

# Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening

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Accepted 13 May 2005

## Abstract

The objective of the present study was to develop a rat model that replicates the natural history and metabolic characteristics of human type 2 diabetes and is also suitable for pharmacological screening. Male Sprague–Dawley rats (160–180 g) were divided into two groups and fed with commercially available normal pellet diet (NPD) (12% calories as fat) or in-house prepared high-fat diet (HFD) (58% calories as fat), respectively, for a period of 2 weeks. The HFD-fed rats exhibited significant increase in body weight, basal plasma glucose (PGL), insulin (PI), triglycerides (PTG) and total cholesterol (PTC) levels as compared to NPD-fed control rats. Besides, the HFD rats showed significant reduction in glucose disappearance rate (*K*-value) on intravenous insulin glucose tolerance test (IVIGTT). Hyperinsulinemia together with reduced glucose disappearance rate (*K*-value) suggested that the feeding of HFD-induced insulin resistance in rats. After 2 weeks of dietary manipulation, a subset of the rats from both groups was injected intraperitoneally with low dose of streptozotocin (STZ) (35 mg kg<sup>-1</sup>). Insulin-resistant HFD-fed rats developed frank hyperglycemia upon STZ injection that, however, caused only mild elevation in PGL in NPD-fed rats. Though there was significant reduction in PI level after STZ injection in HFD rats, the reduction observed was only to a level that was comparable with NPD-fed control rats. In addition, the levels of PTG and PTC were further accentuated after STZ treatment in HFD-fed rats. In contrast, STZ (35 mg kg<sup>-1</sup>, i.p.) failed to significantly alter PI, PTG and PTC levels in NPD-fed rats. Thus, these fat-fed/STZ-treated rats simulate natural disease progression and metabolic characteristics typical of individuals at increased risk of developing type 2 diabetes because of insulin resistance and obesity. Further, the fat-fed/STZ-treated rats were found to be sensitive for glucose lowering effects of insulin sensitizing (pioglitazone) as well as insulinotropic (glipizide) agents. Besides, the effect of pioglitazone and glipizide on the plasma lipid parameters (PTG and PTC) was shown in these diabetic rats. The present study represents that the combination of HFD-fed and low-dose STZ-treated rat serves as an alternative animal model for type 2 diabetes simulating the human syndrome that is also suitable for testing anti-diabetic agents for the treatment of type 2 diabetes.

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**Keywords:** High-fat diet; Streptozotocin; Type 2 diabetes; Pharmacological screening; Rat model

## 1. Introduction

Type 2 diabetes mellitus is a heterogeneous disorder characterized by a progressive decline in insulin action (insulin resistance), followed by the inability of beta cells to compensate for insulin resistance (pancreatic beta cell dysfunction). Insulin resistance is a characteristic metabolic defect that pre-

cedes overt beta cell dysfunction and is primarily associated with resistance to insulin-mediated glucose disposal at the periphery and compensatory hyperinsulinemia. The beta cells normally compensate insulin resistance by secreting more amounts of insulin to maintain the glucose homeostasis. In the course of time, however, this beta cell function gets impaired leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance and frank diabetes [1,2]. There occurs only a relative insulin deficiency as the day-long circulating insulin concentrations in patients with type 2 diabetes are almost comparable or slightly elevated in absolute terms to the values in normal individuals.

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<sup>1</sup> NIPER Commn. No.: 339.

Despite the role of genetic predisposition, aging, obesity and dietetic/sedentary life style are major risk factors involved in the development of type 2 diabetes. Most of the individuals diagnosed with type 2 diabetes are found to be obese [3,4].

Although there exists a surplus of animal models (spontaneous as well as induced) available for the study of type 2 diabetes, the pattern of disease initiation and development in most of them do not appear to be closely analogous to the clinical situation in humans. However, there are certain genetic models namely Zucker diabetic fatty (ZDF) rat and db/db mouse which develop diabetes spontaneously resembling human type 2 diabetes, the development of diabetes in them is predominantly genetically determined unlike in humans [5,6]. Moreover, the observations derived from these highly inbred genetic strains may not always be satisfactorily extended to the human population as a whole because of the large heterogeneity in the latter. In addition, these animals are expensive and are not easily available for the investigative purposes as well as regular screening experiments. Further, in induced diabetic models, most of the animals (adult or neonates) requires relatively high dose of streptozotocin (STZ;  $>50 \text{ mg kg}^{-1}$ ) [6]. The development of hyperglycemia in these rats following STZ injection is primarily due to the direct pancreatic beta cell destruction, and resulting insulin deficiency rather than the consequence of insulin resistance [6,7]. Thus, they depict symptoms and characteristics typical more of human type 1 than type 2 diabetes and further are not very responsive to the effects of drugs like insulinotropic (e.g. glipizide, tolbutamide) and insulin-sensitizing compounds (e.g. pioglitazone, rosiglitazone) [8–10]. In contrast, the rats fed with high-fat diet (HFD) develop obesity, hyperinsulinemia, and insulin resistance and not frank hyperglycemia or diabetes, thus limiting the screening of agents on controlling the blood glucose level [11,12]. Hence, there exists a continued quest among the investigators for establishing the ideal animal model for type 2 diabetes either by way of modification of the existing methods or by developing new methodologies or a combination of both [6,13].

Thus, we initiated this study with the objective of developing a suitable type 2 diabetic rat model that would on the one hand closely mimic the natural history of the disease events (from insulin resistance to beta cell dysfunction) as well as metabolic features of human type 2 diabetes and on the other hand would be cheaper, easily available and useful for the investigation as well as preclinical testing of various compounds viz. insulin sensitizers and insulinotropics for the treatment of type 2 diabetes. The materialization of the disease pattern was achieved by combining the feeding of HFD which produced insulin resistance and low dose of STZ treatment that caused the initial beta cell dysfunction and subsequently the frank hyperglycemia in non-genetic, out-bred Sprague–Dawley rats.

Though attempts have been made previously by the other investigators for developing suitable animal models for type 2 diabetes by injecting STZ into genetically insulin-resistant animals (spontaneous hypertensive rats) or by a combination

of HFD and STZ treatment in normal rats and mice [13–16], the rat model described in this paper is unique in so far as the approach adopted towards the development of the model as well as its suitability for pharmacological screening is concerned.

## 2. Materials and methods

### 2.1. Materials

STZ was purchased from Calbiochem, Germany. The feed ingredients such as casein and cholesterol (both from Himedia laboratories, Mumbai, India), DL-methionine (Loba Chemie, Mumbai, India), vitamin and mineral mix (Sarabhai chemicals, Baroda, India) and yeast powder (Pet Care, Bangalore, India) were procured from the commercial sources. Lard, insulin (Eli Lilly, Gurgaon, India), heparin (S.D. Fine-Chem Ltd., Mumbai) and sodium carboxy methyl cellulose (Na-CMC) (Loba Chemie, Mumbai) were also obtained from commercial sources. Pioglitazone and glipizide were the gift samples from Ranbaxy research laboratories, India and Prof. H.P.S. Chawla, Department of Pharmaceutical Technology, NIPER, respectively. The compounds were administered orally as suspension by mixing with vehicle 1% Na-CMC at a dose volume of  $2 \text{ ml kg}^{-1}$  body weight of rats.

### 2.2. Animals

Male Sprague–Dawley (SD) rats (160–180 g) were procured from the central animal facility of the Institute. The animals were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature ( $22 \pm 2^\circ\text{C}$ ) and humidity ( $55 \pm 5\%$ ) with 12:12 h light and dark cycle. All the rats were provided with commercially available rat normal pellet diet (NPD) (Amrut Diet, New Delhi) and water ad libitum, prior to the dietary manipulation. The guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed and prior permission was sought from the institutional animal ethics committee for conducting the study.

### 2.3. Development of HFD-fed and STZ-treated type 2 diabetic rats

The rats were allocated into two dietary regimens consisting of 36 and 54 rats by feeding either NPD or HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, respectively, for the initial period of 2 weeks [13]. The composition (Table 1) and preparation of HFD as were described elsewhere [12].

After the 2 weeks of dietary manipulation, a subset of the rats (12 and 30) from each dietary group was injected intraperitoneally (i.p.) with low dose of STZ ( $35 \text{ mg kg}^{-1}$ ; for the effect of other dosage forms of STZ (25, 45 and

Table 1  
Composition of HFD

Ingredients	Diet (g/kg)
Powdered NPD	365
Lard	310
Casein	250
Cholesterol	10
Vitamin and mineral mix	60
DL-Methionine	03
Yeast powder	01
Sodium chloride	01

55 mg kg<sup>-1</sup>), see Section 3.2) while the respective control rats were given vehicle citrate buffer (pH 4.4) in a dose volume of 1 ml kg<sup>-1</sup>, i.p., respectively. The body weight and biochemical estimations (plasma glucose (PGL), triglycerides (PTG), total cholesterol (PTC), and insulin (PI)) were carried out just before and 7 days after the vehicle or STZ injection, i.e., on 3 weeks of dietary manipulation in rats. The rats with the non-fasting PGL of  $\geq 300$  mg dl<sup>-1</sup> were considered diabetic and selected for further pharmacological studies. The feed and water intake of the animals were also measured. The rats were allowed to continue to feed on their respective diets until the end of the study.

#### 2.4. Intravenous insulin glucose tolerance test (IVIGTT)

In order to find out the insulin sensitivity of various groups of rats, a simple IVIGTT was carried out as per the literature method with the following modification [16]. A subset of six rats from different groups was fasted for 3 h before IVIGTT. The animals were anaesthetized with a single dose of a mixture of ketamine (70 mg kg<sup>-1</sup>, i.p.) and xylazine (4 mg kg<sup>-1</sup>, i.p.). The two femoral blood vessels were exposed by incision through the skin in the inguinal area. These vessels were cannulated with plastic fine cannulae and then the IVIGTT was performed. The rats were successively injected with glucose (0.7 g kg<sup>-1</sup>) and insulin (0.175 U kg<sup>-1</sup>) into the femoral vein. Blood samples (0.1 ml) were then withdrawn from the femoral artery at approximately 0 (before glucose), 2, 4, 6, 8, 10, 20 and 30 min after insulin injection for the glucose estimation. The equivalent volume of sterile saline (0.9%) was injected into femoral vein after each sampling to prevent the changes in central compartmental blood volume. Insulin sensitivity was measured by the glucose disappearance rate within 10 min, evident from average slope *K* in the fitting curve. The *K*-value was determined by linear regression over this period [16].

#### 2.5. Effect of anti-diabetic compounds on HFD-fed and STZ-treated diabetic rats

In order to determine the validity and suitability of the fat-fed/STZ rat model for pharmaceutical testing, the response of this model to the classes of anti-diabetic drugs commonly used to treat type 2 diabetes, such as insulin sensitizers (thiazolidinediones (TZDs), e.g. pioglitazone) and insulin secretagogues (sulfonylureas, e.g. glipizide) was tested on this

model. Moreover, these compounds have not been tested for anti-diabetic activity elsewhere in the fat-fed/STZ-treated rat models. The fat-fed/STZ diabetic rats were randomly divided into three groups consisting each of six rats after 3 weeks of dietary manipulation (i.e., after 1 week of STZ injection) such that their mean PGL, PTG and PTC levels were similar to each other. The diabetic rats were either treated with pioglitazone (10 mg kg<sup>-1</sup> once daily for 7 days) or a single dose (s.d.) of glipizide (5 mg kg<sup>-1</sup>) while the control group was given vehicle 1% Na-CMC (2 ml kg<sup>-1</sup>, p.o.). The blood samples were collected 3 h after the administration of the vehicle or test compounds to determine the effects of these agents on plasma biochemical parameters viz. PGL, PTG, PTC, and PI levels in the treated animals.

#### 2.6. Collection of blood and analytical methods

Blood was collected from retro-orbital plexus of the rats under light ether anesthesia using capillary tubes into eppendorf tubes containing heparin (20  $\mu$ l, 200 IU ml<sup>-1</sup>). The plasma was separated by centrifugation (5 min, 5000 rpm) and was analyzed for glucose (GOD-POD), triglycerides (GPO-POD) and total cholesterol (CHOD-POD) levels using commercially available colorimetric diagnostic kits (Accurex Biomedical Pvt. Ltd., Thane, India). The remaining plasma samples were then stored at -20 °C until the insulin determination was made by radioimmunoassay using rat insulin as standard (Linco Research, St. Charles, MO, USA).

#### 2.7. Statistical analysis

The results are expressed as mean  $\pm$  S.E.M. The unpaired Student's *t*-test was used for analyzing the data between two groups where as one-way ANOVA followed by multiple comparison test (Tukey's test) was employed if there were more than two groups. A value of *p* < 0.05 was considered statistically significant.

### 3. Results

#### 3.1. Features of HFD-fed insulin-resistant rats

Table 2 illustrates that the feeding of HFD for 2 weeks resulted in significant increase (*p* < 0.05) in body weight as well as non-fasting PGL, PI, PTG and PTC levels in rats as compared to NPD-fed rats. On IVIGTT, HFD rats exhibited significant reduction in glucose disappearance rate (*K*-value, representing the level of insulin sensitivity) as compared to NPD-fed control rats (Table 2).

#### 3.2. Effect of STZ on NPD-fed and HFD-fed insulin-resistant rats

Injection of STZ (35 mg kg<sup>-1</sup>, i.p.) after 2 weeks of dietary manipulation significantly (*p* < 0.05) increased PGL in HFD rats, thus producing the frank hyperglycemia where as it

Table 2  
Effect of HFD on body weight and biochemical parameters in rats

Parameters	NPD-fed	HFD-fed
Body weight (g)	208.43 ± 4.41	233.54 ± 1.78*
PGL (mg dl <sup>-1</sup> )	105.25 ± 0.74	126.39 ± 2.46*
PTG (mg dl <sup>-1</sup> )	33.57 ± 0.52	60.31 ± 2.13*
PTC (mg dl <sup>-1</sup> )	48.46 ± 1.62	111.53 ± 3.15*
PI (pmol l <sup>-1</sup> )	285.50 ± 23.52	457.50 ± 32.69*
K-value	27.03 ± 1.85	17.70 ± 2.55*

Values are mean ± S.E.M; the abbreviations denote PGL: plasma glucose, PTG: plasma triglyceride, PTC: plasma total cholesterol and PI: plasma insulin.

\*  $p < 0.05$  vs. NPD group ( $n = 10-12$ ) except for finding K-values where  $n = 5-6$ .

caused only a small but statistically significant increase in PGL in NPD-fed rats as compared to vehicle-treated control rats (Table 3). In addition, there was a significant ( $p < 0.05$ ) increase in basal PTG and PTC coupled with significant reduction in PI levels in HFD rats while these parameters remained largely unaltered following STZ injection in NPD-fed rats as compared to the respective vehicle treated control groups. Nevertheless, the reduction of PI level was observed in HFD rats only to a level, which was still comparable with NPD-fed control rats. In addition, STZ (35 mg kg<sup>-1</sup>, i.p.) produced significant reduction in the body weights of the HFD-fed rats, which was still considerably higher than NPD-fed rats. However, the body weights remained largely unaltered between STZ-treated and control NPD-fed rats (Table 3). Further on IVIGTT, it was shown that glucose disappearance rate (K-value) remained significantly low as compared to NPD-fed control rats (Fig. 1; Table 3). Also, these HFD-fed, STZ-diabetic rats developed the symptoms of polyphagia, polydipsia and polyuria as compared to NPD-fed control rats (data not shown).

However, the injection of STZ (45 and 55 mg kg<sup>-1</sup>, i.p.) after 2 weeks of dietary manipulation produced frank hyperglycemia and insulin deficiency both in NPD-fed and HFD-fed rats in a comparable manner (data not shown). Moreover, these fat-fed/STZ (45 and 55 mg kg<sup>-1</sup>, i.p.) diabetic rats exhibited a drastic reduction in the body weights (data not shown). In contrast, the dose of STZ (25 mg kg<sup>-1</sup>) did not produce significant hyperglycemia in NPD- as well as HFD-fed rats (data not shown).

Table 3  
Effect of STZ (35 mg kg<sup>-1</sup>, i.p.) on NPD- and HFD-fed rats

Parameters	NPD	NPD + STZ	HFD	HFD + STZ
Body weight (g)	235.43 ± 4.94	241.00 ± 3.58	268.35 ± 3.50*	253.58 ± 2.72*
PGL (mg dl <sup>-1</sup> )	101.06 ± 3.94	137.11 ± 5.47*	129.33 ± 2.49*	418.42 ± 8.45*#
PTG (mg dl <sup>-1</sup> )	30.36 ± 3.42	35.02 ± 2.07	70.70 ± 2.66*	173.34 ± 8.21*#
PTC (mg dl <sup>-1</sup> )	47.00 ± 3.42	43.00 ± 1.91	116.18 ± 7.14*	178.57 ± 7.75*#
PI (pmol l <sup>-1</sup> )	262.00 ± 23.52	265.50 ± 22.60	467.50 ± 32.43*	217.68 ± 26.67*#
K-value	26.00 ± 2.00	ND	16.19 ± 2.48*	15.53 ± 2.08*

Values are mean ± S.E.M.; ND: not determined. The abbreviations denote PGL: plasma glucose, PTG: plasma triglyceride, PTC: plasma total cholesterol and PI: plasma insulin.

\*  $p < 0.05$  vs. NPD group.

#  $p < 0.05$  vs. HFD group ( $n = 10-12$ ) except for finding K-values where  $n = 5-6$ .

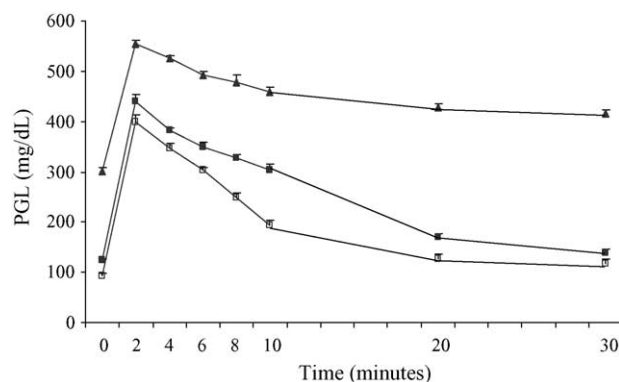


Fig. 1. Intravenous insulin glucose tolerance test on (□) NPD, (■) HFD-fed and (▲) HFD+ STZ-treated rats. Values are mean ± S.E.M. ( $n = 5-6$ ). PGL: plasma glucose.

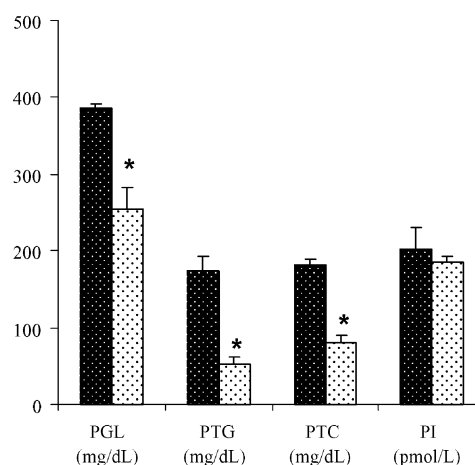


Fig. 2. Effect of pioglitazone (10 mg kg<sup>-1</sup>, p.o. for 7 days) on various biochemical parameters in fat-fed and STZ-treated rats. Values are mean ± S.E.M. \*  $p < 0.05$  vs. vehicle control group ( $n = 5-6$ ). Legends indicate (■) vehicle and (▨) pioglitazone. The abbreviations denote PGL: plasma glucose, PTG: plasma triglyceride, PTC: plasma total cholesterol and PI: plasma insulin.

### 3.3. Effect of anti-diabetic compounds on HFD-fed and STZ-treated diabetic rats

The HFD-fed/low-dose STZ (35 mg kg<sup>-1</sup>, i.p.)-treated type 2 diabetic rat model was validated by the administration of antihyperglycemic compounds. Oral administration of

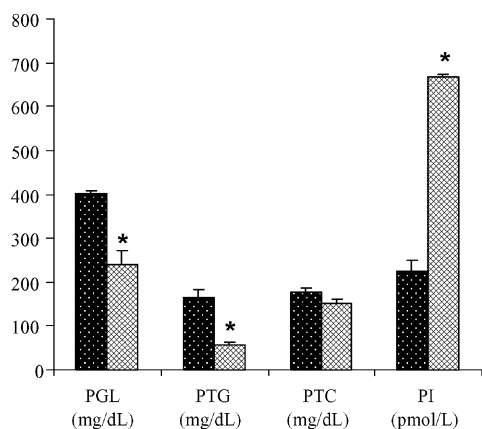


Fig. 3. Effect of glipizide ( $5 \text{ mg kg}^{-1}$ ; p.o.; s.d.) on various biochemical parameters in fat-fed/STZ-treated rats. Values are mean  $\pm$  S.E.M. \* $p < 0.05$  vs. vehicle control group ( $n = 5-6$ ). Legends indicate (■) vehicle and (▨) glipizide. The abbreviations denote PGL: plasma glucose, PTG: plasma triglyceride, PTC: plasma total cholesterol and PI: plasma insulin.

pioglitazone for 7 days significantly ( $p < 0.05$ ) reduced PGL (34.2%) without markedly altering PI levels. In addition, it significantly diminished PTG (69.4%) and PTC (55.5%) levels, respectively, as compared to vehicle-treated diabetic rats (Fig. 2). Interestingly, this was found to be not at the expense of feed intake or body weight alterations (data not shown).

Likewise, glipizide ( $5 \text{ mg kg}^{-1}$ ; p.o.; s.d.) also caused significant reduction in PGL (40.2%), which was, however, associated with significant ( $p < 0.05$ ) increase in PI levels. Further, glipizide significantly decreased PTG (65.8%) without considerable effect on PTC levels as compared to vehicle-treated diabetic rats (Fig. 3).

In contrast, both insulinotropic (glipizide) and insulin sensitizing agents (pioglitazone) failed to elicit any significant effect on PGL in the fat-fed, STZ ( $45$  and  $55 \text{ mg kg}^{-1}$ )-treated diabetic rats (data not shown).

#### 4. Discussion

This study was initiated with the objective of developing an ideal model for type 2 diabetes that would closely reflect the natural history and metabolic characteristics of human type 2 diabetes and respond to the pharmacological treatments. Further, it should be less expensive, easily available, taking relatively shorter period for development and adequate enough to allow for invasive procedures to the investigators.

Thus, our initial attempts were directed towards finding the threshold dose of STZ that is low enough to guarantee the development of type 2 diabetes in HFD rats without much circulating insulin deficiency and further sensitive for pharmacological testing. The different doses of STZ (25, 35, 45 and  $55 \text{ mg kg}^{-1}$ , i.p.) were studied. Injection of STZ (45 and  $55 \text{ mg kg}^{-1}$ ; i.p.; s.d.) after 2 weeks of dietary manipulation caused frank hyperglycemia both in NPD- and HFD-fed rats in a manner to literature reports [13]. Further, these

rats were insulin-deficient as compared to the normal rats. Moreover, these diabetic rats exhibited a drastic reduction in the body weight and some of them died within 2 weeks of STZ administration. Both insulinotropic (glipizide) and insulin-sensitizing (pioglitazone) agents failed to alter the PGL in these fat-fed/STZ ( $45$  and  $55 \text{ mg kg}^{-1}$ ) diabetic rats. Thus, these fat-fed rats with high dose of STZ ( $45$  and  $55 \text{ mg kg}^{-1}$ ) resembled more like type 1 diabetes. In contrast, STZ ( $25 \text{ mg kg}^{-1}$ ) did not produce significant hyperglycemia in NPD- as well as HFD-fed rats, and hence, was not studied further. Interestingly, the dose of STZ ( $35 \text{ mg kg}^{-1}$ , i.p.) that produced frank hyperglycemia in HFD-fed rats failed to produce the same in NPD-fed rats. The HFD rat model with low dose of STZ ( $35 \text{ mg kg}^{-1}$ ) thus can be more considered to represent the pathophysiological state of type 2 diabetes and was accompanied by marginal increase in body weight in contrast to the catabolic loss of body weight, characteristic of diabetic condition produced by high dose of STZ. Hence, HFD in combination with low dose of STZ ( $35 \text{ mg kg}^{-1}$ ) was chosen for generating the rat model for further studies.

The development of a rat model possessing insulin resistance by feeding the rats with HFD prepared in-house has been described in detail elsewhere [12]. The HFD was formulated (58% calories as fat), such that it causes insulin resistance in rats over a short period of time. Thus, the feeding of HFD for a period of 2 weeks produced rats with insulin resistance syndrome as was characterized by the increased body weight (obesity), mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia together with reduced glucose disappearance rate ( $K$ -value), a condition similar to prediabetic, insulin-resistant state in humans [12,17]. HFD has been shown to induce insulin resistance by different mechanisms but considered mainly through Randle or glucose–fatty acid cycle [18]. Briefly, the presence of high level of triglycerides due to excess fat intake could constitute a source of increased fatty acid availability and oxidation. The preferential use of increased fatty acids for oxidation blunts the insulin-mediated reduction of hepatic glucose output and reduces the glucose uptake or utilization in skeletal muscle leading to compensatory hyperinsulinemia, a common feature of insulin resistance [19–21]. The increased body weight found in HFD rats might be due to the consumption of a diet rich in energy in the form of saturated fats (lard) and its deposition in various body fat pads [12] and decreased energy expenditure as compared to NPD-fed animals [11].

The conversion of prediabetes to frank hyperglycemia in patients with type 2 diabetes is associated with decline in secretory capacity of pancreatic beta cells to compensate for the existing insulin resistance. But, there is only a relative insulin deficiency as the circulating day-long insulin concentrations in patients with type 2 diabetes are comparable in absolute terms to the values seen in non-diabetic individuals [13]. The evolution of disease pattern was achieved in insulin-resistant HFD rats upon injection with low dose of STZ ( $35 \text{ mg kg}^{-1}$ , i.p.) which produced frank hyperglycemia

in the presence of circulating insulin concentration almost comparable to normal rats (relative insulin deficiency) where as the same dose did not significantly decrease the insulin secretory capacity enough to cause overt hyperglycemia in rodents fed with NPD. It is interesting and noteworthy that the development of diabetes occurs only in insulin-resistant HFD-fed rats but not in NPD-fed normal rats following low dose of STZ ( $35 \text{ mg kg}^{-1}$ , i.p.). As such, they simulate natural disease progression and metabolic condition of individuals at increased risk of developing type 2 diabetes (because of insulin resistance and obesity). The reasons for the high degree of glycemic difference induced by STZ ( $35 \text{ mg kg}^{-1}$ , i.p.) between these two groups might be that HFD-fed rats were already insulin resistant together with compensatory hyperinsulinemia to maintain glucose homeostasis, and hence, even the slight insult by low dose of STZ that could compromise the beta cell function might lead to drastic hyperglycemic effect as against the NPD-fed normal animals where in the effect could be compensated by normal defense homeostasis mechanisms. Furthermore, in the case of HFD-fed rats, they were already mildly hyperglycemic due to insulin resistance, thus enhancing their susceptibility to diabetogenic effect of STZ and needs further investigation [5].

Apart from glucose, these fat-fed, insulin-resistant STZ animals also showed abnormalities in lipid metabolism as evidenced from increased PTG and PTC levels, as in case of human type 2 diabetic patients which might contribute to various cardiovascular complications. The hypertriglyceridemia observed in these fat-fed/STZ rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of TG-enriched hepatic very low density lipoprotein (VLDL) and decreased TG uptake in peripheral tissues [12]. Hypercholesterolemia may be attributed to increased dietary cholesterol absorption from the small intestine following the intake of HFD in a diabetic condition [6,22]. To determine insulin sensitivity, we adopted IVIGTT in these rats. Rats are larger enough to allow the investigators to perform these kinds of invasive procedures (including certain clamp studies) more easily as compared to mice model. On IVIGTT, the fat-fed/STZ rats exhibited significant reduction in the glucose disappearance rate ( $K$ -value) as compared to control rats. The frank hyperglycemia in the presence of comparable amount of PI concentrations together with reduced  $K$ -value indicated the persistence of insulin resistance even after STZ injection in HFD rats. Despite the well-described effects of hyperlipidemia in affecting the whole-body insulin sensitivity, the development of frank hyperglycemia following STZ injection might further exacerbate insulin resistance as characterized by further decline in the  $K$ -value in fat-fed/STZ rats as compared to the rats fed with HFD alone [1,23]. Hence, this model with the involvement of both insulin resistance and obvious beta cell dysfunction in the development of diabetes could be suitable for studying the pathophysiology of type

2 diabetes as well as for testing new compounds which act through ameliorating insulin resistance and/or by increasing beta cell insulin secretion.

In order to validate the model for further pharmaceutical testing, we chose pioglitazone (an insulin sensitizer) and glipizide (an insulin secretagogue) for the first time to be tested in this model. The administration of pioglitazone for 7 days was able to reduce the PGL, PTG and PTC levels, indicating its potent antihyperglycemic and hypolipidemic activity. The effect of pioglitazone on biochemical parameters was observed without significant alteration in the PI levels, and thus suggesting its mechanism of action might be through improving whole body insulin sensitivity rather than stimulating the beta cell insulin secretion. Pioglitazone, like other TZD-based drugs might have reduced the PGL by sensitizing the insulin action in the target tissues mainly through diminishing lipolysis in adipose tissue and subsequent reduction of glucose production in liver and enhancement of insulin-mediated glucose disposal in skeletal muscle [12]. Further, the attenuating effect of pioglitazone on hyperlipidemia might result either from the inhibition of TG synthesis in liver or increased TG clearance in the periphery (by stimulating the enzyme lipoprotein lipase (LPL)) and/or inhibition of dietary cholesterol absorption from the intestine [12,22]. Its actions are known to be mediated largely by interaction with nuclear peroxisome proliferator-activated receptor (PPAR)  $\gamma$  receptors in target tissues [24].

In contrast to pioglitazone, the glucose-lowering effect of glipizide was, however, associated with significant increase in PI level, thus suggesting its mechanism of action to take place through the stimulation of pancreatic beta cell secretion. Though we did not study the extent of beta cell destruction in this model, the normal basal insulin and elevated PI levels following the administration of insulinotropic agent (glipizide) apparently revealed the presence of sufficient amount of functional beta cell population that was further sensitive to the effects of insulinotropic agent resembling human type 2 diabetes which is in agreement with the earlier report on the other diabetic rat model produced by the combination of STZ and nicotinamide treatment [25]. This property is distinctly different from neonatal-STZ diabetic rats where glipizide fails to alter significantly the circulating glucose and insulin levels in vivo and further their beta cells are completely unresponsive to the insulin stimulatory action of insulinotropic (tolbutamide) agent and glucose in vitro [8,26,27]. However, PTG-lowering effect of glipizide without significantly altering PTC levels in fat-fed/STZ model is in agreement with the clinical reports on human type 2 diabetic patients [28,29].

Since we used low dose of STZ ( $35 \text{ mg kg}^{-1}$ ) for inducing diabetes, we thought that the early reversal of hyperglycemia following the regeneration of the existing beta cells would probably occur in these fat-fed/STZ rats. It was, however, ruled out as the elevated glucose concentrations were relatively stable over a period of 10 weeks when tested on this model. Hence, this model could also be useful for the long-term studies on diabetic complications, such as neuropathy,

nephropathy and hypertension, which are presently underway in our laboratory.

Based on the foregoing observations, an appreciation of the current animal model can be made as compared with the other literature models in the light of following discussion. It was Reed et al. who initially developed the fat-fed STZ rat [13] and was a non-obese, diabetic model unlike the one reported in the present study. Reed et al. used high dose of STZ (50 mg kg<sup>-1</sup>, i.v.) for inducing diabetes in HFD rats, which, however, caused extreme insulin deficiency and overt hyperglycemia in normal control animals. In their method, both the control and HFD-fed rats developed diabetes in contrast to ours where diabetes developed only in insulin-resistant HFD rats following STZ injection, reflecting closely the natural situation of the individuals with increased risk factors (viz. insulin resistance and obesity) being more prone to develop type 2 diabetes than others. Also, their model was not validated with any insulinotropic agent and thus limiting its utility for anti-diabetic screening of such agents. Later, Zhang et al. developed a fat-fed/STZ rat model with a minor modification. Though the authors used relatively low dose of STZ (15 mg kg<sup>-1</sup>) for inducing hyperglycemia in HFD rats, it was more time consuming (4 months) for the model development and was not validated with any antihyperglycemic agent for proving its usefulness in pharmaceutical testing. In addition, the other literature animal models use combination of STZ followed by HFD [30,31]. In the latter, there is initial decrease in circulating insulin concentration caused by STZ and is followed with hyperglycemia. In other words, the development of hyperglycemia in these rats has been mainly because of insulin deficiency rather than the consequence of insulin resistance unlike in humans.

## 5. Conclusions

Our study demonstrates that a combination of HFD and low dose of STZ treatment can be effectively used to generate a rat model that mimic the natural history and metabolic characteristics of the common type 2 diabetes in humans. It is cheap, easy to develop and most suited for studying the pathophysiology of type 2 diabetes and is also useful in evaluating the therapeutic compounds for the treatment of type 2 diabetes.

## Acknowledgement

The authors are immensely thankful to Prof. K.P.R. Kartha, Department of Medicinal Chemistry, NIPER, for proof-reading this article.

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