J. Afr. Ass. Physiol. Sci. 2 (1): 47 - 55

Journal of African Association of Physiological Sciences

Official Publication of the African Association of Physiological Sciences

http://www.jaaps.aapsnet.org

Research Article

Effects of fructose feeding on maternal and amniotic fluid corticotropin releasing hormone and C-reactive protein in pregnant female Sprague-dawley rats

Ogunsola O.A.[1,2] and Arikawe A.P.[2,3]

[1] *Department of Physiology, Ben Carson School of Medicine, Babcock University, Ilishan-Remo, Ogun State, Nigeria.*
[2] *Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria.*
[3]*Laboratory of Neuroendocrinology, Department of Morphology, Physiology and Basic Pathology, School of Dentistry of Ribeirao Preto, University of Sao Paulo, Sao Paulo, Brazil*

Keywords:
Insulin resistance, CRH, CRP, IGF-1 and IGF-2, fetal programming

ABSTRACT

Exposure to a compromised intra uterine environment has been known to alter fetal growth and metabolic processes with long term consequences for health. Maternal nutrition prior to and during gestation plays a role in fetal programming, however the mechanisms underlying these changes have not been fully elucidated. This study was designed to evaluate the effects of fructose feeding on maternal serum and amniotic fluid insulin, corticotropin releasing hormone (CRH), C - reactive protein (CRP), and insulin-like growth factors (IGF-1 and IGF-2) on days 12, 15 and 18 of pregnancy in control and fructose fed rats. Virgin female rats were randomly divided into 2 groups; control group fed with normal rat chow and fructose group fed with 25% w/w fructose orally for 10 weeks. Female rats were mated with proven male rats and the presence of sperm cells in the virginal smear the following morning was taken as day 1 of pregnancy. Maternal serum and amniotic fluid samples were obtained on days 12, 15 and 18 of pregnancy for insulin, CRH, CRP, IGF-1 and IGF-2 measurements using ELISA techniques. Maternal and amniotic fluid insulin, CRH, CRP and IGF-1 levels were significantly higher ($p<0.05$) in the fructose-fed group compared to the control group. IGF-2 level was not significantly different in the maternal and amniotic fluid samples in both groups. The results suggest that stress and inflammatory mechanisms may be implicated in the role maternal nutrition plays in developmental plasticity.

INTRODUCTION

A growing body of empirical evidence suggests that the origins of metabolic syndrome can be traced to intrauterine life, when the developing fetus is acted upon by and responds to suboptimal conditions during critical periods of cellular proliferation, differentiation, and maturation by producing structural and functional changes in cells, tissues and organ systems. These changes, in turn, may have long-term consequences to increase the individual's risk for developing metabolic disorders, which is the concept of fetal programming (Gluckman and Hanson, 2004).

Fetal development is influenced by a complex factor of genetics, maternal nutrition, intra-uterine environment, maternal and fetal hormones. Exposure to a compromised intra-uterine environment has been known to alter fetal growth and metabolic processes

*Address for correspondence:
Email: arikawepaul2002@yahoo.co.uk
Telephone: +234 8023605282

with long term consequences for health. Diabetic mothers have an additional influence of maternal hyperglycaemia, known to cause alterations in circulating maternal and fetal hormones, growth factors and cytokines. Maternal nutrition prior to and during gestation plays a role in fetal programming (Burdge and Lillycrop, 2010) and the mechanisms underlying these changes have not been well elucidated.

CRH is a 41 amino acid peptide hormone first identified in the hypothalamus of mammals, where it acts as the main functional regulator of corticotrophin (ACTH) secretion from corticotropes of the anterior pituitary (Vale *et al.,* 1981). Hypothalamic secretion of CRH is stimulated by many forms of physical or psychological duress and CRH plays a critical role in the coordination of neuroendocrine response to stress. Expression of the gene encoding for CRH has been recognized at a number of other sites, where it serves disparate roles. During pregnancy, the placenta and fetal membranes produce large amounts of CRH, although its function is not yet well understood. The synthesis of CRH in these tissues is known to increase as gestation advances. Some studies have indicated a role for placenta-derived CRH in the pathophysiology of diabetic pregnancies (Gangestad, Caldwell Hooper, and Eaton, 2012) and also in the onset of parturition (Ishimoto and Jaffe, 2011).

CRH release from the hypothalamus is predominantly under neural control, however, the placenta has no nervous tissue, therefore placental CRH secretion is probably regulated exclusively by circulating and locally produced humoral factors. Some studies using either cultured placental trophoblasts or perifused placental villous tissue showed secretion of CRH in vitro (Grino, Chrousos, and Margioris, 1987; Saijonmaa, Laatikainen, and Wahlstrom, 1988) that can be stimulated by a number of neurotransmitters, cytokines, and polypeptide or steroid hormones.

C-reactive protein (CRP) is a known inflammatory marker synthesized by the liver. It is a component of the immune system and one of the acute phase proteins released during systemic inflammation. An increase in serum levels of CRP has been reported to be an independent risk factor for cardiovascular disease (Ridker *et al.,* 1998); insulin resistance (Frohlich *et al.,* 2000), and future development of type 2 diabetes mellitus (Pradhan *et al.,* 2001).

Type 2 diabetes mellitus has been associated with endothelial dysfunction and inflammation as well as increased production of cytokines by the adipose tissue (Balletshofer *et al.,* 2000; Abderrahim-Ferkoune *et al.,* 2003). Experimental and epidemiologic studies have shown an association between increased levels of acute-phase proteins and pathologic conditions, such as type 2 diabetes mellitus, metabolic syndrome, obesity, and cardiovascular diseases (Pickup and Crook, 1998; Lagrand *et al.,* 1999). Studies have also linked biomarkers such as CRP, plasminogen activator inhibitor-1, and interleukin -6 with type 2 diabetes mellitus in different populations (Pradhan *et al.,* 2001; Festa *et al.,* 2002).

Insulin-like growth factors (IGFs) play a vital role during pregnancy by modulating fetal growth through their actions on the mother and placenta. Levels of circulating IGFs influence maternal metabolism thereby regulating nutrient availability for the fetus. Maternal IGFs also regulate placental morphogenesis, substrate transport and hormone secretion, all of which influence fetal growth either via indirect effects on maternal substrate availability, or through direct effects on the placenta and its capacity to supply nutrients to the fetus (Sferruzzi-Perri *et al.,* 2011). Altered fetal development is associated with increased perinatal morbidity and mortality and a greater risk of developing metabolic diseases in adult life, understanding the role of IGFs during pregnancy is essential to identify mechanisms underlying altered fetal programming. Perturbations in insulin-like growth factors have been implicated in diabetic pregnancies (Hiden *et al.,* 2009).

Fructose feeding induces insulin resistance and subsequently type 2 diabetes mellitus in rodents (Suga *et al.,* 2000; Arikawe *et al.,* 2006; Arikawe *et al.,* 2008; Arikawe *et al.,* 2012). We considered the potential role stress and inflammatory responses may play in diabetic pregnancies using corticotrophin releasing hormone (CRH), a stress marker produced by the placenta and C - reactive protein (CRP) an inflammatory response marker by evaluating the effect of fructose-induced insulin resistance on maternal serum and amniotic fluid insulin, CRH, CRP, IGF-1 and IGF-2 on days 12, 15 and 18 of pregnancy in female rats.

MATERIALS AND METHODS

Animals

Virgin female Sprague-Dawley rats aged 6 weeks, weighing 110 – 120 g were obtained from the laboratory animal department of the College of Medicine, University of Lagos. The animals were housed in clear polypropylene cages lined with wood chip beddings. Animals were kept under standard conditions of temperature 27ºC – 30ºC, with 12h light/dark cycle and were randomly divided into 2 groups. Group 1 served as control group and was fed with normal rat chow. Group 2 served as the fructose group and was fed *ad libitum* on a special diet containing 25% fructose (SIGMA Aldrich, USA)

mixed with 75% normal rat chow weight/weight for 10 weeks (Arikawe and Olatunji-Bello, 2004). At this fructose concentration, insulin resistance state was 100% with zero mortality rate. Body weight and blood glucose levels were measured at the beginning of the experiment and weekly from the tail vein of the rats in both groups using Dextrostix Test Strips (Bayer Corporation, U. K.) after an overnight fast till diabetes mellitus was induced in group 2 rats. Hyperglycaemia was confirmed using the glucose oxidase method (Hugget and Nixon, 1957). All animals had free access to drinking water throughout the duration of the study. The procedures were performed in accordance with guidelines of the College Ethical Committee on the use of laboratory animals for research.

Vaginal smears and Induction of pregnancy

The stage of cyclicity in the two groups was determined by microscopic analysis of the predominant cell type in vaginal smears obtained daily from the 8th week (Marcondes, Bianchi, and Tanni, 2002) till the 10th week of feeding i.e. a two-week period. Only female rats showing two consecutive estrous cycles of the same length were used (Cruz, *et al.,* 1990). Pregnancy was induced at the end of the 10th week by mating at night a pro-estrous female rat with mature and proven adult male rat. This was to ensure that copulation occurred at estrus, the only time the female rat is receptive to the male rat. Successful mating was confirmed by the presence of sperm cells in the vaginal smear, and was regarded as day 1 of pregnancy. Animals were further divided into 3 subgroups namely Day 12, 15 and 18 for both groups.

Sample collection and assays of parameters

Cervical dislocation was carried out on the animals in the two groups on days 12, 15 and 18 of pregnancy. Summarily, the animals were placed supine on the dissecting board following dislocation of the spine at the cervical region. With a pair of forceps and scissors, the lower abdominal region was cut open. This incision was extended upwards into the upper abdominal region and subsequently into the thoracic region, to expose the contents of the abdomen and the thorax. Blood was quickly collected by cardiac puncture into plain sample bottles and allowed to clot. Blood samples were centrifuged at 3,000 rpm for 15 minutes to get clear serum samples, which were subsequently kept frozen (-20ºC). Laparatomy was done and the gravid uterus exposed, clear amniotic fluid was obtained from the gestational sacs using a 28G needle and 1ml syringe. Maternal serum and amniotic fluid samples were obtained on different days of pregnancy namely days 12, 15 and 18 to assess the trend of insulin, CRH, CRP, IGF-1and IGF-2 levels as pregnancy advanced.

Analysis of parameters

Maternal serum and amniotic fluid samples were analyzed for insulin, CRH, CRP, IGF-1 and IGF-2 using ELISA technique. Insulin was analyzed using Diagnostic Automation Inc. USA kit with minimum detection limit of 0.03 IU/L, IGF-1 and IGF-2 were measured using DSL kit; Diagnostics Systems Laboratories, TX. Maternal plasma and amniotic fluid CRP and CRH concentrations were measured using DRG kits, USA. The intra- assay coefficient of variation for CRP was 7.5% and the inter assay coefficient of variation for CRP was 4.1%.

Assessment of placental and fetal parameters

On day 18 of pregnancy in both groups, laparatomy was done to expose the uterus and assess the fetus and the placenta tissue. The gestational sacs was carefully opened after amniotic fluid has been collected using a 23G needle and 1ml syringe, placental weight, fetal weight, cord length, litter size and number of resorption sites. The placentae and fetuses were isolated carefully with the aid of forceps without cutting the umbilical cord; the length of the cord was measured using thread from the umbilicus to the point of attachment on the placenta, marked and the length read from a graduated ruler. The number of resorption site was determined by counting the number of implantation sites in the uterus that failed to develop.

Statistical analysis

Results are expressed as means ± S.E.M. The significance of differences among groups was analyzed statistically using Student's unpaired t – test. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Fasting blood glucose concentration (mg/dl) at the beginning of the experiment, 2nd, and 4th week in Control rats was (78.5 ± 6.3, 80.8 ± 5.4, 82.2 ± 4.6 mg/dl) and in fructose-fed rats was (79.8 ± 5.5, 82.4 ± 3.4, 98.6 ± 3.0 mg/dl). Fasting blood glucose at the 6th, 8th, and 10th week in Control rats was (84.6 ± 6.5, 85.8 ± 3.2, 86.0 ± 6.4 mg/dl) and in fructose-fed rats was (120.6 ± 6.8, 128.9 ± 4.6, 142.6 ± 5.8 mg/dl) respectively. There was no significant difference in fasting blood glucose concentration in the 2 groups at the beginning of the experiment till the 4th week (Table 1). However, blood glucose level was significantly higher in the fructose-fed rats from the 4th week till the 10th week and this was confirmed by hyperglycemia following an overnight fast.

Table 1. Fasting blood glucose (mg/dl) concentration in Control and Fructose-fed

	Control	**Fructose-fed**
Week 0	78.0 ± 6.3	79.8 ± 5.5
Week 2	80.8 ± 5.4	82.4 ± 4.4
Week 4	75.2 ± 4.2	98.6 ± 3.0*****
Week 6	75.8 ± 6.5	120.6 ± 6.8*****
Week 8	76.0 ± 3.2	124.9 ± 4.6*****
Week 10	82.0 ± 6.0	142.6 ± 5.8*****

Data are expressed as mean ± SEM (n = 30) * p<0.05 Vs Control

Body weight (gm) at the beginning of the experiment, 2nd, and 4th week in Control rats was (90.0 ± 1.1gm, 94.8 ± 1.9 gm, 105.2 ± 1.6 gm) and fructose –fed rats was (95.0 ± 1.2 gm, 109.0 ± 1.3 gm, 115.8 ± 1.0 gm). Body weight (gm) at the 6th, 8th, and 10th week in Control rats was (112.8 ± 1.5gm, 123.3 ± 1.5 gm, 134.0 ± 1.4 gm) and fructose-fed rats was (109.8 ± 1.0 gm, 103.8 ± 1.1 gm, 95.2 ± 0.9 gm) respectively. There was no significant difference in body weight amongst the two groups at the beginning of the experiment till the 6th week. However, body weight began to significantly decline (P < 0.05) in the fructose-fed group when compared to the control group from the 6th week till the end of experiment (Table 2).

Table 2. Body weight (gm) in Control and Fructose-fed rats

	Control	**Fructose-fed**
Week 0	110.5 ± 1.1	111.0 ± 1.3
Week 2	118.4 ± 1.9	119.5 ± 1.6
Week 4	126.2 ± 1.6	117.7 ± 1.5
Week 6	132.8 ± 1.2	90.8 ± 1.9*****
Week 8	137.3 ± 1.8	80.5 ± 1.7*****
Week 10	142.0 ± 1.6	67.7 ± 2.1*****

Data are expressed as mean ± SEM (n = 30) * p<0.05 Vs Control

Maternal serum and amniotic fluid insulin was significantly higher (p<0.05) in the fructose-fed group compared to the control during pregnancy. There was however a progressive increase in maternal insulin levels as pregnancy advanced in both groups but insulin levels in the fructose-fed rats were over 2 folds that of the control rats (Table 3). IGF-1 levels in maternal serum and amniotic fluid of fructose-fed rats were increased significantly compared to the control and this also increased as pregnancy advanced (Table 4) while IGF-2 levels were not significantly different in the maternal serum and amniotic fluid of both groups (Table 5).

Table 3. Insulin concentrations (IU/L) in maternal serum and amniotic fluid of Control and Fructose-fed pregnant rats.

Insulin (IU/L)	Day 12 (n=10)	Day 15 (n=10)	Day 18 (n=10)
Maternal serum Control	21 ± 1.2	24 ± 0.8	27 ± 0.7
Maternal serum Fructose-fed	38 ± 2.1†	44 ± 2.3†	54 ± 2.1†
Amniotic fluid Control	2.1 ± 0.1	2.8 ± 0.1	3.1 ± 0.2
Amniotic fluid Fructose-fed	6.3 ± 0.6*	7.2 ± 0..4*	8.8 ± 0.2*

Data are expressed as mean ± SEM † P<0.001 Vs. Control (Maternal serum); *P<0.05 Vs. Control (Amniotic fluid)

Placental weight increased significantly (p<0.05) in the fructose-fed group (0.40±0.08gm) compared to the control group (0.28 ± 0.03gm) on day 18 of pregnancy, placental diameter was also significantly increased in

Table 4. IGF-1 (pg/ml) concentrations in maternal serum and amniotic fluid of Control and Fructose-fed pregnant rats.

	Day 12 (n=10)	**Day 15 (n=10)**	**Day 18 (n=10)**
Maternal serum Control	0.99 ± 0.14	1.24±0.21	1.67±0.22
Maternal serum Fructose-fed	2.21±0.10†	3.66±0.30†	4.61±0.10†
Amniotic fluid Control	0.21±0.06	0.33±0.03	0.39±0.02
Amniotic fluid Fructose-fed	0.63±0.05*	0.98±0.04*	1.02±0.02*

Data are expressed as mean ± SEM † P<0.001 Vs. Control (Maternal serum); *P<0.05 Vs. Control (Amniotic fluid)

the fructose-fed group (1.64 ± 0.44cm) compared to the control group (0.82 ± 0.26cm). There was a significant decrease in fetal weight on day 18 of pregnancy in the fructose-fed group (2.2 ± 0.49gm) compared to the control (3.9 ± 0.38gm). Litter size, cord length and number of resorption sites were however not significantly different in both groups (Table 6).

Maternal serum and amniotic fluid CRH levels were significantly higher (p<0.05) in the fructose-fed group compared with the control group. Maternal and amniotic fluid CRH levels increased progressively as pregnancy advanced in both groups. However, CRH levels in maternal and amniotic fluid of the fructose-fed group were significantly higher (p<0.001) compared to the control group (Table 7).

CRP levels were significantly (p<0.05) increased in the maternal serum of fructose-fed group on days 12,

15 and 18 of pregnancy compared to the control group. There was however no significant difference in maternal serum CRP levels in the control and fructose-fed groups as pregnancy advanced. A significant progressive increase in amniotic fluid CRP was observed in the fructose-fed group with no significant (p=0.15) corresponding increase in maternal serum levels on days 12, 15 and 18 of pregnancy (Table 8).

DISCUSSION

Fructose feeding through insulin resistance mechanism induces Type 2 diabetes mellitus in female rats, evident by hyperglycemia, hyperinsulinemia and body weight loss observed in the present study. This finding is in line with the view of Suga *et al.,* 2000. The result also indicates that pregnancy is a diabetogenic state (Vannini, 1994).

Table 5. IGF-2 concentrations **(x10^4 pg/ml)** in maternal serum and amniotic fluid of control and fructose-fed pregnant rats.

	Day 12 (n=10)	**Day 15 (n=10)**	**Day 18 (n=10)**
Maternal serum Control	3.59 ± 0.30	3.65±0.29	3.68±0.22
Maternal serum Fructose-fed	3.43±0.21	3.66±0.30	3.86±0.26
Amniotic fluid Control	1.29±0.16	1.46±0.13	1.50±0.22
Amniotic fluid Fructose-fed	1.44±0.05	1.54±0.04	1.59±0.02

Data are expressed as mean ± SEM

Body weight increased progressively in both groups as anticipated until the 4th week, when it became significantly higher in the fructose-fed group compared to control group. It then declined in the fructose group till the 10th week when it became significantly lower compared to the control group. The body weight pattern in this study is in line with our earlier observations (Arikawe *et al.,* 2006; Arikawe *et al.,* 2008; Arikawe *et al.,* 2012) that body weight in insulin resistant diabetic rats begins to decline from the 4th week. This decline is suggested to be due to the onset of diabetes in rats in the fructose-fed group, which is characterized by gluconeogenesis leading to muscle waiting and weight loss. Our result on body weight is also in line with the view of Catena *et al.,* (2003), who reported that fructose feeding has no significant effect on body weight of virgin female rats in the first two weeks of feeding.

Serum insulin level is a crucial factor to control normal blood glucose level (Islam and Choi, 2008). It was as expected significantly higher in fructose-fed group compared to the control group. Chronic fructose consumption caused structural alterations in pancreatic β cells (Lee at al., 2010; Van Assche, Aerts, and de Prins, 1983) to cause hyperinsulinaemia. During pregnancy, maternal serum and amniotic fluid insulin levels increased progressively as pregnancy advanced in both groups, an indication of pregnancy-induced insulin resistance; however insulin levels in the fructose-fed rats were over two folds the control rats in which the physiological insulin resistance of pregnancy was observed. A similar result was reported by Saito *et al.,* 2010. Amniotic fluid insulin is of fetal origin, since maternal insulin cannot cross the placental barrier (Dickinson and Palmer, 1990). Fetal insulin is excreted via fetal urine into the amniotic fluid, and this has been reported to increase as fetal insulin production increases (Illsley, 2000). Increased amniotic fluid insulin has been associated with fetal morbidity or mortality (Buchanan and Coustan, 1994) and macrosomia (Jansen, Greenspoon, and Palmer, 2003).

Table 6. Placental and fetal parameters in Control and Fructose-fed pregnant rats on day 18 of pregnancy

Parameters	Control	Fructose-fed
Placental weight (gm)	0.28 ± 0.03	0.40 ± 0.08*
Placental diameter (cm)	0.82 ± 0.26	1.64 ± 0.44*
Fetal weight (gm)	3.9 ± 0.38	2.2 ± 0.49*
Fetal/Placenta weight	13.9 ± 3.65	5.5 ± 1.24*
Cord Length (cm)	1.44 ± 0.28	1.68 ± 0.34
Litter size (n)	7.3 ± 2.1	6.2 ± 1.4
Resorption sites (n)	2.82 ± 0.06	3.24 ± 0.49

Data are expressed as mean ± SEM (n = 10) * p<0.05 Vs Control

Maternal serum and amniotic fluid IGF- 1 and IGF-2 of the fructose fed pregnant rats are consistent with the findings of Fowden (2003). Serum and amniotic fluid IGF-1 levels were significantly higher in the fructose group compared to the control, while there was no significant difference in maternal and amniotic fluid IGF-2 levels, although a progressive increase was observed as pregnancy advanced in both groups. This is in line with the view of Sferruzzi-Perri *et al.* (2011). IGF-1 is reported to have mitogenic actions including

stimulation of cellular growth, proliferation and differentiation (Sara and Hall, 1990), enhancement of glucose and amino acid uptake (Turkalj, Keller, and Ninnis, 1992) and appears to be the dominant growth promoting factor during the rapid phase of somatic growth in late gestation (Baker *et al.,* 1993).

Table 7. Maternal serum and amniotic fluid CRH (pg/ml) concentrations in control and fructose-fed pregnant rats.

CRH (pg/ml)	Day 12 (n=10)	Day 15 (n=10)	Day 18 (n=10)
Maternal serum Control	34.1 ± 3.2	42.6±2.9	50.8±2.2
Maternal serum Fructose-fed	42.3±2.8†	58.4±3.0†	76.8±2.6†
Amniotic fluid Control	2.6±0.14	3.8±0.16	6.5±0.52
Amniotic fluid Fructose-fed	8.3±0.25*	15.4±0.84*	21.9±0.86*

Data are expressed as mean ± SEM † P<0.001 Vs. Control (Maternal serum); *P<0.05 Vs. Control (Amniotic fluid)

Table 8. Maternal and amniotic fluid CRP (mg/dl) concentrations in Control and Fructose-fed pregnant rats.

CRP (mg/dl)	Day 12 (n=10)	Day 15 (n=10)	Day 18 (n=10)
Maternal serum Control	0.25 ± 0.08	0.32±0.11	0.38±0.12
Maternal serum Fructose-fed	3.2±0.10†	3.7±0.17†	4.1±0.19†
Amniotic fluid Control	0.11±0.03	0.15±0.06	0.16±0.04
Amniotic fluid Fructose-fed	0.44±0.05*	0.78±0.04*	1.23±0.08*

Data are expressed as mean ± SEM † P<0.001 Vs. Control (Maternal serum); *P<0.05 Vs. Control (Amniotic fluid)

Furthermore, maternal serum and amniotic fluid CRH levels were significantly higher in fructose- fed rats compared to the control; there was however a progressive increase of maternal and amniotic fluid CRH in both groups as pregnancy advanced. This suggests that chronic fructose feeding leading to hyperglycemia and hyperinsulinemia could induce both maternal and fetal distress during pregnancy. Intrauterine exposure to stress may interact with the altered nutritional milieu which may represent an underlying mechanism mediating the effects of intrauterine malprogramming. Wadhwa, 2005; Entringer *et al.,* 2012 also support this view from their findings.

From the findings of this study, maternal serum and amniotic fluid CRP levels were significantly higher in the fructose-fed pregnant rats compared to the control, and increased amniotic fluid CRP levels was observed as pregnancy advanced with no significant corresponding increase in maternal serum CRP levels. Since an increase in this marker has been shown to be an indication of cellular inflammation, it may suggest that chronic fructose feeding could lead to the activation of inflammatory cascade during pregnancy in rats. This finding is consistent with reports of Rota *et al.,* (2005) who reported higher CRP levels in patients with gestational diabetes. Edalat *et al.,* (2013) also reported a similar finding in women with previous gestational diabetes mellitus and metabolic syndrome. Retnakaran *et al.,* (2003) who measured CRP at various gestational ages in pregnant women however found inconsistent results regarding the association between inflammatory markers and type 2 diabetes mellitus. The interpretation of the results was influenced by coexistence of hypertension, preeclampsia, and different racial groups or small sample size in some studies. Inconsistent results regarding the association between inflammatory markers and diabetic pregnancies are related to different racial groups or small sample size and different methods of researches.

The reduction in fetal weight recorded in the fructose-fed rats may suggest intra uterine growth restriction, which may be attributed to the over insulinisation and poor maternal glycemic control, as also reported by Parikh *et al.,* (2007). Increased placental weight and diameter observed in the fructose group may be a placental adaptation to the increased influx of glucose from maternal compartment to the fetus, as reported by Vitoratos, Vrachnis, and Valsamakis, (2010) in the placenta of gestational diabetic women. The weight of the placenta has been used to assess its functions and fetal development. A correlation between placental weight, maternal diabetes, preeclampsia, birth weight and fetal distress has been reported in humans (Hindmarsh *et al.,* 2000). Increase in placental size and low birth weight has been indicated as a risk factor for developing metabolic diseases in adulthood (Lo *et al.,* 2002). Placental hypertrophy have also been postulated as an adaptation to maintain placental function in diabetic pregnancies, resulting in small for gestational age neonates with increased placental to birth weight ratio when compared to non-diabetic pregnancies with appropriate for gestational age neonates (Asgharnia *et al.,* 2008).

In conclusion, the study shows that stress and inflammatory mechanisms may be implicated in the role maternal nutrition plays in developmental plasticity as indicated by elevated CRH and CRP levels observed in the fructose-induced diabetic pregnant rats.

REFERENCES

Abderrahim-Ferkoune, A., Bezy, O., Chiellini, C., et al. (2003). Characterization of the long pentraxin PTX3 as a TNF alpha-induced secreted protein of adipose cells. *Journal of lipid research,* 44 (5): 994 – 1000.

Arikawe, A. P., and Olatunji-Bello, I. I. (2004). Insulin resistance induced by short term fructose feeding may not affect fertility in female rats. *Nigerian Journal of Health and Biomedical Sciences*. Vol. 3 (1). 17 – 19.

Arikawe, A. P., Daramola, A.O, Odofin, A. O., and Obika, L. F. O. (2006). Alloxan-induced and insulin resistance diabetes mellitus affect semen parameters and impair spermatogenesis in male rats. *African Journal of Reproductive Health,* 10 (3): 106 – 113.

Arikawe, A. P., Daramola, A.O, Morakinyo, A. O., and Obika, L. F. O. (2008). Effects of diabetes and insulin resistance on estrous cycle, corpus luteum function and pregnancy in female rats. *Pakistan Journal of Pathology*, 19 (2): 38 – 43.

Arikawe, A. P., Iranloye, B. O., Ogunsola, A. O., and Daramola, A. O. (2012). Chronic fructose consumption as a model of Polycystic Ovary Syndrome in pregnant female Sprague-Dawley rats. *African Journal of Biomedical Research*, Vol. 15 (1): 7 – 13

Asgharnia, M., Esmailpour, N., Poorghorban, M., Atrkar-Roshan, Z. (2008). Placental weight and its association with maternal and neonatal characteristics. *Acta Med. Iran*. 46: 467 - 472.

Balletshofer, B. M., Rittig, K., Enderle, M. D., et al. (2000). Endothelial dysfunction is detectable in young normotensive first-degree relatives of subjects with type 2 diabetes in association with insulin resistance. *Circulation,* 101 (15): 1780 – 1784.

Barker, D. J., Gluckman, P. D., Godfrey, K. M., et al. (1993). Fetal nutrition and cardiovascular disease in adult life. *Lancet*. 341: 938 – 941.

Buchanan, T. A. and Coustan, D. R. (1994). Diabetes mellitus. In: Medical complications during pregnancy. *4th Ed. Philadephia, Pa: WB Saunders;* 29 – 61.

Burdge, G.C. and Lillycrop, K. A. (2010). Nutrition, epigenetics, and developmental plasticity: implications for understanding human disease, *Annual Rev of Nutrition*, (30): 315 – 39

Catena, C., Giacchetti, G., Novello, M., *et al.* (2003). Cellular mechanisms of insulin

resistance in rats with fructose-induced hypertension. *Am. J. Hypertens.* 16 (11 Pt. 1): 973 – 978.

Cruz, M. E., Moran, J. L., Jaramillo, L. P., and Dominquez, L. (1990). Differences in spontaneous ovulation in rats with unilateral lesion of the hypothalamus. *Brain Res. Bull,* Jun 24: 739 – 742.

Dickinson J. E., and Palmer S. M. (1990). Gestational diabetes: pathophysiology and diagnosis. *Semin. Perinatol*. 14 (1): 2 – 11.

Edalat, B., Sharifi, F., Badamchizadeh, Z., et al. (2013). Association of metabolic syndrome with inflammatory mediators in women with previous gestational diabetes mellitus. *Journal of Diabetes and Metabolic Disorders,* doi: 10.1186/2251-6581.

Entringer, S., Buss, C., Swanson, J. M., et al. (2012). Fetal programming of body composition, obesity and metabolic function: the role intrauterine stress and stress biology. *Journal of Nutrition and Metabolism*, doi: 10.1153/2012/632548

Festa, A., D'Agostino, R., Tracy, R. P., and Haffner, S. M. (2002). Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes. *Diabetes*, 51 (4): 1131 – 1137.

Fowden, A.L. (2003). The Insulin-like growth factors and feto-placental growth *Placenta*, 24: 803 – 812.

Frohlich, M., Imhof, A., Berg, G., et al. (2000). Association between C reactive protein and features of the metabolic syndrome: a population-based study. *Diabetes Care,* 23 (12):1835 – 1839.

Gangestad, S. W., Caldwell Hooper, A. E., and Eaton, M. A. (2012). On the function of placental corticotropin-releasing hormone: a role in maternal-fetal conflicts over blood glucose concentrations. *Biol. Rev. Camb. Philos. Soc.* 87(4): 856 – 873.

Gluckman, P. D. and Hanson, M. A. (2004). Living with the past: evolution, development, and patterns of disease, *Science*, Vol. 305 (5691): 1733 – 1736.

Grino, M., Chrousos, G. P., and Margioris, A. N. (1987). The CRH gene is expressed in the human placenta. *Biochem. Biophys. Res. Commun.* 148: 1208 – 1214.

Hiden, U., Glitzner, E., Hartmann, M., Desoye, G. (2009). Insulin and the IGF system in the human placenta of normal and diabetic pregnancies. *J. Anat.* 215 (1): 60 – 68.

Hindmarsh, P. C., Geary, M. P., Rodeck, C. H., et al. (2000). Effect of early maternal iron stores on placental weight and structure. *Lancet.* 356 (9231): 719 – 723.

Hugget, A. S. G., and Nixon, D. A. (1957). Use of glucose oxidase, peroxidase and O-
diamisidine in determination of blood and urinary glucose. *Lancet.* 1: 368 – 370.

Illsley, N. P. (2000). Placental glucose transport in diabetic pregnancy. *Clin. Obstet. Gynecol.* 43:116 – 126.

Ishimoto, H., and Jaffe, R. B. (2011). Development and function of the human fetal adrenal cortex: a key component in the feto-placental unit. *Endocr. Rev.* 32 (32): 317 – 355.

Islam, M. S., and Choi, H. (2008). Comparative effects of ginger (Zingiber officinale)
and garlic (Allium Sativum) investigated in a Type 2 diabetes model of rats. *J. Med. Food* 11 (1): 152 – 159.

Jansen, C., Greenspoon, J. S., Palmer, S. M. (2003). Diabetes mellitus and pregnancy. In: Decherney A.H., Nathan N., eds. *Current obstetric and Gynecologic diagnosis and treatment*. NY: Lange Medical Books/ McGraw Hills.

Lagrand, W. K., Visser, C. A., Hermens, W. T., et al. (1999). C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation,* 100 (1): 96 – 102.

Lee, J. H., Yang, S. H., Oh, J. M., and Lee, M. G. (2010). Pharmacokinetics of drugs in rats with diabetes mellitus induced by Alloxan or Streptozotocin: comparison with those in patients with types I diabetes mellitus. *J. Pharmacy and Pharmacology*, 62: 1 – 23.

Lo, Y. F., Jeng, M. J., Lee, Y. S., et al. (2002). Placental weight and birth characteristics of healthy singleton newborns. *Acta Paediatr Taiwan*. 43 (1): 21 – 25.

Marcondes, F. K., Bianchi, F. J., and Tanno, A. P. (2002): Determination of the estrous cycle phases of rats: some helpful considerations. *Braz. J. Biol.* 62: 609 – 614.

Parikh, R. M., Joshi, S. R., Menon, P. S., Shah, N. S. (2007). Index of central obesity- A novel parameter. *Med Hypotheses*, 68: 1272 - 1275.

Pradhan, A.D., Manson, J.A.E., Rifai, N., Buring, J.E., Ridker, P.M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. JAMA, 286 (3): 327 – 334.

Pickup, J. C., and Crook, M. A. (1998). Is type II diabetes mellitus a disease of the innate immune system? *Diabetologia*, 41 (10): 1241 – 1248.

Retnakaran. R., Hanley, A. J. G., Raif, N., et al. (2003). C-reactive protein and gestational diabetes: the central role of maternal obesity. *Journal of Clinical Endocrinology & Metabolism;* 88 (8): 3507 – 3512.

Ridker, P. M., Buring, J. E., Shih, J., Matias, M., Hennekens, C. H. (1998). Prospective study of C reactive protein and the risk of future cardiovascular events among apparenly healthy women. *Circulation,* 98: 731– 733.

Rota, S., Yildirim, B., Kaleli, B., et al. (2005). C-reactive protein jevels in non-obese pregnant women with Gestational Diabetes. The Tohoku Journal of Experimental Medicine; 206: 341 -5.

Saijonmaa, O., Laatikainen, T. and Wahlstrom, T. (1988). Corticotrophin releasing factor in human placenta: localization, concentration and release in vitro. *Placenta;* 9 (4): 373 – 385.

Saito, F. H., Damasceno, D. C., Kempinas, W. G., et al. (2010). Repercussion of mild diabetes on pregnancy in Wistar rats and on the fetal development. *Diabetology and Metabolic Syndrome*, 2: 26 – 34.

Sarah V. R. and Hall, K. (1990). Insulin-like growth factors and their binding proteins and their binding proteins. *Physiol. Rev.* 70: 591 – 614.

Sferruzzi-Perri, A. N., Owens, J. A., Pringle, K. G. and Roberts, C. T. (2011). The neglected role of insulin-like growth factors in the maternal circulation regulating fetal growth. *J Physiol.* 589 (Pt 1): 7 – 20.

Suga, A., Hirano, T., Kageyama, H., et al. (2000): Effects of fructose and glucose on plasma leptin, insulin and insulin resistance in lean and VMH-

leisoned obese rats. *Am. J. Physiol. Endocrinol. Metab.* 278 (4): E677 – E683.

Turkalj, I., Keller, U., and Ninnis, R. (1992). Effect of increasing doses of recombinant human insulin-like growth factor-I on glucose, lipid and leucine metabolism in man. *J Clin Endocrinol Metab.* 75 (5): 1186 – 1191.

Vale, W., Spiess, J., Rivier, C., and Rivier, J. (1981). Characterization of a 41- residue ovine hypothalamic peptide that stimulates secretion of corticotropin and B-endorphin. *Science,* 213**:** 1394 – 1397.

Van Assche, F. A., Aerts, L., and de Prins, F. (1983). Degranulation of the insulin-producing beta cells in an infant of a diabetic mother. Case report. *Br J Obstet Gynaecol.* 90 (2): 182–185

Vannini, P. (1994): Pregnancy and diabetes mellitus: physiopathological aspects.*Endocrinology*; 19 (2): 45 – 50. *Psychoneuroendocrinology,* 30: 724 – 743.

Vitoratos, N., Vrachnis, N., Valsamakis, G. (2010). Perinatal mortality in diabetic pregnancy. *Annals of the New York Academy of Sciences*. 1205: 94 – 98.

Wadha, P. D. (2005). Psychoneuroendocrine processes in human pregnancy influence fetal development and health. *Psychoneuroendocrinology,* 30 (8): 724 – 743.