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Research Article

Chronic Fructose Consumption As a Model of Polycystic Ovary Syndrome In Pregnant Female Sprague-Dawley Rats

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ABSTRACT: Virgin female Sprague-Dawley rats aged 6 weeks, weighing 110 – 120 g were randomly divided into 2 groups. Group 1 served as control group and was fed with normal rat chow. Group 2 served as Chronic fructose group and was fed *ad libitum* on a special diet containing 25% fructose mixed with 75% normal rat chow weight/weight for 4 weeks and continued till the 8th week. Daily vaginal smear was used to assess estrous cycle for two weeks after which, pregnancy was induced. Cervical dislocation followed by laparatomy was carried out on day 19 of pregnancy and blood sample was obtained by cardiac puncture for measurement of serum insulin, estradiol, progesterone, testosterone, DHEAS and Inhibin using Enzyme-linked immunosorbent assay (ELISA). The ovary was isolated, fixed in 10% formal saline and processed for histological assessment. The serum sex steroid and inhibin profiles of chronic fructose fed pregnant rats are consistent with findings in other models of PCOS. This study shows that chronic fructose consumption in pregnant rat recapitulates ovarian and some metabolic features of PCOS including polycystic ovary morphology, hyperandrogenism, and insulin resistance.

Keywords: Hyperandrogenism, Fructose consumption, Inhibin, DHEAS.

INTRODUCTION

The Polycystic Ovary Syndrome (PCOS) is a hyperandrogenic disorder associated with chronic oligo-anovulation and polycystic ovary morphology (Rotterdam, 2004; Azziz *et al*., 2006). In humans, it is often associated with psychological impairments, including depression and other mood disorders and metabolic derangements, chiefly insulin resistance and compensatory hyperinsulinaemia, which is recognized as a major factor responsible for altered androgen production and metabolism (Escobar-Morreale et al., 2005). Thus, the major marker of polycystic ovary morphology is hyperandrogenism (Gilling-Smith, et al., 1994; Nelson, et al. 2001) and the theca cells are the major source of androgen excess (Gilling-Smith, et al., 1997).

The etiology of PCOS is unclear. One hypothesis is that PCOS is a genetically determined ovarian disorder in which excessive androgen production early in life may provide a hormonal insult that leads to PCOS in adulthood (Apter, 1998; Crosignani and Nicolosi, 2001; Franks, et al., 2006). Furthermore, it has been estimated that 25% of androstenedione and testosterone production is of ovarian origin, 25% is of adrenal origin and 50% is produced in peripheral tissues, while the adrenal cortex accounts almost uniquely for the synthesis of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulphate (DHEAS) as well as that of androstenediol and 11β-hydroxy androstenedione (Piltonen et al., 2002).
Dehydroepiandrosterone (DHEA) and Dehydroepian drosterone sulfate (DHEA-S) as precursors of androgens and estrogens (Bácsi, et al., 2007) are the most abundant steroid hormones in the body and their

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effects on insulin resistance though assumed to be positive is yet to be confirmed (Talaei, et al., 2010).

Several rat models of PCOS have been developed. For example, induction of PCOS in rats using estradiol valerate (Lara, et al., 1993), resulted in acyclicity and ovarian morphology resembling PCOS (Stener-Victorin, et al., 2000; Stener-Victorin and Lindholm, 2004; Manni, et al., 2005; Lara, et al., 2000), but without the typical metabolic disturbances of human PCOS (Stener-Victorin, et al., 2005).

In another rat model, PCOS was induced by daily prepubertal exposure to testosterone for 7–35 days (Beloosesky, et al., 2004). In addition to typical PCOS morphology and a majority of apoptotic follicles, the rats had disturbed glucose and insulin levels, indicating that high levels of androgens can lead to insulin resistance in this model. Likewise, Letrozole, a non-steroidal aromatase inhibitor that blocks the conversion of testosterone to estradiol, also induces PCOS in 6 week old female rats (Kafali, et al., 2004). Locally, we have also shown that a model of insulin resistance in pregnant rats predisposes to PCOS (Arikawe, et al., 2008).

In all these models, endocrine disturbances similar to those in human PCOS were observed, but the metabolic characteristics of the syndrome were not investigated (Fassnacht, et al., 2003). The heterogeneity of the syndrome is reflected in the many animal models of PCOS. However, few of these models have focused on the analysis of predisposing conditions that increase the risk of PCOS, particularly genetic background and environmental factors, such as endocrine disruptors and lifestyle (Pasquali, et al., 2011), of which fructose (sugar) consumption is one of such.

In this study virgin female Sprague-Dawley rats were used. Our hypothesis was that early and chronic fructose consumption in pregnant rats will predispose to PCOS through the insulin resistance mechanism. Testosterone, DHEAS and inhibin are hypothesized to be implicated in this mechanism.

MATERIALS AND METHODS

Animals

Virgin female Sprague-Dawley rats aged 6 weeks, weighing 110 – 120 g were obtained from the Laboratory Animal Department. 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 Chronic fructose group and was fed *ad libitum* on a special diet containing 25% fructose mixed with 75% normal rat chow weight/weight for 4 weeks (Arikawe and Olatunji-Bello, 2004) and continued till the 8th week. At this fructose concentration, insulin resistance state was 100% with zero mortality rate. Hyperglycaemia was confirmed using Dextrostix Test Strips (Bayer Corporation, U. K.) following the glucose oxidase method (Hugget and Nixon, 1957).

Polydipsia, polyuria and polyphagia were observed (Jelodar, et al., 2010) and confirmed in the chronic fructose group (group 2). All animals had free access to drinking water throughout the duration of the study and were also weighed weekly throughout the duration of the experiment. 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 12 week of age (Marcondes, et al., 2002) till the 14th week of age (8th week of chronic fructose consumption) 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 8th week of chronic fructose feeding 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.

Hormone Assay

Cervical dislocation was carried out on the animals in the two groups on day 19 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, allowed to clot and centrifuged at 3,000 rpm for 15 minutes to get clear serum samples, which were subsequently kept frozen (-20ºC) until

measurement of the different hormones (Insulin, Estradiol, Progesterone, Testosterone, DHEAS and Inhibin) using Enzyme-linked immunosorbent assay (ELISA) methods procured from the Diagnostic Automation, Inc.

Ovarian morphology

The ovaries from the two groups were carefully isolated, washed in buffered saline, fixed in 10% formalin, passed through ascending series of ethanol baths, embedded in paraffin, sectioned (5 µm thick); mounted on slides and stained with Haematoxylin and Eosin. This was to observe the various stages of follicular differentiation and to determine the cytoarchitectural changes in cells following the method reported by Pedersen, 1970. The slides were subsequently viewed under the light microscope and photomicrographs taken at different magnifications.

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 $P < 0.05$.

Fasting blood glucose concentration was significantly higher ($P < 0.05$) in the Chronic fructose group at the 8th week and day 19 of pregnancy (145.1 ± 1.8 mg/dl; 285.5 ± 6.0 mg/dl) compared with the control group (78.2 ± 2.5 mg/dl; 94.8 ± 3.0 mg/dl) (Figure 1).

Body weight

Body weights in the control group at zero week; 2nd week; 4th week; 6th week and 8th week were (116.6 ± 5.0 gm; 120.0 ± 4.6 gm; 128.8 ± 2.6 gm; 132.5 ± 2.3 gm; and 137.2 ± 2.6 gm) while in the chronic fructose group it was (118.8 ± 2.5 gm; 121.0 ± 5.0 gm; 134.2 ± 2.9 gm; 129.8 ± 2.8 gm; and 126.4 ± 1.6 gm). This shows that body weights was significantly higher ($P < 0.05$) in the chronic fructose group at the 4th week and significantly lower ($P < 0.05$) at the 8th week compared to the control group (Figure 2).

Body weight in the control group at day 6; day 13 and day 19 of pregnancy was (146.2 ± 2.3 gm; 148.0 ± 2.0 gm; and 165.0 ± 5.6 gm) while in the chronic fructose group it was (130.8 ± 2.8 gm; 133.0 ± 1.6 gm; and 162.0 ± 3.7 gm). This shows that body weight was significantly lower ($P < 0.05$) in the chronic fructose group at day 6 and day 13 of pregnancy compared to the control group (Figure 2).

RESULTS

Blood glucose

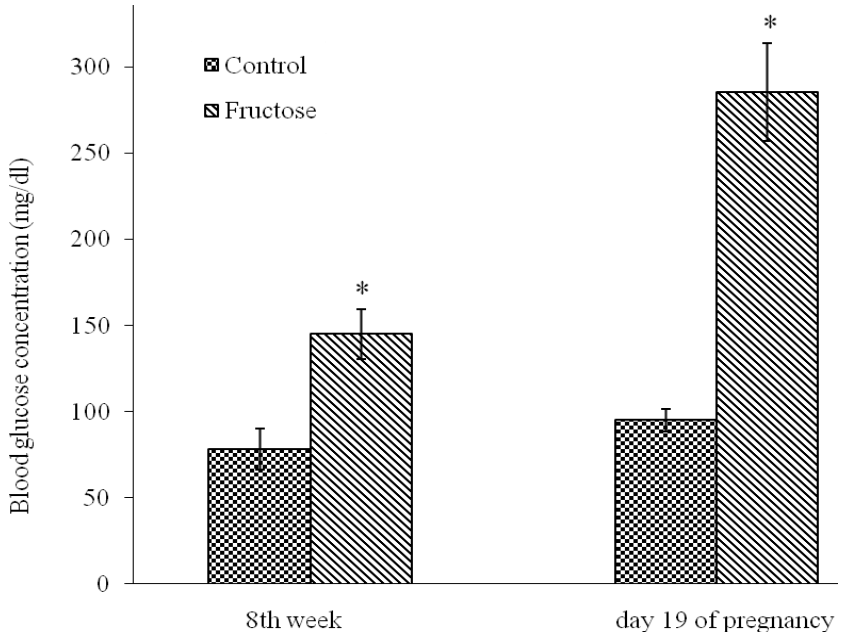

Figure 1

Blood glucose level in both groups at 8th week and 19 day of pregnancy. *P< 0.05 Vs. Control

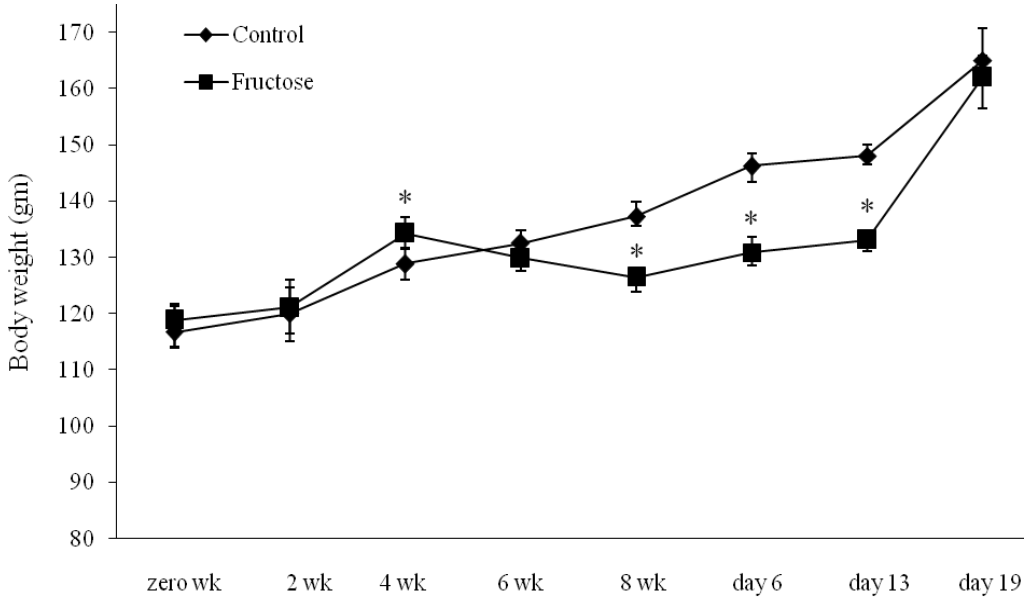

Figure 2. Body weight in both groups weekly and during pregnancy.

*P< 0.05 Vs. Control

Table 1.
Serum concentrations of Insulin, Estradiol, Progesterone, Testosterone, DHEAS and Inhibin in the Control and Chronic Fructose groups

	Group I Control	Group II Chronic Fructose
Serum Insulin (MIU/ml)	33.0 ± 0.7	38.8 ± 1.0*
Serum Estradiol (pg/ml)	169.0 ± 0.7	182.5 ± 4.2*
Serum Progesterone(ng/ml)	27.2 ± 0.3	27.9 ± 1.4
Serum Testosterone (ng/ml)	0.68 ± 0.01	4.88 ± 0.01*
Serum DHEAS (µg/ml)	1.80 ± 0.01	3.66 ± 0.03*
Serum Inhibin B (pg/ml)	29.4 ± 0.24	10.3 ± 0.02*

*All results presented in mean ± S. E. M. *P < 0.05 Vs. Control*

Hormonal assays

Serum insulin and estradiol was significantly higher ($P < 0.05$) in the chronic fructose group (38.8 ± 1.0 MIU/ml; 182.5 ± 4.2 pg/ml) compared to the control group (33.0 ± 0.7 MIU/ml; 169.0 ± 0.7 pg/ml). Likewise, serum testosterone and DHEAS was significantly higher ($P < 0.05$) in the chronic fructose group (4.88 ± 0.01 ng/ml; 3.66 ± 0.03 µg/ml) compared to the control group (0.68 ± 0.01 ng/ml; 1.80 ± 0.01 µg/ml).

On the other hand, serum inhibin was significantly lower ($P < 0.05$) in the chronic fructose group (10.3 ± 0.02 pg/ml) compared to the control group (29.4 ± 0.24 pg/ml). Serum progesterone though slightly higher in chronic fructose group (27.9 ± 1.4 ng/ml) compared to the control group (27.2 ± 0.3 ng/ml), this increase was not statistically significant (Table 1).

Ovarian morphology

Histological sections of the chronic fructose fed and control rats differed moderately in morphology. The follicles in the ovary of the fructose group were similar in number to those in the control but had larger follicles than the control (Figures 3 and 4). Most of the follicles in the chronic fructose group were found in the sub-capsular area of the cortex giving the ovary a nodular appearance

DISCUSSION

The results on fasting blood glucose concentration support the views that chronic fructose consumption is effective in inducing experimental type 2 diabetes mellitus (Arikawe and Olatunji-Bello, 2004; Arikawe et al., 2006). This is also in line with the view of Suga et al., (2000). This result also indicates that pregnancy is a diabetogenic state (Vannini, 1994).

Body weight increased progressively in both groups as anticipated until the 4th week, when it became significantly higher ($P < 0.05$) in the chronic fructose group compared to control group. It then declined in the chronic fructose group till the 8th week when it became significantly lower ($P < 0.05$) compared to the control group. Afterwards, it gradually increased in the chronic fructose group during the pregnancy period with this increase still significantly lower ($P < 0.05$) compared to the control group at days 6 and 13. At day 19 of pregnancy, body weight was not significantly different between the two groups.

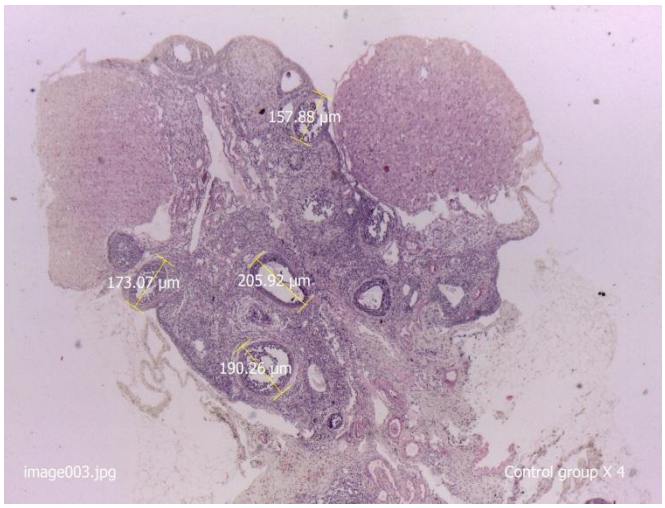

Figure 3
Photomicrograph of Control pregnant rat ovary at day 19 of pregnancy

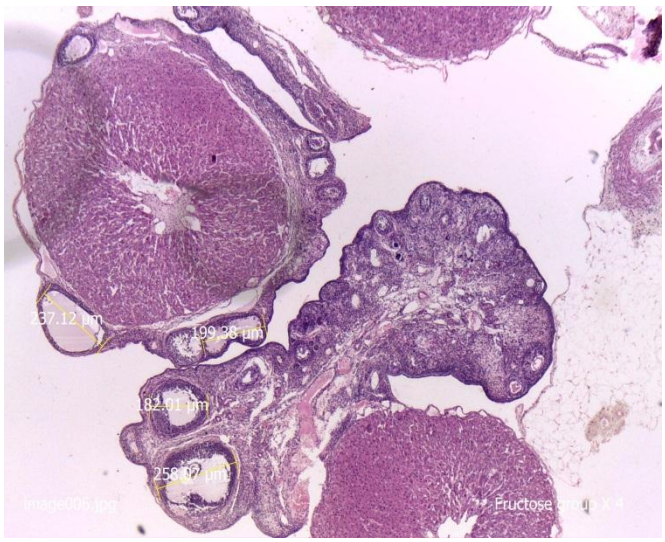

Figure 4
Photomicrograph of Chronic Fructose pregnant rat ovary at day 19 of pregnancy

The body weight pattern in this study is in line with our earlier observations (Arikawe et al., 2006; Arikawe et al., 2008; and Arikawe et al., 2011) 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 chronic fructose group, which is characterized by gluconeogenesis leading to muscle waiting and weight loss (Guyton and Hall, 2000). 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. The results also show that the increase in body weight during pregnancy was not due to insulin resistance per se rather that it was due to the state of pregnancy itself i.e. fluid retention and increased cell proliferation of the developing embryo in the uterus. This is justified because the body weight at term (unpublished data) was significantly higher in the chronic fructose group compared to the control group.

Serum insulin level is a crucial factor to control normal blood glucose level (Islam and Choi, 2008). It was as expected significantly higher ($P < 0.05$) in chronic fructose group compared to the control group (Table 1). Chronic fructose consumption caused structural alterations in pancreatic β cells (Lee at al., 2010; Van Assche et al., 1983) to cause hyperinsulinaemia.

The serum sex steroid and inhibin profiles of chronic fructose fed pregnant rats are consistent with findings in other models of PCOS (Kanazawa, et al., 2011; Soto, et al., 2009; Codner, et al., 2007; and Chan, et al., 2006). Serum estradiol level was significantly higher ($P < 0.05$) in the chronic fructose group compared to the control group. This is in line with the view of Vasudevan, et al., (2005). Furthermore, serum estradiol and progesterone concentrations as observed in this study are in line with our earlier report (Arikawe, et al., 2008). Serum inhibin level measured is in line with the view of Fujiwara et al., (2001) who reported that inhibin was lower in the granulosa cells of women with PCOS compared with granulosa cells from normal women. This is important because inhibin as glycoprotein is produced by the granulosa and theca cells of the ovary. Serum inhibin level in this study also correlates with FSH and LH concentrations which were higher in fructose fed rats compared to control rats in our earlier report (Arikawe et al., 2008).

Testosterone levels were markedly higher in the chronic fructose fed rats than in control rats, presumably because insulin resistance might block the conversion of androgen substrates to estradiol (Holte, 1996). This fact could be justified because the DHEAS concentration has a positive correlation with testosterone level in this study.

In conclusion, this study shows that chronic fructose consumption in pregnant rat recapitulates some metabolic features of PCOS including hyperandrogenism and insulin resistance.

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