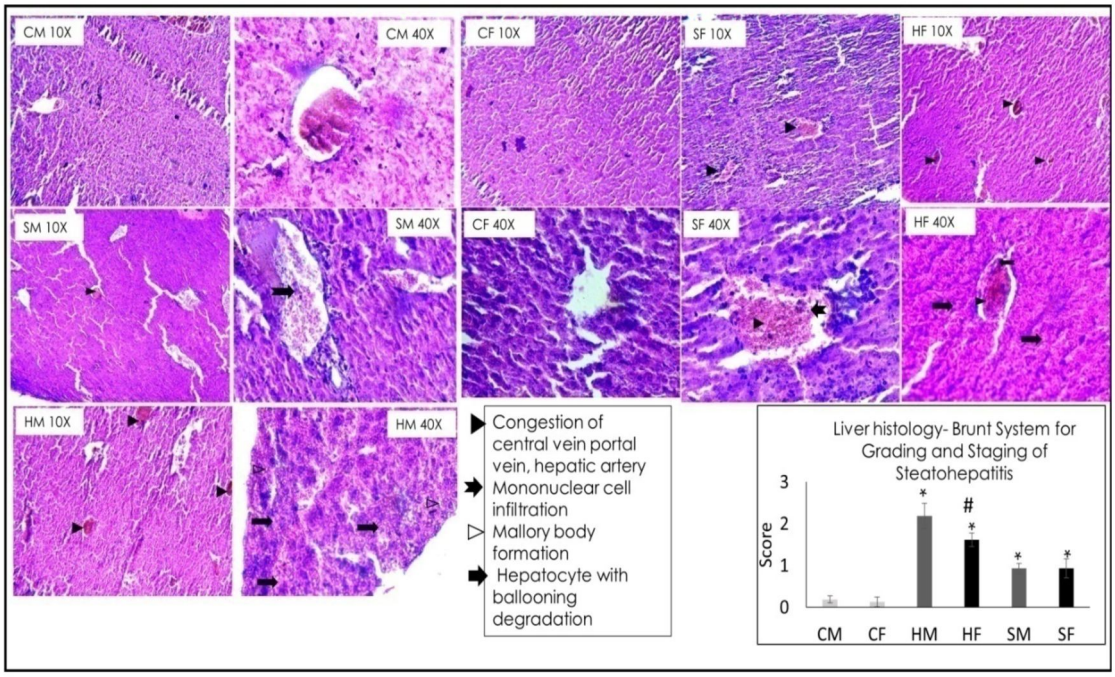


Fig. 12. Histology on liver (A) STZ induced type I diabetes mice. (B) HFD induced type II *p < 0.05, compared to control #p < 0.05, compared to male diseases diabetes mice

exhibit differential responses to STZ-induced diabetes, emphasizing the importance of sex-specific analyses and gender-based considerations in experimental protocols. The research reveals that male mice tend to develop more severe hyperglycemia, faces increased metabolic and morphometric changes. The male display greater susceptibility to STZ-induced diabetes compared to females. Male mice became diabetic with a lower dose of STZ whereas the female mice needed a higher dose of STZ for proper induction of the disease. This gender bias has important implications for understanding the pathophysiology of diabetes and evaluating potential therapeutic interventions. It suggests that there may be underlying biological, metabolic, and inflammatory factors contributing to the differential response, which warrant further investigation

The investigation on the gender bias of high-fat diet (HFD) induced obesity mice models provides valuable insights into the influence of gender on the development and progression of type II diabetes. The findings reveal that male and female mice exhibit differential responses to HFD, highlighting the importance of considering gender as a significant variable in obesity research. The research demonstrate that male mice tend to have a higher susceptibility to HFD-induced obesity compared to females. The male mice exhibited more significant weight gain, increased adiposity, and metabolic disturbances such as insulin resistance and dyslipidemia. These gender differences in response to STZ and HFD have significant implications for unrevealing the underlying mechanisms of diabetes and metabolic disorders. The prevalent significant gender bias in the study emphasizes the need for gender- specific analysis and consideration of both male and female animals in experimental design. It challenges the prevailing notion that male mice are the default choice in metabolic research and calls for a more balanced approach to investigating the complex interactions between gender, diet, and metabolic health.

This study explores the gender bias present in two commonly used diabetes models of Streptozotocin induced type I diabetes and high fat diet (60% of the calories from fat) induced type II diabetes and obesity in Swiss albino mouse by investigating the various metabolic and inflammatory factors contributing to the differential response between male and female animals. In this study demonstrate that male Swiss albino mice were more prone to development of both type I and type II diabetes compered to female Swiss albino mice. This differential response to STZ and high fat diet induced diabetes emphasizes the importance of sex-specific analyses and gender-based considerations in experimental protocols.

The majority of fundamental studies using animal models only considers males; females are not included. This creates three major problems Because female animals are underutilized, there is less knowledge about the illness processes that affect them. Results from research on male animals are frequently and unjustifiably applied to female animals, and several conditions that are more common in female animals are even studied in primarily male animals. There is a discrepancy between the percentage of female test animals and the percentage of female patients in patient populations. Sex has frequently been shown to be a significant factor, such as in the control of immunological response. Lost chances to investigate phenomena unique to female such menopause or pregnancy that may interact with disease progression. Convention and practicality may be the only explanations for the use of only male animal models. The examined population's heterogeneity is increased by the female oestrous cycle is one of the main reasons for avoiding female animals in preclinical research. Evidences suggests, rather than over-reliance on male-only animal model, balancing both sexes in the preclinical studies and drug discovery study is important as gender specific personalized dosage regimen and gender specific therapeutic intervention might be an emerging option for better management of the disease.

Conflict of interest: There are no conflicts of interest for the authors of this work.

Author's contribution: AM: Involved in investigation, data generation, preparing original draft, statistical analyses, methodology and editing; ERB: Engaged in conceptualization, data curation, supervision and final editing.

Data availability statement: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Ethical approval: All experiments were conducted in accordance with the established guidelinesset forth by the department's and institution's animal ethics committee. The University of Calcutta's Department of Zoology's Animal House provided the animals with specialised, pathogen-free housing.

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