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Effects of Maternal Dexamethasone Exposure During Lactation on Metabolic Imbalance and Oxidative Stress in the Liver of Male Offsprings of Wistar Rats

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Summary: It has been reported in human and animal studies that early exposure to glucocorticoids could retard growth and subsequent development of cardio metabolic diseases. Chronic exposure to glucocorticoids induced oxidative stress. Therefore, the role of oxidative stress in some of the observed metabolic imbalance needs to be elucidated. This study examined the effects of lactational dexamethasone exposure on metabolic imbalance and oxidative stress marker in the liver of male offspring of exposed mother. Twenty lactating dams were divided into 4 groups of 5 animals each. Group 1 was administered 0.02 ml/100gbwt/day normal saline through lactation days 1-21. Group 2, 3, and 4 were administered 100 µg/kgbwt/day dexamethasone for lactation days 1-7, 1-14, and 1-21 respectively. The male offspring were thereafter separated and sacrificed at 12weeks of age for evaluation of lipid profile and oxidative stress marker in the liver.

Results from this study indicate that Total Cholesterol (TC), Triglycerides (TAG) and LDL- cholesterol (LDL-C) were significantly ($p<0.001$) higher in the Dex 1-7, Dex 1-14 and Dex 1-21 groups when compared with the control. HDL-Cholesterol (HDL-C) was significantly ($p<0.001$) reduced in the Dex 1-7, Dex 1-14 and Dex 1-21 groups relative to the control. Basal Fasting Blood Sugar (FBS) was also significantly ($p<0.001$) higher in the Dex 1-14 and Dex 1-21 groups when compared with the control. Liver malondialdehyde was significantly ($p<0.001$) higher in the Dex1-14 and Dex1-21 group compared to the control. However, liver catalase and SOD activity were all significantly ($p<0.001$) lower in Dex 1-7, Dex 1-14 and Dex 1-21 groups relative to control. Liver protein was significantly ($p<0.001$) lower in the Dex1-14 and Dex1-21 treatment groups when compared with the control. Findings from this study suggest that there is possible increase in metabolic imbalance in the offspring of mother exposed to dexamethasone during lactation and these effects may be secondary to increase oxidative stress in the liver.

Keywords*:* Dexamethasone; Lactation; Oxidative Stress; Liver; offspring.

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INTRODUCTION

Exposure to stress and glucocorticoids hormone mediator exert influences on organ growth, developments and subsequent offspring physiology and pathophysiology (Drake et al., 2005). Sources of exposure to synthetic glucocorticoids during development can occur if the mother has a medical condition requiring glucocorticoids treatments, such as asthma (Wang et al., 2010). Prevention or lessen the morbidity of chronic lung disease in preterm infants (Wang et al., 2010) also required glucocorticoids treatment. The treatment regimen for chronic lung diseases typically consists of high doses of dexamethasone for several weeks (Singh et al., 2012).

Evidence from epidemiological studies and animal experiment suggest a strong link between weight during early neonatal life and subsequent development of cardio-metabolic diseases, (Hales et al, 1991; Barker et al., 1993; Lithell et al., 1996). These developments appear to be independent of classical adult lifestyle risk factors. In rodent, glucocorticoids exposure during neonatal life causes reduce weight as well as retard growth (Wang et al., 2010). In explaining these observations, it has been proposed that a stimulus or insult acting during critical periods of growth and development permanently alters tissue structure and function, a phenomenon termed "Fetal Programming" (Drake et al., 2005).

The programming effect of synthetic glucocorticoids is mediated through programming of HPA axis (Kapoor et al., 2006). The hallmark of this programming is an increase in the circulating corticosterone. However excess circulating corticosterone in prenatal life has been shown to induce oxidative stress in cerebellar granule (Ahlbom et al., 2000). In addition, chronic administration of glucocorticoids in rats causes increased lipid peroxidation and decreased antioxidant activity (Orzechowski et al., 2002; Mcintosh et al., 1998).

The role of oxidative stress in the pathogenesis of metabolic imbalance such as insulin resistance, diabetes mellitus and hyperlipidaemia has also been well reported in literature' (Collins, 2005). Numerous studies dealing with maternal dexamethasone exposure and programming of metabolic diseases have focused on exposure during prenatal life. The neonatal effect of dexamethasone exposure have only been observed through direct administration of dexamethasone in pups (Wang et al., 2010), but if administration of dexamethasone in mother during lactation will affects the pup is not known. Tilbrook et al (2006) reported that maternal stress during lactation suppresses HPA activities in the mother. The clinical use of the synthetic glucocorticoids as anti-inflammatory agents called for the understanding of the possible role of maternal exposure through lactation to dexamethasone in the programming of metabolic effects and oxidative stress in the offspring. It is therefore hypothesize that exposure to dexamethasone during lactation may induce increase metabolic imbalance in the offspring and this may be associated with increase in oxidative stress in the liver, since liver is the major metabolic organ.

MATERIALS AND METHODS

Drug: Dexamethasone 21- Phosphate disodium salt was purchased from Sigma Aldrich Chemical, UK. A dose of 100 pg dexamethasone /Kg/day was administered to the treated groups (Drake et al., 2005).

Experimental Animal: Twenty female Wistar rats weighing (150-180 g) were purchased from Central Animal House of University of Ibadan, Ibadan, Nigeria. The animals were housed in the Department of Physiology Animal House and they had free access to water and food. After two weeks of acclimatization, animals in proestrous were exposed to matured male overnight and the presence of sperm in their vaginal in the next morning mark gestation day 1(GD1). After pregnancy has been established, animals were randomly divided into four groups of 5 animals each and they were treated accordingly during lactation (Table 1). Administration was between 09.00am and 10.00am daily. 100 pg/kgbwt/day of dexamethasone was administered for the drug treated groups and 0.02 ml/100gbwt/day normal saline was administered in the control. Administration was done subcutaneously in the dams. The litter size was standardized to 5pups/litter. Biochemical analysis was done on the male offspring at 12weeks of Age. All animal experiments were conducted in accordance with ethical norms acceptable at the University of Ibadan. The male offspring were allowed to grow to adulthood (12wk of age). Blood were thereafter collected from the ocular sinus into plane tube. This was centrifuge at 3000rpm for 10minutes for the preparation of serum for subsequent evaluation of biochemical parameters.

Table 1: Treatment of animals and number of offspring collected.

Group	Treatment	Number of dams (No of male Offspring)
Control	0.02ml/100gbw/day Normal saline	5 (6)
Dex 1-7	100µg/kgbw/day Dexamethasone (PND 1-7)	5 (6)
Dex 1-14	100µg/kgbw/day Dexamethasone (PND 1-14)	5 (6)
Dex1-21	100µg/kgbw/day Dexamethasone (PND 1-21)	5 (6)

Dex (Dexamethasone), PND (postnatal days)

Rats were sacrifice through cervical dislocation. During dissection, the liver was carefully collected and rinsed with ice cold 1.15% KCl solution. Dry weight of the tissue was recorded. They were thereafter placed in 0.1M Potassium phosphate buffer pH 6.5 and homogenize using homogenizer, after which the sample was centrifuge in cold centrifuge at 10,000rpm for 10minutes. The homogenate was removed and stored in a refrigerator for analysis of oxidative stress. Biochemical analysis was done within 48 hours of sample collection.

BIOCHEMICAL ANALYSIS

Determination of Tissues lipid peroxidation: Level of lipid peroxidation (MDA) was evaluated by method of Buege and Aust (1978). Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde (MDA) has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give a red species absorbing at 535nm.

Determination of Tissues Catalase activities: Catalase activity was evaluated by method of Sinha (1971). This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchromic acid as an unstable intermediate. The chromate acetate then produced is measured colorimetrically at 570-610 nm. Since dichromate has no absorbance in this region, the presence of the compound in the assay mixture does not interfere at all with the colorimetric determination of chromic acetate. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate colorimetrically after heating the reaction mixture.

Determination of Tissues Superoxide Dismutase (SOD) Activities: SOD activity was evaluated by method of Mistra and Fridovich (1972). The ability of superoxide dismutase to inhibit the auto oxidation of adrenaline at pH 10.2 makes this reaction a basis for the SOD assay. Superoxide anion (O_2) generated by the xanthine oxidase reaction is known to cause the oxidation of adrenaline to adrenochrome. The yield of adrenochrome produced per superoxide anion increased with increasing pH and also with increasing concentration of adrenaline. These led to the proposal that auto oxidation of adrenaline proceeds by at least two distinct pathways, one of which is a free radical chain reaction involving superoxide radical and hence could be inhibited by SOD.

Determination of Tissues protein activities: Protein estimation was done by method of Lowrey et al (1951). The Folin-Ciocalteau reagent was used in the quantification of proteins by Lowry (1951). In its simplest form the reagent detects tyrosine residues due to their phenolic nature. The reaction of a protein in solution with the Folin reagent occurs in two stages: Reaction with Cu++ in alkaline medium and Reduction of the phosphomolybdic-phosphotungstic reagent by the Cu++ - protein complex. The reduced complex gives a blue solution with an absorption in the red portion of the visible spectrum (600¬800 nm).

Determination of Fasting blood glucose, Lipid profile and serum corticosterone level: Total cholesterol(TC), HDL-Cholesterol, LDL-cholesterol and Triglyceraldehyde (TAG) were evaluated using Randox Kits (Randox laboratory, United Kingdom). Fasting Blood glucose (FBS) was determined (after animals have been fasted for 12hours overnight) using Accu-Check Active glucometer (Roche diagnostics Germany). Serum corticosterone level was assessed using Cloud Clone corticosterone Kit (United State). Serum was collected between 8.00am and 9.00am in the morning to accommodate for the diurnal peak in serum corticosterone level.

Statistical Analysis

Data are expressed as mean ± standard error of mean (SEM) n= 6 Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare the means of the different treatment groups. Differences between the treatment groups with a p- value < 0.05 were considered significant. Data were analysed with the use of Graphpad Prism Version 5.0 for Windows (GraphPad® Software, San Diego, CA, USA.

RESULTS

Effects of Lactational exposure to dexamethasone on growth pattern

The birth weight and weight at postnatal day 7 were not significantly different in all the treatment groups when compared with the control (Table 2). The mean weight at postnatal day (PND) 14 was significantly reduced ($p<0.001$) in the Dex 1-7, Dex 1-14 and Dex 1-21when compared with the control. In addition, the mean weight at PND 21 was significantly reduced ($p<0.001$) in Dex 1-7, Dex 1-14 and Dex 1-21when compared with the control (Table 2). The mean weight at necropsy (PND 12 weeks) was also significantly reduced in the Dex 1-7 ($p<0.05$), Dex 1-14 ($p<0.05$) and Dex 1-21($p<0.001$) when compared with the control (Table 2).

Effects of Lactational exposure to dexamethasone on serum corticosterone: The mean serum corticosterone concentration was significantly increased in the Dex 1-7 ($p<0.05$), Dex 1-14 ($p<0.01$) and Dex 1-21($p<0.01$) when compared with control (Fig. 1).

Table 2: Effects of maternal dexamethasone treatment during lactation on body weight

	Weights (g)				
	Birth	PND 7	PND 14	PND 21	PND 12weeks (g)
Control	4.98±0.05	7.77±0.44	17.5±0.65	25.39±1.08	203.84±8.4
Dex 1-7	5.47±0.26	8.41±0.57	10.3±0.26**	16.27±0.35**	167.5±7.5*
Dex 1-14	5.14±0.08	9.99±1.21	12.7±0.19**	16.95±0.06**	167.5±7.5*
Dex 1-21	5.26±0.17	9.46±1.39	12.7±0.17**	17.99±0.31**	143.75±5.3**

*p<0.01, **p<0.001; *, shows significant different between the group and the control. PND (Postnatal days)

Table 3: Comparison of lipid profile level in the male offspring at 12 weeks of age following maternal treatment with dexamethasone during lactation in Wistar rats.

	Total Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-Cholesterol (mg/dl)	LDL- Cholesterol (mg/dl)
Control	65.67±1.34	71.28±4.45	32.41±2.30	18.95±2.35
Dex 1-7	118.00±1.01***	185.25±16.5***	27.33±1.50***	53.62±4.21***
Dex 1-14	122.00±1.10***	293.39±11.00***##	9.79±0.46***###	53. 59±3.64***
Dex 1-21	87.50±1.50***###	210.84±1.38***	3.20±0.65***###	42.30±2.19***

Values are expressed as mean ± SEM, n=6. *p< 0.05, **p<0.01, ***p<0.001; # p< 0.05, ## p<0.01, ### p<0.001; *, shows significant different between the group and the control; #, shows significant different between the group and Dex 1-7.

Effects of Lactational exposure to dexamethasone on Fasting blood sugar (FBS) and lipid profile: FBS showed a significant ($p<0.001$) increase in the treatment groups' Dex 1-14 days and Dex 1¬21 days when compared with control. FBS in Dex 1-21days and Dex 1-14 days were all significantly ($p<0.001$) higher than Dex 1-7 (Fig. 2). The total cholesterol (TC) increased significantly ($p<0.001$) in all the test groups administered with dexamethasone when compared with the control. TC was however significantly reduced in Dex 1-21 group ($p< 0.001$) when compared with Dex 1-7 and Dex 1-14 (Table 3).

Table 4: Relative liver weight following lactational exposure to dexamethasone

Treatments	Relative liver weight (g tissue/kg bwt)
Control	19.06±1.326
Dex 1-7	20.07±1.621
Dex 1-14	27.21±1.13 **#
Dex 1-21	31.32± 2.1*** ##

**p<0.01, ***p<0.001; # p< 0.05, ##p<0.01,*, shows significant different between the group and the control; #, shows significant different between the group and Dex 1-7

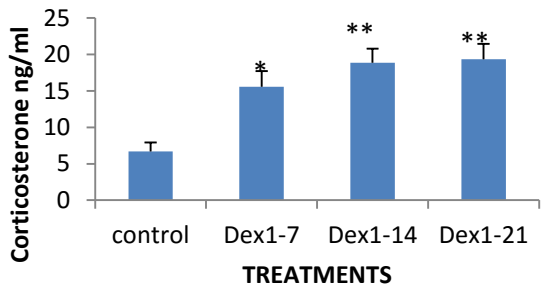

Fig. 1: Serum corticosterone level in the male offspring following maternal dexamethasone treatment during lactation. *p<0.05, **p<0.01, ***p<0.001; N=6, *, shows significant different between the group and the control.

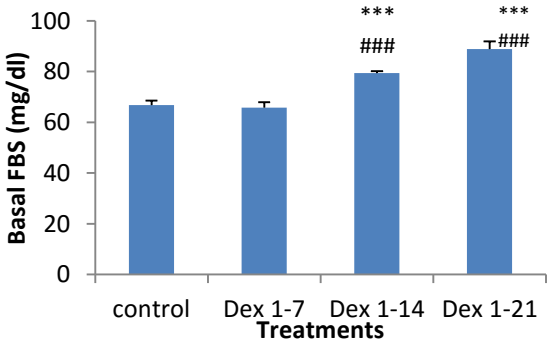

Fig. 2: Comparison of Fasting Blood Sugar (FBS) level in the male offspring at 12 weeks of age following maternal treatment with dexamethasone during lactation in Wistar rats. Values are expressed as mean ± SEM, N=6. *p< 0.05,**p<0.01, ***p<0.001; #p< 0.05, ##p<0.01, ### p<0.001, *, shows significant different between the group and the control; #, shows significant different between the group and Dex 1-7

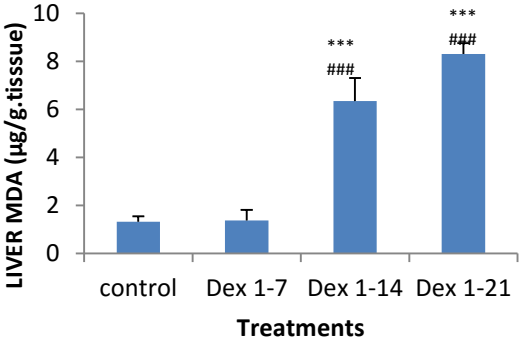

Fig. 3: Comparison of Liver MDA level in the male offspring at 12 weeks of age following maternal treatment with dexamethasone during lactation in Wistar rats. Values are expressed as mean ± SEM, n=6. *p< 0.05, **p<0.01, ***p<0.001; # p< 0.05, ## p<0.01, ### p<0.001; *, shows significant different between the group and the control; #, shows significant different between the group and Dex 1-7.

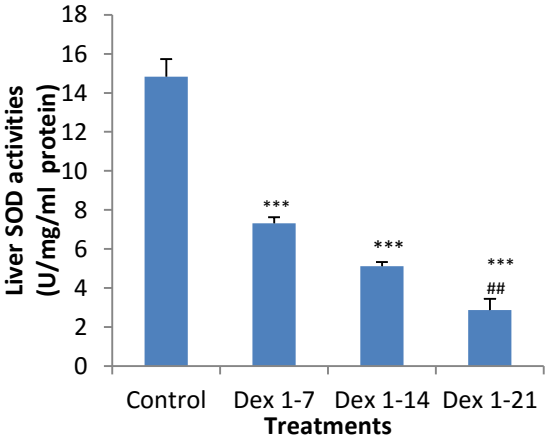

Fig. 4: Comparison of liver SOD activities in the male offspring at 12 weeks of age following maternal treatment with dexamethasone during lactation in Wistar rats. Values are expressed as mean ± SEM, n=6. *p< 0.05, **p<0.01, ***p<0.001; # p< 0.05, ## p<0.01, ### p<0.001; *, shows significant different between the group and the control; #, shows significant different between the group and Dex 1-7

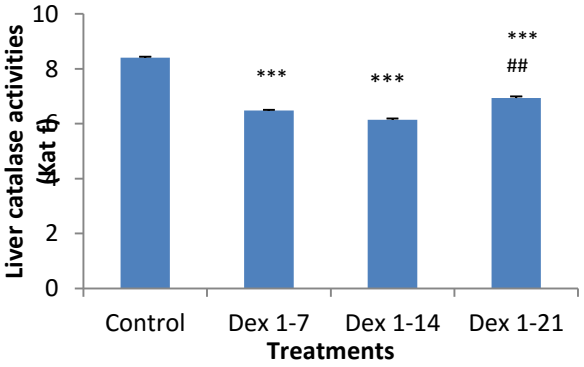

Fig. 5: Comparison of liver catalase activities in the male offspring at 12 weeks of age following maternal treatment with dexamethasone during lactation in Wistar rats. Values are expressed as mean ± SEM, n=6. *p< 0.05, **p<0.01, ***p<0.001; # p< 0.05, ## p<0.01, ### p<0.001; *, shows significant different between the group and the control; #, shows significant different between the group and Dex 1-7.

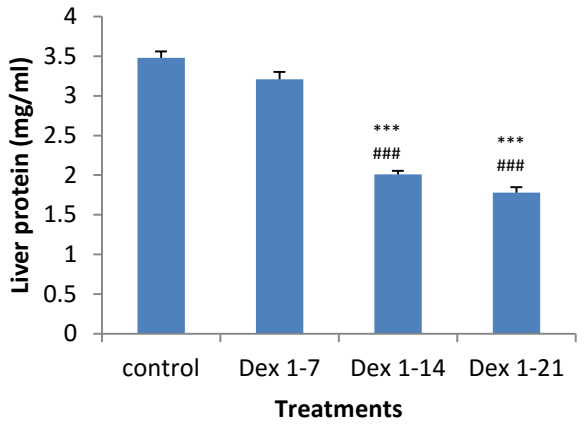

Fig. 6: Comparison of liver total protein level in the male offspring at 12 weeks of age following maternal treatment with dexamethasone during lactation in Wistar rats. Values are expressed as mean ± SEM, n=6. *p< 0.05, **p<0.01, ***p<0.001; # p< 0.05, ## p<0.01, ### p<0.001; *, shows significant different between the group and the control; #, shows significant different between the group and Dex 1-7

The triglyceride concentration levels showed a significant (p<0.001) in all treatment groups when compared with the control. TAG in Dex 1-14 group was however significantly higher than Dex 1-7 and Dex 1-21 (p<0.01) (Table 3). The HDL-cholesterol levels reveal a significant (p<0.001) decrease in all treatment groups when compared with the control. HDL-cholesterol was also significantly (p < 0.001) reduced in Dex 1-21 and Dex 1-14 when compared with the Dex 1-7 (Table 3).

The LDL-Cholesterol levels showed a significant (p<0.001) increase in all treatment groups when compared with the control. It was however, significantly (p<0.01) lower in Dex 1-21 group when compared with Dex 1-7 (Table 3).

Effects of Lactational exposure to dexamethasone on relative liver weight

The relative liver weight was significantly increased in the Dex 1-14 (p<0.01) and Dex 1- 21(p<0.001) group when compared with control (Table 4).

Effects of Lactational exposure to dexamethasone on makers of oxidative stress in the liver: The Liver MDA levels shows a significant (p<0.001) increase in the treatment groups' 1-14 days and 1-21 days, compared with the control Dex 1-14 and Dex 1-21 liver MDA were also significantly (p <0.001) higher than Dex 1-7 group (Fig. 3).

The liver of SOD activities showed a significant (p<0.001) decrease in all treatment groups when compared with the control. Also, Dex 1-21 group liver SOD activities was significantly (p<0.01) lower than Dex 1-7 group (Fig. 4).

The liver catalase activities showed a significant (P<0.001) decrease in all test groups when compared with the control. However, Dex 1-7 and Dex 1-14 groups showed a significant (p<0.01) decrease in liver catalase activities when compared with Dex 1-21. (Fig. 5) The liver protein level was significantly (p<0.001) decrease in Dex 1-14 and Dex 1-21 groups when compared with the control and Dex1-7. (Fig. 6).

DISCUSSION

The study examined the effect of maternal exposure to dexamethasone during lactation on metabolic imbalance and oxidative stress marker in the liver of male offspring in Wistar rats.

The results from this study demonstrated that maternal exposure to 100 pg/Kgbwt/day dexamethasone during lactation could leads to dyslipidaemia in the offspring. The total cholesterol (TC), Triglycerides (TAG) and LDL-cholesterol (LDL-C) levels were raised by the treatments compared to control. However, serum HDL-Cholesterol (HDL-C) was significantly reduced by the treatment. Associated with this observation is the increased serum basal corticosterone level in the treatment groups. Contrary to the finding of this study, exposure to glucocorticoids have direct effects on the circulating lipids and lipoproteins; increasing LDL-C, TAG and HDL-C and total cholesrterol (Ettinger and Hazzard, 1988). Glucocorticoids decrease concentration of LDL-C receptors on hepatocyte (Rainey et al., 1992) leading to higher LDL-C level. It also increases VLDL production and secretion from the liver thereby causing hypertriglyceridemia (Brindley, 1995). Increase in the liver VLDL level may leads to low HDL-C level (Masharani and German, 2007). Another possible explanation is increase circulating glucocorticoids as seen in chusing syndrome leads to insulin resistance (Aron et al., 2007). Insulin resistance could lead to visceral obesity that is presented with this kind of lipid profile. Basal fasting blood glucose (FBS) was also significantly increased in the offspring of the treated mother. However, the increase in FBS is within normal range. According to Sapolsky et al., (2000) chronic exposure to glucocorticoids could lead to muscle and hepatic insulin resistance and precipitate hyperglycemia. Maternal treatment with dexamethasone during first week, second or throughout lactation significantly reduces the body weight of the offspring at PND 7, PND 14 and PND 21. This growth retardation was sustained till adulthood. Evidence is accumulating from epidemiological study in human that early life dexamethasone exposure negatively affects the somatic growth (Wang et al., 2010). Normal somatic growth is the result of the proper interactions between genetic, nutritional, metabolic and endocrine factors (Lin et al., 2006; Huang et al., 2007; Berry et al., 1997; Shrivastava et al.,2000). Although the cause of the lasting growth retardation observed in Dex treated groups offspring remain unclear, this adverse effect may be linked, at least in part to alteration in the maternal HPA activity as a result of the Dex treatment.

Tilbrook et al., (2006) has reported similar alteration in HPA activity due to maternal stress during lactation. Since the neuroendocrine system in the pulps are still undergoing maturation7, altered HPA activity in the dam may programme stress related glucocorticoids secretion in the offspring. Chronic increase in the glucocorticoids level may raise tissue catabolism or protein breakdown (Leitch et al., 1999; Neal et al., 2004). This may lead to increase muscle waste and reduce muscle mass.

It was also found from this study that neonatal dexamethasone administration significantly reduced the antioxidant enzyme Catalase and SOD in the liver and raises the level of lipid peroxidation. Increase lipid peroxidation with reduced antioxidant enzyme level is indicators of oxidative stress (Shcafer and Buettner, 2001). In humans, oxidative stress is thought to be involved in the development of many diseases or may exacerbate their symptoms (Whitworth et al., 2000). Consistent with this finding, is the report that oxidative stress may be especially pronounced with prolonged glucocorticoids exposure (Iuchi et al.,2003). Patients with Cushing syndrome may have increased nitrotyrosine levels (a measure of increased oxidative stress) in vascular tissue and decreased brachial artery reactivity (Celsi and Aperia, 1999). Human umbilical vein endothelial cells exposed to dexamethasone also generate reactive oxygen species via stimulation of NADPH oxidase and xanthine oxidase (Celsi and Aperia, 1999). Increase oxidative stress leads to DNA damage and may also result in cell death. These two effects of oxidative stress on the liver may impaired the liver metabolic functions.

Numerous studies both in human population and animals have reported that prenatal exposure to glucocorticoids can leads to subsequent development of hyperlipidamea, glucose intolerance, insulin resistance and other cardio-metabolic diseases (Drake et al.,2005). This study extends the observation of metabolic imbalance in offspring to maternal Dex treatment during lactation. Indeed, glucocorticoids hormones modulate tissue development during both the prenatal and the weaning period regulating organ maturation at different times in a synchronized and orderly fashion (Celsi and Aperia, 1999). Rats are born relatively immature, with several organs maturation during the weaning period (Kapoor et al., 2006). Therefore, our data suggest a possible increase metabolic imbalance in the offspring of mother xposed to Dex during lactation and these effects may be secondary to increase oxidative stress in the liver.

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