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Effect of Feeding High-Fat with or Without Sucrose on the Development of Diabetes in Wistar Rats

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Abstract: The aim of the study is to know the effect of high-fat and sucrose in the development of type 2 diabetes in Wistar rats. Several factors including high caloric diets, chronic positive caloric balance and the sedentary lifestyles are proposed as the causative factors in the development of type 2 diabetes but the exact mechanism involved is not known. Numerous studies on genetic models of obesity and diabetes induced by chemical destruction of pancreas are available, but the metabolic syndrome and diabetes observed in general population is mostly different from the genetic models and chemically induced diabetes. In this study we analyzed the effect of feeding, high-fat with or without sucrose, orally for 12 weeks. The rats were divided into three groups of twelve animals each. The groups are control, high-fat and high-fat with sucrose. After 12 weeks of treatment, fasting glucose, fasting plasma insulin, glucose tolerance test, plasma triglycerides, cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol were analyzed. Insulin resistance was analyzed by euglycemic clamp technique. Both high-fat diet and high-fat diet with sucrose impaired the lipid profile, glucose tolerance and have caused severe insulin resistance in the normal Wistar rats.

Key words: Diabetes, high-fat diet, sucrose, insulin, fatty acids, triglycerides

INTRODUCTION

In recent years metabolic syndrome and type 2 diabetes are most prevalent and reaching epidemic proportions worldwide (Misra *et al*., 2007; Firdaus *et al*., 2006). Many causative factors are proposed in the development of metabolic syndrome and type 2 diabetes (Wilkin and Voss, 2004; Avramoglu *et al*., 2006). Genetic and environmental factors play a major role (Bays *et al*., 2004) and they act independently as well as synergistically in the development of metabolic syndrome and type 2 diabetes. The following are some of the causative factors implicated in the development of metabolic syndrome and type 2 diabetes: thrifty genotype, high-fat diet (Ai *et al*., 2005), high-sucrose or fructose diet (Basciano *et al*., 2005), chronic positive caloric balance (Mcauley and Mann, 2006), sedentary life styles, excess cortisol as observed in Cushing's disease (Wang, 2005), hormonal changes observed in Poly Cystic Ovarian Syndrome (PCOS) (Salehi *et al*., 2004) and drugs such as cortisol analogs (Severino *et al*., 2002), danazol (Wynn, 1977) and nicotinic acids (Schwartz, 1993) etc., excluding single gene mutations and other specific forms of diabetes. Although several factors are implicated the exact mechanism underlying the pathogenesis of metabolic syndrome and type 2 diabetes is not clear (Kretschmer *et al*., 2005; Popov *et al*., 2003; Jazet *et al*., 2003).

It is observed in nature that the accumulation of fat or percentage of adiposity vary between animal species and also within same species living in different geographical locations (Schreider, 1950).

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In human populations it is observed that android obesity is more prone to develop type 2 diabetes than gynoid obesity. The Native, Hispanic and African American are prone to develop type 2 diabetes and tend to store visceral fat than do Caucasians (Vague, 1956; Firdaus *et al*., 2006). The mechanism of variations in the tolerance/intolerance to store more fat in some species or within species is not known. The best example for the variation in the tolerance of high-fat or high-caloric diet is observed in the desert sand rats and spiny mice, *Acomys cahirinus* (Spiny Mice) and (*Psammomys obesus*) (Shafrir *et al*., 2006). When the sand rats provided the laboratory chow diets, develop hyperinsulineamia, hypertriglyceridemia and diabetes mellitus more easily than Wistar rats. Understanding the mechanisms of tolerance/intolerance of high fat or high-caloric diet by different species or within the same species may pave the way to develop preventive and treatment modalities.

There are numerous animal models, both genetic models as well as chemically induced diabetes are available to study the pathogenesis of type 2 diabetes (Ahren *et al*., 1996; Rossini *et al*., 1977). They are however not directly related to the pathophysiological changes induced by food intake and furthermore suffer from compensatory effect induced by the early onset of malfunctioning that cannot be controlled (Kretschmer *et al*., 2005). Previous studies have proved that high-caloric diets induce insulin resistance in animal models (Storlien *et al*., 1997, 1986), but there are contrasting reports on these diets in the development of diabetes. Several reports have shown that animals fed a high-fat diet induce diabetes mellitus (Han *et al*., 1997), but Chalkley *et al*. (2002) reported that rats fed high-fat diet for 10 months induced only insulin resistance and impaired glucose tolerance but not diabetes. Thus the effects of high-fat/caloric diet in the development of type 2 diabetes need further studies.

Reports have shown that Saturated Fatty Acids (SFA) and Trans-Fatty Acids (TFA) induce insulin resistance (Clandinin *et al*., 1993). Indian vanaspati contains high levels of trans-fatty acid. It contains >20% TFA and >60% of SFA (Ibrahim *et al*., 2005). Coconut oil also contains high proportion of saturated fatty acids. In parts of India, trans fats from hydrogenated vegetable oil in the form of vanaspati and coconut oil are consumed in greater quantity than in the United States (Willett and Ascherio, 1994; Singh *et al*., 1996; Boden *et al*., 2001; Kraegen *et al*., 1991). In India and world-over sucrose is consumed along with high-fat in the form of bakery foods, sweets etc.

In this study, we analyzed the effect of oral administration of Indian vanaspati and coconut oil with or without sucrose for 12 weeks in the development of diabetes mellitus in Wistar rats. It was reported that when the high-fat is administered through diet the rats modulate the intake based on the calorific value of the foods. So we decided to feed the experimental diet orally by intra-gastric tube (Ai *et al*., 2005).

MATERIALS AND METHODS

Male Albino rats, Wistar strain of body weight ranging 220-240 g bred in Central Animal House, Rajah Muthiah Medical College, Tamil Nadu, India, fed on pellet diet (Agro Corporation Private Limited, Bangalore, India) were used for the study and water and food was given *ad libitum*. The study was conducted during February to June, 2006. The standard pellet diet comprised 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamin and 55% nitrogen free extract (carbohydrates). The animals were housed in plastic cages under controlled conditions of 12 h light/12 h dark cycle, 50% humidity and at 30±2°C. The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India and approved by the Animal Ethical Committee, Annamalai University, Tamilnadu, India.

Insulin was purchased from Sigma Chemicals Company, St Louis, USA. Hydrogenated fat, Vanaspati (Indian, partially hydrogenated vegetable oil) and the coconut oil were purchased from the

local market, Chidambaram, India. The fatty acid composition of vanaspati is as follows: 14:0-0.9, 16:0-38.5, 18.0-5.2, 18:1 trans n-9-16.2, 18:1cis-34.0 and 18:2n-6-5.0. And the 18:1 the trans n-9 is the major trans-isomer present in Indian vanapati (Saravanan *et al*., 2005). The fatty acid composition of coconut oil is as follows: Lauric acid 51%, Myristic acid 18.5%, Caprilic acid 9.5%, Palmitic acid 7.5%, Oleic acid 5%, Capric acid 4.5%, Stearic acid 3%, Linoleic acid 1% (Hilditch, 1956). All the other chemicals and biochemicals used for the experiments were of analytical grade.

The vanaspati was mixed with coconut oil in 3:1 ratio and boiled for 10 min and cooled. (The solid vanaspati is mixed with coconut oil to get a viscous mixture for the easy administration by intragastric tubes, orally). This is mentioned as high-fat in this study. A saturated solution of sucrose was prepared by dissolving sucrose in distilled water.

The animals were divided into three groups of 12 rats each.

Group-1 (Control)	=	Control rats fed with the laboratory diet *ad libitum*.
Group-2 (High-fat (HF))	=	Rats were given 5 mL of high-fat (vanaspati and coconut oil 3:1 ratio).
Group-3 (High-fat/Sucrose (HF/S))	=	Rats were given high-fat and sucrose (3 mL of high-fat and 2 mL of saturated sucrose solution).

All the animals in HF and HF/S were given the experimental diet by intragastric tube daily at a particular time for 12 weeks. The dose was increased gradually from 1 to 5 mL in the first week of the experiment and fixed at 5 mL throughout the experimental period. All animals were given free access to food and water. The food intake and water intake (daily) and body weight gain (weekly) were monitored.

Oral Glucose Tolerance Test (OGTT)

After 12 weeks of oral feeding, the animals were fasted overnight (15 h) and blood was drawn from the tail vein using hematocrit tubes for estimation of fasting insulin and glucose. Insulin was determined by rat insulin Enzyme Linked Immonosorbant Assay (ELISA) kits (LINCO laboratories) and glucose was estimated by assay kit (Boehringer Mannheim GmbH, Germany). And oral glucose tolerance test was conducted by administering glucose (2 g kg^{-1} body weight) with intragastric tube (Buckley *et al*., 2005). Blood samples were obtained from tail vein at 30, 60, 90 and at 120 min after glucose administration for the estimation of insulin and glucose. Insulinogenic index, defined as the ratio of the change in circulating insulin to the change in the corresponding glycemic stimulus was calculated using the following equation with slight modifications: = (30 min plasma insulin (pmol L^{-1})-Fasting plasma insulin (FPI)/(30 min plasma glucose (mmol L^{-1})-Fasting Plasma Glucose (FPG), the final unit is expressed as pmol $mmol^{-1}$.

After two days of OGTT, blood was drawn after overnight fasting by sino-ocular puncture (Subramanian *et al*., 2000) for the estimation of plasma triglycerides, cholesterol, high-density lipoprotein cholesterol (HDL-C) and free fatty acids. Plasma cholesterol and triglycerides were estimated by assay kits (Erba diagnostics Mannheim, Germany). And HDL-C was analyzed in the supernatant obtained after precipitation of plasma with phosphotungstic acid/Mg^{2+}. Plasma Low-density Lipoprotein Cholesterol (LDL-C) was calculated from total cholesterol, HDL-C and Triglycerides values using the Friedwald equation (Friedwald *et al*., 1972). Plasma free fatty acid concentration was estimated by the method of Falholt *et al*. (1973).

Euglycemic Clamp Technique

After two days of the previous blood drawn for lipid profile analysis, the rats were fasted for 15 h and blood was drawn from tail vein for the estimation of insulin and glucose. The rats were

anaesthetized by giving intra peritoneal injection of amobarbital sodium (25 mg kg^{-1}). Under anesthesia, euglycemic clamp were conducted by cannulating in the jugular vein for the infusion of glucose and insulin. Ten percent glucose and insulin 1 IU mL^{-1} was administered. To keep the blood sugar in a relatively steady state, the rate of glucose infusion was continuously adjusted. Glucose Injection Rate (GIR) (mg kg^{-1} min^{-1}) was measured under homeostasis five times during the experiment at 15 min intervals; Glucose levels were determined by glucometer (One touch-horizon-Lifescan. Inc.) (Kraegen *et al*., 1991).

Finally, the rats were sacrificed by cervical decapitation under anesthesia, tissues were collected and stored for further studies. Statistical analysis was done by analysis of variance (ANOVA) followed by Duncan's multiple range test (Bennet and Franklin, 1967) by means of the SPSS version 9.0 for Windows. p-value of less than 0.05 was considered to be statistically significant.

RESULTS

Changes in Food and Water Intake and Body Weight Gain in Control, HF and HF/S Groups

In the HF and HF/S groups water intake was increased and food intake was decreased though out the study period (data not shown) compared to control. The body weight was increased steadily (Fig. 1.) in the HF and HF/S groups for about seven weeks then it started decline from 8th week onwards. But in control animals the body weight increased steadily throughout the study period. A significant increase in body weight was noted in the 4, 5, 6 and 7 weeks in the HF and HF/S animals, thereafter the body weight started decline steadily compared to control.

Oral Glucose Tolerance Test (OGTT)

OGTT was carried out after 12 weeks of administration of HF or HF/S. Variations in the plasma glucose and plasma insulin levels are given the Fig. 2. The FPG was significantly increased in both HF and HF/S when compared to control. The plasma glucose level was increased significantly at all time point during OGTT, in the HF and HF/S groups compared to the controls.

No significant difference in FPI was noted in the HF and HF/S groups compared to controls. In control rats the peak level of insulin was noted at 30 min after glucose load and decreased steadily thereafter, showing a normal curve (Fig. 2). In HF and HF/S groups the insulin level at 30 min was

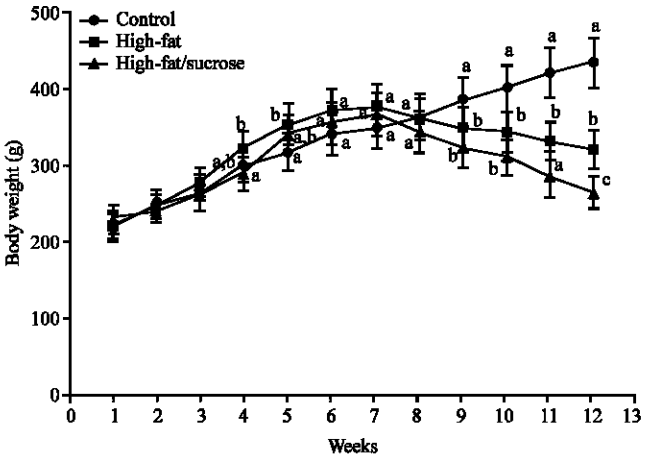

Fig. 1: The body weight changes in HF, HF/S and control rats. Values are mean±SD of 12 rats in each group $p<0.05$. Significant difference was denoted by the letters a, b and c

Table 1: Plasma lipid profile, GIR and insulinogenic index in HF, HF/S and control animals

Parameters	Control	High-Fat (HF)	High-Fat/Sucrose (HF/S)
Insulinogenic index (pmol mmol^{-1})	28.73±3.20^{a}	8.90±1.00^{b}	7.65±1.20^{b}
GIR (mg kg^{-1} min^{-1})	26.30±2.00^{a}	12.70±0.97^{b}	11.86±0.90^{b}
Tgl (mmol L^{-1})	1.09±0.08^{a}	3.85±0.29^{b}	4.40±0.34^{c}
Free fatty acid (mmol L^{-1})	0.49±0.02^{a}	0.94±0.03^{b}	0.86±0.02^{c}
Total cholesterol (mmol L^{-1})	2.26±0.17^{a}	3.87±0.29^{b}	3.92±0.30^{b}
HDL-Cholesterol (mmol L^{-1})	1.00±0.08^{a}	0.69±0.05^{b}	0.66±0.05^{b}
LDL-Cholesterol (mmol L^{-1})	0.77±0.06^{a}	1.41±0.11^{c}	1.24±0.09^{b}

Values are mean±SD for 12 rats in each group, Statistical differences between groups are shown by superscripts, $p<0.05$, Significant difference was denoted by letter a, b and c $p<0.05$

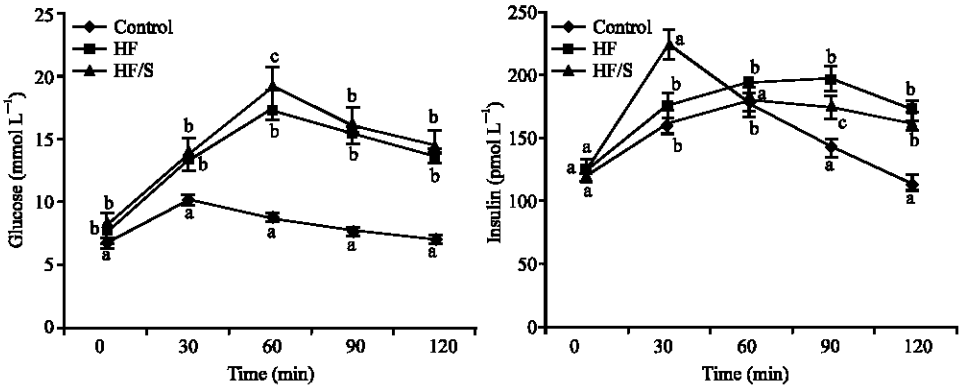

Fig. 2: Glucose and insulin levels in rats during oral glucose tolerance test (OGTT) in the HF, HF/S and control rats after 12 weeks of treatment. Values are mean±SD for 12 rats. Significant difference was denoted by letter a, b and c $p<0.05$

significantly lower and reached its maximum only at 60 min. Thereafter the insulin level was significantly higher at 90 and 120 min compared to control, but the peak insulin level was significantly lower in HF and HF/S compared to control.

Table 1 show the values for insulinogenic index, GIR and lipid profile in HF, HF/S and control rats. The insulinogenic index was markedly reduced in HF and HF/S groups compared to controls. We observed lipemic plasma in the HF as well as in HF/S groups compared to control rats. A significant increase in triglycerides, cholesterol and free fatty acid was observed in HF and HF/S groups compared to control rats. The HDL cholesterol was decreased while LDL cholesterol was increased in HF and HF/S groups compared to control. LDL cholesterol was increased more significantly in the HF/S rats compared to HF rats.

From the euglycemic clamp studies the GIR was calculated and expressed as mg of glucose required per kg body weight per min (mg/kg/min) to maintain the steady state. The GIR was reduced significantly in both experimental groups compared to control (Table 1).

DISCUSSION

In this study the administration of High Fat (HF) and high-fat with sucrose (HF/S) have caused severe insulin resistance, hyperlipidemia and hyperglycemia in the HF and HF/S rats compared to control. Increased water intake observed in the HF and HF/S rats might be the phenotype of metabolic complications induced by the experimental diet. The daily feeding of 5 mL of high-fat or high-fat/sucrose in the HF and HF/S rats might have alleviated the hunger that may be the reason for the decreased food intake noted in these groups.

The body weight was increased in all the three groups steadily until seventh week. During 4, 5, 6 and 7th weeks the body weight was significantly higher in the HF and HF/S groups compared to control rats (Fig. 1). The increase in body weight observed in HF and HF/S rats during the 4, 5, 6 and 7th weeks might be due to the fat accumulation (Kretschmer *et al*., 2005; Storlien *et al*., 1997, 1986). After seventh week the body weight was decreased steadily in the HF and HF/S groups, but in control animals the body weight increased steadily throughout the study period. The reduction in body weight after 7th weeks in the HF and HF/S rats might be due the establishment of metabolic complications such as insulin resistance and hyperglycemia (Wang, 2005).

Twelve weeks of high-fat (vanaspati and coconut oil) or high-fat with sucrose feeding have caused hyperglycemia with altered glucose tolerance as shown by OGTT (Fig. 2) in the HF and HF/S rats. The criteria for diabetic type are a peak plasma glucose concentration > 302.2 mg dL^{-1} or 120 min post load plasma glucose concentration >201 mg dL^{-1} (Wang, 2005). According to this criterion, it is observed in our study, that HF and HF/S groups have developed metabolic syndrome and diabetes.

The experimental feeding in the HF and HF/S has caused marked changes in the lipid profile causing severe hyperlipidemia in both groups. Insulinogenic index, as a measure of insulin response to glucose, and GIR as a measure of insulin resistance, (Table 1) also reduced significantly in both HF and HF/S rats which is indicating the development of severe insulin resistance and impaired glucose tolerance in the HF and HF/S rats (Ai *et al*., 2005).

Numerous reports have suggested that high-fat and high- caloric diets have caused insulin resistance, hyperinsulinemia, hypertriglyceridemia and obesity in humans and in laboratory animals (Storlien *et al*., 1997; Barnard *et al*., 1993, 1998). Human studies have linked TFA intake with insulin resistance indices in obese patients and increased risk of type 2 diabetes (Ibrahim *et al*., 2005; Christiansen *et al*., 1997). Previous reports have suggested that trans fatty acids and saturated fatty acids independently increase plasma insulin and triglyceride levels (Ibrahim *et al*., 2005; Piatti *et al*., 1995). Triglyceride-rich particles have been reported to influence binding of insulin to its receptors or interfere with insulin signal transduction steps (Gumbiner *et al*., 1996). And high-fat diet was demonstrated to reduce the insulin secretion from β-cells in laboratory animals (Ahren *et al*., 1996). These reports are in consistence with our findings in HF and HF/S rats compared to control. High-sucrose diet has been attributed to an elevation of plasma triglyceride concentration and causes insulin resistant independently (Anai *et al*., 1999; Pagliassotti *et al*., 2002). Although the amount of fat fed in the HF/S is less compared to HF rats the caloric equivalence of sucrose and fructose present in the sucrose might have acted synergistically in the development of insulin resistance and hyperglycemia.

Although there is severe insulin resistance and hyperglycemia, we don't observe any overt diabetic symptoms, as observed in alloxan, strptozotocin model of diabetes or type-1 diabetes (Ganda *et al*., 1976; Dunn and McLetchie, 1943). Chalkley *et al*. (2002) reported that high-fat feeding for 10 months have caused mild fasting hyperglycemia (Chalkley *et al*., 2002), but not overt diabetes. It has been reported that, the physical symptoms actually herald the advent of the metabolic syndrome because it develops so slowly overtime and the features of the metabolic syndrome have been known to exist in humans for 10 years before the detection of type 2 diabetes (Zimmet and Alberti, 1997). It is not known whether the duration of present study is short to develop overt diabetes or Wistar rats will ever present overt diabetes with such a diets need further studies.

The Native, Hispanic and African American are prone to develop type 2 diabetes than do Caucasians (Vague, 1956; Firdaus *et al*., 2006). *Psammomys obesus*, a seasonal desert rats develop severe insulin resistance and type 2 diabetes when maintained on normal laboratory diet (Shafrir *et al*., 2006). But the non seasonal Wistar rats do not develop any overt diabetic symptoms even after 12 weeks of feeding high-fat diet alone or with sucrose. There is a greater variation observed in the development of type 2 diabetes and tolerance towards high caloric diets between different human

race and rat strains. It can be assumed from these observations that other factors, apart from high-fat/caloric diets, also involved in the development of type 2 diabetes and the role of other factors or its interaction with high-fat/caloric diets in the development of type 2 diabetes need to be studied.

CONCLUSION

In conclusion, the oral feeding of vanaspati and coconut oil with or without sucrose for 12 weeks have caused severe metabolic syndrome and hyperglycemia but not overt diabetes in normal Wistar rats.

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