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Fatty-Sucrosed Diet/Minimal Dose of Streptozotocin-Treated Rat: A Novel Model of Gestational Diabetes Mellitus, Metabolic and Inflammatory Insight

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Abstract

To date, a variety of experiments were done to get animal models of diabetes types. The current study is a trial to get a Gestational Diabetes Mellitus (GDM) model which is comparable to that in human, using a minimal dose of Streptozotocin (STZ). Female albino rats were divided into two groups; one fed Normal Diet (ND) and the other fed Fatty-Sucrosed Diet (FSD) from 60 days of age onward. After five weeks on the diets (pregestational period), rats were mated and STZ (25 mg/kg b.wt.) was injected intraperitoneally to FSD-fed dams at the 7th day of gestation. During pregestational period, FSD-fed rats exhibited significant increase in body weight that reduced significantly after STZ injection in comparable to ND-fed rats. Frank hyperglycemia with mild decrease in serum insulin level of Gestational Diabetic (GD) dams showed a state of insulin resistance that clarified by the increase in Homeostasis Model Assessment Of Insulin Resistance (HOMA-IR) and subsequent decrease in Quantitative Insulin Sensitivity Check Index (QUICKI) values. Also, blood glycated hemoglobin (HbA1c) and fructosamine were significantly increased while hepatic glycogen content was decreased. In addition, lipid profile of GD-dams showed a significant increase in levels of Triglycerides (TG), total-cholesterol (Total-Ch.), LDL-cholesterol (LDL-Ch.) and vLDL-cholesterol (vLDL-Ch.) while HDL-cholesterol (HDL-Ch.) was decreased. Furthermore, serum adipokines levels showed a significant increase in leptin and tumor necrosis factor-alpha (TNF-α) while adiponectin level was significantly decreased. On the other hand, the diabetic dams exhibited high rate of implantation loss and impaired fetal glycaemia. In conclusion the combination of FSD and a minimal dose of STZ can effectively induce gestational diabetes analogue to that of human and suitable for further investigations of physiological and molecular abnormalities in GDM.

Keywords: Fatty-sucrosed diet; Streptozotocin; Gestational diabetes; Insulin resistance; Adipokines; Rat model

Introduction

Diabetes mellitus is a multi-systemic disorder, affects almost every cell in the body and considered one of the most important health problems worldwide [1,2]. As the global epidemic of diabetes continues to expand, the prevalence of Gestational Diabetes Mellitus (GDM) increased from 1.4% to 25.5% over the last twenty years [3].

Gestational Diabetes Mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy, irrespective of the glycemic status after delivery [4]. Insulin resistance and inadequate insulin secretion founded to play a central role in the pathophysiology of GDM [5]. Gestational diabetic women have increased risk of Type 2 Diabetes Mellitus (T2DM) and heart disease later in life [6], and their offspring have greater incidence of perinatal complications and increased risk of obesity and diabetes in adulthood [7].

Several models were developed to study gestational diabetes. The preferred and most often used experimental models are on rodents because of their convenient maintenance, short length of pregnancy, multiparty (enabling studies on multiple fetuses and generations), and lack of special problems in termination of pregnancy and fetus recovery [8]. GD-models include the genetic altered obese rats, high fat feeding rats and Streptozotocin (STZ) induced gestational diabetes, but these models do not reflect the metabolic characteristics of human GDM. However, the observations derived from the highly inbred genetic rat strains may not always be satisfactorily extended to the human population as a whole because of the large heterogeneity in the latter; these strains are expensive and are not easily available for the investigative purposes as well as regular screening experiments [9]. Moreover, the rats fed with High Fat Diet (HFD) develop obesity, hyperinsulinemia and insulin resistance but not cause frank hyperglycemia or diabetes, thus limiting the screening of agents on controlling the blood glucose level [10]. STZ-induced gestational diabetes either by a low (30 mg/kg) or high (50 mg/kg) dose of STZ on day one of gestation creates mild or severe maternal diabetes, respectively, resulting in direct pancreatic beta cell destruction and insulin deficiency rather than the consequence of insulin resistance, and causing various degrees of fetal resorption and malformed fetuses [11]. This aimed to study initiates an animal model that develops a suitable state closely mimic to the natural history (from insulin resistance to beta cell dysfunction) and metabolic features of human gestational diabetes.

Materials and Methods

Animals

Female virgin white albino rats (*Rattus norvegicus*) weighing about 100 ± 10 g, 60 day old, were used as experimental animals in this study. They were obtained from the animal house of Helwan town, Cairo, Egypt. The chosen animals were housed individually in standard polypropylene cages and maintained under normal atmospheric room temperature (25 ± 5°C), humidity (55 ± 5%), illumination (12 h light/12

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h dark cycles) for one week before the onset of the experiment to be acclimatized. Rats had free access to water and to two dietary regimens.

Dietary formula

The rats were allocated into two dietary regimens by feeding either normal diet (ND; 60% starch, 5% corn oil and 20% casein protein as g%) or fatty-sucrosed diet (FSD; 25% sucrose, 40% beef tallow and 20% casein protein as g%). Diet ingredients were purchased from Oxford Laboratories, Mumbai, India. Diets were prepared in the Department of Nutrition, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt, at intervals according to requirements and stored at 4°C till use.

Experimental design and animal grouping

A total 40 rats were randomly assigned into two groups. ND group (received the normal diet) and FSD group (received fatty-sucrosed diet).

Five weeks after dietary manipulation, both groups were time mated overnight with males and the presence of sperms in the vaginal smear checked in the morning will be considered zero day of pregnancy. Female rats with negative detected sperms in the vaginal smear were excluded. Time before mating is referred to pre-gestational period while that after mating is referred to gestational period.

At the 7th day of pregnancy FSD-feeding rats were fasted for 16 hours and then injected intraperitoneally (i.p.) with low dose of STZ (25 mg/kg b.wt. in citrate buffer; pH 4.5) (preliminary study for the effect of other doses of STZ, 20, 30 and 35 mg/kg, with FSD-feeding were performed). ND-feeding rats were i.p. injected only with the vehicle (citrate buffer; pH 4.5). Glucose and insulin levels were evaluated on 6th (pre-STZ), 13th and 20th (post-STZ) days of pregnancy. At the 21st day of pregnancy, overnight fasted dams were sacrificed. Blood and tissues sampling and their all measured parameters were referred to main two groups (n=10, each):

- NP + ND group: normal pregnant dams received normal diet.

- GD + FSD group: gestational diabetic dams received FSD/minimal dose of STZ (25 mg/kg b.wt.).

The experimental design and animal grouping is summarized in Figure 1.

Blood sampling and tissue preparation

Two blood samples were collected from each dam. The first was collected into a tube containing Ethylene Di-Amine Tetra-Acetic Acid (EDTA) as anticoagulant and used for determination of HbA1c. The second blood sample was allowed to coagulate, centrifuged and sera were kept at -20°C for subsequent analysis. After dissection of the sacrificed rats, each dam uterine horns exposed to count fetus numbers and implantation loss sites. Fetuses were delivered and weighed. Fresh maternal liver samples were excised for determination of glycogen content. Blood pool was collected from the axillary vein of newborns of each dam and sera were separated for the immediate measurement of glucose and insulin.

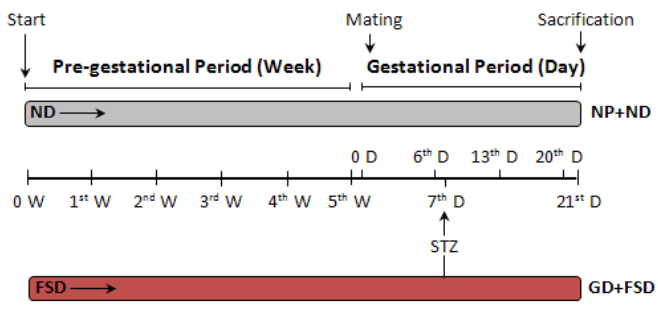

Figure 1: Experimental design and animal grouping. NP+ND: Normal pregnant dams received normal diet, GD+FSD: Gestational diabetic dams received fatty-sucrosed diet/minimal dose of streptozotocin (STZ; 25 mg/kg b.wt.).

Food intake and body weight changes

Food was provided in standard stainless steel hoppers. Food intake was calculated daily at the same time by subtracting the amount of food left over in the cage of each rat from the measured amount of food provided at the previous day. The mean of food consumption per each rat was considered by dividing the amount of food eaten in a week by 7 [12]. The average of food consumptions were represented in g/day/rat. Body weight for each rat was determined once a week (g).

Biochemical measurements

Serum levels of glucose, fructosamine, triglycerides, total-cholesterol and HDL-cholesterol were determined using reagent kits obtained from Spinreact Company (Spain). Insulin was assayed in serum by Sandwich ELISA method using reagent kit purchased from BioSource Europe S.A. (Belgium), while serum leptin, TNF-α and adiponectin were estimated using reagent kits procured from RayBiotech, Inc. (USA). Because abnormalities in insulin action are poorly detected by a single determination of glucose or insulin levels [13], the maternal glucose-insulin homeostasis state were described by calculating HOMA-IR [14] and QUICKI [15]. HbA1c was determined using reagent kit purchased from BioSystems S.A. (Spain). Also, hepatic glycogen content was detected [16]. Serum LDL-cholesterol, vLDL-Cholesterol, Cardiovascular Risks (CVR1 & CVR2) and Anti-Atherogenic Index (AAI) were estimated [17-20].

Statistical analysis

All results are expressed as the mean ± SEM. Two-way Analysis of Variance (ANOVA) followed by Duncan's method for post-hoc analysis was performed to compare the data of maternal body weight, food intake, and serum glucose and insulin levels. Student's *t* test was used for analyzing other parameters. All results were analyzed using Statistical Package for Social Science (SPSS) version 20 software with significance set at P<0.05 [21].

Results

Body weight and food intake changes

A) During pre-gestational period (Tables 1a and b and Figures 2 A and B)

FSD-feeding rats showed an obvious increase in their body weight up to the 5th weeks accompanied with marked decrease in the food intake onward as compared to ND-fed rats. Two-way ANOVA revealed significant effect of time, diet (P<0.001) and time-diet interaction (P<0.01) on the body weight. However, the effect of time and diet were insignificant on the food intake, their interaction depicted significant effect (P<0.001).

B) During gestational period (Tables 2 a and b and Figure 3 A and B)

At the 6th day, GD+FSD dams showed marked increase in their body weight when compared to the normal ones. This increase is no

Parameter	Group	Pre-gestational period (Time)					
		0 week	1st week	2nd week	3rd week	4th week	5th week
Body weight (g)	ND	115.66 ± 3.64^{a}	142.00 ± 3.60^{b}	155.21 ± 4.17^{bc}	166.28 ± 1.86^{cd}	176.91 ± 4.47^{def}	189.86 ± 4.76^{ef}
	FSD	119.75 ± 2.59^{a}	160.41 ± 3.68^{c}	176.08 ± 2.66^{de}	191.75 ± 1.54^{fg}	205.58 ± 1.76^{gh}	214.06 ± 1.41^{h}
Food intake (g/day/rat)	ND	--	12.40 ± 0.31^{a}	14.42 ± 0.58^{abc}	14.53 ± 0.34^{abc}	15.93 ± 0.45^{bc}	16.17 ± 0.62^{c}
	FSD	--	16.65 ± 0.23^{c}	15.82 ± 0.74^{bc}	14.47 ± 0.47^{abc}	13.73 ± 0.6^{ab}	12.72 ± 0.28^{a}

Data shown as mean ± SEM of six rats
For each parameter, values with the same superscript letter are similar (non-significant, P>0.05) whereas others aren't (significant, P<0.05). ND: Normal Diet-fed rats, FSD: Fatty Sucrosed Diet-fed rats.

Table 1(a)**:** Body weight and food intake of female rats during pre-gestational period.

Parameter		Effect of time	Effect of Diet	Time-Diet interaction
Body weight	F calculated	178.046	118.601	3.640
	P-value	P<0.001	P<0.001	P<0.01
Food intake	F calculated	0.948	0.002	19.293
	P-value	P>0.05	P>0.05	P<0.001

P<0.001 and P<0.01 show significant effects at α=0.001 and α=0.01, respectively, while P>0.05 is insignificant.

Table 1(b): Two-way ANOVA to test the effect of time (pre-gestational period), diet and their interaction on body weight and food intake of female rats.

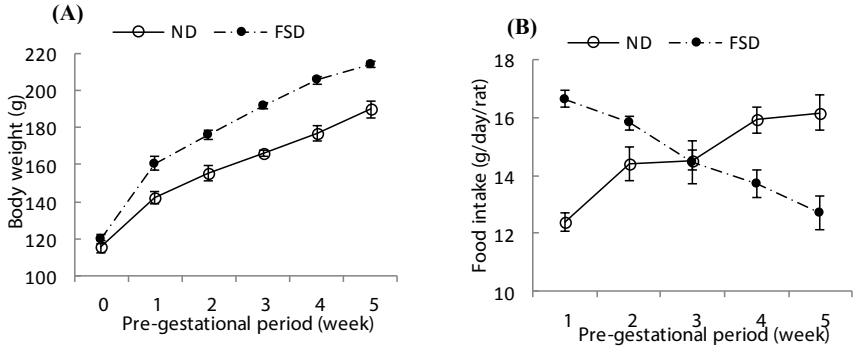

Figure 2: Body weight (A) and food intake (B) of female rats during pre-gestational period. FSD-feeding rats (closed circle) exhibited significant increase (P<0.05) in the body weight with a significant decrease (P < 0.05) in their food intake than ND-feeding rats (open circle). Data shown as mean ± SEM of six dams.

longer continuing after injection of STZ that cause obvious decrease in their body weight at the 13th and the 20th day of gestation as compared to NP+ND dams at the same periods. There was a non-notable change in food intake between both groups at the 6th and the 13th days, while it elevated at the end of the gestational period for GD+FSD dams. Two-way ANOVA analysis revealed significant effect (P<0.001) of time, diet and time-diet interaction on both of the body weight and food intake.

Fasting and two hours (2hr) postprandial glucose level

Dams of GD+FSD group showed elevation of fasting and 2hr. postprandial glucose level at mid-gestation and late-gestation after streptozotocin injection at the dose of 25 mg/kg b.wt. Two-way ANOVA analysis revealed significant effect (P<0.001) of time, diet and time-diet interaction on both of serum fasting and 2hr. glucose level (Tables 2a and b and Figures 3c and d).

However, the injection of STZ doses (35 and 30 mg/kg b.wt.) to FSD-fed dams at the 7th day of gestation was found to produce a drastic reduction in their body weight, extremely elevated hyperglycemia and led finally to death. In contrast, the dose of STZ (20 mg/kg b.wt.) did not cause significant hyperglycemia in FSD-fed dams (data not shown).

Fasting and two hours postprandial insulin level

The recorded values in Tables 2a and b and Figure 3e and f indicated a significant increase in fasting and postprandial insulin level at early gestation (the 6th day) in GD+FSD dams that turned to a notable decrease post-STZ injection in comparison to the control ones at mid and late gestation. Two-way ANOVA analysis revealed significant effect (P<0.001) of time, diet and time-diet interaction on both of serum fasting and 2hr insulin level.

HOMA-IR and QUICKI indices

Table 3 illustrated a significant increase (P<0.001) in HOMA-IR value of GD+FSD group accompanied with a significant decrease in QUICKI (P<0.001) as compared to normal pregnant group.

HbA1c, Fructosamine and liver glycogen

The recorded values of glycated hemoglobin (HbAc1) and fructose amine showed a significant increase (P<0.001) in the gestational diabetic dams compared to the normal ones, while there was a significant decrease in the liver glycogen content values (P<0.001) showed in GD+FSD rats.

The lipid profile and the cardiovascular indices

The data describing the changes of lipid profile between GD+FSD and NP+ND groups clarify an overall significant increase (P<0.001) in triglyceride, total cholesterol, LDL-cholesterol and vLDL- cholesterol values, while HDL-cholesterol was significantly decreased (P<0.05) in the gestational diabetic rats. Cardiovascular risk index 1 and 2 exhibited the same behavioral pattern, where they were significantly increased

Parameter	NP+ND			GD+FSD		
				Pre-STZ	Post-STZ	
	6th day	13th day	20th day	6th day	13th day	20th day
Body weight (g)	203.13 ± 1.25^{a}	222.20 ± 0.82^{c}	241.38 ± 1.14^{d}	221.88 ± 2.91^{c}	209.60 ± 2.38^{ab}	214.50 ± 1.40^{bc}
Food intake (g/day/rat)	14.78 ± 0.37^{a}	18.76 ±0.35^{b}	23.16 ± 0.42^{c}	12.30 ± 0.36^{a}	18.81 ± 0.45^{b}	31.15 ± 1.11^{d}
Fasting glucose (mg/dL)	82.70 ± 1.64^{a}	76.08 ± 3.34^{a}	64.70 ± 2.11^{a}	122.54 ± 1.54^{b}	283.17 ± 5.42^{c}	267.44± 9.53^{c}
2hr. glucose (mg/dL)	114.70 ± 1.66^{b}	102.62±1.50^{ab}	92.32 ± 2.90^{a}	141.35 ± 2.51^{c}	372.00 ± 3.84^{d}	357.05 ± 9.02^{d}
Fasting insulin (µIU/ml)	19.43 ± 0.54^{b}	20.78 ± 0.49^{bc}	27.12 ± 0.91^{d}	23.12 ± 0.35^{c}	14.38 ± 0.43^{a}	21.56 ± 0.48^{bc}
2hr. insulin (µIU/ml)	22.30 ± 0.28^{b}	24.78 ± 0.16^{cd}	30.45 ± 0.46^{e}	26.03 ± 0.30^{d}	18.71 ± 0.4^{a}	24.25 ± 0.24^{c}

Data shown as mean ± SEM of six dams

For each parameter, values with the same superscript letter are similar (non-significant, P>0.05) whereas others aren't (significant, P<0.05). NP+ND: Normal pregnant dams received normal diet, GD+FSD: Gestational diabetic dams received fatty sucrosed diet/minimal dose of STZ (25 mg/kg b.wt.).

Table 2(a): Female rats body weight, food intake, and serum glucose and insulin levels during the gestational period.

Parameter		Effect of time	Effect of Diet	Time-Diet interaction
Body weight	F calculated	40.009	21.787	82.848
	P-value	P<0.001	P<0.001	P<0.001
Food intake	F calculated	276.893	15.070	43.765
	P-value	P<0.001	P<0.001	P<0.001
Fasting glucose	F calculated	110.359	807.884	133.355
	P-value	P<0.001	P<0.001	P<0.001
2hr. glucose	F calculated	959.571	5752.604	1165.918
	P-value	P<0.001	P<0.001	P<0.001
Fasting insulin	F calculated	72.777	36.086	49.719
	P-value	P<0.001	P<0.001	P<0.001
2hr. insulin	F calculated	152.254	116.904	155.971
	P-value	P<0.001	P<0.001	P<0.001

P<0.001 shows significant effect at α=0.001

Table 2(b): Two-way ANOVA to test the effect of time (gestational period), diet and their interaction on body weight, food intake, and serum glucose and insulin levels of female rats.

(P<0.01) in gestational diabetic dams while the antiatherogeic index showed an opposite behavior.

Leptin, TNF-α and adiponectin

According to the data represented in Table 3, serum leptin and TNF-α were significantly increase (P<0.001) in the GD+FSD dams as compared to the normal ones. In contrast, adiponectin showed a significant decrease (P<0.001) in diabetic rats compared to NP+ND ones.

The maternal reproductive outcome and fetal glycemic state

Table 4 indicated that the average number of lived fetuses was significantly decreased (P<0.05) in the gestational diabetic dams when compared to those of normal ones. In turn the implantation loss sites of diabetic dams showed a significant increase (P<0.01). The fetal weight recorded in the same table indicated a significant increase (P<0.05) in the fetuses of GD+FSD rats. Corresponding the fetal glucose and insulin concentrations of both groups, there were an obvious significant increase (P<0.001) in these parameters for GD+FSD-fetuses in comparison to those of normal ones.

Discussion

As mentioned, several animal models were developed to study gestational diabetes but the pattern of disease initiation and development in most of them do not appear to be closely analogous to the clinical situation in humans. High Fat Diet (HFD)-fed with low dose of Streptozotocin (STZ)-treated rat is a well-known model used to develop type II diabetes [22]. Our studies modify this model to initiate a novel rat model that mimics the pathophysiology of human gestational diabetes and more sensitive to the pharmacological testing. FSD-feeding, that results in insulin resistance through glucose-fatty acid cycle [23], and injection of a very low dose of STZ (25 mg/kg b.wt.) at the 7th day of gestation, that makes partial dysfunction of maternal beta cells with a low percentage of fetal malformations [24], founded to be suitable for this purpose.

In view of our results, the pregestational period showed a significant increase in body weight of dams fed FSD. Consumption of this diet facilitates the development of a positive energy balance leading to an increase in visceral fat deposition [25]. The increase in body weight was accompanied with a significant decrease in the food intake that might be due to the elevated plasma leptin level which set the received calorie by the rats.

During gestational period and post-STZ injection, gestational diabetic dams indicate marked decrease in their body weights. The given low dose of STZ causes moderate destruction of pancreatic β-cells. Together, with FSD-feeding, caused impairment in glucose stimulated insulin release and insulin resistance [26] that divert the body toward the catabolism of fats and proteins resulting in body weight loss [27]. It is relevant here to mention that dams of both groups showed fluctuating increase in their body weight at the 20th day of gestation as a result of the complete development of their fetuses and the probable labor during the next day.

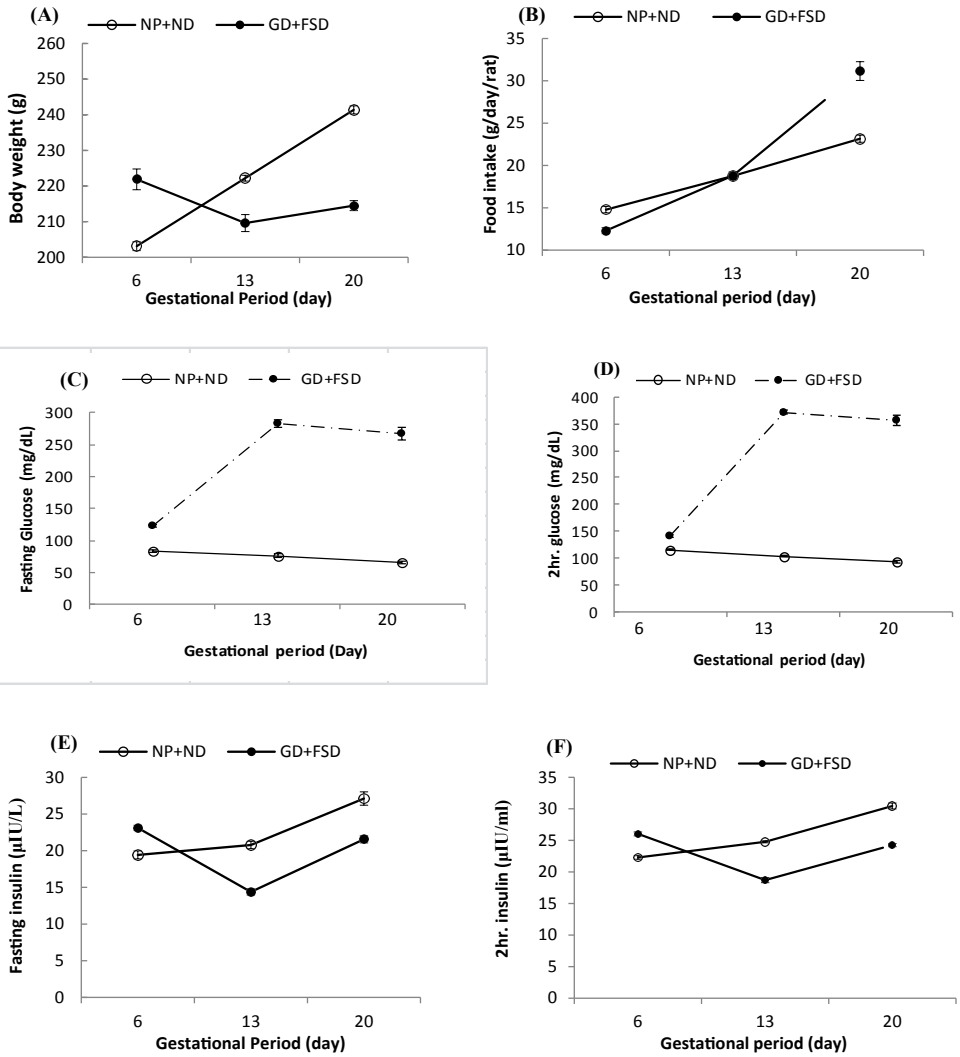

Figure 3: Body weight (A), food intake (B), and serum fasting glucose (C), 2 hours postprandial glucose (D), fasting insulin (E) and 2 hours postprandial insulin levels (F) of female rats during the gestational period. Data shown as mean ± SEM of six dams. NP+ND: Normal pregnant dams received normal diet, GD+FSD: Gestational diabetic dams received fatty sucrosed diet/minimal dose of STZ (25 mg/kg b.wt.). GD+FSD group (closed circle) exhibited significant increase ($P<0.05$) in the food intake and serum glucose level while it have a significant decrease ($P<0.05$) in their body weight and serum insulin level as compared to NP+ND group.

The hyperglycemia observed in the gestational diabetic dams could be due to a state of insulin resistance, confirmed by the elevation of HOMA-IR value that accompanied with a decrease in QUICKI level, and the injection of a minimal dose of STZ.

The observed insulin resistance state may be a result of feeding of FSD and the hormonal production of placenta. FSD-feeding founded to increase serum Free Fatty Acids (FFAs) level that plays a key role in promoting loss of insulin sensitivity by increasing serine phosphorylation of insulin receptor (IR) at the expense of its tyrosine phosphorylation causing reduction in the Glucose Transporter-4 (GLUT4) translocation to the plasma membrane and the consequent decrease in the peripheral tissue glucose utilization [28,29]. At the same level, Placental Growth Hormone (PGH) and Placental Lactogen (PL) are the two main placental hormones that implicated in the state of insulin resistance, where they attenuating phosphatidylinisitol 3-kinase (PI 3-kinase) activation which led to depression in translocation of GLUT4 and the resultant decrease in insulin-stimulated glucose uptake to skeletal muscle [30]. Moreover, PL stimulates lipolysis leading to an increase in circulating free fatty acids that interferes with insulin-directed entry of glucose into cells. Therefore, PL is considered as a potent antagonist to insulin action during pregnancy [31].

Injection of STZ selectively destructs β-cells of the islets of Langerhans in the pancreas [32]. The cytotoxic action of STZ is associated with its deoxyglucose moiety that facilitates the transport across the cell membrane of the pancreatic β-cells via glucose transporter-2, and with its DNA alkylating activity through methylnitrosourea moiety; where the transfer of the methyl group from streptozotocin to the DNA molecule causes its fragmentation. STZ, also, increases the Reactive Oxygen Species (ROS) generation and inhibits the free radical scavenger-enzymes enhancing the pancreatic β-cells destruction [33].

Marked alterations in lipid metabolism have been reported in the gestational diabetes [34]. The observed hypertriglyceridemia and hypercholesterolemia may be due to increased dietary triglycerides and cholesterol absorption from the small intestine following the

Parameter	NP+ND	GD+FSD
HOMA-IR [[(Fasting Insulin, μIU/ml) × (Fasting Glucose, mmol/L)]/22.5]	4.27 ± 0.15	25.50 ± 0.51*
QUICKI [1/ [Log (Fasting Insulin, μIU/ml) + Log (Fasting Glucose, mg/dL)]]	0.31 ± 0.001	0.25 ± 0.001*
HbA1c (%)	4.87 ± 0.12	6.86 ± 0.08*
Fructosamine (mmol/L)	45.39 ± 3.05	185.70 ± 5.25*
Liver glycogen content (mg/g tissue)	12.69 ± 0.57	3.55 ± 0.19*
Triglycerides (mg/dL)	58.72 ± 1.44	189.64 ± 1.34*
Total Cholesterol (mg/dL)	81.12 ± 1.45	185.13 ± 2.51*
HDL-Ch.(mg/dL)	58.06 ± 1.54	52.52 ± 1.18#
LDL-Ch. (mg/dL) [Total-Ch – T.G/5 – HDL-Ch.]	11.95 ± 0.51	94.59 ± 2.74*
vLDL-Ch. (mg/dL) [T.G./5]	11.74 ± 0.29	37.93 ± 0.26*
CVR1 [Total-Ch./HDL-Ch.]	1.37 ± 0.05	3.53 ± 0.09*
CVR2 [LDL-Ch./HDL-Ch.]	0.20 ± 0.01	1.80 ± 0.08*
AAI [HDL-Ch.×100 / Total Ch. – HDL-Ch.]	290.85 ± 20.96	40.19± 1.47*
Leptin (pg/ml)	574.65 ± 46.38	1265.40 ± 42.15*
TNF-α (pg/ml)	210.77 ± 4.42	374.40 ± 7.19*
Adiponectin (pg/ml)	946.68 ± 4.54	693.95 ± 16.73*

Data shown as mean ± SEM of six dams
#P<0.05, *P<0.001 versus NP+ND group NP+ND: Normal pregnant dams received normal diet, GD+FSD: Gestational diabetic dams received fatty sucrosed diet/minimal dose of STZ (25 mg/kg b.wt.).

Table 3: Maternal glucose-insulin homeostasis, lipid profile and adipocytokines of NP+ND and GD+FSD after scarification.

Parameter	NP+ND	GD+FSD
Live fetuses	8.75 ± 0.52	4.25 ± 0.22#
Implantation loss	3.50 ± 0.2	9.50 ± 0.38ı
Fetal weight (g)	3.08 ± 0.03	3.40 ± 0.10#
Fetal glucose (mg/dL)	24.94 ± 1.46	161.50 ± 4.12*
Fetal insulin (μIU/ml)	3.60 ± 0.14	6.20 ± 0.16*

Data shown as mean ± SEM of six dams #P< 0.05, ıP < 0.01, *P < 0.001 versus NP+ND group NP+ND: Normal pregnant dams received normal diet, GD+FSD: Gestational diabetic dams received fatty sucrosed diet/minimal dose of STZ (25 mg/kg b.wt.).

Table 4: Maternal reproductive outcome, and fetal glucose and insulin levels.

intake of FSD, decreased T.G. uptake in peripheral tissues as a result of dysfunction of insulin-dependent Lipoprotein Lipase (LPL), increased hepatic production of triglycerides enriched very Low Density Lipoprotein (vLDL-TG) or through activation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is a rate limiting enzyme involved in cholesterol synthesis [10,35,36].

Adipose tissue is an active participant in controlling the pathophysiology of GDM by releasing a variety of adipokines in the blood stream that are directly or indirectly involved in the state of insulin resistance accompanied GDM [37]. Our study revealed a significant increase in both serum leptin and TNF-α level in GD+FSD group. These findings are in agree with who stated that these adipokines were significantly high in gestational diabetic mothers and are conversely correlated with insulin secretion and sensitivity [38]. On the other hand, hypoadiponectinemia was reported in the diabetic group that illustrates the severe state of insulin resistance [39]. Leptin, an antiobesity adipocyte-derived hormone, is supposed to regulate body weight through a negative feedback signal between adipose tissue and the hypothalamic satiety center causing a decrease in food intake and an increase in energy expenditure [40]. The elevated leptin concentrations may actually represent a state of leptin resistance. Hyperleptinemia proved to cause insulin resistance through impairment of tyrosine phosphorylation of IR, increasing peroxynitrite-mediated oxidative stress and stimulation of T-helper cell proliferation that control release of the most important proinflammatory cytokine TNF-α [41-43]. TNF-α is thought to induce insulin resistance by number of mechanisms involving the increase in serine phosphorylation of the Insulin Receptor Substrate-1 (IRS-1) that impaired the insulin-signaling cascade, and mediating lipolysis through suppression of the peroxisome proliferated activated receptor-γ (PPAR-γ) expression in adipose tissues that is essential in fat cell differentiation and lipid storage [44,45]. In contrast to other adipokines, adiponectin has insulin sensitizing effect which is attributed primarily to decreasing expression of gluconeogenic enzymes (as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) which in turn suppress the hepatic glucose production and enhancing fatty acid oxidation and energy dissipation causing decrease in tissue T.G. accumulation and increase in insulin sensitivity [46,47].

Regarding the offspring, the maternal glucose intolerance showed in GD+FSD dams as a result of FSD-feeding not prevent embryo implantation. Elsewhere, injection of STZ at the 7th day of gestation revealed higher implantation loss sites in these dams as compared to normal ones. This result confirmed by the decrease in the number of live fetuses that may be attributed to intrauterine growth restriction [48]. As placental transfer of glucose is carried out by facilitated diffusion according to concentration-dependent kinetics, fetuses of FSD+GD-rats showed high glycaemia level accompanied by fetal hyperinsulinism [11,49]. It is known that insulin is one of the main growth factors during fetal life, thus the hyperinsulinemia leads to macrosomia [50].

Conclusion

The present model proved that the combination of fatty-sucrosed diet and the minimal dose of streptozotocin serve as alternative model for GDM study and is suitable for testing different maternal pathophysiological alterations and the attributed fetal development errors.

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