GLUCOCORTICOIDS AS REGULATORY SIGNALS DURING INTRAUTERINE DEVELOPMENT

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ABBREVIATIONS

ACTH, Adrenocorticotrophic hormone
AII, Angiotensin II
AT1, Angiotensin II type 1 receptor
ACE, Angiotensin converting enzyme
CRH, Corticotrophin releasing hormone
GH, Growth hormone
GR, Glucocorticoid receptor
HPA, hypothalamic-pituitary-adrenal axis
11β-HSD, 11β-hydroxysteroid dehydrogenase type 1 or 2
IGF, Insulin-like growth factor –I or –II
MR, Mineralocorticoid receptor
PG, Prostaglandin E_2 or $F_{2\alpha}$
PGDH, 15-hydroxy prostaglandin dehydrogenase
PGHS. Prostaglandin H_2 synthase
PNMT, Phenylethanolamine-N-methyl-transferse
POMC, Pro-opiomelanocorticotrophin
T_4, Thyroxine
T_3, Tri-iodothyronine
UCP, Uncoupling protein

NEW FINDINGS

What is the topic of this review?

- This review discusses the role of the glucocorticoids as regulatory signals during intrauterine development.

- It examines the functional significance of these hormones as maturation, environmental and programming signals in determining offspring phenotype.

What advances does it highlight?

- It focuses on the extensive nature of the regulatory actions of these hormones
- It highlights the emerging data that these actions are mediated, in part, by the placenta, other endocrine systems and epigenetic modifications of the genome.

ABSTRACT

Glucocorticoids are important regulatory signals during intrauterine development. They act as maturational, environmental and programming signals that modify the developing phenotype to optimise offspring viability and fitness. They affect development of a wide range of fetal tissues by inducing changes in cellular expression of structural, transport and signalling proteins, which have widespread functional consequences at the whole organ and systems levels. Glucocorticoids, therefore, activate many of the physiological systems that have little function *in utero* but are vital at birth to replace the respiratory, nutritive and excretory functions previously carried out by the placenta. However, by switching tissues from accretion to differentiation, early glucocorticoid overexposure in response to adverse conditions can program fetal development with longer term physiological consequences for the adult offspring which can extend to the next generation. The developmental effects of the glucocorticoids can be direct on fetal tissues with glucocorticoid receptors or mediated by changes in placental function or other endocrine systems. At the molecular level, glucocorticoids can act directly on gene transcription via their receptors or indirectly by epigenetic modifications of the genome. This review examines the role and functional significance of glucocorticoids as regulatory signals during intrauterine development and discusses the mechanisms by which they act *in utero* to alter the developing epigenome and ensuing phenotype.

INTRODUCTION

In adults, glucocorticoids are stress hormones with a wide range of physiological effects which aid survival in environmental conditions that challenge homeostasis. They maintain blood flow and a supply of nutrients and oxygen to tissues when these resources are either scarce or in increased demand. In the fetus, glucocorticoids have an even broader range of functions during normal and adverse conditions (Fowden *et al.,* 1998). Towards term, they act as the primary maturational signal in the developmental sequence that prepares the fetus for the new challenges of extra-uterine life. Earlier in gestation, they can act as environmental cues that alter fetal development in relation to resource availability for intrauterine growth (Fowden & Forhead, 2009). This improves viability both before and at birth, particularly when conditions are sub-optimal for survival. However, by changing fetal tissue development, early exposure to excess glucocorticoids modifies the phenotype with life-long physiological consequences (Fowden *et al.,* 1998; 2006; Harris & Seckl, 2011; Moisiadis & Matthews, 2014). Glucocorticoids are, therefore, also programming signals that adapt intrauterine development to optimise offspring fitness (Fowden & Moore, 2012). This review examines the role

and functional significance of glucocorticoids as regulatory signals during intrauterine development and discusses the mechanisms by which they act *in utero*.

GLUCOCORTICOID BIOAVAILABILITY DURING DEVELOPMENT

Glucocorticoids are present in both the maternal and fetal circulation and increase in concentration towards term in most species, even in normal conditions (Fowden & Forhead, 2009). Generally, concentrations are higher in the mother than fetus so maternal glucocorticoids enter the placenta and fetus down their concentration gradient for most of gestation. In sheep and mice, for example, 70-80% of the glucocorticoid in the fetal circulation is of maternal origin when the fetal adrenal cortex is relatively inactive or incapable of steroidogenesis (Hennessy *et al.* 1982; Huang *et al.,* 2011). Consequently, environmental stressors that raise maternal glucocorticoids levels also increase feto-placental glucocorticoid exposure (Fowden & Forhead, 2009). Once the fetal hypothalamic-pituitary-adrenal (HPA) axis is activated in late gestation, feto-placental glucocorticoid exposure can be increased independently of the mother by elevating glucocorticoid secretion from the fetal adrenal cortex. This occurs towards term as part of the normal maturational process and earlier in gestation in response to circadian cues and environmental stressors like fetal hypoglycaemia and hypoxaemia (Fowden *et al..,* 1998). In fetal mice, the adrenal cortices are sufficiently active in late gestation to supply glucocorticoids to the mother as significant amounts of corticosterone are detected in the circulation of adrenalectomised dams (Cottrell *et al.,* 2011). Glucocorticoids can, therefore, cross the placenta in both directions depending on the concentration gradient. Finally, at the tissue level, glucocorticoid bioavailability can be altered independently of maternal or fetal glucocorticoid concentrations by changes in tissue 11β-hydroxysteroid dehydrogenase (11β-HSD) activity (Chapman *et al.,* 2013). This enzyme exists in two isoforms; 11β-HSD1 which predominantly regenerates active glucocorticoids from their inactive metabolites and is expressed in a wide range of fetal tissues and 11β-HSD2, which converts active glucocorticoids to their inactive forms and is high in activity in the placenta and fetal kidney (Chapman *et al.,* 2013). In addition, P-glycoprotein, a member of the ABCB family of multidrug resistance transporters, is expressed in the placenta and fetal brain where it transports glucocorticoids out of the cells (Pappas *et al.,* 2014). Placental P-glycoprotein and 11β-HSD2, therefore, act as barriers to placental glucocorticoid transfer and normally limit fetal exposure to the higher maternal glucocorticoid concentrations. However, in several species, placental expression of these two proteins declines towards term and during adverse conditions earlier in gestation (Fowden & Forhead, 2004; Kalabis *et al.,* 2005; Mark *et al.,* 2009). This will further increase placental glucocorticoid exposure alongside

the concomitant increases in fetal and maternal glucocorticoid concentrations with consequences for placental gene expression and transport phenotype. In addition, ontogenic and environmentally induced changes in the activity of the two isoforms in fetal tissues exert fine control over glucocorticoid bioavailability locally in a tissue specific fashion (Fowden *et al.,* 2008; Harris & Seckl, 2011; Chapman *et al.,* 2013) Ultimately, the actions of the glucocorticoids are controlled by the glucocorticoid (GR) and mineralocorticoid receptors (MR), which change in abundance developmentally and in response to environmental cues in a tissue specific manner (Brown *et al.,* 1996; Speirs *et al.,* 2004; Cuffe *et al.,* 2012).

Clinically, synthetic glucocorticoids are given to pregnant women to treat asthma, arthritis and adrenal insufficiency, and to improve neonatal viability in threatened preterm delivery (McKinlay *et al.,* 2014). These drugs are also given to mares to treat laminitis and to cattle to induce delivery at or near term (Johnson *et al.,* 2002; Mansell *et al.,* 2006). Synthetic glucocorticoids are up to 20 times more potent than their natural counterparts and are poorly inactivated by 11β-HSD2 (Chapman *et al.,* 2013). They also bind predominantly to GR whereas natural glucocorticoids bind to both GR and MR. Clinical and experimental treatment with synthetic glucocorticoids, therefore, also alters fetal growth and development but the specific effects, mechanisms of action and long term outcomes of this treatment often differ from those seen in response to natural glucocorticoids (Jellyman *et al.,* 2015).

DEVELOPMENTAL EFFECTS OF THE GLUCOCORTICOIDS

Glucocorticoids have a wide range of developmental effects in normal and adverse conditions, particularly in tissues essential for survival immediately at birth (Figure 1). They induce changes in tissue expression of cytostructural proteins, receptors, transporters, ion channels and enzymes (Fowden & Forhead, 2004; 2009). These changes lead to alterations in the morphology, metabolism, hormone sensitivity and biochemical composition of fetal tissues with widespread functional consequences at the whole organ and systems levels (Fowden *et al.,* 2006; Harris & Seckl, 2011; Moisiadis & Matthews, 2014; Rog-Zielinska *et al.,* 2014). Glucocorticoids, therefore, activate many of the physiological processes that have little or no function *in utero* but which are vital to extra-uterine life, such as pulmonary gas exchange, hepatic gluconeogenesis, gastrointestinal digestion and thermogenesis (Figure 1). For instance, the effects of cortisol in increasing hepatic glycogen deposition, glucogneogenic enzyme activity and adrenoreceptor abundance mean that the neonatal

liver can produce glucose endogenously in response to hypoglycaemia and other stresses (Fowden et al., 1998; 2006). However, by stimulating differentiation, glucocorticoids limit the degree of further cell proliferation in many fetal tissues. For example in fetal sheep, maturational concentrations of cortisol stimulate terminal differentiation of proliferative mono-nucleated cardiomyocytes to their binucleated form that can still hypertrophy but not divide (Thornburg *et al.,* 2011). Certainly, in sheep, the prepartum cortisol surge decreases the fetal growth rate overall in parallel with the maturation of individual fetal tissues (Fowden et al., 1996). In addition to visceral tissues essential for neonatal survival, glucocorticoids also affect growth and development of tissues like the brain, heart and skeletal muscle that are important to offspring viability and fitness in the longer term (Champagne *et al.,* 2006; Brown, 2014; Rog-Zielinska *et al.,* 2014). In the fetal heart, both basal glucocorticoid concentrations and preterm cortisol infusion have been shown to increase cardiac weight relative to body weight during late gestation (Rog-Zielinska *et al.,* 2014). At maturational cortisol concentrations, this cardiac growth is believed to reflect the concomitant hypertension but infusion of cortisol at subpressor doses directly into the coronary vessels stimulates cardiomyocyte expression of proliferative markers in association with increased cardiac weight in fetal sheep during late gestation (Giraud *et al.,* 2006). Thus, in some fetal tissues, glucocorticoids appear to stimulate cell proliferation while also activating the cellular pathways which eventually switch the cell cycle to differentiation, perhaps at a critical concentration or duration of increased exposure.

Early overexposure to glucocorticoids in response to environmental insults appears to induce this switch from tissue accretion to differentiation prematurely in several fetal tissues. While this has beneficial effects on neonatal viability if pre-term delivery occurs, it reduces fetal growth overall and decreases total cell numbers in certain tissues (Fowden *et al.,* 1996; 1998). These effects of early glucocorticoid overexposure are tissue specific and dose dependent. They are also influenced by gestational age at the time of overexposure. For instance, increasing fetal cortisol levels to prepartum values enhances activity of most of the key rate limiting enzymes in the hepatic gluconeogenic pathway at 130 days but not at 115 days (Fowden & Forhead, 2009). Similarly, the fetal cardiac and pulmonary transcriptomes induced by early glucocorticoid exposure are distinct from those seen at term (McGillick *et al.,* 2013; Richards *et al.,* 2014). The effects of early glucocorticoid exposure, therefore, do not recapitulate entirely the maturational effects of the prepartum cortisol surge.

By altering fetal growth and development, early exposure to natural or synthetic glucocorticoids has long term effects on the physiological phenotype of the offspring. The changes in tissue structure and function induced *in utero* may persist throughout life or emerge at natural transitions in the life course such as birth, puberty or pregnancy (Wada, 2008). Alternatively, they may become apparent only after postnatal environmental challenges such as undernutrition and hypoxia (Daskalakis *et al.,* 2013). In experimental animals, prenatal glucocorticoid overexposure by fetal or maternal administration affects the same range of tissues and cellular processes in adulthood as seen prenatally (Harris & Seckl, 2011; Moisiadis & Matthews, 2014; Rog-Zielinska *et al.,* 2014). This leads to adult dysfunction of multiple physiological systems including the cardiovascular, metabolic, endocrine and nervous systems as well as organs not functional *in utero* like the reproductive tract (Fowden *et al.,* 2006; Harris & Seckl, 2011). There are also changes in adult behaviour, memory and appetite regulation after prenatal glucocorticoid exposure in rodents (Huang, 2011; Bouret *et al.,* 2015). These adult phenotypic changes tend to be more pronounced with prenatal overexposure to synthetic than natural glucocorticoids and become more obvious with advancing age, possibly due to the reduced functional reserve capacity of adult tissues prematurely switched from accretion to differentiation *in utero* by glucocorticoid overexposure (Somm *et al.,* 2012; Jellyman *et al.,* 2015).

Early glucocorticoid exposure *in utero* affects at least two generations. In rodents, guinea pigs and sheep, the adult F1 metabolic and endocrine phenotype induced by F0 maternal glucocorticoid administration has been shown to be inherited to the F2 generation without further intervention (Drake *et al.,* 2005; Iqbal *et al.,* 2012; Long *et al.,* 2013a&b). In rodents, these intergenerational effects are transmitted through both the maternal and paternal line, which may reflect physiological changes in the pregnant F1 mother and/or a germ line epigenetic component (Drake *et al.,* 2005). However, the effects of F0 glucocorticoid treatment do not persist to the F3 generation never exposed to glucocorticoid excess, which indicates that any glucocorticoid-induced epigenetic marks are not stably inherited (Drake *et al.,* 2005). In humans, prenatal treatment with synthetic glucocorticoids also alters blood pressure and indices of insulin resistance postnatally but the adult physiological outcomes of early life glucocorticoid overexposure in humans appear to be less pronounced than in experimental animals and, to date, have unknown intergenerational consequences (Harris & Seckl 2011; McKinlay *et al.,* 2014). This relates to the longer human lifespan as the majority of infants clinically exposed to synthetic glucocorticoids *in utero* are still relatively young adults. The outcomes of clinical glucocorticoid treatment of pregnant women for their infants are also likely to depend on the type and dose of synthetic glucocorticoid given, its route of

administration and on the timing and duration of treatment during pregnancy (Brownfoot *et al.,* 2013; Aiken *et al.,* 2014; Romeijko-Wolniewicz *et al.,* 2014).

MECHANISMS OF ACTION

The glucocorticoids can regulate intrauterine development via a wide range of different mechanisms from the systems to the gene level. Their actions may be direct or mediated indirectly via changes in placental function or production of other growth regulatory hormones and growth factors (Vaughan *et al.,* 2011; Fowden & Forhead, 2014). Together these actions alter the availability of substrates for intrauterine growth, their metabolic fate and the expression of many nutrient and hormone sensitive genes that control intrauterine development.

Placental effects

Maternal glucocorticoids administration during late gestation reduces placental weight in all species studied to date (Vaughan *et al.,* 2011). In sheep, fetal or maternal glucocorticoid treatment also alters the gross placentome morphology in association with reduced expression of proliferative markers and increased expression of apoptotic factors (Ward *et al.,* 2002; Braun *et al.,* 2015). In rodents, maternal administration of natural or synthetic glucocorticoids leads to reduced vessel volumes and/or surface area in the placenta, which decreases the potential for transplacental transfer of substances via simple and facilitated diffusion as well as by active transport (Vaughan *et al.,* 2012; 2013; O'Connell *et al.,* 2013). In several species, direct measurements of placental nutrient transport have shown that raising fetal or maternal glucocorticoid concentration reduces placental transfer of glucose and amino acids during treatment (Vaughan *et al.,* 2011; 2012; 2015a; Audette *et al.,* 2014). These changes are often accompanied by alterations in expression of the respective transporters and in nutrient consumption by the placenta (Fowden *et al.,* 2015). Increased glucocorticoid exposure of the placenta from either the maternal or fetal circulation also alters its expression of *Vegf*, 11β-HSD2, the renin-angiotensin system and a range of other genes involved in its endocrine function (Fowden *et al.,* 2008; 2015). In rodents and humans, there is also evidence for sexual dimorphism in these placental responses (Stark *et al.,* 2011; Cuffe *et al.,* 2011; 2012). Glucocorticoids can, therefore, induce alterations in placental morphological, transport and metabolic phenotype that contribute to the fetal growth restriction observed when glucocorticoid concentrations are raised. Moreover, the intergenerational effects of the glucocorticoids may also

involve modification of the placental transport phenotype as amino acid transport and transporter expression are altered in the F2 placenta of F0 dexamethasone treated rodent dams (Drake *et al.,* 2011; Vaughan *et al.,* 2015b).

Effects on other endocrine systems

Glucocorticoids affect the development and function of many other endocrine systems in the fetus and placenta, particularly during late gestation (Fowden & Forhead, 2004; Fowden *et al.,* 2015). In the placenta, they affect production of sex steroids, eicosanoids, lactogenic hormones and adipokines (Table 1). In the fetus, they affect almost all of the endocrine systems functional during late gestation including the HPA axis itself (Table 1). Even in endocrine systems like the endocrine pancreas where there is little direct experimental evidence for maturational effects of glucocorticoids, there are ontogenic changes in fetal β cell function that parallel the prepartum cortisol increment (Aldoretta *et al.,* 1998; Fowden *et al.,* 2004). In contrast, in rodents, glucocorticoids are essential for normal development of the pancreatic β cells before the maturational increase in their concentration towards term (Gesina *et al.,* 2006; Blondeau *et al.,* 2012).

Glucocorticoids can act either directly via altered transcription of hormone genes or indirectly via the enzymes involved in hormone synthesis and metabolism (Table 1). They also alter expression of several growth factors and hormone receptors in feto-placental tissues including the insulin like growth factors (IGFs), growth hormone (GH) receptor, adrenoreceptors, angiotensin (AII) receptors, MR and GR themselves (Table 1) . In addition, they can affect components of the intracellular signalling pathways for hormones and growth factors and, hence, have local tissue effects independent of the circulating concentrations. For example, glucocorticoids affect the abundance of several proteins in the insulin signalling pathway in fetal skeletal muscle near term although not expression of the insulin receptor itself (Jellyman *et al.,* 2012; Blanco *et al.,* 2014). Maturationally, these effects of the glucocorticoids enable the sensitivity of the endocrine axes to be set appropriately for extrauterine life and, in some species, also ensure that fetal maturation is co-ordinated with the onset of parturition.

The glucocorticoid-induced changes in fetal endocrine function lead to prepartum increases in the fetal concentration of several other hormones including T_3, IGF-I, leptin and adrenaline, which, in

turn, have independent effects on fetal tissue growth and function (Fowden & Forhead, 2009). Indeed, the increases in plasma T_3 and leptin and the decreases in tissue IGF-II abundance may mediate, in part, the maturational effects of the prepartum cortisol surge. Thyroid hormones, in particular, have been shown to be essential for aspects of glucocorticoid-stimulated maturation of the renin-AII system, the somatotrophic axis, hepatic glucogenic capacity, lung liquid reabsorption and terminal differentiation of the cardiac myocytes in fetal sheep during late gestation (Fowden & Forhead, 2014). Similarly, leptin appears to have an important role in modifying the actions of cortisol on gluconeogenic enzyme activities in ovine fetal liver near term (Forhead *et al.,* 2008). It may also be involved in the developmental changes in cardiac function and hypothalamic appetite regulatory circuits during the perinatal period (Vickers & Sloboda, 2012; Bouret *et al.,* 2015). Indeed, ontogenic and environmentally induced changes in the circulating concentration and tissue receptor abundance of these hormones may explain, in part, the gestational dependence of some of the developmental outcomes associated with raised glucocorticoid levels *in utero*.

During adverse conditions earlier in gestation, the glucocorticoid-induced endocrine changes have immediate benefits to fetal survival by maintaining pregnancy and modifying fetal growth to match the more limited supply of oxygen and nutrients. Development of certain fetal tissues like the brain are preserved in these circumstances at the expense of others such as the liver and skeletal muscle. However, if the endocrine changes persist after restoration of normal conditions, they may become more detrimental to intrauterine development and compromise the ability of the fetus to respond to subsequent environmental challenges. For example, fetal HPA responses to stressful stimuli such as hypoxia are known to be altered by prior exposure to glucocorticoids (Fletcher *et al.,* 2003; Jellyman *et al.,* 2004). Similarly, early activation of the switch in the somatotrophic axis from local GH independent IGF-I synthesis to GH dependent hepatic production of endocrine IGF-I is likely to affect growth of many fetal tissues long after normal fetal glucocorticoid levels are restored. Indeed, changes in endocrine function induced by early glucocorticoid exposure *in utero* are known to persist after birth to alter the adult endocrine environment (Moisiadis & Matthews, 2014). For instance, prenatal glucocorticoid exposure alters adult HPA function at every level of the axis from the brain to tissue glucocorticoid bioavailability (Jellyman *et al.,* 2015). In turn, these programmed changes in HPA function may contribute to the adult cardiometabolic dysfunction associated with prenatal glucocorticoid overexposure. The regulatory effects of glucocorticoids on intrauterine development, therefore, involve multiple interactions between different endocrine systems, when glucocorticoids are acting both as maturational and environmental signals.

Epigenetic effects

At the molecular level, glucocorticoids act via several different mechanisms to alter gene expression. Bound to their receptors they act as enhancer binding proteins that activate or repress gene expression via interaction with glucocorticoid response elements (GRE) in promotor or other regulatory regions of the genome (Adcock *et al.,* 2004). For example, cortisol-stimulated down-regulation of *IGF2* gene transcription in ovine fetal liver is mediated preferentially by a GRE in the 5' regulatory region of the untranslated leader exon 7 containing the P4 promotor (Li *et al.,* 1998). In contrast, cortisol-induced up-regulation of *IGF1* gene transcription in fetal liver is likely to be more indirect as there are no GREs in the vicinity of the promotor regions of this gene (Fowden *et al.,* 2011). Postnatally, glucocorticoids are also known to alter gene expression more indirectly via epigenetic modifications of the genome and chromatin structure (Weaver, 2009). These include DNA methylation, histone modifications and changes in abundance of non-coding long and microRNAs (Adcock *et al.,* 2004; Weaver, 2009). However, relatively little is known about the epigenetic effects of glucocorticoids *in utero.*

Glucocorticoids have been shown to alter GRE methylation of the tyrosine aminotransferase gene in fetal rat hepatocytes treated *in vitro* (Thomassin *et al.,* 2001). They also affect methylation of the differentially methylated region (DMR) and the imprinted control region (ICR) of the *Igf2* gene in fetal liver of the F1 and F2 generation, respectively, of F0 rat dams dexamethasone exposed during late pregnancy (Drake *et al.,* 2011). In guinea pigs, maternal betamethasone treatment changes global methylation of the placenta, liver, kidney and adrenal gland of fetuses delivered 1 and 14 days after ending treatment (Crudo *et al.,* 2012). This treatment also alters DNA methylation and histone h3 lysine 9 acetylation in the fetal hippocampus (Crudo *et al.,* 2013b). The glucocorticoid-induced methylomes are tissue specific and change with time after treatment (Crudo *et al.,* 2012). In the placenta and fetal kidney, the epigenetic changes were accompanied by altered expression of DNA methyltransferase 1 and 3b involved in maintenance and *de novo* DNA methylation, respectively (Crudo *et al.,* 2012). Changes in global methylation were also seen in adult tissues of the F1 and F2 offspring of betamethasone treated pregnant guinea pigs, although the methylation patterns differ from those seen prenatally (Crudo *et al.,* 2012). More specifically, demethylation of the GR promotor and increased GR gene expression are seen in kidneys of adult rats dexamethasone overexposed during late gestation (Wyrwoll *et al.,* 2007). Betamethasone treatment of guinea pigs in late pregnancy also causes differential GR binding to a large number of different gene promotors

and methylation of specific GREs in the fetal hippocampal MR gene (Crudo *et al.,* 2013a). Taken together, these studies indicate that glucocorticoids alter DNA methylation through a range of different mechanisms from alterations in chromatin structure and global methylation pathways to more specific changes in the methylation state of CpG islands and individual CpG dinucleotides within promotors or other more distant regulatory regions of the genes (Grange *et al.,* 2001; Zhang *et al.,* 2013).

Glucocorticoids may also act by changing the imprint status of growth regulatory imprinted genes like *IGF2,* which are expressed from only one allele in a parent of origin manner. In both human and ovine liver, the *IGF2* gene switches from solely paternal to biallelic expression in parallel with the prepartum cortisol surge (Fowden *et al.,* 2011). Whether these changes in *IGF2* expression are due to altered expression of the *H19* derived non-coding RNA or to changes in methylation at the DMR and/or ICR of the *IGF2-H19* locus remains unknown. Nor it is clear whether any of the epigenetic effects are due directly to the glucocorticoids or mediated indirectly by changes in placental function or other hormone concentrations. Certainly, maternal treatment with synthetic glucocorticoids in rats alters placental transfer of methyl donors essential for DNA methylation (Wyrwoll *et al.,* 2012). In addition, T_3 is a key component of the epigenetic mechanism regulating GR promotor methylation in the rat brain in response to neonatal stresses (Zhang *et al.,* 2013). Glucocorticoid exposure in early life, therefore, affects the developing epigenome through a number of different routes with dynamic consequences for epigenetic marks throughout the lifespan of the offspring. Indeed, the long term outcomes of prenatal glucocorticoid overexposure are likely to be modified continually by postnatal factors, often independent of the physical environment, like the level of maternal care and the reproductive history of the mother reflected in the quality and glucocorticoid content of the milk during lactation (Zhang *et al.,* 2006; Hinde *et al.,* 2015).

CONCLUSIONS

Glucocorticoids act as maturational, environmental and programming signals in regulating intrauterine development (Figure 2). Towards term, they activate the physiological systems that replace the respiratory, nutritive and excretory functions of the placenta immediately at birth. Earlier in gestation, they act as environmental signals that modify the fetal epigenome and optimise the phenotype for the prevailing conditions *in utero*. At the tissue level, these maturational and developmental effects are achieved largely by switching tissues from accretion to differentiation

(Figure 2). When this glucocorticoid-triggered switch is activated prematurely, there can be permanent changes in cell type, tissue morphology and organ function that have long term physiological consequences for the offspring, particularly as it ages. If the postnatal environment differs from that signalled *in utero*, the glucocorticoid-induced changes in offspring phenotype may become maladaptive and lead to accelerated ageing with early onset of degenerative cardiometabolic diseases characteristic of old age (Figure 2). Nevertheless, the developmental adaptations induced *in utero* by glucocorticoids maximise the chances of survival to reproductive age and, hence, transmission of genes onto the next generation. However, the molecular mechanisms involved in these processes remain largely unknown. Nor is it clear to what extent the regulatory actions of the glucocorticoids are sex-linked or modifiable by postnatal interventions when outcomes are likely to be detrimental to adult health.

FIGURE LEGENDS

Figure 1: Schematic diagram of the developmental effects of the glucocorticoids on visceral tissues of fetal sheep during late gestation. Data from Fowden & Forhead, 1998; 2004; 2009; 2014 and Fowden *et al.,* 1998; 2015.

Figure 2: Schematic diagram of the regulatory roles of glucocorticoids during intrauterine development and their functional significance for offspring fitness in pre- and post-natal life.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRBUTION

Both authors contributed equally to the compiling of the literature, its analysis and the writing of the paper.

Figure 1

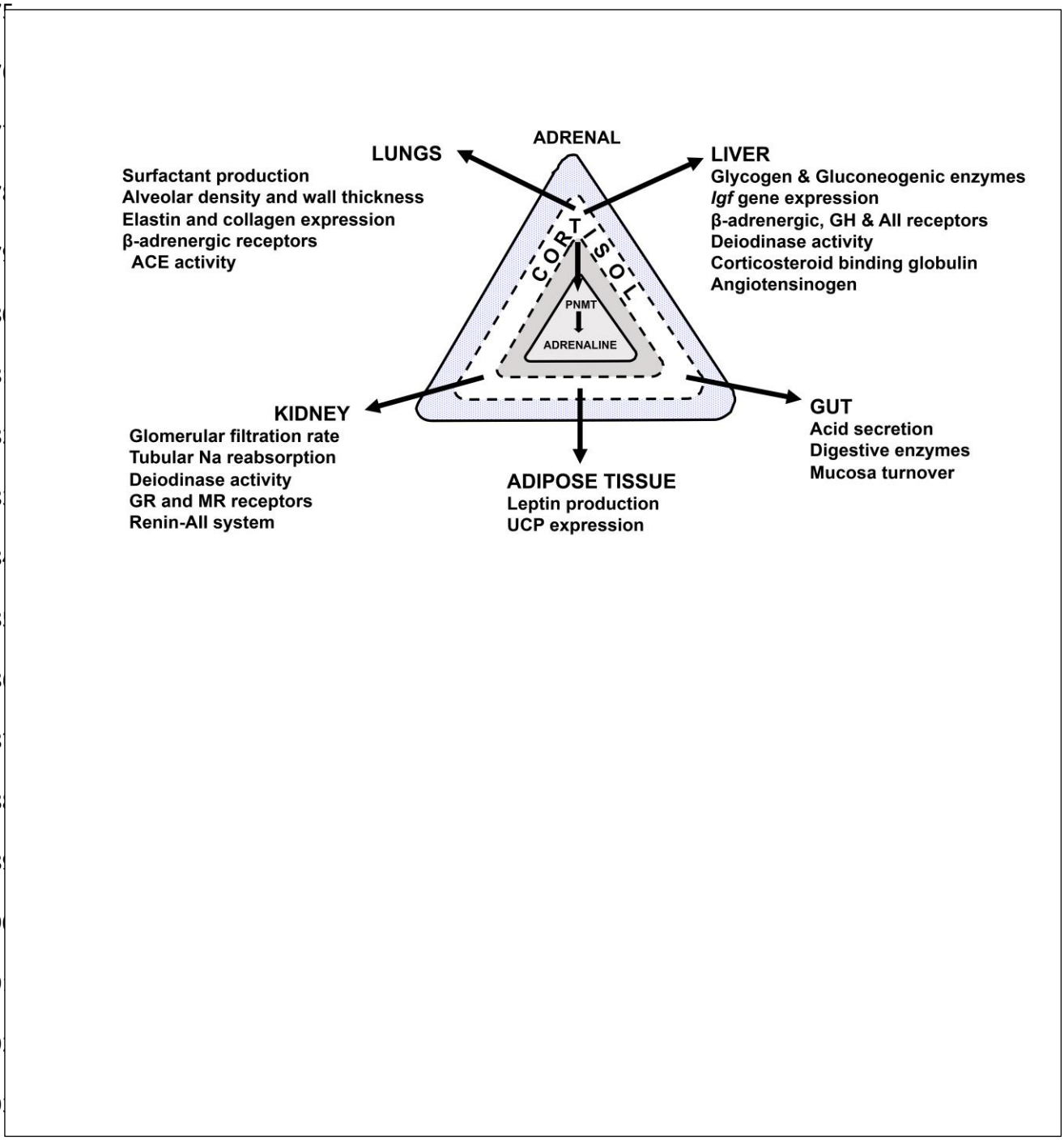
ADRENAL
LUNGS
Surfactant production
Alveolar density and wall thickness
Elastin and collagen expression
β-adrenergic receptors
ACE activity
LIVER
Glycogen & Gluconeogenic enzymes
Igf gene expression
β-adrenergic, GH & AII receptors
Deiodinase activity
Corticosteroid binding globulin
Angiotensinogen
CORTISOL
PNMT
ADRENALINE
KIDNEY
Glomerular filtration rate
Tubular Na reabsorption
Deiodinase activity
GR and MR receptors
Renin-AII system
GUT
Acid secretion
Digestive enzymes
Mucosa turnover
ADIPOSE TISSUE
Leptin production
UCP expression

Figure 2

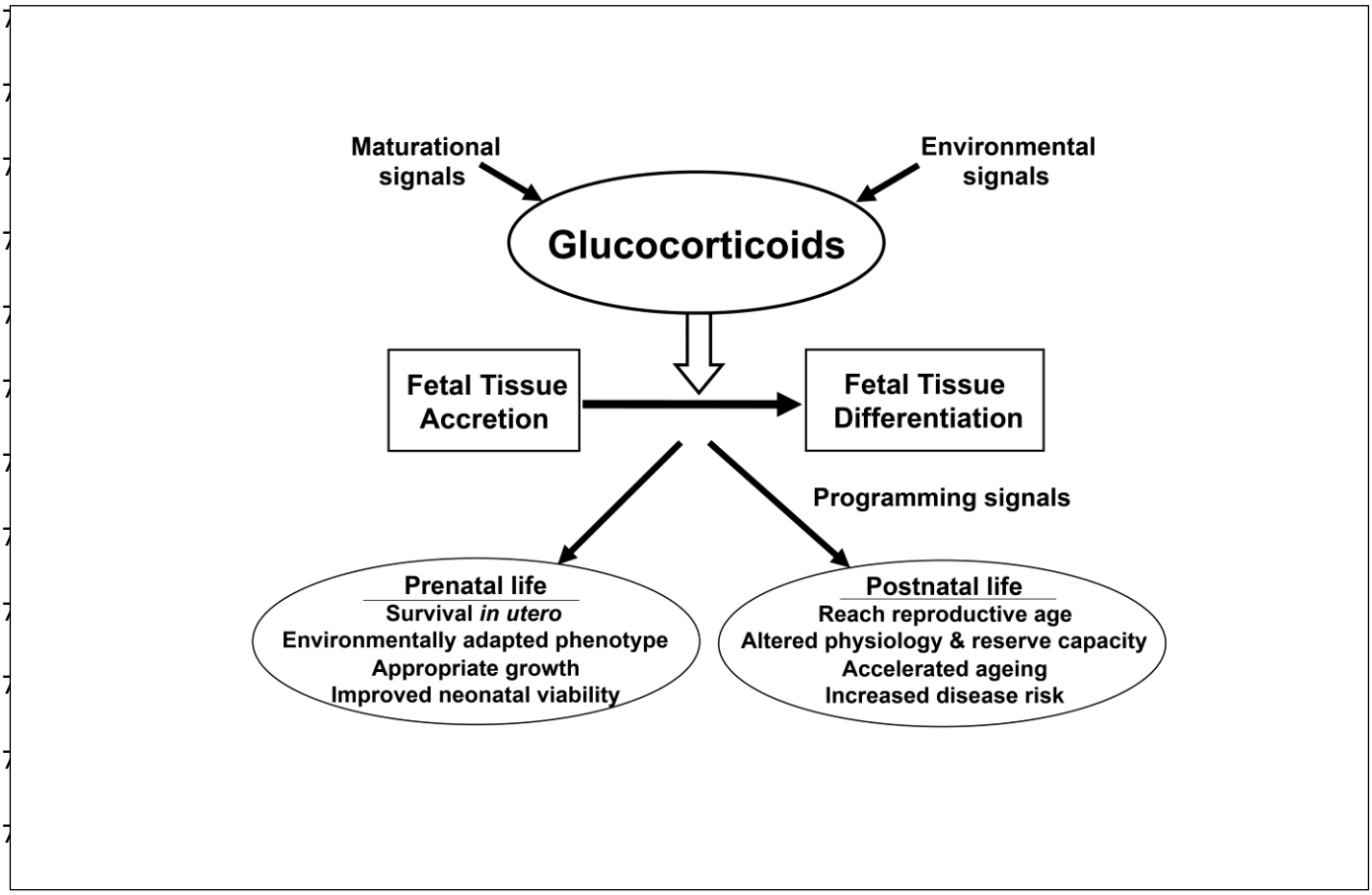
Maturational signals
Environmental signals
Glucocorticoids
Fetal Tissue Accretion
Fetal Tissue Differentiation
Programming signals
Prenatal life
Survival *in utero*
Environmentally adapted phenotype
Appropriate growth
Improved neonatal viability
Postnatal life
Reach reproductive age
Altered physiology & reserve capacity
Accelerated ageing
Increased disease risk

Table 1: Endocrine systems affected by maternal and/or fetal glucocorticoids in sheep and rodents			
Endocrine system	**Hormones**	**Processes involved**	**Tissue**
Sex steroids	Progesterone	Cytochrome $P450_{17\alpha}$	Placenta
	Estrogens	C17-20 lyase, Aromatase	
Eicosanoids	$PGE_{2,}$ $PGF_{2\alpha}$	PGHS, PGDH	Placenta
Lactogenic hormones	Placental lactogen	Hormone mRNA	Placenta
	Prolactins	Hormone mRNA	
Adipokines	Leptin	Hormone mRNA/protein	Plasma, Placenta, Adipose tissue
HPA axis	CRH	Hormone mRNA	Hypothalamus
	ACTH/POMC	Prohormone convertases	Pituitary
	Glucocorticoids	ACTH receptors, Cytochrome $P450_{17\alpha}$ 11βHSD1, 11βHSD2, GR	Adrenal cortex Placenta, Brain, Peripheral tissues
Renin- Angiotensin System	Angiotensin II	Renin protein Angiotensinogen protein Angiotensin converting enzyme AT1 receptors mRNA	Plasma Liver, Plasma Lungs Kidney
Catecholamines	Adrenaline	Phenylethanolamine N-methyl-transferase Adrenoreceptors	Adrenal medulla Liver, Heart
Somatotrophic axis	GH IGF-I IGF-II	GH receptor mRNA Peptide mRNA Peptide mRNA	Liver Liver , Skeletal muscle Liver , Skeletal muscle
Thyroid Hormone axis	T_4, T_3	Deiodinase D1, D2, D3	Placenta, Liver

724 Data from Fowden & Forhead, 2004; 2009; 2014; Fowden *et al.,* 2015; Jellyman *et al.,* 2015

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