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Citation for published version:
Stirrat, LI & Reynolds, RM 2016, 'The Effect of Fetal Growth and Nutrient Stresses on Steroid Pathways' The Journal of Steroid Biochemistry and Molecular Biology, vol. 160. DOI: 10.1016/j.jsbmb.2015.07.003

Digital Object Identifier (DOI):
10.1016/j.jsbmb.2015.07.003

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
The Journal of Steroid Biochemistry and Molecular Biology

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Download date: 05. Apr. 2019

The Effect of Fetal Growth and Nutrient Stresses on Steroid Pathways

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Keywords: pregnancy, HPA axis, cortisol, fetal programming

Word count: 3257 words

ABSTRACT

The early life environment is a crucial time for establishing the trajectory of future health. Low birthweight is considered a marker of an adverse *in utero* environment and predisposes to cardio-metabolic disease later in life. It has been proposed that this is mediated by glucocorticoids, with life-long activation of the HPA axis. Here we review the evidence to support this hypothesis, with particular emphasis on the effects of fetal growth and nutrient stresses *in utero* on steroid pathways of the HPA axis. A better understanding of the mechanisms underlying these processes could help to optimize *in utero* health, and identify individuals at greatest risk of future disease.

1. INTRODUCTION

The early life environment is established as a crucial time for influencing the trajectory of future health. Epidemiological studies reported in the past two decades have highlighted the importance of fetal adaptations to a poor intrauterine environment for longer-term health outcomes[1, 2]. A growing body of evidence has shown associations between low birthweight, a surrogate marker of an adverse intrauterine environment and subsequent cardio-metabolic disease, and mental health problems. This concept is termed 'early life programming' or the 'developmental origins of health and disease' hypothesis[3]. The developing fetus is thought to respond to insults *in utero* with adaptations in structure, physiology and metabolism. Although these adaptations may initially be beneficial, later in life they may become maladaptive and predispose to disease.

One mechanism proposed for these adaptations, is a 'programming' of the offspring hypothalamic-pituitary-adrenal (HPA) axis in utero. The HPA axis is highly susceptible to programming during fetal and neonatal development[4]. The ability of the early environment to modify HPA development and subsequent function was first described over 40 years ago[5]. However, recent epidemiological evidence indicating that the fetal environment can profoundly influence disease processes later in life[6] has rejuvenated research in the field. Robust correlations between birth weight, plasma cortisol concentrations and the development of hypertension and type 2 diabetes have been identified[7-9]. Changes in the HPA axis are also postulated to underlie some of the programmed changes that may occur, secondary to nutrient stresses, such under or over nutrition, or secondary to maternal stress. In this review, we focus on the evidence from human studies linking fetal growth and *in utero* stresses on the steroid pathways of the offspring HPA axis, and the implications this may have for health later in life.

2. THE HPA AXIS AND ITS CHANGES DURING PREGNANCY

Glucocorticoids are vital for life, acting upon and influencing virtually all tissues and physiological functions. These include metabolism, blood pressure, fluid and electrolyte homeostasis and the immune system. They also mediate increasing energy demands in response to stress. In response to both physiological and psychological stress, cortisol is secreted by the adrenal gland, which is regulated by the HPA axis. The release of cortisol follows a circadian rhythm, which is characterized by peak levels of cortisol prior to activity in the morning and a

subsequent decline throughout the rest of the day. Circadian release of adrenocorticotrophic hormone (ACTH) from the pituitary is stimulated by the action of corticotrophin releasing hormone (CRH) and vasopressin (AVP) from the hypothalamus, under control of the hypothalamus. This circadian rhythm of cortisol release can be interrupted by stressors, which cause a premature release of glucocorticoids. Cortisol release also follows a pulsatile fashion, with an ultradian rhythm of hormone oscillations. These oscillations are thought to be important for optimal transcriptional regulation[10]. Circulating glucocorticoids are predominantly protein bound[11] to corticosteroid binding globulin (CBG). At high physiological levels, binding proteins are saturated which allows greater diurnal fluctuation in levels of free, bioavailable cortisol.

Glucocorticoids are also required for normal fetal growth and development. A number of endocrine changes occur during pregnancy, causing a dramatic activation of the maternal HPA axis. As a result of this, maternal cortisol levels increase exponentially[12, 13]. The placenta, which has significant endocrine properties, secretes CRH from the second trimester onwards, and this activates the maternal HPA axis to stimulate cortisol production (figure 1). A complex feed-forward loop is created, with cortisol also stimulating placental CRH synthesis[13, 14]. The placenta also secretes estrogen and progesterone, both of which may mediate levels of free, bioavailable cortisol[14, 15]. Estrogen stimulates the hepatic synthesis of CBG during pregnancy[16]. Progesterone has also been shown to displace cortisol from CBG[15].

The circadian rhythm of cortisol release is maintained during pregnancy[17], but as gestation advances, the cortisol awakening response and normal physiological responses to stressors (both markers of basal HPA activity) are attenuated[13]. Glucocorticoids are lipophilic, and can therefore freely cross the placenta from the maternal to the fetal compartments, and levels of maternal cortisol are therefore thought to influence fetal cortisol levels. Maternal plasma cortisol has been shown to be a significant predictor of amniotic fluid cortisol, independent of maternal age, gestation age and time of collection[18]. A pattern of increasing positive correlation between maternal plasma and amniotic fluid cortisol with increasing gestation has also been reported[18-21].

Fetal glucocorticoid levels are up to 10-fold lower than maternal levels, due to the actions of placental 11-beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2); an

enzyme-based barrier that deactivates active cortisol into inactive cortisone[22]. Levels of 11β-HSD2 in the human placenta increase with advancing gestation[23], thus limiting fetal glucocorticoid exposure during critical stages of development during pregnancy. However, this barrier is not complete as up to 20% of maternal glucocorticoids cross to the fetal compartment[24]. Although the regulation of 11β-HSD2 is not fully understood, both human and animal studies have suggested that the efficiency of placental 11β-HSD2 varies considerably, and may be weakened by diet, infection, inflammation, hypoxia and stress. Reduced efficiency of this enzyme would therefore allow a greater transplacental passage of cortisol to the developing fetus[25, 26]. Given that maternal cortisol levels are considerably higher than fetal levels, even modest changes in placental 11B-HSD2 activity can significantly alter fetal glucocorticoid exposure[20, 27].

Glucocorticoids bind to glucocorticoid and mineralocorticoid receptors[28]. These receptors are activated upon ligand binding and the receptor-ligand complex translocates to the nucleus, where it binds to glucocorticoid response elements in the promoter region of target genes, and can then influence gene transcription. Glucocorticoids can also exert non-genomic effects by direct actions on membrane lipid and cytoplasmic proteins via membrane-located receptors[29]. Glucocorticoid receptor (GR) is expressed from mid-gestation onwards in fetal tissues, the placenta and fetal membranes[30]; and mineralocorticoid receptor (MR) is more limited and tends to be present later in gestation[31].

Glucocorticoids play a key role in organ maturation to prepare the fetus for extra-uterine life, and the rise in maternal cortisol levels in later gestation parallels the increasing maturity of fetal organs[32]. This is also the rationale for administering synthetic glucocorticoids to women who are suspected to deliver at preterm gestations, to accelerate fetal lung maturation and reduce the risk of respiratory complications for the premature neonate[33].

3. FETAL GROWTH

a. Glucocorticoid overexposure *in utero* is associated with lower birthweight

In addition to promoting maturation of fetal organs, increased cortisol levels may increase availability of glucose for the developing fetus, by mobilizing substrates for hepatic gluconeogenesis[34]. This premise would suggest that increased fetal glucocorticoid exposure might lead to increased fetal growth. However, contrary to

this suggestion both human and animal studies indicate that although glucocorticoids are crucial for normal growth and development, a fine balance of fetal glucocorticoid exposure is required, as fetal over-exposure to glucocorticoids is in fact linked with fetal growth restriction[35], and recently, epidemiological studies have also linked the association with increased cardiovascular risk later in life[36, 37]. Timing of fetal glucocorticoid exposure also appears critical, as for example, amniotic fluid cortisol levels (a surrogate marker for fetal glucocorticoid exposure) measured at 15-18 weeks are negatively correlated with birthweight ($r=-0.25$, $P<0.01$) and gestational age ($r=-0.18$, $p<0.05$)[19], suggesting that cortisol levels in the second trimester of pregnancy may influence fetal growth.

Maternal cortisol levels have also been shown to predict offspring birthweight. In a large cohort (n=28,010), high maternal total serum cortisol at 20 weeks gestation was negatively associated with offspring birthweight ($\beta=-0.35$; $p<0.01$), and was positively associated with risk for 'small for gestational age' (SGA) (Odds ratio [OR]=1.00; $p=0.027$), although blood samples were obtained at different times of day, and significance was lost after controlling for covariates[38]. This pattern of a negative correlation with birthweight has also been observed in maternal saliva samples, which are reflective of free cortisol levels. A smaller study of 70 pregnant women found that even after adjusting for covariates, that mothers with a higher salivary cortisol awakening response at 13-18 weeks gestation had babies of lower birthweight who were also shorter at birth[39]. In this study, morning cortisol levels explained 19.8% of the variance in birth weight and 9% of the variance in body length[39]. Another small study of 98 women[40], showed that maternal saliva cortisol at 36 weeks found that that both morning cortisol and a steeper morning decline were associated with lower birthweight. The heaviest neonates were born to women with a relatively flat diurnal rhythm characterized by a blunted morning peak[40]. Taken together, these studies suggest that a flattening of the diurnal rhythm of cortisol secretion may protect the fetus from overexposure to glucocorticoids during the morning peak.

b. Fetal glucocorticoid overexposure and low birthweight are associated with increased activity of the HPA axis

In human studies altered glucocorticoid exposure *in utero* is associated with increased activity of the offspring HPA axis. For example, higher maternal cortisol levels in mid-late gestation are associated with increased cortisol response in the

newborn to the stress of a heel prick test[41]. This study suggests the window of susceptibility of the fetal HPA axis to excess maternal glucocorticoids is in mid-late gestation. By 24 weeks gestation, both GR and MR are present in the human hippocampus, which is an important site for glucocorticoid central negative feedback[42]. Animal studies have shown that excessive glucocorticoids can alter the density of these receptors in both the hippocampus and amygdala, with consequences for HPA axis feedback sensitivity[43, 44]. Thus, cortisol exposure in late pregnancy may influence the neonatal HPA axis response. Consistent with this is the observation that lower maternal cortisol levels, particularly in the third trimester, in women with post traumatic stress disorder, which itself is associated with low cortisol, are associated with lower cortisol levels at awakening and bed-time in their offspring at aged 1 year[45].

Observational studies of women who consume large amounts of licorice during pregnancy (which contains glycyrrhizin, and 11β-HSD2 inhibitor)[46] also serve as proof of concept that 11β-HSD2 is relevant for fetal glucocorticoid exposure, and subsequent fetal outcome. These studies found that although high licorice consumption during pregnancy did not significantly affect birthweight, there was evidence of earlier gestation at delivery and altered HPA axis function of the offspring. At aged 8 years, offspring of women with high licorice consumption during pregnancy had raised fasting cortisol levels, and an increased cortisol secretion in response to psychological stress, compared to the offspring of women with lower licorice consumption[46]. *In utero* licorice exposure was also associated with significant increases in externalizing symptoms, attention, rule breaking and aggression problems with notably a 2.26 fold increase in attention deficit hyperactivity disorder, which may be mediated by altered glucocorticoid exposure *in utero*[47].

There is also evidence that changes in offspring HPA axis activity associated with low birthweight may also persist across the lifespan. Low birthweight is associated with increased fasting cortisol concentrations in populations of men and women ranging from young adulthood to older life[7, 9, 48]. A meta-analysis of 11 studies including data from fasting cortisol measurements from 2301 subjects reported that cortisol concentrations fell on average by 25.3nmol/kg increase in birthweight[9]. The measurement of fasting cortisol is itself considered a ‘stress’ response due to the reaction to venepuncture, particularly in the context of attending a research clinic in an unfamiliar situation after an overnight fast. This argument has been used to

explain the lack of association between cortisol and birthweight in adult studies reporting integrated cortisol exposure over the day[49-51]. However studies measuring cortisol when stimulated by ACTH, or in response to psychological stress suggest that *the response* of the HPA axis is susceptible to fetal programming[7, 52-57]. Salivary cortisol responses to the psychological stress of the 'Trier Psychosocial Stress Test' (TSST) in 106 male twins at age 18 years were significantly and inversely related to the subjects' birthweight, and the low birthweight twins had higher salivary cortisol levels in response to the stress[56]. Dynamic function studies of the HPA axis have demonstrated that lower birthweight was associated with increased responsiveness to synthetic ACTH, which is strongly suggestive of increased activation of the HPA axis[52-54]. In accord with this, children, adolescents and adults with lower birthweight have been reported to excrete more cortisol and its metabolites in urine[53, 58, 59]. There is currently no evidence that the association between high fasting cortisol and low birthweight is explained by altered central negative feedback sensitivity of the HPA axis. Studies using dexamethasone suppression, which tests the GR component of central negative feedback, found no differences in the central negative feedback sensitivity in association with birthweight[53, 54]. However, further tests of the contribution of both MR and GR demonstrated altered central negative feedback sensitivity in obesity[60]. Similar studies are required to test whether this also occurs in association with low birthweight. Taken together, these studies suggest that the HPA axis response to stress may be susceptible to fetal programming, and that the resultant increased HPA axis activity persists into adult life. Importantly raised fasting and ACTH stimulated cortisol levels are associated not only with low birthweight, but also with an adverse metabolic profile[7, 48, 53, 54]. This supports the hypothesis that fetal programming could result in long-term changes in cortisol secretion, which in turn could lead to increased glucocorticoid exposure over a lifespan, and a predisposition to cardio-metabolic disease later in life.

4. FACTORS INFLUENCING FETAL GLUCOCORTICOID EXPOSURE

A number of factors have been proposed to alter fetal glucocorticoid exposure, including maternal stress, maternal diet and nutrient stresses.

a. Maternal stress may alter fetal glucocorticoid exposure

It is well established that prenatal stress is associated with low birthweight [61, 62] and it has been suggested that this may be due to increased fetal glucocorticoid

exposure, as a result of altered circulating levels of maternal cortisol associated with stress and anxiety during pregnancy and/or changes in placental gene expression. However, the data are inconsistent as while some studies report increased cortisol levels when mothers experience higher levels of stress[63] or anxiety[18, 21], others found no such findings[64, 65]. There is also some evidence that exposure to stress *in utero* may also program the offspring HPA axis[66-69]. One potential mechanism proposed for this is alteration of placental biology (figure 2). In late pregnancy, maternal anxiety is associated with down regulation of 11β-HSD2 mRNA expression[70], which may increase fetal glucocorticoid exposure; and maternal depression is associated with increased mRNA expression of GR and MR, which may increase placental glucocorticoid sensitivity[71]. Further, there is some evidence that prenatal exposure to maternal depression is associated with increased DNA methylation of the GR (*NR3C1*), which suggests that there may be a potential epigenetic process that links antenatal maternal mood and HPA stress reactivity[72].
Maternal plasma and amniotic fluid cortisol measurements have shown that correlation coefficients between maternal and amniotic fluid cortisol increased with increasing maternal anxiety scores, suggesting that maternal anxiety may reduce the efficiency of placental 11β-HSD2[18], which has also been shown to occur after prenatal stress in animal studies[73]. Consistent with this, urine morning cortisol at 20 weeks in 300 depressed women found that higher maternal urinary cortisol was associated with lower birthweight in the offspring, and a higher incidence of prematurity[74]. Prenatal stress is also associated with an altered circadian rhythm of cortisol, with children exposed to *in utero* stress with high morning cortisol levels and then flattening of the day curve[75, 76]. Infants of mothers with symptoms of depression have been noted to have higher urinary cortisol within the first week of life, consistent with overall increased HPA axis activity[77].

b. Maternal diet and nutrient stresses may alter fetal glucocorticoid exposure

In animal models altered fetal glucocorticoid exposure has been proposed as one mechanism linking the robust observations of changes in maternal diet during pregnancy with adverse programmed offspring outcomes including lower birthweight[78], raised blood pressure[79, 80] and reduced lifespan[81-89]. For example, maternal malnutrition may cause a stress response in the mother and the fetus[78, 90], and increased stress may simultaneously limit food intake[90]. In humans, epidemiological evidence suggests that intrauterine experience of maternal undernutrition plays a major role in the aetiology of cardiovascular diseases[91] and

developmental programming[92], but in these studies it is harder to dissect effects of maternal diet *per se* on outcome from other potential confounders.

Further it is challenging to study the effects of malnutrition during human pregnancy, as this is often not restricted to pregnancy alone. The Dutch famine has provided the opportunity to study the long-term health effects for offspring of women exposed to malnutrition at different stages in pregnancy. In this cohort, there were increased rates of coronary heart disease in those exposed to famine in utero, and those exposed in later gestation had decreased glucose tolerance[93]. People exposed to malnutrition in early gestation had higher systolic blood pressure responses to stress[94], but no changes in HPA axis responses to stress, ACTH stimulation or psychological stress were documented in relation to prenatal famine exposure at any stage in pregnancy[95, 96]. The authors argued that although there was no evidence of fetal programming at the adrenal level, it is possible that the HPA axis may be altered at the level of the hippocampus or hypothalamus, which has been observed in studies of rats subjected to malnutrition during pregnancy[86, 97]. However, these findings may also suggest that prenatal exposure to famine linked to fetal programming of the autonomic nervous system, and that this is more important the HPA axis in terms of increasing susceptibility of offspring to cardio-metabolic disease. The lack of response of the HPA axis in response to famine also contrasts with observations of higher cortisol secretion in response to psychological stress in adult offspring of women who consumed an unbalanced diet during late pregnancy [98]. This supports the notion that nutrient stressors *in utero* can program the offspring HPA axis.

Maternal obesity and in utero exposure to overnutrition

At present the effects of over-nutrition during human pregnancy on the offspring HPA axis are not known. Obesity in non-pregnancy is associated with dysregulation of the HPA axis, notably with activation of the axis but with associated increased hepatic metabolism and renal excretion of cortisol, ultimately leading to normal or lower levels of circulating cortisol[99-101]. If this dysregulation of the HPA axis is maintained during pregnancy, it is possible that the offspring of obese women may be exposed to altered glucocorticoid levels, and that this could impact upon fetal size and risk of disease later in life. Indeed, maternal obesity is associated with increased fetal size[102], and with increased risk of cardiovascular events and premature death in adult offspring, compared with offspring of mothers with normal body mass index[103]. As up to 35% of women of reproductive age in the United States and

Europe are obese[104-106], a better understanding of the effects of maternal over-nutrition and increased birthweight on offspring HPA axis and subsequent disease risk is urgently required.

5. CONCLUSIONS

There is a growing body of evidence to support the hypothesis that fetal glucocorticoid overexposure is associated with low birthweight, and may predispose to subsequent cardio-metabolic disease, via altering the activity of the HPA axis. Exposure to maternal stress and nutrient stresses *in utero* have the potential to impact on maternal HPA axis activity and/or pathways within the placenta regulating fetal glucocorticoid exposure. Whether or not interventions during pregnancy to regulate HPA axis activity would be beneficial, is currently unknown, though preliminary data suggests use of stress reduction instructions in pregnancy may reduce maternal perceived stress as well as morning cortisol levels[107]. Further, activity of 11β-HSD2 may itself, be a suitable target for modification. A greater understanding of placental metabolism of cortisol, and transport of glucocorticoids between the maternal and fetal compartments, may also help identify modifiable targets for mediating fetal glucocorticoid exposure.

Acknowledgements

We acknowledge the support of Tommy's and the British Heart Foundation.

Conflict of interest

None to declare

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Figure Legends

Figure 1. The Hypothalamic Pituitary Adrenal Axis (HPA) in Pregnancy. Figure shows interaction between maternal and placental compartments during pregnancy which contribute to an increase in maternal cortisol levels. Placental release of CRH drives the maternal HPA axis increasing cortisol production, which further stimulates placental CRH release via a positive feed-forward loop. Placental estrogen production also stimulates hepatic synthesis of CBG, to which free cortisol binds.
ACTH – adrenocorticotropic hormone, CRH – corticotrophin releasing hormone, CBG – corticosteroid binding globulin.

Figure 2. Modifications to placental biology that may alter fetal glucocorticoid exposure. Altered maternal HPA axis may alter fetal glucocorticoid exposure. Maternal stress and nutrient stresses may downregulate placental 11β-HSD2 mRNA expression, which may increase transplacental passage of glucocorticoids. Maternal depression may increase placental GR and MR mRNA expression, which may increase placental sensitivity to glucocorticoids. All these placental modifications may increase fetal glucocorticoid exposure, which is associated with activation of the fetal HPA axis. This may predispose to cardio-metabolic disease later in life.
HPA – hypothalamic pituitary adrenal. mRNA – messenger ribonucleic acid, 11β-HSD2 – 11beta-hydroxysteroid dehydrogenase, GR – glucocorticoid receptor, MR – mineralocorticoid receptor.