Diabetogenic Effect of Pregnancy in Sprague-Dawley (SPD) Rats: Potential use as Experimental Model of Human Gestational Diabetes

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Abstract: The effect of pregnancy on the pattern of oral glucose tolerance was investigated using Sprague-Dawley (SPD) rats. Adult virgin, timed-pregnant and non-pregnant rats were subjected to brief ether anaesthesia after 18-hour overnight fasting period to allow for oro-gastric administration of glucose load at 3.0g/kg body weight (b. wt.) as 30% solution. Glucose concentration determined from the tail blood shows that the starting glucose concentration of the pregnant rats was 6.9±0.4 mmol/l, a significantly higher ($P<0.05$) value than 5.8 mmol/l, the starting blood glucose concentration of the non-pregnant animals (Controls). The peak blood glucose level attained at the 60th minute was significantly higher ($p<0.05$) in the pregnant rats (13.5±0.3 mmol/l) as compared to that of the non-pregnant rats (8.5±0.3 mmol/l). After 120minutes, the blood glucose level of the non-pregnant rats dropped to a near starting level while the corresponding value in the pregnant rats remained comparatively higher ($P<0.05$). Assessment of the rate of appearance and disappearance of glucose in the blood and the determination of glucose response and glucose tolerance indexes (GRI and GTI) respectively showed that pregnancy caused poor glucose utilization in the rats. The results of this short-term study suggest that pregnancy is largely diabetogenic in Sprague-Dawley (SPD) rats. The diabetogenic effect of pregnancy did not necessitate administration of any other diabetogenic agent such as streptozotocin or fructose. Thus, pregnancy induced diabetes in this strain of rats may have potential value as model of gestational diabetes in human. [Nature and Science 2010; 8(4):107-111]. (ISSN: 1545-0740).

Keywords: Gestational diabetes; glucose response index; glucose tolerance index; insulin resistance

1.0 Introduction

Diabetes is a life-threatening disorder characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. (Odom et al, 2004). The vast majority of diabetes fall into two broad categories namely type 1 and type 2 diabetes. Type 1 diabetes is caused by an absolute deficiency of insulin secretion due to pancreatic β-cell destruction. The other major category, type 2 diabetes, is more prevalent especially in developing countries where there are gradual changes to western life styles. Type 2 diabetes forms a spectrum of pathophysiological conditions ranging from predominantly an insulin resistance state with relative insulin deficiency to predominantly an insulin secretory defect combined with insulin resistance (Alberti and Zimmet, 1998).

In many developing countries like Nigeria, these two categories of diabetes are well known, and considerable attention is being focused on their prevention and proper management. Another important class of diabetes that has not been given comparable level of attention particularly in developing countries is gestational diabetes(Alberti and Zimmet, 1998). It is characterized by carbohydrate intolerance of variable severity with onset or first recognition during pregnancy. In many developing countries, several fetal and neonatal deaths are associated with diabetes-related pregnancy. Babies born of diabetic pregnant mothers are macrosomic (large), and unhealthy. In the United States and other developed countries, the frequency of gestational diabetes is increasing. The long-term implications for developing countries like Nigeria are important. In view of the increasing prevalence of gestational diabetes and the associated risk of maternal and neonatal morbidity and mortality, gestational diabetes remains a significant challenge and increasing attention should be focused on the problem.

Experimental animal models of gestational diabetes are of immense value in this respect. It is generally known that the use of human subjects in biomedical research sometimes has obvious limitations which include ethical and other considerations. Development of experimental animal

models remains an important way of enhancing understanding the pathophysiological mechanisms of conditions that affect human. Considering gestational diabetes and its associated problems, experimental findings from animal models would enhance the development of preventive and management strategies with a view to improving diabetic pregnancy outcomes. Previous studies relating to animal models of gestational diabetes involved administration of streptozotocin (Lopez-Soldado and Herrera, 2003) or fructose (Olatunji-Bello and Nwachukwu, 2000) before or during pregnancy. However the adequacy of these models in reproducing human gestational diabetes has been questioned (Caluwaerts et al., 2003) partly due to the fact that these agents (STZ and fructose) are themselves diabetogenic (Rakieten et al., 1963; Zavaroni et al., 1980); thus, the diabetogenic effect of pregnancy is complicated by the effect of these agents. Thus, induction of diabetes in animals by pregnancy without administering other diabetogenic agents may represent more appropriate animal models of human gestational diabetes in human. The aim of this study is to assess susceptibility of Sprague-Dawley (SPD) rats to pregnancy-induced diabetes with a view to evaluating the use of this strain as experimental models of gestational diabetes.

2.0 Materials and Methods

2.1 Experimental Animals

Sprague-Dawley albino rats of both sexes weighing 160-180g were obtained from the Laboratory Animal Unit of the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos, and transferred to the Animal Room of the Biological Garden, University of Lagos, where they are kept in plastic cages with mesh grid floors for acclimatization and mass breeding in the new environment. The cages were thoroughly cleaned and the animals examined on a daily basis. Clean tap water and rat feed were made available *ad libitum*. The temperature of the animal room was $30\pm3^{o}C$ with 12h:12h light darkness cycle. On this regime, the animals remained uniformly healthy and active. Rats that became pregnant were separated into solid floor maternity cages. Fine sterilized wood shavings were provided in the cages as bedding and nesting materials. Immediately after the offspring were weaned (i.e. before attaining sexual maturity), they were transferred into new cages where they were kept separately as females or males only to avoid mating before induction of pregnancy. This was to ensure that the animals were still virgin males and females before induction of pregnancy. In this study, strict adherence of experimental procedures to ethics in animal experimentation was ensured.

2.2 Induction of Pregnancy

After 90 days of life, 20 virgin rats (10 females:10 males) obtained from mass breeding above were housed in 10 mating groups of monogamous pairs (1 females: 1 male per cage). At this period of life, the animals would have attained sexual maturity, and the virgina would have opened. To check for successful mating, the virgina was examined every morning, and virgina smears were obtained to see if sperm cells were present. In addition, the virgina and the floor of the cages were observed to check for the presence of cornified plug. The presence of sperm cells in the virgina smear or the availability of cornified plug in the virgina or on the floor of the cage indicated successful mating, and this was regarded as Day-1 of gestation. Such females were separated into maternity cages to constitute pregnant rats for oral glucose tolerance test (OGTT) which was carried out on D-17 of gestation.

2.3 Oral Glucose Tolerance

Since successful mating does not imply successful pregnancy, the criterion for inclusion in oral glucose tolerance test (OGTT) among the rats placed n the Pregnant Group was successful pregnancy. Female rats (n=18) consisting of 8 pregnant rats (treatment) and10 non-pregnant rats (control) were used for OGTT on Day-17 as described in previous reports (Odeigah et al., 1994). Essentially, the animals were fasted for 18 hours before the test, and a glucose load of 3.0/kg body weight (b. wt.) was delivered into the stomach through the buccal cavity as 30% solution by oro-gastric intubation under light ether anaesthesia. Blood glucose was obtained from cut tail tips for the determination of blood glucose concentration (glucose-oxidase method) using an automated digital blood glucose analyzer, glucometer (Accu-Chek Advantage, Roche, USA), just before oral glucose loading (0 minute) and at 30, 60, and 120 minutes of OGTT to obtain the glucose tolerance curve of each rat.

2.4 Analysis of Data

2.4.1 Statistical Analysis

All data were input into the computer for statistical analysis using a software package – GraphPad Prism Version 5.00. The results are expressed as mean$\pm$SEM. Statistical difference between means was determined by Student's t-test,

and P<0.05 was considered significant while P<0.01 was taken to be highly significant.

2.4.2 Rate of Appearance and Disappearance of Glucose from the Blood

The mean rate of appearance of glucose in the blood was obtained by determining the positive slope of the glucose tolerance curve from the starting (0-minute) blood glucose level to the peak level according to the formular below:

Rate of appearance/disappearance of glucose (mmol/min) = ΔG/ΔT (Note: ΔG=change in blood glucose concentration in mmol. while ΔT=time in minutes (mins.).

The rate of disappearance of glucose was similarly calculated using the negative slope of the curve from the peak level to the final glucose level.

2.4.3 Glucose Response Index (GRI) and Glucose Tolerance Index (GTI)

Glucose utilization was assessed by Glucose response index (GRI). The GRI for each rat was estimated as the incremental area under its glucose tolerance curve. It is calculated by summation of the areas of the trapezoids defined by individual points on the curve (Lebovitz and Feinglos, 1983).This area which is above the fasting blood glucose level during the two-hour oral glucose tolerance determination is inversely related to glucose tolerance index (GTI) which gives a direct assessment of glucose utilization in each animal. Thus, GTI was estimated as the reciprocal of GRI.

3. Results

Figure 1 depicts the plasma glucose profiles of the pregnant and the non-pregnant rats as revealed by their glucose tolerance curves. It could be observed that the starting blood glucose concentration of the pregnant rats was 6.9±0.4, a significantly higher (P<0.05) value than 5.8 mmol/l, the starting blood glucose concentration of the non-pregnant animals. After 60 minutes of OGTT, the blood glucose concentration reached a peak level in both groups. However, the peak plasma glucose concentration in the pregnant rats (13.5±0.3 mmol/l) was significantly higher (P<0.05) than that of the non-pregnant rats (8.5±0.3 mmol/l). This blood glucose rise resulted in a rate of appearance of glucose of 0.11mmol/min. and 0.05 mmol/min respectively in the pregnant and the non-pregnant rats respectively (Table 1).The rate of disappearance of glucose after attaining the peak concentration was, however, higher in the non-pregnant rats. After 120minutes, the blood glucose level of the non-pregnant rats dropped to 6.2±0.3, a value which was near the starting level; however, the corresponding in the pregnant rats remained significantly high (P<0.05). Determination of glucose tolerance index (GTI) as revealed by Figure 2 showed that pregnancy caused a very significantly higher GTI of 483.3.0±35.0 mmol.min/l (P<0.01) when compared to the non-pregnant state (GTI=175.5±25.0 mmol.min/l).

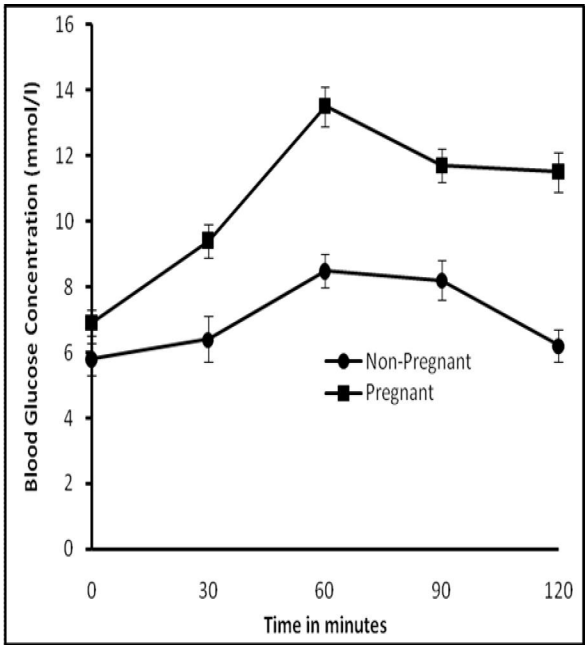

Figure 1. Increased Glycaemic Response in Pregnant Rats as Compared to non-Pregnant Rats.

Table 1. Rate of Appearance and Disappearance of Glucose (mmol/min.) in the Blood of Pregnant and Non-Pregnant Rats

Animal Groups	Rate of Appearance (mmol/min.)	Rate of Disappearance (mmol/min.)
Non-Pregnant	0.05	0.05
Pregnant	0.11	0.03

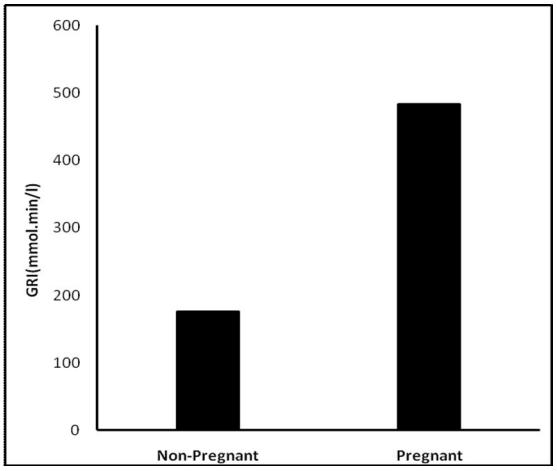

Figure 2. Higher Glucose response Index (GRI) in the pregnant Rats as Compared to that of Non-Pregnant Rats

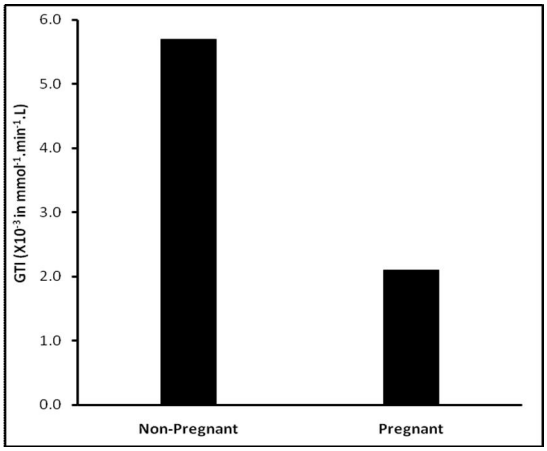

Figure 3. Higher Glucose Tolerance in Pregnant Rats than the Non-Pregnant Rats as Assessed by Glucose Tolerance Index

4.0 Discussion

In the present study, oral glucose tolerance test or OGTT was performed on the 17th day of gestation in the rats, a period that is equivalent to the third trimester of pregnancy in human. Although the cause of pregnancy induced diabetes is not fully known, there are some theories explaining the etiology of the condition. According to a popular theory, some hormones (oestrogen, cortisol and human placental lactogen) produced during pregnancy are responsible for insulin resistance observed in gestational diabetes. Normally, the pancreas produces additional insulin to overcome insulin resistance, but when insulin production is not enough to overcome the effect of these hormones, gestational diabetes results. Xiang et al. (1999) suggested that the degree of insulin resistance increases with gestational period and that insulin resistance plays a major role in the development of diabetes mellitus during pregnancy. Subsequent studies in rats by Olatunji-Bello and Nwachukwu (2000) agreed with the findings of Xiang et al. (1999). Thus, on Day-17 of pregnancy in rats, insulin resistance or, possibly, diabetes associated with pregnancy would have fully developed.

The elevated blood glucose concentration at every time-point of OGTT in the pregnant rats indicated the presence of impaired glucose tolerance. It was therefore not surprising that glucose tolerance index or GTI was significantly higher in the pregnant rats than in those that were not pregnant. Several reports have indicated that in late gestation, women with gestational diabetes have increased fasting insulin concentrations and less suppression of hepatic glucose production during insulin infusion, thereby indicating decreased hepatic insulin sensitivity in women with gestational diabetes compared with a weight-matched control group (Catalano et al., 1993). It is not clear whether impaired glucose tolerance due to pregnancy was associated with hyperinsulinemia in SPD rats. Direct determination of insulin concentration concurrently with glucose measurement would be elucidating.

The underlying pathophysiology of pregnancy-induced diabetes is associated with decreased maternal insulin resistance, a situation whereby a defined concentration of insulin is unable to effect a predictable biological response of nutrient metabolism at the target tissue (Catalano et al., 2003). This agrees with the observation of Xiang et al. (1999) that significant alteration of glucose metabolism occurs in women who develop gestational diabetes. The increased rate of appearance of glucose in the blood of pregnant rats and its slow rate of disappearance as observed in this study was an indication of poor glucose metabolic state resulting from insulin resistance during pregnancy in the rats. Decreased maternal insulin sensitivity in women with gestational diabetes may increase nutrient availability to the foetus, possibly accounting for an increased risk of foetal overgrowth and adiposity. This explains why babies of diabetic pregnant women are macrosomic (Catalano et al., 2003).

Considering the significantly elevated starting glucose level and the general blood glucose profile during OGTT in the rats used in this study, it may be suggested that pregnancy is largely diabetogenic particularly in SPD rats. Unlike other studies where

the diabetogenic effect of pregnancy is complicated by other agents like streptozotocin (STZ) and fructose (Lopez-Soldado and Herrera, 2003; Olatunji-Bello and Nwachukwu, 2000) that are themselves diabetogenic (Rakieten et al., 1963; Zavaroni et al., 1980), the glucose tolerance pattern observed in the present study was due to pregnancy alone. This does not rule out the presence of underlying genetic factors predisposing the animals to pregnancy diabetes. Similar observation in human had suggested that the metabolic stress of pregnancy may unmask a genetic susceptibility that causes alterations in glucose metabolism leading to gestational diabetes. If this observation is confirmed by other workers, one may suggest the presence of genetic factors in the susceptibility of SPD rats to pregnancy-induced diabetes.

In order to characterize the genetic component more accurately, selective breeding is in progress in our laboratory to create two strains of rats having different susceptibility to gestational diabetes. Other genetic and molecular studies are also in progress to determine the heritability of this trait and possible association of molecular markers such as RFLPs which may lead to easy identification of people at risk.

Conclusion

Pregnancy is largely diabetogenic in SPD albino rats, and they may serve as models for human gestational diabetes.

Acknowledgement

We appreciate the support and advice of the entire staff of the department of Cell Biology and Genetics, university of Lagos, Akoka, Lagos.

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10/03/2010