RESEARCH ARTICLE PHARMACOLOGIA

DOI: 10.5567/pharmacologia.2013.157.163

High Carbohydrate Diet with 30% Sucrose Solution: An Optimized Rodent Model for Simulating Human Insulin Resistance

[1]Aruna S. Theerthahalli, [2]Rekha R. Shenoy, [2]Pawan G. Nayak, [2]Jayesh Mudgal, [2]Nitesh Kumar, [2]Mallikarjuna C. Rao and [2]Gopalan N. Kutty

[1]Manipal Acunova, Shirdi Sai Baba cancer research hospital, Manipal- 576104, Karnataka, India

[2]Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal- 576104, Karnataka, India

ABSTRACT

Background: The development of an appropriate animal model that can mimic the actual human disease condition is an important consideration of a successful pharmacological experiment. **Methods:** To achieve type-2 diabetic conditions similar to that of human, we performed an optimization experiment, taking in to consideration the previously reported methods of inducing type-2 diabetes such as high fat diet, high sucrose diet and high calorie diet models with low dose Streptozotocin (STZ). We adopted High Carbohydrate Diet (HCD) model with some modifications. Post weaned male albino rats were chosen for the experiment and were fed HCD for six months prior to the experiment. The rats were later grouped, treated with sucrose and fructose solutions and STZ was administered at two different doses. The animals were subjected to OGTT. Plasma glucose, insulin, total cholesterol, triglycerides, AST, ALT levels were measured. The comparison was done with both normal pellet diet and HCD rats. **Results:** The findings demonstrated that HCD induced frank obesity with increased circulating TG levels. The low dose STZ treatment was not encouraging as significant mortality was observed with 35 mg kg^{-1} STZ and reversal to normoglycemia was observed with 20 mg kg^{-1} STZ. **Conclusion:** The group with sucrose supplementation simulated the conditions desirable to mimic human insulin resistance syndrome.

Key words: Glucose excursion, triglycerides, total cholesterol, insulin assay, albino rats

Pharmacologia 4 (3): 157-163, 2013

INTRODUCTION

Type-II diabetes (T2D) is a condition characterized by insulin resistance followed by progressive decrease in insulin secretion. In T2D research, genetic models offer a better picture of the disease to study different areas of diabetic pathology such as diabetic nephropathy, retinopathy, cardiomyopathy and neuropathy, but these models are neither easy to maintain, nor widely available amongst researchers[1]. The epidemiological evidence indicates the fact that most of the type-II diabetics are generally obese and have abnormal lipid profile. Many reports have established the association between altered food habits such as high intake of fat, carbohydrate regardless of need and a sedentary lifestyle[2,3]. To develop an appropriate animal model, which can mimic the actual human conditions is an important consideration for any pharmacological experiment. Literature search revealed that there have been attempts at developing such models for insulin resistance and using them for different pharmacological experiments[4]. The current fat-fed and fructose-fed models require a long diabetes induction time which enhances the cost of the experiment[5]. It is a well-known fact that disease conditions affect the pharmacokinetics and pharmacodynamics of drugs. The same stands valid for herb-drug interactions. In India, transformation of lifestyle from traditional to western with a drastic change in food habits and lack of exercise has led to increased prevalence of Impaired Glucose Tolerance (IGT) and Insulin Resistance (IR). Obesity is one of the immediate and direct fallouts of changed lifestyle. It has significant effects on the pharmacology of drugs[6]. Considering all these observations, we attempted to optimize an appropriate animal model which can simulate the actual clinical scenario of obesity associated with impaired glucose tolerance and insulin resistance.

MATERIALS AND METHODS

Animals: All the animal experiments were performed at Central Animal Research Facility, Manipal University, Manipal. The animal studies were initiated after obtaining written approval from IAEC (IAEC/KMC/07/2008-2009). Healthy, inbred, post-weaned, male albino rats of Wistar strain weighing

Corresponding Author: Gopalan N. Kutty, Department of Pharmacology, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal- 576104, Karnataka, India Tel: +91820 2922482, Extn: 322 Fax: +91820 2571998

around 30 to 35 g were selected for the study. They were maintained under controlled conditions of temperature (23±2°C), humidity (50±5%) and day/night cycles (14 h light and 10 h dark). The animals were put on custom-made HCD, except for the control group which was fed NPD and water *ad libitum*. Two rats were housed in each autoclave-sterilized polypropylene cage containing autoclave sterilized paddy husk as bedding. The animals were maintained for about six months with scheduled monitoring of food, water intake and body weight measurement.

The animals selected for the optimization experiments were fed HCD except for a group of six, which were fed NPD (control). The environmental conditions were maintained uniformly for all the rats for the next six months. After ensuring reasonable stabilization of the body weight, the optimization experiments were initiated.

Chemicals: The required ingredients for feed such as DL-Methionine, cholesterol, choline bitartrate and streptozotocin were procured from Sigma-Aldrich Pvt. Ltd., Bangalore, India. Casein, cellulose and fructose were procured from Suvedhanath Laboratories, Baroda, India. Vitamin mineral mix (ostovet/virbac) was purchased from a local drug house and lard was purchased in local market, Udupi, India. AST, ALT, TC, TG and glucose estimation kits were sourced from Aspen labs, New Delhi, India. Rat insulin Elisa kit procured from Mercodia diagnostics, Sweden, was used for insulin estimation. Centrifugation was done with Mikro refrigerated centrifuge (Hettich, Germany) to obtain plasma for various biochemical estimations which were performed by using ELx-800 micro plate reader, Bio Tek, USA.

Composition of high carbohydrate diet: High-carbohydrate diet preparation was adapted from the earlier experiments[7] with some modifications to include the component of fat (1.5% lard) with high carbohydrate (55% sucrose) in to the diet.

The measurements of food, water intake and body weight were done fortnightly. The previous day, known amounts of food (in g) and water (100 mL) were provided for each cage. Next day at the same time, the remaining food was weighed, water was measured and recorded. The difference was calculated for each animal, which gave the consumption. On the same day, the body weight was also recorded. Graphs were plotted with mean change in the parameter versus time.

Treatment: The animals (n = 6) were allocated to five different groups namely, normal control [NPD+water], HCD [HCD+water], HCD+30% sucrose, HCD+25% fructose, HCD+STZ 35 mg kg^{-1} i.p. and HCD+STZ 20 mg kg^{-1} i.p. The control group received i.p. injection of citrate buffer[8]. All the procedures including weighing, preparation of dose and administration of STZ were performed under dim light. The weighed quantity of STZ was dissolved in freshly prepared citrate buffer (50 mM sodium citrate at pH 4.4 maintained at 2 to 8°C. The drinking water was replaced with 10% sucrose solution for the next 12 h and the animals were monitored subsequently for 7 days for mortality due to hypoglycemia or hyperglycemia. The extent of induction of diabetes was assessed on 7th day.

Collection of blood and tissue samples: The rats were fasted overnight and were administered with glucose 2 g kg^{-1} orally as 40% solution. Blood samples were collected by retro orbital puncture method before the administration of glucose solution (0 min) and at 15, 30, 60, 90 and 120 min. The collected samples were centrifuged and the plasma was separated and refrigerated at 2-8°C until the determination of glucose and insulin. Additional blood samples were collected for the estimation of TC, TG and liver function tests. All the biochemical estimations were performed as per the respective kit inserts.

Statistical analysis: Glucose excursion, AUC was analyzed with Winnonlin Professional version 5.3, Pharsight Corporation, St.Louis, USA. The statistical tests of significance were applied with GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. The obtained glucose values were normalized to percentage values. The data analyses were performed for both actual and percentage normalized data. Two-Way analysis of variance (2-way ANOVA) followed by Bonferroni post-test was applied for OGTT assessment. For the rest, one way ANOVA with Dunnett's test was used. NPD was used as comparator (control group) for glucose excursion and AUC analyses of non-STZ groups, whereas HCD was used as comparator for STZ treated groups. The obtained data are presented with descriptive statistics (Mean, SD, SEM and n) or graphically.

RESULTS

Food, water intake and body weight: The food intake was relatively higher in HCD fed rats than rats on NPD. However, the difference was not statistically significant. There was a significant weight gain in HCD group from seventh week onwards with mean body weight of 174.83±2.47 g as compared to NPD fed rats with mean body weight of 149.50±12.00 g (Table 1). The difference became statistically significant ($p<0.05$) after 11th week with mean body weight of 239.67±3.07 g and 166.33±13.53 g for HCD and NPD fed rats, respectively.

Table 1: Food, water intake and body weight measurements of albino rats treated with NPD and HCD

	Food intake (g)		Water intake (mL)		Body weight (g)	
Duration (weeks)	NPD	HCD	NPD	HCD	NPD	HCD
1	21.00±1.00	29.22±0.89	25.00±3.00	23.75±1.18	29.50±1.28	37.92±1.53
3	29.50±2.50	36.53±0.88	28.50±4.50	39.09±2.93	75.67±5.39	83.28±2.61
5	30.50±1.50	39.03±1.08	41.00±1.00	37.66±2.98	135.50±11.39	142.48±2.27
7	34.00±3.00	39.34±0.83	35.00±2.00	37.97±2.62	149.50±12.00	174.83±2.47
9	39.00±2.00	41.03±1.06	54.00±5.00	43.06±3.57	175±11.75	201.00±3.00
11	35.50±5.50	38.06±0.84	59.50±1.50	52.78±3.23	166.33±13.53	239.67±3.07*
15	34.00±1.00	38.06±0.85	44.50±7.50	45.94±2.45	194.67±10.57	273.27±3.08*
19	34.50±3.50	38.19±0.84	44.00±6.00	44.47±4.07	214.33±13.53	306.61±3.82*
27	37.00±2.00	44.00±2.19	47.50±2.50	36.07±2.03	231.67±14.21	340.82±4.01*

*p<0.05, ANOVA followed by Dunnet's posttest, n = 64 for HCD rats, n = 6 for NPD rats

Table 2: Effect of the various diets and supplementation on plasma glucose levels (mg dL^{-1})

Time	NPD	HCD	HCD+25% Fructose solution	HCD+30% Sucrose solution	HCD+STZ (20 mg kg^{-1})	HCD+STZ (35 mg kg^{-1})
0	88.00±2.898	91.50±2.861	94.50±2.405	94.33±3.565	124.67±26.894	233.75±12.951†
15	144.83±1.905	137.50±5.195	155.33±2.692	159.17±7.618	204.17±38.448	307.25±15.212†
30	127.83±1.302	159.00±8.718*	130.50±2.291	147.33±9.193*	198.50±39.484	455.00±19.870†
60	115.33±1.667	136.50±4.766*	135.50±5.915*	153.33±6.672*	215.83±48.518	560.00±18.708†
90	104.83±1.600	125.00±6.202*	126.83±5.400*	145.83±6.529*	191.67±40.204	540.00±23.541†
120	96.00±1.932	118.33±7.370*	122.00±3.540*	148.67±5.903*	164.50±28.530	461.75±21.631†

*p<0.05 Vs NPD, †p<0.05 Vs HCD, n=6 for all the groups except HCD+STZ (35 mg kg^{-1}) where n = 4; 2-way ANOVA followed by Bonferroni test

OGTT: After an overnight fast, a known amount of glucose (2 g kg^{-1} as 40% solution) was given and the glucose levels were measured before glucose administration and after the glucose administration at scheduled time intervals. The obtained values were tabulated with descriptive statistics, giving mean, SD and SEM for each group. HCD and HCD+Sucrose in comparison with NPD showed significant difference ($p<0.05$) in the OGTT profile from 30 min onwards for all time points. HCD+Fructose group showed significant difference in the OGTT from 60 min onwards. The STZ treated groups were compared against HCD instead of NPD and HCD+STZ (20 mg kg^{-1}) did not show any significant change in the profile with respect to HCD whereas HCD+STZ (35 mg kg^{-1}) showed marked difference ($p<0.05$). The glucose (mg dL^{-1}) excursions observed (Mean±SEM) were 159.00±8.718,135.50±5.915,153.33±6.672,215.83±48.518 and 560.00±18.708, respectively for HCD, HCD+Fructose, HCD+Sucrose, HCD+STZ (20 mg kg^{-1}) and HCD+STZ (35 mg kg^{-1}) as compared to excursion of 144.83±1.905 mg dL^{-1} in NPD (Table 2). The fructose supplemented group and the group treated with STZ at the dose of 20 mg kg^{-1} did not show appreciable difference as compared to their respective control groups. HCD+STZ (35 mg kg^{-1}) did show prompt induction of diabetes, which appeared to be type-I as the animals were entirely dependent on external insulin for the survival. We also observed that the animals eventually lost body weight and became very lean. HCD+STZ (35 mg kg^{-1}) resulted in substantial mortality with 30 deaths out of 34 animals. Autopsy revealed fluid accumulation in the intestine and inflamed kidneys. The HCD+STZ (20 mg kg^{-1}) treated group initially showed hyperglycemia by 7th day and eventually turned normoglycaemic by 15th day. The variability was very high with standard deviation ranging from 65.878 to 118.845 as compared to HCD alone or HCD with fructose and glucose supplementation which remained at 7.007 to 21.354, 5.612 to 14.488 and 8.733 to 22.518, respectively.

Statistical evaluation of percentage normalized OGTT data showed that HCD+ Sucrose group significantly affected the OGTT from 30 min onwards at all the time points with values (% increase) 56.02±7.133, 63.55±8.913, 55.26±7.544, 58.45±7.844 as compared to NPD values of 45.83±3.559, 31.62±3.868, 19.57±3.077, 9.50±3.205, respectively. Percentage normalized OGTT values of HCD+STZ (20 mg kg^{-1}) did not vary significantly from respective control group.

It can be observed that in both the cases of comparison, HCD+Sucrose group demonstrated significant disruption of glucose tolerance.

The AUC of OGTT profile was calculated by applying linear trapezoidal rule for each group and compared with one-way ANOVA followed by Dunnett's test. NPD served as control group for all sugar supplementation groups and HCD for both the STZ add-on groups. HCD+Sucrose group caused significant difference for peak glucose value when compared with NPD group. The results are presented in Table 3.

Plasma Insulin and OGTT: Plasma insulin levels were determined after glucose challenge in all groups. A six

point calibration curve was constructed for the estimation of insulin. The calibration range was 0.2 to 10 μg L^{-1}. The calibration curve was accurate and precise with coefficient of determination (r^2) being 0.9995. The obtained insulin levels were tabulated and group-wise comparison was performed with two-way ANOVA followed by Bonferroni post-test. The NPD group served as control for all group comparisons. HCD group served as control to perform a differential assessment of effect with sucrose and fructose supplementation. All the groups, except the group with add-on STZ (35 mg kg^{-1}) treatment, showed significant rise in degree of insulin release as compared to NPD group as depicted in Fig. 1. The group with STZ had lower insulin levels as

Table 3: Effect of the various diets and supplementation on peak glucose, AUC and median time of peak glucose

Parameter	Units	NPD	HCD	HCD+25% Fructose	HCD+30% Sucrose	HCD+STZ 20 mg kg^{-1}	HCD+STZ 35 mg kg^{-1}
PGL	mg dL^{-1}	144.83±1.905	162.5±7.117	155.5±2.655	165.667±5.129	238.167±46.938	560.00±18.708
AUC	min*mg dL^{-1}	13753.75±161.13	15946.25±400.477*	15675±390.317*	17615±667.957*	23156.25±4521.22	56525.62±1591.716†
TPGL	Min	15	30	15	15	15	60

*p<0.05 vs. NPD, One way ANOVA with Dunnett's test, PGL: Peak Glucose Level, AUC: Area Under the Curve, TPGL: Time of PGL, †p<0.05 vs. HCD, One way ANOVA with Dunnett's test, PGL: Peak Glucose Level, AUC: Area Under the Curve, TPGL: median time of PGL

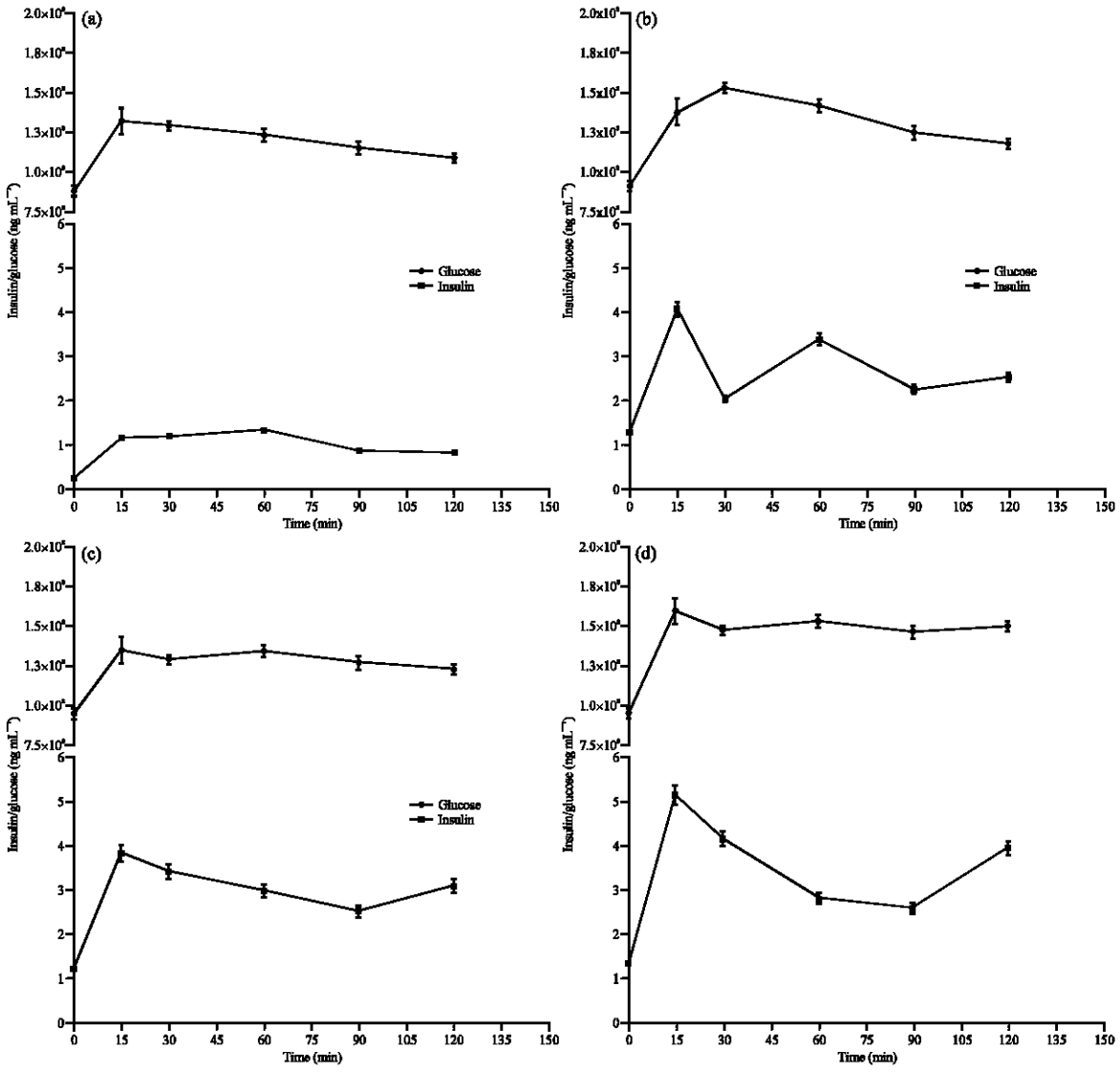

Fig. 1(a-e): Contineu

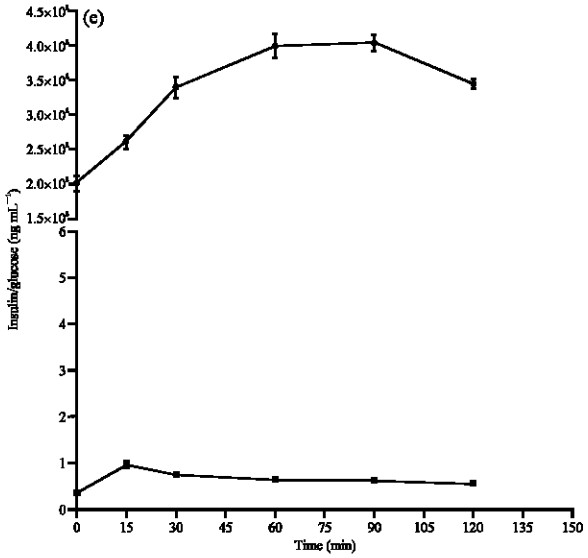

Fig. 1: A comparative representation of insulin levels and corresponding plasma glucose levels (a) NPD, (b) HCD, (c) HCD+fructore, (d) HDC sucruse and (e) HCD+STZ (ng mL^{-1}) (n = 6)

Table 4: Effect of the various diets and supplementation on TG, TC AST and ALT levels in the albino rats

Treatment (Diet)	Triglycerides (mg dL^{-1})	Total Cholesterol (mg dL^{-1})	AST (IU L^{-1})	ALT (IU L^{-1})
NPD	123.9±2.21	92.5±3.32	90.2±1.83	46.5±1.82
HCD	155.9±9.15	108.5±3.85	103.7±3.48	54.5±2.23
HCD+Fructose	190.6±4.48*	105.7±2.91	137.0±3.18*	66.2±3.36*
HCD+Sucrose	194.1±28.16*	89.4±4.09	138.3±6.46*	74.0±3.31*
HCD+STZ (35 mg kg^{-1})	138.4±14.29	117.7±11.38*	160.7±6.18*	79.5±2.01*

*p<0.05 vs. NPD, One-Way ANOVA with Dunnett's test, n = 6

compared to NPD group. The differential assessment for sucrose and fructose supplementation groups revealed that the group with sucrose supplementation fared better than HCD alone.

Biochemical evaluation: Other biochemical investigations such as TG, TC, AST and ALT were performed along with insulin estimation. There was a clear difference in TG levels across different diet groups and add-on STZ treatment. TG levels were significantly high ($p<0.05$) in both fructose and sucrose supplemented groups and low in STZ-treated groups. However, TC levels were increased significantly ($p<0.05$) as compared to NPD as presented in Table 4. No statistically significant changes were observed in rest of the groups with respect to TC levels. Both AST and ALT levels were high in all HCD treated groups. Fructose, sucrose and add-on STZ groups showed a significant ($p<0.05$) increase in the liver enzymes as compared to NPD. The rise in the liver profile was highest in the STZ group.

DISCUSSION

Considering the epidemiological and etiological aspects and the risk factors for human T2D, it becomes very important that we choose the right animal model, which can mimic the actual pathophysiological condition for the experimentation with the anti-diabetic drugs, used in pre-diabetics and initial stages of T2D patients. Being aware of the pathogenesis of IGT and insulin resistance, it was necessary to induce obesity which would further initiate a chain of events. Increased visceral adipose tissue raises circulating FFA due to insulin refractory adipocytes associated with decreased adiponectin. FFAs in turn get deposited in liver and muscles, impairing the glucose uptake[3,9].

We chose two reported models as the basis for this experiment, sucrose-induced insulin resistance model by Wright *et al.*[7] and high fat diet+low dose STZ model for T2D by Srinivasan and Ramarao[10]. We carefully studied the reported diet composition and modified to incorporate both high carbohydrate and high fat components. We raised the sucrose content to 55% and optimized fat content to 1.5% of the total diet composition. Another important modification was the selection of animals and duration of feeding. We selected post-weaned rodents and put them on HCD diet for 6 months. This was to ensure that the animals were exposed to the altered food habit early in their life so that food avoidance problem could be eliminated and, at the

same time, they were exposed to HCD for a reasonable period of their lifespan. Two rats per cage (12×8×8 inches) were maintained since the beginning of the experiment. Cages allowed enough mobility initially but, eventually as the rats grew in size, mobility of the rats got restricted making them more sedentary. We felt that these were some important considerations to mimic the actual disease progression. As a part of the optimization experiment, once the body weights were reasonably high and stabilized indicating reasonable degree of obesity, the rats were subjected to supplementation with 30% sucrose solution, 25% fructose solution as substitute to drinking water in addition to HCD for one month. Simultaneously, two separate groups were administered with STZ i.p. at the doses of 20 and 35 mg kg^{-1}. The former sets of trials were done to inflict profound stress on beta cells to create additional insulin demand which mimicked condition of insulin resistance. The low-dose STZ administration was done to partially damage the beta cells, compromising the insulin secretion to mimic the phenomenon of inadequate secretion of insulin.

Our experiments revealed that the food and water intake did not change significantly and the intake pattern was almost similar to that of the control (NPD). However, there was a significant change in the body weight, which started becoming apparent after the seventh week and became statistically significant by eleventh week. This clearly indicates that the initial growth phase was unaffected by diet modification and eventually HCD diet did cause weight gain. The reduced mobility of the rats due to the restricted space might also have contributed to the weight gain.

We did not find encouraging results with low-dose STZ (35 mg kg^{-1}) when administered i.p. in HCD fed rats. There was significant mortality (88.23%) associated with the experiment despite monitoring. The rats eventually became insulin dependent within 7 days of STZ injection and lost weight significantly by 11th day. All the deaths occurred between 4th day and 11th day. We tried another dose of STZ (20 mg kg^{-1}), much lower than the earlier one. The rats did show the symptoms of STZ intervention characterized by an initial fall in blood glucose levels and subsequent hyperglycemia. On day 1 of STZ treatment, rats were maintained on 10% glucose solution for initial 12 h to counter hypoglycemia. The blood glucose levels increased for the next two days. However, by seventh day the rats turned out to be normo-glycemic. The OGTT profile of the group treated with add-on STZ at the dose of 35 mg kg^{-1} showed very high glucose values with 233 mg dL^{-1} at 0 h and excursion of 560 mg dL^{-1} and by 120 min, the levels were still at around 461 mg dL^{-1}. Insulin estimation showed that the levels were much lower than the control (NPD) group. The glucose and insulin levels indicated insulin dependent diabetes. The model could not be taken forward because of two important factors. Firstly, high mortality rate and secondly, unpredictable responses to STZ post diet manipulation. STZ at 20 mg kg^{-1} was ineffective and 35 mg kg^{-1} was toxic in HCD fed rats. In addition, there was a progressive loss of body weight making the rats lean precluding the induction of IGT and insulin resistance. Both the STZ treatments failed to achieve the initial objectives of either partial destruction of beta cell mass or insulin resistance.

HCD sans sugar supplementation produced adverse changes in glucose tolerance marked by increase in peak glucose levels coupled with significant rise in insulin levels showing the indications of insulin resistance syndrome. To make the symptoms more pronounced and to bring in reproducible IGT, we chose to introduce additional stress through sugar supplementation. The sugars, sucrose (30% solution) and fructose (25% solution) in drinking water were provided instead of normal drinking water. The sugar solutions at the chosen strength did not lead to reduced fluid consumption. OGTT performed after one month showed that there was no much change in the peak glucose concentration achieved after glucose challenge at 2 g kg^{-1} but the glucose disposition pattern was significantly altered and was at abnormally elevated level as compared to NPD at 60, 90 and 120 min despite increased insulin secretion. This observation clearly demonstrated that the peripheral glucose utilization is being dampened because of insulin resistance. This sluggish disposition of the circulating glucose clearly indicated the pre-diabetic stage. The degree of OGTT alteration was not the same with the two sugars. OGTT alteration was more with sucrose supplementation than fructose. This was demonstrated when the sugar supplemented groups were compared against HCD group. By considering % glucose excursion, AUC, insulin levels, it can be said that HCD+30% sucrose supplementation reasonably answers the objectives of the model optimization experiments for insulin resistance model.

Additional biochemical evaluations for TG and TC were performed to understand the extent of lipid abnormalities caused by HCD diet and HCD with add-on interventions. The group with HCD alone showed increased levels of TG and TC as compared to NPD group. However, the rise in TG became statistically significant with add-on sugar supplementations, which was not observed with STZ treated group.

The HCD fed animals with sugar supplementation demonstrated all the vital indications of development of IGT and IR. Increased visceral fat accumulation, abnormally high TG levels, AST and ALT levels[11], fatty liver established abnormal response to glucose load and

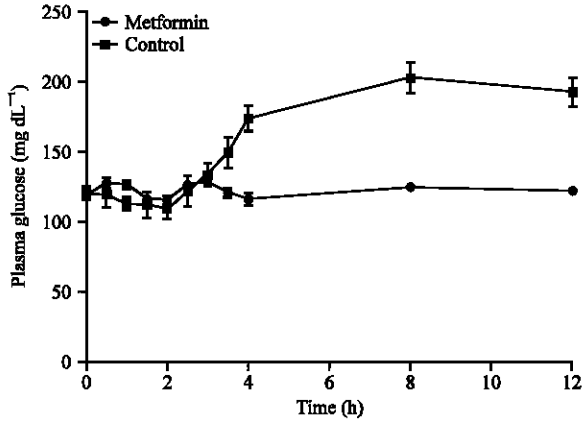

Fig. 2: Application of the optimized insulin resistance model as demonstrated with metformin treatment

presence of raised insulin levels supported this finding HCD for six months failed to cause any elevation of FBS in rats. Recently, it has been reported that FBS cannot be always considered as an authentic marker for the diagnosis of T2D[12]. However, continued sucrose supplementation for one more month raised FBS significantly. This was demonstrated when the applicability of model was verified with single dose metformin (90 mg kg^{-1}; p.o.). It was clear that HCD and sucrose supplementation could create a condition of 'insulin resistance as evidenced by hyperinsulinemia, glucose intolerance, hyper triglyceridemia and fatty liver. Literature mentions that in human beings, the transition from impaired glucose tolerance to frank diabetes is not a rapid process. Going by the estimates presented in the literature, about 70% of the people with IGT eventually develop diabetes[13]. It may also be possible that the species difference and effect of duration of study affected the results especially in case of low dose STZ[14]. The optimized insulin resistance model (HCD+30% sucrose solution supplementation) was successfully applied to PK-PD experiments with metformin (Fig. 2).

CONCLUSION

High carbohydrate diet induced frank obesity with increased circulating triglyceride levels. Sucrose (30%) supplementation inflicted desired stress on the glucose homeostasis resulting in sustained hyperinsulinemia, impaired glucose tolerance and liver abnormalities, simulating the conditions of human insulin resistance syndrome.

ACKNOWLEDGMENT

The authors are thankful to Manipal University and All India Council for technical education (AICTE), New Delhi, India for providing the necessary facilities and equipment to perform this project.

REFERENCES

1. Bell, R.C., J.C. Carlson, K.C. Storr, K. Herbert and J. Sivak, 2000. High-fructose feeding of streptozotocin-diabetic rats is associated with increased cataract formation and increased oxidative stress in the kidney. Br. J. Nutr., 84: 575-582.
2. Diamond, J., 2011. Medicine: Diabetes in India. Nature, 469: 478-479.
3. Kahn, B.B. and J.S. Flier, 2000. Obesity and insulin resistance. J. Clin. Invest., 106: 473-481.
4. Jang, E.H., H.K. Kim, C.S. Park and J.H. Kang, 2010. Increased expression of hepatic organic cation transporter 1 and hepatic distribution of metformin in high-fat diet-induced obese mice. Drug Metab. Pharmacokinet., 25: 392-397.
5. Reuter, T.Y., 2007. Diet-induced models for obesity and type 2 diabetes. Drug Discovery Today: Dis. Model., 4: 3-8.
6. Blouin, R.A. and G.W. Warren, 1999. Pharmacokinetic considerations in obesity. J. Pharm. Sci., 88: 1-7.
7. Wright, D.W., R.I. Hansen, C.E. Mondon and G.M. Reaven, 1983. Sucrose-induced insulin resistance in the rat: Modulation by exercise and diet. Am. J. Clin. Nutr., 38: 879-883.
8. Srinivasan, K., B. Viswanad, L. Asrat, C.L. Kaul and P. Ramarao, 2005. Combination of high-fat diet-fed and low dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. Pharmacol. Res., 52: 313-320.
9. Kahn, S.E., R.L. Prigeon, R.S. Schwartz, W.Y. Fujimoto, R.H. Knopp, J.D. Brunzell and D. Jr Porte, 2001. Obesity body fat distribution insulin sensitivity and Islet beta-cell function as explanations for metabolic diversity. J. Nutr., 131: S354-360S.
10. Srinivasan, K. and P. Ramarao, 2007. Animal models in type 2 diabetes research: An overview. Indian J. Med. Res., 125: 451-472.
11. Harris, E.H., 2005. Elevated liver function tests in type 2 diabetes. Clin. Diabetes, 23: 115-119.
12. Islam, M.S., 2011. Fasting blood glucose and diagnosis of type 2 diabetes. Diabetes Res. Clin. Pract., 91: E26-E26.
13. Nathan, D.M., M.B. Davidson, R.A. DeFronzo, R.J. Heine, R.R. Henry, R. Pratley and B. Zinman, 2007. Impaired fasting glucose and impaired glucose tolerance: Implications for care. Diabetes Care, 30: 753-759.
14. Rodrigues, B., M.C. Cam, J. Kong, R.K. Goyal and J.H. McNeill, 1997. Strain differences in susceptibility to streptozotocin-induced diabetes: Effects on hypertriglyceridemia and cardiomyopathy. Cardiovasc. Res., 34: 199-205.