

Short and long term effects of exposure to natural and synthetic glucocorticoids during development

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Summary

1. Glucocorticoids are necessary for fetal development but clinical and experimental studies suggest excess exposure may be detrimental to health in the short and in the longer term.

2. Exposure of the fetus to synthetic glucocorticoids can occur if the mother has a medical condition requiring glucocorticoid therapy (such as asthma) or if she threatens to deliver her baby prematurely. Synthetic glucocorticoids can readily cross the placenta and treatment is beneficial, at least in the short term, for maternal health and fetal survival.

3. Maternal stress during pregnancy can raise endogenous levels of the natural glucocorticoid, cortisol. A significant proportion of the cortisol is inactivated by the placental "glucocorticoid barrier". However, exposure to severe stress during pregnancy can result in increased risk of miscarriage, low birth weight and behavioural deficits in children.

4. Animal studies have shown that excess exposure to both synthetic and natural glucocorticoids can alter normal development of organs including the heart, brain and kidney. The nature and severity of the organ impairments is dependent upon the timing of exposure and in some cases, the type of glucocorticoid used and the sex of the fetus.

5. In animal models, exposure to elevated glucocorticoids during pregnancy has been associated with adult onset diseases including elevated blood pressure, impaired cardiac and vascular function and altered metabolic function.

Introduction

Glucocorticoids (GCs) are known to be important for normal embryogenesis¹ and while exposure to GCs may be beneficial or even necessary for fetal survival, evidence is accumulating to suggest that excessive exposure may have long term detrimental consequences. Women (and their fetuses) may be exposed to increased levels of GC at any stage of gestation. Fetal exposure to synthetic GCs commonly occurs in mid-late gestation in women threatening to deliver their baby prematurely. However, women are given synthetic GCs at other stages of pregnancy for a variety of medical conditions, including asthma (refer to Table 1). Animal studies have shown that prenatal exposure to synthetic GCs can have deleterious effects on the development of organs (such as the kidney, brain and the heart) which may in the longer term

contribute to adult onset disease, including hypertension (details in following sections). Outcomes following prenatal synthetic GC exposure are likely to be highly dependent upon the duration of GC exposure and in part on fetal sex. In relation to effects on specific organs, outcomes may be dependent upon a specific stage of development or 'critical window' at which the organ is most susceptible. Developing fetuses may also be exposed to inappropriate levels of naturally occurring GCs (cortisol in the human and sheep, corticosterone in rodents) if the mother experiences significant stress during pregnancy. Whilst maternal cortisol is largely inactivated within the placenta (as discussed in the later sections), a proportion is likely to reach the fetus, particularly if maternal concentrations are high. This review will consider when and why the human fetus is likely to be exposed to synthetic and natural GCs and in particular, we shall highlight the similarities and differences in outcomes following exposure to natural and synthetic GC.

Human fetal exposure to glucocorticoids

Synthetic Glucocorticoids

Pregnant women, and thus their fetuses, are most commonly treated with synthetic GCs when at risk of preterm delivery to ensure survival of the preterm infant. Liggins² discovered the beneficial effects of synthetic GC administration on the preterm infant following investigations of the effects of GC administration on premature delivery in the sheep. When GCs were given to pregnant ewes at doses 0.06-4mg/d on gestational days 100-121, preterm parturition occurred after approximately 48h. At these gestational ages, the lungs of the fetal sheep are very immature making postnatal survival impossible. However, it was found that GC administration accelerated fetal lung maturation resulting in some viable lambs. Liggins² suggested that GCs advanced lung development by affecting the production of lung surfactant. In 1972, Liggins *et al.*³ first demonstrated that maternal administration of betamethasone in humans substantially reduced the incidence of respiratory distress syndrome in preterm infants born before 32 weeks of gestation. It has in the past been common practice for women at risk of preterm delivery (post 24 weeks gestation) to receive a course (two injections 12-24 hours apart) of synthetic GCs;⁴ most commonly betamethasone (92%), dexamethasone (DEX, 4%) or combinations of other synthetic GC including prednisolone.⁵ A recent large scale Cochrane review reported that GC administration to mothers at risk of

Table 1. Reasons for glucocorticoid treatment to pregnant women at different times in pregnancy and outcomes on growth and health of the child.

| Glucocorticoid | Reason for exposure | Timing of exposure | Benefit | Consequence |
|-------------------------|--|---------------------------------|---|---|
| Betamethasone | Improve fetal outcome in women at risk of preterm delivery (92% ⁵) | 24-36 weeks | Reduce preterm infant mortality, Reduce systemic infections ⁴ | Increase blood pressure in children ⁹ |
| | | | Reduce incidence of RSD (24-32 weeks) ³ | Repeat dose shortly before birth reduces protection from RSD ⁸ |
| Dexamethasone | Improve fetal outcome in women at risk of preterm delivery (4% ⁵) | 24-36 weeks | As above | As Above |
| | congenital-adrenal hyperplasia | Week 7 until term ¹¹ | Reduces female genital virilisation | Maternal weight gain and oedema ¹⁰ Fetal/offspring- Unknown |
| Prednisolone | Moderate idiopathic thrombocytopenic purpura | Whole of pregnancy | Increase platelet count (prevent autoimmune attack of platelets) ⁸⁷ | Unknown Case study: methylprednisolone caused low birth weight and fetal adrenal suppression ⁸⁸ |
| | systemic lupus erythematosus | Whole of pregnancy | Maternal- Reduce disease activity Fetal-Improve rate of live birth ¹⁹ | Increase rate of premature birth ¹⁹ |
| | Acute asthma | Any time during pregnancy | Reduce asthma symptoms, prevent fetal hypoxia, reduce severe fetal outcomes ¹⁶ | Increased risk of preeclampsia ¹⁷ Preterm birth, low birth weight ¹⁶ |
| | hyperemesis gravidarum (severe form of morning sickness) | Any time during pregnancy | Reduce symptoms <i>e.g.</i> nausea ²⁰ | Unknown |
| Inhaled corticosteroids | Asthma (affects about 11% of Australian women ¹²) | Whole of pregnancy | Prevent asthma exacerbations, reduce adverse fetal outcomes ¹⁴ | High dose linked to congenital malformation ¹⁵ |
| Cortisol | Stress | Any stage | NA | Impaired mental and motor development in infant ²¹ Miscarriage ²² Low birth weight and reduced birth length ⁸⁹ |
| | Depression | Any stage | NA | Increased infant cortisol. Impaired neonatal behavioural development ²³ |
| | Physical abuse | Any stage | NA | Reduce fetal birth weight ²⁴ |
| | Disaster | Any stage | NA | Reduced head circumference ²⁵ |

RSD, respiratory distress syndrome, NA (not applicable)

preterm delivery significantly reduces neonatal death.⁴ A 30 year follow-up of the initial randomized control trial investigating the effects of antenatal corticosteroid

treatment on adult health outcomes of subjects has revealed some suggestions of insulin resistance but minimal effects on other health parameters.⁶ What is of concern is that it

has been shown that 83% of obstetricians give a second course of GC to women at risk of preterm delivery and 50% would give women weekly doses of GC until birth of the infant or until the risk of preterm delivery no longer remains.⁵ There is little evidence to support that these repeated and prolonged exposures to maternal GCs are more beneficial to the fetus than the recommended two doses 24h apart. Recent guidelines put forward by the Australian Royal College of Obstetricians and Gynaecologists suggest that although weekly doses of GCs are not recommended, a single rescue course of GCs may be given if the first dose was given prior to 26 weeks of gestation.⁷ While the additive negative effects of increased GC exposure on the developing fetus are unknown, one study has suggested that an additional dose of betamethasone close to birth can impair the respiratory adaptations caused by an earlier betamethasone dose.⁸ In addition, while the immediate protective effects of GC on fetal survival are quite clear, the long term consequences of GC exposure are less well understood, although a study by Doyle *et al.*⁹ has reported a link between antenatal exposure to GC and increased blood pressure in adolescence.

Pregnant women who are at risk of delivering a child with congenital adrenal hyperplasia are likely to receive doses of the synthetic GC, DEX that are 60 fold higher than normal mid-gestation GC levels¹⁰ to reduce genital virilisation of the female fetus.¹¹ These GCs are given as soon as pregnancy is confirmed at about week 5 of pregnancy or from about week 7 when genital development begins. However, confirmation of congenital adrenal hyperplasia can only be made by fetal sampling (chorionic villus sampling from week 9 or amniocentesis-from week 14) thus many fetuses will be exposed to high levels of GC before confirmation of the condition.¹¹ As this condition is autosomal recessive and only half of affected fetuses are female, there is only a 1 in 8 chance of the individual benefitting from the GCs¹⁰ with the remaining fetuses receiving GCs for several weeks unnecessarily.

Synthetic GCs are also frequently given to pregnant women for a number of autoimmune conditions, not unique to, but still present throughout pregnancy. The most common of these conditions is asthma which affects approximately 11% of all Australian women.¹² Inhaled GCs are frequently prescribed to women both before and during pregnancy to manage asthma and prevent severe exacerbations. It has been shown that the use of inhaled GC reduces the risk of having a low birth weight baby compared to asthmatics who have not taken inhaled corticosteroids.¹³ Furthermore, studies have shown that inhaled GC do not affect fetal GC regulated pathways and are relatively safe for the fetus during pregnancy.¹⁴ However, some studies have reported a link between high doses of inhaled GC and fetal abnormalities.¹⁵ In addition to inhaled GC, pregnant women are often prescribed oral synthetic GCs such as prednisolone in response to severe asthma exacerbations. While minimising the effects of an asthma exacerbation and preventing further attacks is important for preventing severe fetal outcomes including preterm birth, membrane related disorders and hypertensive

disorders,¹⁶ prednisolone has been associated with an increased risk of preeclampsia,¹⁷ premature delivery and low birth weight.¹⁸ Prednisolone is also often prescribed to women suffering from idiopathic thrombocytopenic purpura and systemic lupus erythematosus. Prednisolone improves patient platelet counts in idiopathic thrombocytopenic purpura but the effects on the developing fetus are largely unknown. While GCs are routinely used to treat systemic lupus erythematosus and have been shown to improve the rate of live births, GCs have also been shown to increase the incidence of premature delivery.¹⁹ Prednisone is even used to treat severe forms of morning sickness which can itself lead to maternal weight loss which may in turn lead to fetal growth restriction.²⁰ Synthetic GCs are often administered to women with medical conditions which are likely to result in an impaired intrauterine environment. As such, in the human it is often difficult to distinguish the effects of GC exposure from the condition itself.

Natural Glucocorticoids

Elevated levels of cortisol are produced endogenously in times of stress and as such the effects of cortisol on fetal development are likely to be hard to separate from the effects of the stimuli causing the increase in plasma cortisol levels. Due to the wide array of stimuli that can lead to an increase in cortisol levels, the timing and duration of increased cortisol exposure is wide and varied and fetal outcome is likely to be dependent on both of these factors. Stress during pregnancy in association with increased maternal cortisol levels has been shown to impair mental and motor development in the young infant²¹ and lead to low birth weight and reduced birth length, while miscarriages in early pregnancy have been associated with increased cortisol levels in the first three weeks after conception.²² Women suffering from depression have increased cortisol levels and are more likely to have children with increased cortisol levels who also have inferior behavioural scores in orientation, excitability, reflex and withdrawal based on the Brazelton Neonatal Behavioral Assessment scale.²³ Similarly, women exposed to extremely stressful events such as physical partner abuse and disasters such as the 11th of September 2001 terrorist attack on the World Trade Centre, have babies with reduced birth weight²⁴ and head circumference,²⁵ respectively.

The role of the placenta and fetal sex on glucocorticoid exposure

The passage of GCs from mother to fetus is regulated largely by the actions of the placental "glucocorticoid barrier". The main constituent of this barrier is the enzyme 11 β hydroxysteroid dehydrogenase type 2 (11 β HSD-2) which converts GCs such as cortisol, corticosterone and prednisolone into their inactive 11 keto metabolites, cortisone, 11-dehydrocorticosterone and prednisone, respectively. Additionally, a placental transporter known as the multidrug resistant protein (MDR1-also known as p-glycoprotein 1, p-gp1), actively transports GCs away from the fetal blood supply, thus protecting the fetus from excess

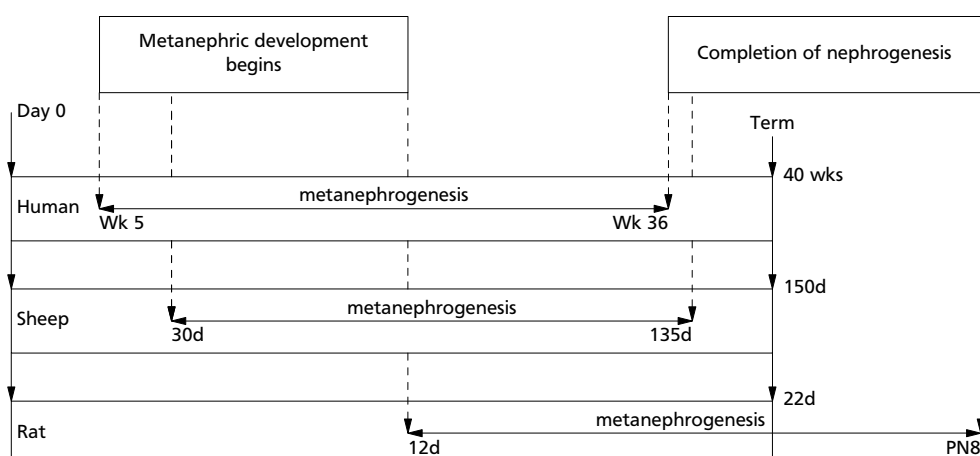


Figure 1. Representation of the period of kidney development in the human, the sheep and the rat. Metanephric (permanent kidney) development commences at week 5 of gestation in the human, at 30 days in the sheep and at day 12 in the rat. Note that metanephrogenesis is completed in the human at week 36 of gestation and in sheep at 130d, some weeks prior to term birth (human; 40 weeks (wks), sheep (150d). Importantly after the period of nephrogenesis, no new nephrons will form. In contrast, the rat is born quite immature and as can be seen, the rat kidneys continue to form nephrons after term birth at 22 days with completion of nephrogenesis at postnatal day (PN) 8. It can be seen that if glucocorticoids were administered at the commencement of the nephrogenic period, this would indeed be at very different times of gestation in the different species, for example, it would be administered at 1/8 of gestation in the human, 1/3 of gestation in the sheep and mid-gestation in the rat.

maternal GC exposure. Collectively these components minimize materno-fetal GC transfer in a sex specific and GC specific manner. It has been known for a long time that 11 β HSD-2 has different efficiencies at metabolising different GC, particularly in reference to natural compared to synthetic GC. Cortisol inactivation by 11 β HSD-2 is thought to be high under normal physiological condition with approximately 90% of cortisol being converted into cortisone²⁶ (although this value varies greatly). A number of studies have investigated the efficiency of 11 β HSD-2 in metabolising various synthetic GCs and while the absolute values vary considerably, as a general rule they all show that synthetic GCs are metabolized but much less efficiently by placental 11 β HSD-2. One of the early studies investigating the capacity of the human placenta to inactivate synthetic GCs used an *in-vitro* 11 β HSD-2 activity assay and determined that 67% of cortisol, 51% of prednisolone, 2% of DEX and 7% of betamethasone were broken down into their 11-keto metabolites.²⁷ A more recent study using purified placental 11 β HSD-2 rather than a placental homogenate has reported that inhaled GCs such as budesonide and fluticasone are not metabolized at all by the placenta and that prednisolone (23%), betamethasone (approximately 20%) and DEX (13%) are all partially metabolized.²⁸ Results using this more recent *in-vitro* technique suggests that 11 β HSD-2 is more efficient at metabolising synthetic GCs than previously thought. The sex of the fetus also impacts the 11 β HSD-2 activity of the placenta. It has been shown that placentas from female fetuses born within 72 hours of betamethasone administration had higher 11 β HSD-2 activity levels

compared to placentas from male fetuses.²⁹ Thus, it has been proposed that placentas from female fetuses respond more appropriately to a GC exposure providing greater protection from excess exposure than do placentas from male fetuses.

Experimental studies of glucocorticoid exposure

Experimental studies in a wide-variety of animals including sheep, rabbits, rats, mice and baboons have shown that prenatal GC administration at various doses and at different time-points during pregnancy have differential effects on the fetus, the developing organs, and on long-term physiology of the adult offspring (see Table 2 for summary). These studies can largely be divided into those that examined the *direct* effects of GC exposure on the growth and development of the fetus and those that examined the long term or *fetal programming* effects of GC exposure on adult health. Most animal studies have administered synthetic GC during a specific part of pregnancy. Of critical importance when interpreting outcomes following GC exposure in animal studies, is an appreciation of the differences between species in the *timing* of organ development. This is especially true for rodents that are born with relatively immature organs, particularly brains and kidneys, compared to the human. As an example, Figure 1 demonstrates the period of renal development during gestation in the human, sheep and rat. If GC were administered at 0.6 gestation (corresponding to 24 weeks in the human and day 13 in the rat), the relative development of the kidneys would be very different with the human kidney relatively developed and undergoing

rapid nephrogenesis whilst the rat would be in the very first stages of renal development.

Although there is a large body of literature examining the effects of various environmental stresses (which would be expected to raise endogenous GCs) on fetal development, in this review we shall focus on the animal studies which have administered a known dose of natural GCs in a manner similar to that of synthetic GCs.

Effects of maternal glucocorticoid on placental structure and expression of placental transporters

As noted above, the ability of natural and synthetic GCs to cross the placenta can vary markedly. The presence of 11 β HSD-2 in the placenta protects the fetus from excess maternal GC exposure, however this barrier is incomplete and levels of 11 β HSD-2 decrease towards late gestation, therefore making the fetus more vulnerable to effects of excess maternal GC.³⁰ Additionally, GCs themselves can have direct effects on placental development which may indirectly affect the fetus. In the rat, maternal DEX (1 μ g/ml in maternal drinking water) from 13-22d of gestation (term=22d) impairs fetal growth at term in association with decreased placental labyrinth VEGF-a gene expression, labyrinth weight, labyrinth volume and maternal and fetal blood space surface area and volume³¹ suggesting that DEX can impair placental vasculogenesis which in turn may affect placental and fetal growth. A similar study using the same DEX treatment protocol found that DEX reduced placental leptin transport and placental leptin receptor expression (Ob-Rb, long form of leptin receptor) and proposed that reduced fetal leptin levels may contribute to reduced fetal growth.³² GC exposure may also impair fetal development by affecting placental nutrient transfer, particularly by reducing glucose and amino acid transporter abundance, although studies have also shown improved placental transport as a compensatory mechanism for improved fetal outcome in GC exposed foetuses.³³

In the spiny mouse, mid-gestation DEX administration (23d of gestation, term=40d) has been shown to alter placental structure and gene expression, where following 60h of treatment, male and female fetuses had similar reductions in placental expression of insulin-like-growth factor1 and its receptor (IGF1, IGF1R) and in solute carrier family 2, facilitated glucose transporter 1 (SLC2A1).³⁴ In contrast on 37d gestation, male DEX-exposed fetuses exhibited an *increase* in maternal blood sinusoids (blood spaces) and a *decrease* in expression of SLC2A1, whereas the female fetuses had a *decrease* in maternal blood sinusoid spaces and an *increase* SLC2A1 and mitogen activated protein-kinase 2 (MAP2K) expression.³⁴ In the C57/BL6 strain mouse, we have shown that maternal DEX administration (1 μ g/kg/h at 12d gestation for 60h) during the period of rapid placental growth caused a significant reduction in placental weight and protein expression of MAPK but an elevation in expression of 11 β HSD-2 and vascular endothelial growth factor-a (VEGF-a) in the placenta from female fetuses only.³⁵ Very recently we have reported that administration

of equipotent doses of the natural GC, CORT (33 μ g/kg/h at 12d gestation for 60h) has markedly different effects to the synthetic DEX on the murine placental development.³⁶ In contrast to DEX treatment, maternal CORT did not directly affect placental weight during the period of treatment but resulted in increased weight of placentas from males some 2 days after exposure. In contrast to the effects of DEX, MAP2K1 gene expression was increased and VEGF-a mRNA decreased during exposure in placentas from males only. Similar to DEX, CORT increased expression of 11 β HSD-2 protein in females only.

Some of these studies lend support to the hypothesis that the placenta plays a major role in determining the sex specific outcomes following maternal GC exposure.

Effects of maternal GC on fetal growth

Numerous studies have shown that maternal administration of synthetic GCs can alter fetal growth but this phenotype is strongly dependent on the dosing and timing of exposure during gestation.³⁷ Repeated administration of betamethasone (0.1mg/kg) to the pregnant rabbit either early in pregnancy (19-20d) or late in pregnancy (25-26d) significantly reduced fetal survival, fetal birth weight and placental weights on 27d gestation (term=30d).³⁸ Furthermore, while fetal viability was worse with repeated administration *early* in pregnancy fetal growth was more adversely affected following treatment in *late* gestation. In the sheep, Ikegami *et al.*³⁹ showed that repeated administration of betamethasone (0.5 mg/kg) in increasing doses commencing on 104d gestation to the pregnant ewe caused a dose-dependent increase in postnatal lung function in male and female lambs. However, increasing doses also caused significant fetal growth restriction.³⁹ Prolonged maternal DEX administration (100 μ g/kg/day) in the rat in late gestation (15-20d) caused a reduction in body weights of offspring at birth while administration early in gestation (1-10d) had no effect on body weights at birth.⁴⁰ In the sheep, prolonged DEX administration early in gestation (25-45d), caused a transient reduction in fetal body weights at 135d,⁴¹ however weights were normalized by 2 months of age and there were no long-term effects on renal function or arterial pressure of the offspring.⁴² Furthermore, short periods of maternal DEX (0.2mg/kg) administration on 11-12, 13-14, 15-16, 17-18, 19-20d in the rat, caused moderate reductions in body weight in the very late gestation treated groups only (17-18 and 19-20).⁴³ Our own studies using short DEX administration early in gestation (0.48mg/h/d, 26-28d) in sheep showed no effect on offspring birth weight⁴² whilst in the mouse DEX (1 μ g/kg/h) at 12.5-14.5d caused a transient reduction in fetal size at 14.5d that was fully restored by 17.5d.³⁵ Johnson *et al.*⁴⁴ have shown that apart from fetal weight, administration of 2 mg/d of betamethasone to the pregnant rhesus monkeys from 120-133d, reduced weights of multiple organs, including the lung, liver, brain, heart, pancreas and adrenals at 165 days of gestation (term=165d).

A recent study in the marmoset monkey has shown a significant effect of variation in maternal cortisol on

Glucocorticoid exposure during development

Table 2. Effects of maternal glucocorticoid treatment at different times of gestation on overall growth and development of organs and health outcomes in the offspring.

| Species | Type of Glucocorticoid administered | Timing of exposure | Phenotype |
|---|--|--|---|
| Mice (C57/BL6 term=19d) | Dexamethasone | 12-14.5d | transient ↓ body weight at 14.5d, normalized at 17.5d ³⁵ |
| Rat (term=22d) | Dexamethasone | 1-10 or 15-20d | ↓body weight (15-20d only) ⁴⁰ |
| Rabbit (term=30d) | Betamethasone | 19-20d or 25-26d | ↓fetal survival, birth weight, placental weight; (19-20d) ↓↓fetal survival; (25-26d) ↓↓ fetal growth ³⁸ |
| Sheep (term=150d) | Betamethasone | 104d-124d (weekly intervals) | ↑lung function, ↓body weight at delivery ³⁹ |
| Sheep | Dexamethasone | 25-45d | Transient reduction in body weight at 135d, normalized in 2 month old offspring ⁴² |
| Marmosets | Natural variation in cortisol across gestation | across gestation | Decrease BMI change in early postnatal life, increased catch-up growth in adolescence ⁴⁵ |
| Effects on placenta | | | |
| Mice (C57/BL6) | Dexamethasone | 12-14.5d | ↓placental weight and MAPK, ↑VEGF-A ³⁵ |
| Spiny mouse (term=40d) | Dexamethasone | 23-26.5d | 37d fetus; Males (↑blood space, ↓SLC2A1), Females (↓blood space, ↑SLC2A1, MAPK) ³⁴ |
| Rat | Dexamethasone (maternal drinking water) | 13-22d | ↓placental labyrinth VEGF-A gene expression, volume, ↓maternal and fetal blood space ³¹ |
| Effects on the heart | | | |
| Rat | Dexamethasone | 17-22d | ↑cardiomyocyte proliferation ⁴⁸ |
| Sheep | Dexamethasone | 114-128d | ↑fetal heart weights, ↑ LV/ RV wall thickness ⁴⁹ |
| Effects on metabolic organs | | | |
| Rat | Dexamethasone | 15-20d | ↑BP, ⁵⁷ Glucose intolerance, hyperglycemia, hyperinsulinemia, ⁶⁵ ↑liver triglycerides, fatty liver on a high-fat diet ⁶⁶ |
| Sheep | Betamethasone | 104d-124d (weekly intervals) | altered gene expression of glucocorticoid-dependent hepatic enzymes ⁶⁴ |
| Effects on brain and behaviour | | | |
| Rat | ↑CORT induced <i>via</i> maternal stress | 15-20d | ↑plasma CORT in offspring in response to novelty ⁶¹ |
| Rat | Dexamethasone or CORT | 18-19d | ↓sexual behaviour; altered hippocampal structures in male Milder phenotype with CORT ⁶³ |
| Sheep | Repeated maternal stress <i>via</i> isolation from flock | 30-100d or 100-150d | ↑stress responses in offspring (greater effects in the 30-100d group) ⁶² |
| Rhesus monkey (term=165d) | Dexamethasone (single or multiple courses) | 132-133d | ↓hippocampal size, ↓number of pyramidal neurons ^{54,55} |
| Baboons (term=187d) | Betamethasone | 0.6, 0.65, 0.7 of gestation | Attention disorders in 3 year old female offspring only ⁶⁰ |
| Effects on kidney structure, function and blood pressure | | | |
| Rat | Dexamethasone | 1-10d-15-20d | ↑BP in both sexes (15-20d only) ⁴⁰ |
| Rat | Dexamethasone | Day 1-term | ↓GFR, ↑proteinuria (20d offspring), ↑BP (60d offspring) ⁶⁹ |
| Rat | Dexamethasone | Multiple 11-12, 13-14, 15-16, 17-18, 19-20 | ↓nephron number in male and female (15-16d or 17-18d), ↑BP male (15-16d or 17-18d) and female (17-18d) ^{43,67} |
| Spiny mouse | Dexamethasone | 23-26.5d | 20 week offspring: ↓ Nephron number, ↔ BP Male and Female ⁶⁸ |
| Sheep | Betamethasone | 80-81d | ↓Nephron number ↓GFR in males only, ↑BP male & female, ⁷⁰ Impaired baroreflex in female only, ⁷¹ altered tubular sodium response to Ang1-7 in 6 month old male and female ⁷² |
| Rat | CORT | 14-15d | ↓nephron number 30d, ↑BP 120d male and female offspring ⁴⁷ |

BP (blood pressure), LV (left ventricle), RV (right ventricle), MAPK (mitogen activated protein kinase), VEGF (vascular endothelial growth factor), SLC2A1 solute carrier family 2, facilitated glucose transporter 1, CORT (corticosterone or cortisol), GFR (glomerular filtration rate)

postnatal growth of the offspring. Mustoe *et al.*,⁴⁵ reported that while exposure to high levels of cortisol during gestation did not alter body mass index (BMI) at birth, the

offspring that were exposed to high levels of cortisol exhibited a slower change in BMI shortly after birth, followed by a significant catch-up period in adolescence,

with high cortisol during the first trimester having a stronger relationship to postnatal growth compared to third trimester cortisol elevation. In contrast, early gestational short-term administration of cortisol (5mg/h/d, 26-28d) in the sheep⁴⁶ or corticosterone (0.8mg/kg/d, 14-15d) in the rat⁴⁷ did not alter birth weights or weights of the offspring.

Effects of glucocorticoids on cardiac structure and function

Maternal DEX (0.48µg/d) administration from 17d in the rat resulted in larger hearts with an increase in cardiomyocyte proliferative index.⁴⁸ Maternal hydrocortisone (80mg/d) administration from 119d in sheep has also been shown to increase fetal heart weight and thickness of left and right ventricular walls.⁴⁹ Cortisol administration directly to the near-term sheep fetus has been shown to increase heart mass that is associated with increased cardiomyocyte proliferation rather than hypertrophy.⁵⁰ Miller *et al.*⁵¹ have shown that following induction of intrauterine growth restriction (IUGR) in late gestation sheep fetuses by single umbilical artery ligation (SUAL), antenatal betamethasone administration caused a greater decrease in body weight of IUGR fetuses compared to controls with female fetuses being more growth retarded. Recent studies in this model showed that while maternal betamethasone administration did not interfere with cardiac adaptations in the IUGR fetus (increases in left ventricular pressure and contraction and relaxation), betamethasone administered IUGR fetuses did have an enhanced responsiveness of the left ventricle β -adrenoreceptors⁵² which could increase susceptibility to adult cardiac dysfunction. In the rat, late gestation DEX exposure (100-200 µg/kg/d) causes upregulation in expression of Ca²⁺ binding proteins; proteins that have been shown to cause cardiac impairment and premature death; in both the 21 day old fetus and in adulthood suggesting that upregulation in expression of these genes could impair adult cardiac function in offspring exposed to elevated maternal GC.⁵³ All these findings taken together suggest GC administration either *via* the mother or directly to the fetus causes structural adaptations that may impair cardiac function in adulthood.

Brain structure and behaviour

A multitude of studies have shown that the brain is particularly susceptible to the effects of exogenous maternal GC. Administration of DEX (5mg/kg) either in single or multiple doses on 132-133d to the pregnant rhesus monkey reduced the number of pyramidal neurons in the hippocampus and the number of granular neurons in the dentate gyrus of 135d and 162d old foetuses.⁵⁴ A reduction in hippocampal size and volume in 9 month old offspring was also observed.⁵⁵ A single injection of maternal betamethasone at 114d has been shown to increase both basal and stimulated cortisol levels in 1 year old sheep offspring whereas repeated injections did not.⁵⁶ Late gestation maternal DEX in the rat^{57,58} as well as mid-gestation administration in the primate⁵⁹ has been shown to permanently elevate basal GC levels and cause anxiety-like

behaviour in the offspring. A recent study showed that repeated betamethasone (175µg/kg/d) administration at 0.6, 0.65 and 0.7 of gestation in the pregnant baboon results in impaired learning and attention disorders in the 3 year old female offspring only, which was possibly due to alterations in hippocampal structure.⁶⁰

Similarly, maternal stress in the last third of gestation in the rat has been shown to cause elevations in corticosterone in response to novelty in the offspring.⁶¹ In sheep, repeated maternal stress induced by isolation from the flock during early (30-100d) and in late gestation (100-150d) augmented the stress response in the late-gestation fetus, however the effects were greater in the early gestation treatment group compared to the late treatment.⁶² A study examining adult male sexual behaviour in the Wistar rat, showed that maternal DEX (1mg/kg) and equipotent corticosterone (25mg/kg) administration very late in pregnancy (18-19d), caused a significant decrease in mounts and intromission latencies in the DEX group which was associated with reduced levels of plasma testosterone and dopamine and higher levels of androgen receptors in the hypothalamus, and increased dopamine receptors in both the hypothalamus and *nucleus accumbens*.⁶³ In contrast, the phenotype was much milder in the corticosterone group in which only a decrease in nucleus accumbens dopamine levels were observed.⁶³ The milder phenotype may be due to more corticosterone being inactivated at the level of the placenta by 11 β HSD-2 as mentioned earlier, thus less getting to the fetus. These studies indicate that prenatal GC administration at various stages of pregnancy have consequences on the developing brain, resulting in adverse effects on behaviour and learning ability similar to what has been observed in humans²¹ as mentioned earlier.

Metabolic type effects

Weekly betamethasone administration from 104d in the pregnant ewe causes alterations in the liver, particularly in gene expression of glucocorticoid-dependent hepatic enzymes (increased fetal hepatic 11 β HSD-1 gene and protein and increased corticosteroid-binding protein gene) in 146d old fetuses, indicating impaired glucose production.⁶⁴ Maternal DEX administration in the last third of pregnancy in the rat, results in hypertension,⁵⁷ hyperglycemia, glucose intolerance and hyperinsulinemia⁶⁵ in adult offspring. Further studies in this model have shown that while prenatal DEX treatment does not cause obesity, it does increase liver triglycerides and susceptibility to fatty liver when offspring are placed on a high-fat diet⁶⁶ indicating prenatally programmed tissue-specific fat deposition.

Kidney structure, function and adult cardiovascular physiology

Ortiz and colleagues^{43,67} demonstrated that short-periods (2d) of exposure of the pregnant rat to DEX at specific times during gestation had varying effects on kidney structure and adult arterial pressure. Maternal DEX

(0.2mg/kg) administration on 15-16d or 17-18d caused a reduction in glomerular number in 2 month old male and female offspring. Both sexes from the 15-16d group but only male offspring from the 17-18d group were hypertensive, whilst glomerular filtration rates were normal in both sexes at 3 months of age.⁴³ In another study, Ortiz *et al.*⁶⁷ showed that only male offspring from the maternal DEX exposure at 13-14, 15-16 and 17-18d groups were hypertensive at 6 months of age but both male and female offspring from the 15-16d group demonstrated glomerulosclerosis at 6-9 months of age, with the severity being greater in male offspring.⁶⁷ However, a similar period of maternal DEX administration in mid-gestation to the spiny mouse caused a reduction in nephron number in the offspring, but had no effect on arterial pressure.⁶⁸ Woods & Weeks⁴⁰ found that maternal DEX administration (100µg/kg/d) in late gestation (15-20d) in the rat resulted in elevation in mean arterial pressure and a tendency for glomerular filtration rates per kidney weights to be lower in 20 week old male and female offspring, whereas administration early in gestation (1-10d) had no effect on arterial pressure or kidney function in adulthood. Celsi *et al.*⁶⁹ found that DEX (0.1mg/kg/d) from day 1 to parturition in the rat resulted in a significant reduction in glomerular number in 20 day offspring and reduction in glomerular filtration rate, increased proteinuria and arterial pressure in 60 day old offspring. In the sheep, short-term mid-gestation betamethasone administration (0.17mg/kg, 80-81d) decreased nephron number in the 135d fetus and in adult male and female offspring, but decreased glomerular filtration rate in the 17 month old male offspring only, while both male and female offspring had elevated arterial pressure.⁷⁰ In this same model, Shaltout *et al.*⁷¹ have reported enhanced sympathetic and hypothalamic-pituitary responses associated with impaired baroreceptor reflex in 42 day old betamethasone exposed female offspring and Tang *et al.*⁷² observed altered renal hemodynamic and tubular sodium excretion responses to Ang (1-7) in 6 month old male and female offspring.

Renal effects of exposure to excess natural GC have been less well studied. Celsi *et al.*⁶⁹ examined the effects of a natural GC (hydrocortisone) administration throughout gestation in the rat and finding no effect on fetal development and birth weights did not examine renal function or cardiovascular profile in these offspring. To our knowledge, our group has been the only one to-date to investigate the effects of the natural GC, corticosterone on kidney structure and adult blood pressure in the rat. We found that administration of corticosterone (0.8 mg/kg/d) on 14-15d caused a significant reduction in nephron number in 30 day old male and female offspring and elevations in arterial pressure in both sexes at 120 days of age.⁴⁷

Comparison of effects of short term synthetic or natural glucocorticoid exposure in sheep

Most of the experimental studies discussed above examined the effects of either synthetic or natural GC but not within a single animal model. In addition, the timing

and length of exposure varied considerably. This led us to ask: *would short term exposure to DEX or cortisol, at similar stages of gestation, have similar effects on development and long term outcomes?* Examining the effects of short-periods of elevation in the natural glucocorticoid in pregnancy is essential as women are likely to undergo significant periods of stress especially in early pregnancy which may be associated with getting used to the fact that they are pregnant or related to financial and partner support especially if the pregnancy was unplanned.²³⁻²⁵ Over the past 10-12 years, we have used a sheep model of early prenatal GC exposure to answer this question. Although our original studies were conducted using exposure to DEX (0.48mg/d for 48h between 26-28 days of gestation), our recent studies have used both DEX and cortisol (5mg/d between 26-28 days of gestation). This has enabled us to directly compare the outcomes of maternal exposure to these two GC. We hypothesized that the two GC would have similar effects but given the higher potency of DEX for the glucocorticoid receptor (GR) and the ability of the placenta to inactivate cortisol, *via* 11βHSD-2 as mentioned in earlier sections, the effects of DEX would be more profound. As shown in Table 3, although this hypothesis was found to be correct for some outcomes, in many instances, the phenotype observed was found to be GC specific and despite being less potent than DEX, the cortisol exposure elicited strong programming effects.

Both cortisol and DEX result in male and female offspring developing elevated blood pressure without changes in heart rate.^{42,46,73} However, the cardiovascular mechanism underlying this hypertension is GC specific. In the DEX treatment offspring, we found increases in cardiac output⁷⁴ whilst in the cortisol exposed offspring, peripheral resistance was increased.⁷⁵ Blood pressure responses to peripheral infusions of angiotensin II were not different⁷⁶ however, direct central (intracerebroventricular) administration of angiotensin II, caused an increased pressor responsiveness in animals exposed prenatally to DEX but not in those exposed to cortisol.⁷⁷ These differences in pressor responses were likely associated with differences in angiotensin II type 1 receptor (AT1R) expression as DEX treated sheep had an increased AT1R expression in the medulla oblongata whereas cortisol treated sheep did not.^{42,46}

We have thoroughly explored effects of GC exposure on the developing kidney. Whilst in the kidney, many outcomes appear to be relatively similar there are also subtle differences: both GC treatments resulted in fetuses with a nephron deficit^{78,89} increased fetal renal expression of components of the renin-angiotensin system⁸⁰ and increased expression of renal sodium channels.⁷⁸ However, differences were seen in renal GR gene expression which was increased in late gestation fetal kidneys of the DEX but not cortisol group.⁸¹ The prenatal GC treatment did not cause impairments in basal renal function (such as glomerular filtration rate, renal blood flow or sodium excretion) however, in response to a salt load, the DEX exposed offspring did not increase urine flow or decrease fractional sodium reabsorption to the same degree as

Table 3. Effects of prenatal exposure to DEX (0.48mg/h) or cortisol (5mg/h) between 26-28d of gestation on outcomes in sheep. Responses are compared to a saline infused control group.

| | DEX | Cortisol |
|--|--|--|
| Mean Arterial blood pressure | Increased in adult offspring (male & female) ⁴² | Increased in adult offspring (male & female) ⁴⁶ |
| Cardiac output | Increased in adult offspring ⁷⁴ | Unchanged ⁷⁵ |
| Total peripheral resistance | Unchanged ⁷⁴ | Increased in adult offspring ⁷⁵ |
| Pressor response to angiotensin II (peripheral intravenous infusion) ⁷⁶ | Unchanged | Unchanged |
| Pressor response to angiotensin II (intracerebroventricular) ⁷⁷ | Increased | Unchanged |
| Nephron number | Reduced ⁷⁹ | Reduced ⁷⁸ |
| Renal gene expression- (late gestation fetus) Renin-angiotensin system ⁸⁰ : | | |
| - Renin | Unchanged | Unchanged |
| - Angiotensinogen | Increased | Unchanged |
| - AT1R | Increased | Increased |
| - AT2R | Increased | Increased |
| Steroid receptors ⁸¹ | | |
| - GR | Increased | Unchanged |
| - MR | Increased | Unchanged |
| Sodium channels ⁷⁸ | Increased | Increased |
| Renal Function (adult): ⁷⁸ | | |
| - GFR | Unchanged | Unchanged |
| - Urinary sodium excretion | Unchanged | Unchanged |
| - Urinary response to dietary salt load | Impaired response | Unchanged |

DEX (dexamethasone), AT1R (Angiotensin II type 1 receptor), AT2R (Angiotensin II type 2 receptor), GR (glucocorticoid receptor), MR (mineralocorticoid receptor), GFR (glomerular filtration rate)

control or cortisol exposed animals.⁷⁸ Finally, we have found subtle differences in the metabolic outcomes following prenatal GC. Those offspring exposed to cortisol had elevated fasting glucose concentrations and hyperinsulinemia during the second phase of a glucose tolerance test. In contrast, prenatal maternal DEX administration induced a first-phase hyperinsulinemia and improved glucose tolerance.⁸²

These studies highlight that high levels of natural GC early in gestation are able to cross the placental glucocorticoid barrier and are thus capable of eliciting a range of deleterious effects on the developing fetus. In addition these findings provide strong evidence that excess exposure to synthetic and natural glucocorticoids early in gestation do not have identical effects on fetal development and long term outcomes for offspring.

Future areas for research

The evidence from animal studies makes it imperative for there to be long term follow up of babies and children that have been exposed to prenatal GCs, particularly known doses of synthetic GC. Given GC

treatment prior to premature delivery has only been commonplace in the last 30-40 years, these adults are now of an age where an increased risk of disease such as hypertension, may become more apparent. The mechanisms underlying the differential effects of synthetic and natural GCs are not fully understood and require further investigation. Apart from having different potencies on the GR, both the natural and synthetic GCs can bind to other receptors. For example, cortisol can bind to and activate the mineralocorticoid receptor (MR) whereas DEX can bind to, but not activate the MR.⁸³ Conversely, DEX, but not cortisol, exerts some of its effects through the pregnane-X receptors (PXR), especially PXR2.⁸⁴ In addition, GCs have been shown to cause epigenetic changes⁸⁵ and it has been shown that betamethasone administration in the late-gestation guinea pig changes DNA methylation states in multiple organs in the male offspring which are also present in the next generation.⁸⁶ This raises the possibility that increased levels of maternal stress or factors increasing maternal cortisol levels could have similar multigenerational effects as observed in the presence of the synthetic GC. Finally, detailed investigation of the placenta

following exposure to naturally occurring GCs is required and all analysis must take into account the sex of the fetus.

Conclusion

It is undoubtable that administration of synthetic GC to women threatening preterm labour has resulted in increased survival of preterm babies over the past decade. However, mounting evidence from experimental studies suggest that fetal exposure to short or long-term periods of GCs at various stages of development has detrimental effects on metabolic, behavioural, renal and cardiovascular function. The challenge facing obstetricians is balancing the short-term favourable effects with long-term adverse outcomes. As was discussed earlier, there is significant variation in how much GC a particular obstetrician may prescribe to their patients, with courses ranging from the standard 2 injections, 24h apart to multiple courses over weeks until birth or until the risk of preterm delivery passes. From animal studies, we know that increasing courses of GC have more detrimental effects on the fetus and adult offspring, thus strict guidelines for GC administration during pregnancy should be set-out and adhered to.

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