

ORIGINAL ARTICLE

Prenatal Stress Induces Metabolic Impairment in Adolescent Male Wistar Rat

Fatemeh Rostamkhani^{1*}, Homeira Zardooz^{2,3}, Kazem Parivar¹, Nasim Hayati Roodbari¹

¹ Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

² Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³ Department of Physiology, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

E-mail: shirinrostamkhani@yahoo.com, rostamkhani@iausr.ac.ir

ABSTRACT

A large number of studies have reported associations between prenatal stress and offspring lifetime consequences. Chronic gestational stress alters maternal glucocorticoids and subsequently disturbs intrauterine environment which may lead to metabolic disorders in the offspring. The aim of this study was to investigate the effects of chronic prenatal stress on the metabolic parameters in adolescent male Wistar rat. We examined the effects of maternal 8 and 20 days foot-shock stress on body weight, plasma corticosterone, insulin, glucose, triglyceride and cholesterol concentrations of dams and offspring. Stress was induced by a foot-shock box twice a day (1 h/session) for 8 consecutive days beginning on E8 in 8-day stressed group and for 20 consecutive days beginning on E1 in 20-day stressed group. The results obtained from this investigation indicate that gestational chronic foot-shock stress arises maternal plasma corticosterone concentration. In addition, maternal plasma triglyceride and cholesterol concentrations significantly elevated following 20-day gestational stress. Prenatal stress induces lower birth weight and body weight gain in offspring. Furthermore, prenatal stressed offspring had significant elevation in plasma glucose concentration without marked alteration in plasma insulin, corticosterone, triglyceride and cholesterol concentrations. These data suggest that prenatal stress could result in impaired glucose metabolism in the adolescent rats which is independent of timing of the stress exposure.

Keywords: Prenatal stress, corticosterone, insulin, glucose, triglyceride, cholesterol

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INTRODUCTION

The concept of prenatal stress (PS) refers to the developmental changes that occur to the fetus in-utero if the mother experiences stress during pregnancy. The stress exposure during pregnancy has a profound effect on the maternal hormone concentrations and can result in developmental alterations in the offspring [1]. If the insult occurs at the time of organogenesis, the changes may be severe and lead to a permanent developmental deficit [2]. Certainly, in-utero changes lead to the structure and function modification of placenta to adapt to the maternal environment, and consequently the substrate and hormonal supply to the fetus is altered therefore the placenta affects fetal growth [3]. In addition, glucocorticoids are able to cross the placenta subsequently maternal glucocorticoid changes may be reflected in fetus [4]. Therefore, elevated maternal glucocorticoid concentrations during prenatal stress can cross the placental barrier causing alterations in the development of the hypothalamic-pituitary-adrenal (HPA) axis [1], metabolic tissues (such as liver, skeletal muscle, pancreas) and brain of the prenatally stressed offspring, which consequently can have long term effects [5] on body weight, neuroendocrine function [6], glucose intolerance [7], incidence of cardiovascular [8] and metabolic [9,10] diseases [11] in later life.

The aim of the present study is to evaluate the effects of chronic maternal foot shock stress during development of pancreas and whole pregnancy period on the body weight, plasma corticosterone, insulin, glucose, triglyceride and cholesterol concentrations of both mother and male offspring rats.

MATERIALS AND METHODS

Animals and housing

Adult virgin Wistar female rats (180-200 g) were housed in groups of 4 per cage for 2 weeks before mating in order to coordinate their estrus cycles. They were then separately mated with a sexually experienced male (400g) for one night. The next day was considered as day 0 of pregnancy if

spermatozoa were found in the vaginal smears. Pregnant females were then transferred to individual cages allowed *ad libitum* access to food (Pars Company of animal food producer, Iran) and water, and maintained on a 12h light/dark cycle (lights on at 0700 am) with constant temperature and humidity. All procedures were approved by the Animal Care and Use Committee of the Neuroscience Research Center, Shahid Beheshti University of Medical Sciences.

Experimental protocol

Pregnant females were divided to prenatal stress (PS) or control (CTL) groups. The animals of the prenatal stress group were subdivided into 8-day prenatal stressed in embryonic day 8-16 (E8-E16) (n=7) and 20-day prenatal stressed in embryonic day 1-20 (E1-E20) (n=7). Pregnant females in the prenatal stress groups were placed for 1h in a foot-shock box twice a day (0900, 1400). A foot-shock box (Borje Sanat, Iran) was used as a stimulus device. This device (48 cm×48 cm×50 cm) is divided into nine compartments (16 cm×16 cm×50 cm) by transparent plastic sheets. In each session, the rats were exposed to the electrical foot-shock (1 mA, 1 Hz) for a 10-s duration every 60 s (1 h) through the stainless steel grids.

The animals of the control group had the same subgroups and served as the control E8-E16 (n=7) and E1-E20 (n=7). These animals were placed in the box (1 h) twice a day without receiving any stress. After laboring male offspring were housed with their mothers (n=7) and left undisturbed for 1 month before the beginning of the next experiments.

Blood Sampling & Assay

Blood samples of pregnant females were obtained by orbital sinus puncture method [12] with a heparinized capillary micro Tube under light isoflurane (Nicholas Primal, UK) anesthesia [13]. To evaluate plasma corticosterone concentration blood sampling for pregnant females of each group were performed before placing in the box (Basal-Before Stress 1:B-BS1) and immediately after removing from the box (After Stress :AS1) on the first day (i.e. E1 for 20-day stressed group and E8 for 8-day stressed group) of the experimental procedure. This process was repeated for each group on the last day (Basal-Before Stress 2:B-BS2) (After Stress 2:AS2) (i.e. E20 for 20-day stressed group and E16 for 8-day stressed group) of the stress exposure. To determine the basal levels of plasma insulin, glucose, triglyceride and cholesterol blood samples were taken on the first and last days of the experimental process before placing the animals in the box. Blood sampling from adolescent (1 month old) [14] male offspring were carried out by collecting trunk blood rapidly after decapitation. Blood was collected into an Eppendorf tube containing 25 IU/5 μ l heparin [15], immediately centrifuged at 3000 rpm for 5 min at 4°C. Plasma aliquot fractions were kept at -80°C until the day of the assay. Plasma glucose concentration was measured using the glucose oxidase method (Pars Azmoon, Iran). Cholesterol and triglyceride concentrations were measured by enzymatic calorimetric method (Pars Azmoon, Iran). Rat insulin ELIZA kit (Mercodia, Sweden) and rat corticosterone ELIZA kit (DRG, Germany) were used to measure plasma insulin and corticosterone concentrations.

Body Weight

Body weight of the pregnant females were measured every 5 days during the whole period of the gestation and offspring's weight gain were evaluated every week by a digital scale (FEW, Japan, sensitivity 0.1 g).

Statistics

All data are presented as means \pm SEM. One-way and two-way analysis of variance (ANOVA) with repeated measures were performed (day was considered as a repeated factor and stress was considered as an independent factor) by SPSS Version 16.0 program package. In each case, $p < 0.05$ was considered statistically significant.

RESULTS

Offspring body weights

All the experimental groups showed an increasing trend so that weights of animals in each study day were significantly higher than the first day ($P < 0.001$) (Figure 1). A significant difference was observed between the control and prenatal stressed rats either in E8-E16 or in E1-E20 groups in whole experimental process ($P < 0.001$) except for day 14 (Figure 1). Moreover, Statistical analysis did not show a marked difference between E8-E16 and E1-E20 of the control and prenatal stressed groups but a significant difference was observed on day 14 ($P < 0.05$), 21 ($P < 0.001$) and 30 ($P < 0.01$) of the prenatal stressed group and on day 21 ($P < 0.001$) of the control (Figure 1).

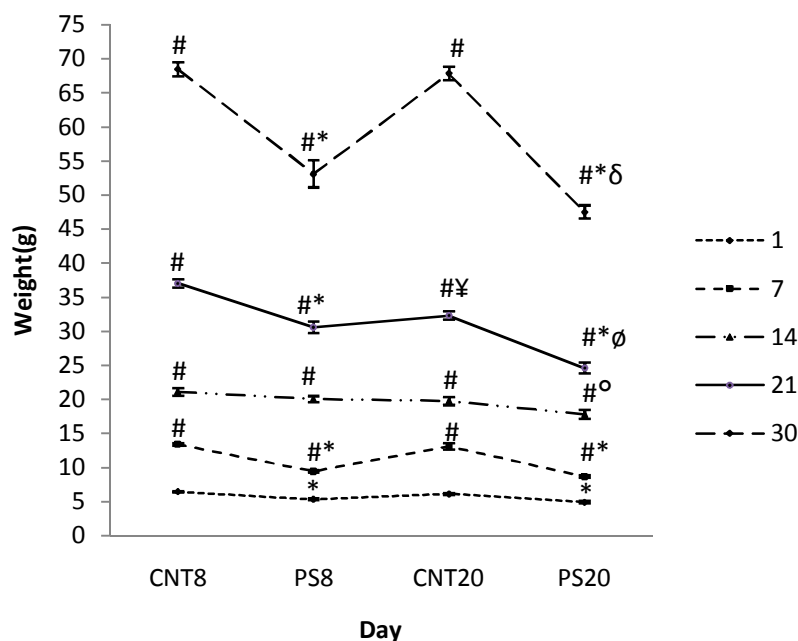


Figure 1: Body weights of the male offspring of control and prenatal stressed groups.

Values are mean±SEM, n=15 offspring in each group

P<0.001 significant difference versus day 1 of the same group

*P<0.001 significant difference versus control group of the same day

°P<0.05, δP<0.01, øP<0.001 significant difference versus PS8 of the same day

¥P<0.001 significant difference versus CN8 of the same day

Effect of prenatal stress on physiological parameters of adolescent male rats

Plasma corticosterone concentration showed an increase trend in prenatal stressed rats, however was not significant in compared with controls (Table1). Prenatal stress had no significant effect on plasma insulin concentration, whereas significantly elevated plasma glucose levels as compared to controls in both prenatal stressed groups (P<0.001) (Table1). Plasma triglyceride and cholesterol did not show any significant difference between prenatal stressed and control groups (Table1).

Table 1: Effects of prenatal stress on physiological parameters of adolescent male rats. Values are mean±SEM from 7 animals/group.

Group	E8-E16		E1-E20	
	control	PS	control	PS
Corticosterone(ng/ml)	76.86±11.49	102.43±11.28	82.57±23.75	141±23.05
Insulin(µg/L)	0.74±0.27	0.74±0.19	0.72±0.13	0.80±0.20
Glucose(mg/dl)	144.71±8.03	182.5±5.81*	156.93±3.22	195.01±3.84*
Triglyceride(mg/dl)	130.28±23.03	119.62±35.85	121.23±15.33	185.6±37.82
Cholesterol(mg/dl)	70.16±2.42	66.91±3.88	73.94±5.01	70.01±3.65

* P<0.001 significant difference versus control group

Maternal weight gain

A significant difference was observed between each experimental day and first day of gestation in all dam groups (P<0.01, P<0.001 for days 5 and 10,15,20 of the control E8-E16 respectively, P<0.001 for days 5,10,15 and 20 of control and prenatal stressed E1-E20 groups, P<0.05 for day 5 and P<0.01 for day 10 of the prenatal stressed E8-E16) (Figure 2). No significant difference was observed between the control and prenatal stressed rats either in E8-E16 or E1-E20 groups (Figure 2).

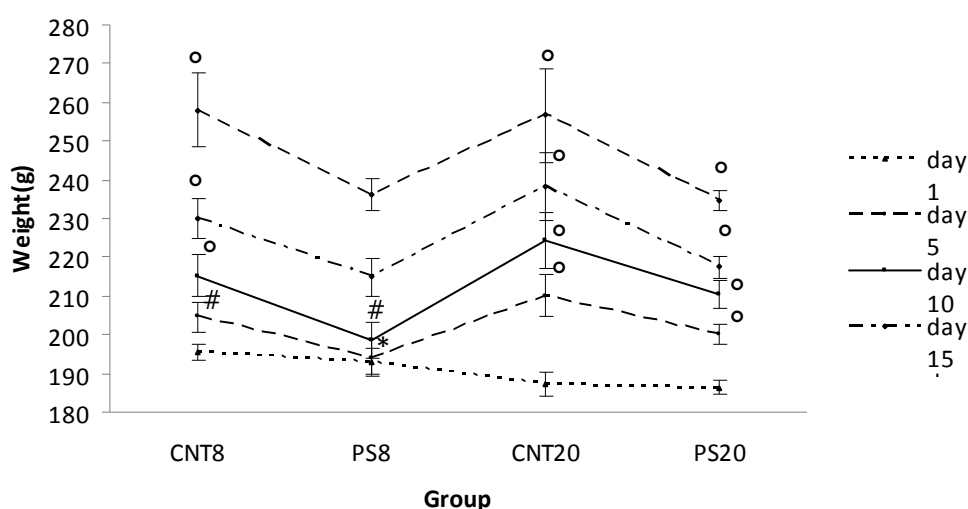


Figure 2: Body weights of control and stressed dams Values are mean±SEM, n=7 dam in each group. * P<0.05, # P<0.01, °P<0.001 significant difference versus day 1 of the same group

Effect of chronic gestational stress on maternal physiological parameters

The AS1, B-BS2 and AS2 plasma corticosterone concentrations of the 8-day stressed dams were significantly higher as compared to the controls (P<0.001, P<0.05 and P<0.001, respectively) (Table 2). Plasma corticosterone concentrations in 8-day gestational stress dams were markedly elevated immediately after removing from the box on the first day (AS1) (P<0.001), before placing into the box on the last day of the process (B-BS2) (P<0.01) as compared to the basal level on the first day (B-BS1) (Table 2). In addition, plasma corticosterone levels of AS2 were higher than B-BS2 (P<0.01). Whereas, no differences were observed between plasma corticosterone concentration of AS1 and AS2. In the control group no significant differences were detected in any of the experimental days.

As shown in table 2, gestational stress induced an increase of plasma corticosterone level after removing from the box (AS1 and AS2) in 20-day stressed dams in compared with their controls (P<0.001). Plasma corticosterone concentrations in 20-day gestational stress dams were significantly increased immediately after removing from the box on the first day (AS1) of the experiment as compared to the B-BS1 (P<0.001) (Table 2). Moreover, plasma corticosterone level of AS2 was significantly increased compared to B-BS2 (P<0.001). On the other hand, corticosterone level of AS2 tended to decrease in compared with AS1 albeit nonsignificantly. In contrast, plasma corticosterone concentrations of the control group showed no marked alteration throughout the experiment.

Table 2: Effect of gestational stress on maternal corticosterone concentrations. B-BS1 (Basal-Before Stress 1) and AS1 (After Stress) on the first day, B-BS2 (Basal-Before Stress 2) and AS2 (After Stress 2) on the last day of the stress exposure.

Group	E8-E16		E1-E20	
	control	PS	control	PS
B-BS1	124.14±15.14	135.15±8.82	113.06±10.9	122.80±15.87
AS1	144.25±11.4	377.31±48.67 ^{bd}	118.24±10.88	428.36±29.66 ^{bd}
B-BS2	140.13±7.24	187.88±20.46 ^{ac}	121.18±9.3	138.4±21.48
AS2	145.08±6.76	339.1±48.42 ^{be}	128.92±12.89	337.24±34.04 ^{bf}

^aP<0.05, ^bP<0.001 significant difference versus the control group

^cP<0.01, ^dP<0.001 significant difference versus B-BS1

^eP<0.01, ^fP<0.001 significant difference versus B-BS2

Letters a and b show differences in row and Letters c to f show differences in column.

8-day gestational stress had no significant effect on plasma glucose and insulin concentrations (Table 3). However, 20-day gestational stress significantly reduced plasma insulin level and increased glucose

level on the last day as compared to the first day of the stress procedure ($P<0.05$) (Table3). Moreover, Plasma glucose concentration on day 20 of the experiment was notably higher in stressed dams as compared to the controls ($P<0.01$) (Table3).

No significant alterations were observed in plasma triglyceride and cholesterol concentrations in 8-day stressed dams and the control group. Whereas, 20-day gestational stress dams showed higher plasma triglyceride and cholesterol concentrations on day 20 as compared to the controls ($P<0.01$ and $P<0.05$ respectively) (Table3). In addition, 20 days stress caused significant increase of plasma triglyceride and cholesterol concentrations on day 20 in comparison to day 1 ($P<0.001$ and $P<0.05$ respectively) (Table3).

Table 3: Effect of gestational stress on maternal physiological parameters. Values are mean \pm SEM from 7 animals/group.

Group	E8-E16			
	control		PS	
	day8	day16	day8	day16
Insulin(μ g/L)	1.16 \pm 0.18	1.17 \pm 0.19	1.51 \pm 0.53	1.07 \pm 0.26
Glucose(mg/dl)	145 \pm 4.77	135.02 \pm 2.28	136.49 \pm 6.99	125.75 \pm 9.04
Triglyceride(mg/dl)	145.56 \pm 36.9	149.67 \pm 32.18	109.94 \pm 20.22	168.08 \pm 51.91
Cholesterol(mg/dl)	61.98 \pm 3.27	58.7 \pm 2.36	59.98 \pm 2.33	51.22 \pm 4.3
Group	E1-E20			
	control		PS	
	day1	day20	day1	day20
Insulin(μ g/L)	1.68 \pm 0.24	0.94 \pm 0.2	1.37 \pm 0.34	0.46 \pm 0.04 ^a
Glucose(mg/dl)	145.08 \pm 4.5	129.47 \pm 7.8	134.74 \pm 4.01	159.04 \pm 7.55 ^{ad}
Triglyceride(mg/dl)	180.69 \pm 6.29	202.87 \pm 11.24	146.83 \pm 17.77	321.32 \pm 41.61 ^{bd}
Cholesterol(mg/dl)	61.59 \pm 3.73	68.09 \pm 1.92	61.35 \pm 5.07	85.50 \pm 7.09 ^{ac}

^a $P<0.05$, ^b $P<0.001$ significant difference versus day 1

^c $P<0.05$, ^d $P<0.01$ significant difference versus control group

Letter s a to d show differences in row.

DISCUSSION

The present study shows prenatal foot-shock stress induces lower birth weight and body weight gain in offspring. Our data showed that prenatal stressed offspring had significant elevation in plasma glucose concentration without an alteration in plasma insulin and corticosterone concentrations. We have demonstrated that gestational stress arises maternal plasma corticosterone concentration. Moreover, maternal plasma triglyceride and cholesterol concentrations significantly elevated following 20-day gestational stress.

As observed in this study chronic gestational stress markedly increased corticosterone levels in pregnant dams. Corticosterone secretion is one of the principal biological responses to stress [16] and this stress procedure could stimulate maternal glucocorticoid secretion. Our observations, coupled with other reports [17; 18].

Our study indicates that maternal chronic stress causes no alteration in offspring corticosterone concentration. In the animal work much research has shown how prenatal stress can alter the function of the HPA axis in the offspring [1,18]. The observations are various and these discrepancies could be a result of the nature and the timing of the stress exposure during pregnancy, the age of testing of the offspring, the genetic strain of rat or mouse used, the sex of the offspring, the time of testing the offspring, and whether basal or stress-induced corticosterone levels were measured [19,20].

The present findings are in agreement with some reports indicated that chronic prenatal stress declines pups birth weight [7,21,22]and reduces the rate of weight gain [23]. Different studies have shown that excess prenatal glucocorticoides exposure reduces birth weight in a variety of mammalian species

[24,25]. Moreover, administration of glucocorticoides during the last week of pregnancy in rats decreases birth weight [26]. Many studies have shown that elevation of maternal plasma corticosterone level of the stressed dams [17,18] could decrease maternal weight gain [27,28, 29], food intake [29] and nutrients and oxygen availability [30] which may be resulted in fetal growth retardation. Reduced offspring birth weight in the present experiment may also be due to the aforementioned altered factors. In addition, the decrement of weight gain in prenatal stressed pups could be the result of postnatal factors such as maternal milk yield [31] or milk intake in newborns. In this regard, it has also been shown that excessive gestational glucocorticoids influence maternal behaviour and care during the suckling period and thus alter development of the offspring [32,33].

According to previous studies stress causes metabolic dysfunction and prenatal stress exposure may lead to metabolic disorders in the offspring [6]. The timing of gestational stress administration induced various maternal metabolic responses so that 8-day gestational stress did not change plasma glucose, insulin, triglyceride and cholesterol concentrations whereas 20-day gestational stress decreased plasma insulin but increased plasma glucose, triglyceride and cholesterol concentrations. Nevertheless, both offspring groups' metabolic responses were similar. It may be concluded that maternal metabolic changes may not directly influence at least adolescent offspring metabolic parameters.

Numerous animals studies have shown that prenatal exposure to glucocorticoids is associated with hyperglycemia [34, 35, 36] and glucocorticoid exposure will lead to long term alterations in glucose metabolism in the offspring [23] which is compatible with our experiment.

Corticosterone is a catabolic hormone that can induce processes leading to increased concentrations of blood glucose. Therefore, it is possible that the offspring plasma glucose elevation is due to hormonal changes in the fetal circulation induced by maternal stress. The mechanisms through which glucocorticoids mediate these effects remain unclear. Prenatal exposure to glucocorticoids may influence glucose metabolism by developmental disorders of skeletal muscle, adipose tissue and pancreas, which are key organs in glucose homeostasis [23].

However, consistent with our report no alteration in plasma insulin concentration of adult rats was observed following prenatal stress [7; 17]. On the other hand, there may be a link between the low birth weight, caused by excessive exposure to glucocorticoids in utero, and metabolic disorders [6].

As a whole, our results suggest an association between exposure to the chronic foot-shock prenatal stress and glucose-insulin metabolism in adolescent male rats.

Our result is in agreement with earlier study showing that plasma triglyceride and cholesterol did not differ between prenatal stressed and control offspring [37]. Therefore, in this study offspring's lipid metabolism may not affected by maternal stress.

This study suggesting a link between prenatal stress exposure and metabolic changes in male adolescent rat.

It can be concluded that prenatal stress might result in impaired glucose metabolism in the adolescent rats which is independent of timing of the stress exposure.

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