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**GLUCOCORTICOIDS AS REGULATORY SIGNALS DURING INTRAUTERINE DEVELOPMENT**

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36 **ABBREVIATIONS**

- 37
- 38 ACTH, Adrenocorticotrophic hormone
- 39 All, Angiotensin II
- 40 AT1, Angiotensin II type 1 receptor
- 41 ACE, Angiotensin converting enzyme
- 42 CRH, Corticotrophin releasing hormone
- 43 GH, Growth hormone
- 44 GR, Glucocorticoid receptor
- 45 HPA, hypothalamic-pituitary-adrenal axis
- 46 11 $\beta$ -HSD, 11 $\beta$ -hydroxysteroid dehydrogenase type 1 or 2
- 47 IGF, Insulin-like growth factor –I or –II
- 48 MR, Mineralocorticoid receptor
- 49 PG, Prostaglandin E<sub>2</sub> or F<sub>2 $\alpha$</sub>
- 50 PGDH, 15-hydroxy prostaglandin dehydrogenase
- 51 PGHS. Prostaglandin H<sub>2</sub> synthase
- 52 PNMT, Phenylethanolamine-N-methyl-transferase
- 53 POMC, Pro-opiomelanocorticotrophin
- 54 T<sub>4</sub>, Thyroxine
- 55 T<sub>3</sub>, Tri-iodothyronine
- 56 UCP, Uncoupling protein

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59 **NEW FINDINGS**

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61 What is the topic of this review?

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- 63 • This review discusses the role of the glucocorticoids as regulatory signals during intrauterine
- 64 development.
- 65
- 66 • It examines the functional significance of these hormones as maturation, environmental and
- 67 programming signals in determining offspring phenotype.
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69 What advances does it highlight?

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

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

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104 **INTRODUCTION**

105

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

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

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## 122 **GLUCOCORTICOID BIOAVAILABILITY DURING DEVELOPMENT**

123 Glucocorticoids are present in both the maternal and fetal circulation and increase in concentration  
124 towards term in most species, even in normal conditions (Fowden & Forhead, 2009). Generally,  
125 concentrations are higher in the mother than fetus so maternal glucocorticoids enter the placenta  
126 and fetus down their concentration gradient for most of gestation. In sheep and mice, for example,  
127 70-80% of the glucocorticoid in the fetal circulation is of maternal origin when the fetal adrenal  
128 cortex is relatively inactive or incapable of steroidogenesis (Hennessy *et al.* 1982; Huang *et al.*,  
129 2011). Consequently, environmental stressors that raise maternal glucocorticoids levels also  
130 increase fetoplacental glucocorticoid exposure (Fowden & Forhead, 2009). Once the fetal  
131 hypothalamic-pituitary-adrenal (HPA) axis is activated in late gestation, fetoplacental glucocorticoid  
132 exposure can be increased independently of the mother by elevating glucocorticoid secretion from  
133 the fetal adrenal cortex. This occurs towards term as part of the normal maturational process and  
134 earlier in gestation in response to circadian cues and environmental stressors like fetal  
135 hypoglycaemia and hypoxaemia (Fowden *et al.*, 1998). In fetal mice, the adrenal cortices are  
136 sufficiently active in late gestation to supply glucocorticoids to the mother as significant amounts of  
137 corticosterone are detected in the circulation of adrenalectomised dams (Cottrell *et al.*, 2011).  
138 Glucocorticoids can, therefore, cross the placenta in both directions depending on the concentration  
139 gradient. Finally, at the tissue level, glucocorticoid bioavailability can be altered independently of  
140 maternal or fetal glucocorticoid concentrations by changes in tissue 11 $\beta$ -hydroxysteroid  
141 dehydrogenase (11 $\beta$ -HSD) activity (Chapman *et al.*, 2013). This enzyme exists in two isoforms; 11 $\beta$ -  
142 HSD1 which predominantly regenerates active glucocorticoids from their inactive metabolites and is  
143 expressed in a wide range of fetal tissues and 11 $\beta$ -HSD2, which converts active glucocorticoids to  
144 their inactive forms and is high in activity in the placenta and fetal kidney (Chapman *et al.*, 2013). In  
145 addition, P-glycoprotein, a member of the ABCB family of multidrug resistance transporters, is  
146 expressed in the placenta and fetal brain where it transports glucocorticoids out of the cells (Pappas  
147 *et al.*, 2014). Placental P-glycoprotein and 11 $\beta$ -HSD2, therefore, act as barriers to placental  
148 glucocorticoid transfer and normally limit fetal exposure to the higher maternal glucocorticoid  
149 concentrations. However, in several species, placental expression of these two proteins declines  
150 towards term and during adverse conditions earlier in gestation (Fowden & Forhead, 2004; Kalabis  
151 *et al.*, 2005; Mark *et al.*, 2009). This will further increase placental glucocorticoid exposure alongside

152 the concomitant increases in fetal and maternal glucocorticoid concentrations with consequences  
153 for placental gene expression and transport phenotype. In addition, ontogenic and environmentally  
154 induced changes in the activity of the two isoforms in fetal tissues exert fine control over  
155 glucocorticoid bioavailability locally in a tissue specific fashion (Fowden *et al.*, 2008; Harris & Seckl,  
156 2011; Chapman *et al.*, 2013) Ultimately, the actions of the glucocorticoids are controlled by the  
157 glucocorticoid (GR) and mineralocorticoid receptors (MR), which change in abundance  
158 developmentally and in response to environmental cues in a tissue specific manner (Brown *et al.*,  
159 1996; Speirs *et al.*, 2004; Cuffe *et al.*, 2012).

160

161 Clinically, synthetic glucocorticoids are given to pregnant women to treat asthma, arthritis and  
162 adrenal insufficiency, and to improve neonatal viability in threatened preterm delivery (McKinlay *et*  
163 *al.*, 2014). These drugs are also given to mares to treat laminitis and to cattle to induce delivery at or  
164 near term (Johnson *et al.*, 2002; Mansell *et al.*, 2006). Synthetic glucocorticoids are up to 20 times  
165 more potent than their natural counterparts and are poorly inactivated by 11 $\beta$ -HSD2 (Chapman *et*  
166 *al.*, 2013). They also bind predominantly to GR whereas natural glucocorticoids bind to both GR and  
167 MR. Clinical and experimental treatment with synthetic glucocorticoids, therefore, also alters fetal  
168 growth and development but the specific effects, mechanisms of action and long term outcomes of  
169 this treatment often differ from those seen in response to natural glucocorticoids (Jellyman *et al.*,  
170 2015).

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## 172 **DEVELOPMENTAL EFFECTS OF THE GLUCOCORTICOIDS**

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

184 liver can produce glucose endogenously in response to hypoglycaemia and other stresses (Fowden  
185 et al., 1998; 2006). However, by stimulating differentiation, glucocorticoids limit the degree of  
186 further cell proliferation in many fetal tissues. For example in fetal sheep, maturational  
187 concentrations of cortisol stimulate terminal differentiation of proliferative mono-nucleated  
188 cardiomyocytes to their binucleated form that can still hypertrophy but not divide (Thornburg *et al.*,  
189 2011). Certainly, in sheep, the prepartum cortisol surge decreases the fetal growth rate overall in  
190 parallel with the maturation of individual fetal tissues (Fowden et al., 1996). In addition to visceral  
191 tissues essential for neonatal survival, glucocorticoids also affect growth and development of tissues  
192 like the brain, heart and skeletal muscle that are important to offspring viability and fitness in the  
193 longer term (Champagne *et al.*, 2006; Brown, 2014; Rog-Zielinska *et al.*, 2014). In the fetal heart,  
194 both basal glucocorticoid concentrations and preterm cortisol infusion have been shown to increase  
195 cardiac weight relative to body weight during late gestation (Rog-Zielinska *et al.*, 2014). At  
196 maturational cortisol concentrations, this cardiac growth is believed to reflect the concomitant  
197 hypertension but infusion of cortisol at subpressor doses directly into the coronary vessels  
198 stimulates cardiomyocyte expression of proliferative markers in association with increased cardiac  
199 weight in fetal sheep during late gestation (Giraud *et al.*, 2006). Thus, in some fetal tissues,  
200 glucocorticoids appear to stimulate cell proliferation while also activating the cellular pathways  
201 which eventually switch the cell cycle to differentiation, perhaps at a critical concentration or  
202 duration of increased exposure.

203

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

216

217 By altering fetal growth and development, early exposure to natural or synthetic glucocorticoids has  
218 long term effects on the physiological phenotype of the offspring. The changes in tissue structure  
219 and function induced *in utero* may persist throughout life or emerge at natural transitions in the life  
220 course such as birth, puberty or pregnancy (Wada, 2008). Alternatively, they may become apparent  
221 only after postnatal environmental challenges such as undernutrition and hypoxia (Daskalakis *et al.*,  
222 2013). In experimental animals, prenatal glucocorticoid overexposure by fetal or maternal  
223 administration affects the same range of tissues and cellular processes in adulthood as seen  
224 prenatally (Harris & Seckl, 2011; Moisiadis & Matthews, 2014; Rog-Zielinska *et al.*, 2014). This leads  
225 to adult dysfunction of multiple physiological systems including the cardiovascular, metabolic,  
226 endocrine and nervous systems as well as organs not functional *in utero* like the reproductive tract  
227 (Fowden *et al.*, 2006; Harris & Seckl, 2011). There are also changes in adult behaviour, memory and  
228 appetite regulation after prenatal glucocorticoid exposure in rodents (Huang, 2011; Bouret *et al.*,  
229 2015). These adult phenotypic changes tend to be more pronounced with prenatal overexposure to  
230 synthetic than natural glucocorticoids and become more obvious with advancing age, possibly due to  
231 the reduced functional reserve capacity of adult tissues prematurely switched from accretion to  
232 differentiation *in utero* by glucocorticoid overexposure (Somm *et al.*, 2012; Jellyman *et al.*, 2015).

233

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

250 administration and on the timing and duration of treatment during pregnancy (Brownfoot *et al.*,  
251 2013; Aiken *et al.*, 2014; Romejko-Wolniewicz *et al.*, 2014).

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253

## 254 **MECHANISMS OF ACTION**

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

261

### 262 **Placental effects**

263 Maternal glucocorticoids administration during late gestation reduces placental weight in all species  
264 studied to date (Vaughan *et al.*, 2011). In sheep, fetal or maternal glucocorticoid treatment also  
265 alters the gross placentome morphology in association with reduced expression of proliferative  
266 markers and increased expression of apoptotic factors (Ward *et al.*, 2002; Braun *et al.*, 2015). In  
267 rodents, maternal administration of natural or synthetic glucocorticoids leads to reduced vessel  
268 volumes and/or surface area in the placenta, which decreases the potential for transplacental  
269 transfer of substances via simple and facilitated diffusion as well as by active transport (Vaughan *et al.*  
270 *et al.*, 2012; 2013; O'Connell *et al.*, 2013). In several species, direct measurements of placental  
271 nutrient transport have shown that raising fetal or maternal glucocorticoid concentration reduces  
272 placental transfer of glucose and amino acids during treatment (Vaughan *et al.*, 2011; 2012; 2015a;  
273 Audette *et al.*, 2014). These changes are often accompanied by alterations in expression of the  
274 respective transporters and in nutrient consumption by the placenta (Fowden *et al.*, 2015).  
275 Increased glucocorticoid exposure of the placenta from either the maternal or fetal circulation also  
276 alters its expression of *Vegf*, 11 $\beta$ -HSD2, the renin-angiotensin system and a range of other genes  
277 involved in its endocrine function (Fowden *et al.*, 2008; 2015). In rodents and humans, there is also  
278 evidence for sexual dimorphism in these placental responses (Stark *et al.*, 2011; Cuffe *et al.*, 2011;  
279 2012). Glucocorticoids can, therefore, induce alterations in placental morphological, transport and  
280 metabolic phenotype that contribute to the fetal growth restriction observed when glucocorticoid  
281 concentrations are raised. Moreover, the intergenerational effects of the glucocorticoids may also



282 involve modification of the placental transport phenotype as amino acid transport and transporter  
283 expression are altered in the F2 placenta of F0 dexamethasone treated rodent dams (Drake *et al.*,  
284 2011; Vaughan *et al.*, 2015b).

285

## 286 **Effects on other endocrine systems**

287 Glucocorticoids affect the development and function of many other endocrine systems in the fetus  
288 and placenta, particularly during late gestation (Fowden & Forhead, 2004; Fowden *et al.*, 2015). In  
289 the placenta, they affect production of sex steroids, eicosanoids, lactogenic hormones and  
290 adipokines (Table 1). In the fetus, they affect almost all of the endocrine systems functional during  
291 late gestation including the HPA axis itself (Table 1). Even in endocrine systems like the endocrine  
292 pancreas where there is little direct experimental evidence for maturational effects of  
293 glucocorticoids, there are ontogenic changes in fetal  $\beta$  cell function that parallel the prepartum  
294 cortisol increment (Aldoretta *et al.*, 1998; Fowden *et al.*, 2004). In contrast, in rodents,  
295 glucocorticoids are essential for normal development of the pancreatic  $\beta$  cells before the  
296 maturational increase in their concentration towards term (Gesina *et al.*, 2006; Blondeau *et al.*,  
297 2012).

298

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

311

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

314 turn, have independent effects on fetal tissue growth and function (Fowden & Forhead, 2009).  
315 Indeed, the increases in plasma T<sub>3</sub> and leptin and the decreases in tissue IGF-II abundance may  
316 mediate, in part, the maturational effects of the prepartum cortisol surge. Thyroid hormones, in  
317 particular, have been shown to be essential for aspects of glucocorticoid-stimulated maturation of  
318 the renin-All system, the somatotrophic axis, hepatic glucogenic capacity, lung liquid reabsorption  
319 and terminal differentiation of the cardiac myocytes in fetal sheep during late gestation (Fowden &  
320 Forhead, 2014). Similarly, leptin appears to have an important role in modifying the actions of  
321 cortisol on gluconeogenic enzyme activities in ovine fetal liver near term (Forhead *et al.*, 2008). It  
322 may also be involved in the developmental changes in cardiac function and hypothalamic appetite  
323 regulatory circuits during the perinatal period (Vickers & Sloboda, 2012; Bouret *et al.*, 2015). Indeed,  
324 ontogenic and environmentally induced changes in the circulating concentration and tissue receptor  
325 abundance of these hormones may explain, in part, the gestational dependence of some of the  
326 developmental outcomes associated with raised glucocorticoid levels *in utero*.

327

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

347

348 **Epigenetic effects**

349 At the molecular level, glucocorticoids act via several different mechanisms to alter gene expression.  
350 Bound to their receptors they act as enhancer binding proteins that activate or repress gene  
351 expression via interaction with glucocorticoid response elements (GRE) in promotor or other  
352 regulatory regions of the genome (Adcock *et al.*, 2004). For example, cortisol-stimulated down-  
353 regulation of *IGF2* gene transcription in ovine fetal liver is mediated preferentially by a GRE in the 5'  
354 regulatory region of the untranslated leader exon 7 containing the P4 promotor (Li *et al.*, 1998). In  
355 contrast, cortisol-induced up-regulation of *IGF1* gene transcription in fetal liver is likely to be more  
356 indirect as there are no GREs in the vicinity of the promotor regions of this gene (Fowden *et al.*,  
357 2011). Postnatally, glucocorticoids are also known to alter gene expression more indirectly via  
358 epigenetic modifications of the genome and chromatin structure (Weaver, 2009). These include DNA  
359 methylation, histone modifications and changes in abundance of non-coding long and microRNAs  
360 (Adcock *et al.*, 2004; Weaver, 2009). However, relatively little is known about the epigenetic effects  
361 of glucocorticoids *in utero*.

362

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

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

386

387 Glucocorticoids may also act by changing the imprint status of growth regulatory imprinted genes  
388 like *IGF2*, which are expressed from only one allele in a parent of origin manner. In both human and  
389 ovine liver, the *IGF2* gene switches from solely paternal to biallelic expression in parallel with the  
390 prepartum cortisol surge (Fowden *et al.*, 2011). Whether these changes in *IGF2* expression are due  
391 to altered expression of the *H19* derived non-coding RNA or to changes in methylation at the DMR  
392 and/or ICR of the *IGF2-H19* locus remains unknown. Nor it is clear whether any of the epigenetic  
393 effects are due directly to the glucocorticoids or mediated indirectly by changes in placental function  
394 or other hormone concentrations. Certainly, maternal treatment with synthetic glucocorticoids in  
395 rats alters placental transfer of methyl donors essential for DNA methylation (Wyrwoll *et al.*, 2012).  
396 In addition, T<sub>3</sub> is a key component of the epigenetic mechanism regulating GR promotor methylation  
397 in the rat brain in response to neonatal stresses (Zhang *et al.*, 2013). Glucocorticoid exposure in  
398 early life, therefore, affects the developing epigenome through a number of different routes with  
399 dynamic consequences for epigenetic marks throughout the lifespan of the offspring. Indeed, the  
400 long term outcomes of prenatal glucocorticoid overexposure are likely to be modified continually by  
401 postnatal factors, often independent of the physical environment, like the level of maternal care and  
402 the reproductive history of the mother reflected in the quality and glucocorticoid content of the milk  
403 during lactation (Zhang *et al.*, 2006; Hinde *et al.*, 2015).

404

## 405 **CONCLUSIONS**

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

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

423

424

#### 425 **FIGURE LEGENDS**

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

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

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#### 656 **COMPETING INTERESTS**

657 The authors have no competing interests to declare.

658

#### 659 **AUTHOR CONTRIBUTION**

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

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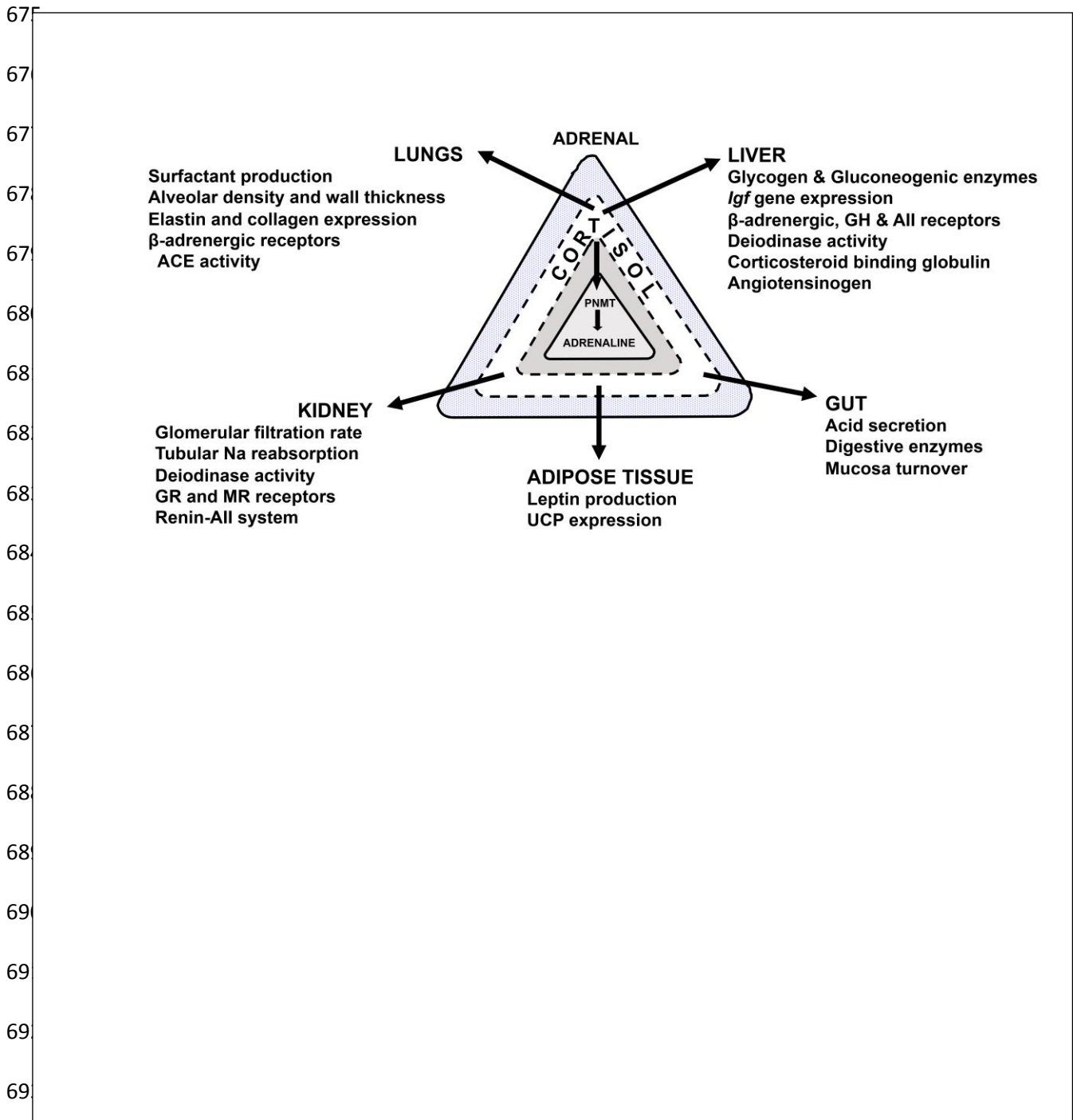
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674 **Figure 1**



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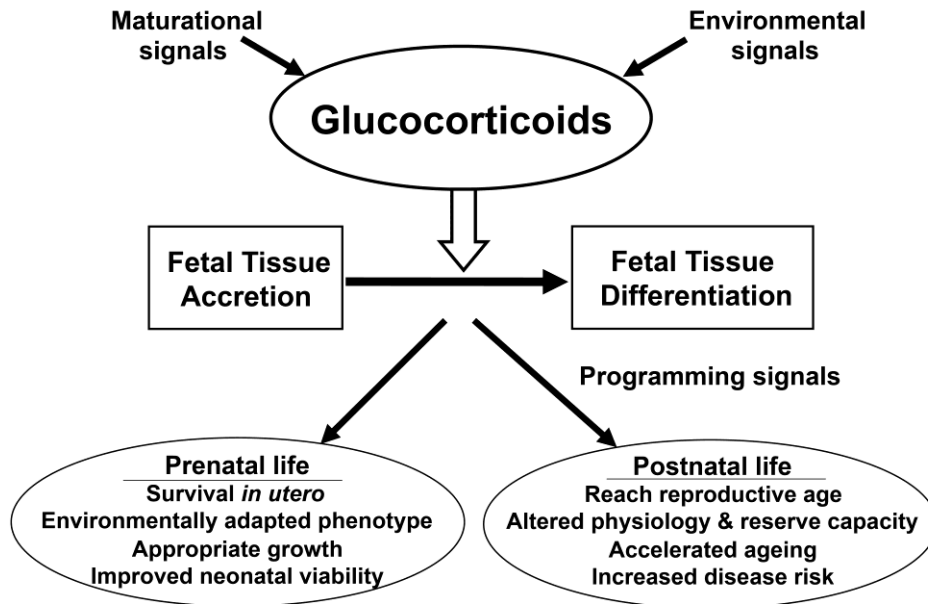
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699 **Figure 2**



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<b>Table 1: Endocrine systems affected by maternal and/or fetal glucocorticoids in sheep and rodents</b>			
<b>Endocrine system</b>	<b>Hormones</b>	<b>Processes involved</b>	<b>Tissue</b>
Sex steroids	Progesterone	Cytochrome P450 <sub>17<math>\alpha</math></sub>	Placenta
	Estrogens	C17-20 lyase, Aromatase	
Eicosanoids	PGE <sub>2</sub> , PGF <sub>2<math>\alpha</math></sub>	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 <sub>17<math>\alpha</math></sub> 11 $\beta$ HSD1, 11 $\beta$ HSD2, 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 <sub>4</sub> , T <sub>3</sub>	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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