Endocrine mechanisms of intrauterine programming

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Abstract

Epidemiological findings and experimental studies in animals have shown that individual tissues and whole organ systems can be programmed *in utero* during critical periods of development with adverse consequences for their function in later life. Detailed morphometric analyses of the data have shown that certain patterns of intrauterine growth, particularly growth retardation, can be related to specific postnatal outcomes. Since hormones regulate fetal growth and the development of individual fetal tissues, they have a central role in intrauterine programming. Hormones such as insulin, insulin-like growth factors, thyroxine and the glucocorticoids act as nutritional and maturational signals and adapt fetal development to prevailing intrauterine conditions, thereby maximizing the chances of survival both *in utero* and at birth. However, these adaptations may have long-term sequelae. Of the hormones known to control fetal development, it is the glucocorticoids that are most likely to cause tissue programming *in utero*. They are growth inhibitory and affect the development of all the tissues and organ systems most at risk of postnatal pathophysiology when fetal growth is impaired. Their concentrations *in utero* are also elevated by all the nutritional and other challenges known to have programming effects. Glucocorticoids act at cellular and molecular levels to alter cell function by changing the expression of receptors, enzymes, ion channels and transporters. They also alter various growth factors, cytoarchitectural proteins, binding proteins and components of the intracellular signalling pathways. Glucocorticoids act, directly, on genes and, indirectly, through changes in the bioavailability of other hormones. These glucocorticoid-induced endocrine changes may be transient or persist into postnatal life with consequences for tissue growth and development both before and after birth. In the long term, prenatal glucocorticoid exposure can permanently reset endocrine systems, such as the somatotrophic and hypothalamic–pituitary–adrenal axes, which, in turn, may contribute to the pathogenesis of adult disease. Endocrine changes may, therefore, be both the cause and the consequence of intrauterine programming.

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Introduction

Epidemiological studies in man have shown that impaired intrauterine growth is associated with an increased incidence of cardiovascular, metabolic and other diseases in later life (Barker 1994). Low birth weight, in particular, has been linked to hypertension, ischaemic heart disease, glucose intolerance, insulin resistance, type 2 diabetes, hyperlipidaemia, hypercortisolaemia, obesity, obstructive pulmonary disease, renal failure and reproductive disorders in the adult (Barker 1994). These associations have been described in populations of different age, sex and ethnic origin and occur independently of the current level of obesity or exercise (Barker 1994, Rhind *et al.* 2001). Detailed morphometric analyses of the human epidemiological data have shown that certain patterns of intrauterine growth can be related to specific adult diseases. For instance, it is the thin infant with the low ponderal index, rather than the symmetrically small baby, that is more prone to type 2 diabetes as an adult (Phillips *et al.* 1994).

These observations have led to the hypothesis that adult disease arises *in utero* as a result of changes in the development of key tissues and organ systems during suboptimal intrauterine conditions associated with impaired fetal growth (Barker 1994).

This hypothesis has been tested experimentally in a number of species using a range of techniques to impair fetal growth (Table 1). Inducing intrauterine growth retardation (IUGR) by maternal undernutrition or placental insufficiency leads to postnatal hypertension, glucose intolerance, insulin insensitivity and to alterations in the functioning of the adult hypothalamic–pituitary–adrenal (HPA) axis in several species (Table 1). Similarly, in naturally occurring IUGR in polytocous species, low birth weight is associated with hypertension and abnormalities in glucose metabolism and HPA function after birth (Table 1). The range of postnatal physiological perturbations observed after induced and naturally occurring IUGR in experimental animals is, therefore, similar to that seen in human populations.

Table 1 Postnatal consequences of naturally and experimentally induced intrauterine growth retardation.

Procedure	Species	Postnatal outcome	Reference
Maternal undernutrition			
Calorie deprivation	Rat	Hypertension, hypercholesterolaemia, obesity, glucose intolerance	Jones & Friedman (1982), Woodall *et al.* (1996*a*), Szitanyi *et al.* (2000)
	Guinea-pig	Hypertension, insulin resistance, obesity	Kind *et al.* (2002, 2003)
	Sheep	Hypertension, altered HPA axis	Hawkins *et al.* (2000), Bloomfield *et al.* (2003)
Protein deprivation	Rat	Hypertension, glucose intolerance	Dahri *et al.* (1991), Langley-Evans (1997)
		Insulin resistance	Burns *et al.* (1997)
Iron deficiency	Rat	Hypertension	Crowe *et al.* (1995)
Placental insufficiency			
Increased litter size	Guinea-pig	Glucose intolerance, insulin deficiency	Kind et al. (2003)
	Pig	Hypertension, glucose intolerance	Poore *et al.* (2001), Poore & Fowden (2002)
		Altered HPA axis	Poore & Fowden (2003)
Restricted blood flow	Rat	Glucose intolerance, insulin deficiency and resistance	Simmons *et al.* (2001)
	Guinea-pig	Hypertension	Persson & Jansson (1992)
Decreased placental size	Sheep	Hypertension, glucose intolerance	Gatford *et al.* (2000)
	Horse	Altered sympathoadrenal function	Giussani *et al.* (2003)
Glucocorticoid exposure			
Maternal dexamethasone treatment	Rat	Hypertension, glucose intolerance	Benediktsson *et al.* (1993), Nyirenda *et al.* (1998)
		Insulin resistance, obesity	Dahlgren *et al.* (2001)
	Guinea pig	Altered HPA axis	Owen & Matthews (2003)
	Sheep	Hypertension, insulin resistance	Moss *et al.* (2001), Dodic *et al.* (2002)
		Altered HPA axis	Sloboda *et al.* (2002*a*)
Inhibition of placental 11βHSD2	Rat	Hypertension, glucose intolerance, insulin resistance	Lindsay *et al.* (1996*a,b*)
Maternal stress	Rat	Altered HPA axis	Barbazangers *et al.* (1996)

11βHSD2, 11β-hydroxysteroid dehydrogenase type 2.

The animal studies have also shown that the timing, duration and exact nature of the insult during pregnancy are also important determinants of the pattern of fetal growth and of the specific postnatal outcomes. In rats, calorie restriction during pregnancy leads to hypertension in the adult offspring when it occurs throughout gestation but not when it is confined solely to the second half of pregnancy (Woodall *et al.* 1996*a*, Holemans *et al.* 1999). Similarly, in rats, the extent to which maternal protein deprivation during pregnancy leads to adult hypertension depends on the severity of the restriction and the precise composition of the low protein diet (Langley-Evans 2000). In sheep, undernutrition for 10 days in late gestation alters postnatal HPA function, but not glucose metabolism, while extending the period of prenatal undernutrition to 20 days alters glucose metabolism, but not HPA function, in the adult offspring (Oliver *et al.* 2002, Bloomfield *et al.* 2003). In addition, maternal nutritional insults which have little or no effect on birth weight have been shown to alter glucose tolerance, blood pressure and HPA function in the fetus during late gestation (Hawkins *et al.*, 2000, Oliver *et al.* 2001). These observations are consistent with the human epidemiological data from the Dutch hunger winter (1944–1945) which showed that the increased risk of specific adult onset degenerative diseases depended on the gestational age at famine exposure and that these associations occurred despite little, if any, reduction in birth weight (Roseboom *et al.* 2001). Together, the animal experiments and human epidemiological data demonstrate that individual tissues and whole organ systems can be programmed during critical periods of intrauterine development with adverse consequences for their function in later life. This programming occurs across the normal range of birth weights and has the worst prognosis at the extreme ends of the birth weight spectrum.

Hormones and fetal development

The role of hormones in regulating fetal growth and the development of individual fetal tissues has been identified using a range of techniques including ablation of the fetal endocrine glands, hormone administration to the fetus and mother, and gene knockout and disruption experiments (Fowden 1995, Efstratiadis 1998). These studies show that hormones affect both tissue accretion and differentiation *in utero* and that specific hormone deficiencies are associated with particular types of IUGR. They also show that hormones act on fetal growth both directly, via genes, and indirectly, through changes in placental growth, fetal metabolism, and/or the production of growth

factors and other hormones by the feto-placental tissues (Fowden & Forhead 2001, Fowden 2003).

The hormones present in the fetal circulation have four main sources. First, they may be secreted by the endocrine glands of the fetus. The fetal pancreas, thyroid, pituitary and adrenal glands are functional from early in gestation and become progressively more responsive to stimuli during late gestation (see Fowden & Hill 2001). Secondly, hormones may be derived from the uteroplacental tissues. These tissues produce a number of hormones including steroids, peptides, glycoproteins and eicosanoids, which are released into both the umbilical and uterine circulations (Challis *et al.* 2001). Thirdly, lipophilic hormones such as the steroids and thyroid hormones may be derived from the mother by transplacental diffusion. The amount of hormone transferred in this way depends on the materno-fetal concentration gradient and the permeability of the placental barrier, both of which vary between species (Sibley *et al.* 1997). Finally, hormones in fetal plasma may be derived from circulating precursor molecules by metabolism in the fetal or placental tissues.

The concentrations of hormones in the fetal circulation change both developmentally and in response to nutritional and other stimuli. Towards term, there are increases and decreases in the concentrations of specific hormones, which act as maturational signals to the fetus (Fowden *et al.* 1998). These developmental endocrine changes occur independently of the nutritional state of the fetus and induce permanent changes in tissue morphology and function in preparation for extrauterine life. Changes in hormone concentrations also occur in response to variations in nutritional state, especially in late gestation when all the fetal endocrine glands are functional (Fig. 1). In general, nutritional challenges that reduce fetal nutrient availability lower anabolic hormones (e.g. insulin, insulin-like growth factor (IGF)-I, thyroxine (T_4)) and increase catabolic hormone concentrations (e.g. cortisol, catecholamines, growth hormone (GH)), whereas challenges that increase the fetal nutrient supply raise anabolic and reduce catabolic hormone levels in the fetal circulation (Fowden &

Figure 1 Diagram illustrating the relationships between nutritional state, hormone concentrations, metabolism and tissue accretion and differentiation in the fetus.

Forhead 2001). The specific combination of endocrine changes depends on the magnitude, duration and precise nature of the insult and alters the pattern of fetal development accordingly. The key hormones involved in these regulatory processes are insulin, the thyroid hormones, the IGFs and the glucocorticoids (Fig. 1).

Insulin

Insulin is derived from the fetal pancreas from early in development. Its concentration *in utero* rises between early and mid gestation and then remains stable until term (Fowden & Hill 2001). Fetal insulin concentrations are also positively related to the fetal glucose levels and to body weight at birth (Fowden 1995). Fetal insulin deficiency leads to a symmetrical type of IUGR with little, if any, developmental abnormalities in individual fetal tissues (see Fowden & Hill 2001). Insulin, therefore, has negligible effects on tissue differentiation or maturation *in utero* but enhances tissue accretion via its anabolic effects on fetal metabolism and by stimulating production of IGF-I (Fig. 1). Thus, fetal insulin is a growth-promoting hormone, which acts as a signal of nutrient plenty.

Thyroid hormones

In sheep, thyroid hormones present in fetal plasma are derived primarily from fetal sources, although in other species such as man and rabbits they can also have a maternal origin. Fetal plasma contains T_4, tri-iodothyronine (T_3) and large amounts of reverse T_3 (rT_3), the biologically inactive metabolite of T_4. Developmentally, fetal plasma T_3 concentrations rise while plasma rT_3 falls towards term as a result of increased peripheral $5'$ monodeiodination of T_4 (Thomas *et al.* 1978). Fetal thyroid hormone concentrations are not related to metabolite concentrations during normal conditions but are reduced during hypoxaemic conditions associated with IUGR (Fowden 1995). Fetal hypothyroidism leads to an asymmetrical type of IUGR with a reduction in muscle mass (see Fowden 1995). It also alters development of the fetal nervous system, appendicular skeleton, skin, lungs and skeletal muscle. Thyroid hormones, therefore, affect both tissue accretion and differentiation, and stimulate these processes via modulation of IGF production and by metabolic actions, which increase fetal O_2 consumption (Fig. 1). Thus, thyroid hormones promote fetal development and act as signals of energy availability.

IGFs

Fetal plasma IGF-I and IGF-II are derived from a range of feto-placental tissues throughout gestation. Their concentrations vary widely between species but are positively correlated with glucose and pO_2 levels in the sheep fetus (Owens 1991). Plasma concentrations and tissue expression of the IGFs are also regulated developmentally and by the other key hormones involved in the control of fetal growth
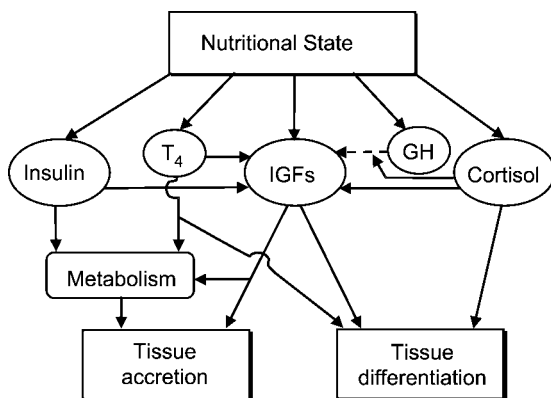
(Fig. 1). In mice, knockout or disruption of the *Igf* genes or the IGF-type 1 receptor leads to severe growth retardation whereas over-expression of the *Igf2* gene results in fetal overgrowth (Efstratiadis 1998). These IGF-induced changes in fetal body weight are accompanied by developmental abnormalities in several individual tissues including bone, skin, respiratory and other muscles. Similarly, in fetal sheep and monkeys, administration of IGF-I has selective effects on the growth of individual tissues but has little effect on body weight (see Fowden 2003). The IGFs stimulate fetal growth by metabolic and non-metabolic mechanisms. They act as progression factors in the cell cycle, prevent apoptosis and increase DNA and protein synthesis in fetal tissues (Hill *et al.* 1998). IGF-I also has anabolic effects similar to insulin *in utero*. Since fetal IGF-I is more nutritionally sensitive than fetal IGF-II (Fowden 2003) IGF-I appears to be the signal of nutrient sufficiency, which regulates tissue accretion in relation to the nutritional conditions *in utero*. Fetal IGF-II may provide a more general stimulus to cell growth, and regulate tissue-specific changes in cell differentiation during late gestation and in response to adverse intrauterine conditions (Fowden 2003).

Glucocorticoids

For most of gestation, glucocorticoids are low in concentration in the fetus and are derived from the mother down a materno-fetal concentration gradient, which varies widely between species (Table 2). This transplacental concentration gradient is maintained by placental 11βHSD2, which converts the active glucocorticoids, cortisol and corticosterone, to their inactive metabolites (Seckl 2001). This enzyme is, therefore, a key factor in limiting fetal and placental exposure to maternal glucocorticoids. Its placental activity is regulated by nutritional and endocrine factors (Clarke *et al.* 2002, Seckl 2001), and varies between species in parallel with the magnitude of the materno-fetal cortisol concentration gradient (Table 2). In sheep, in which this gradient is small (Table 2), 90% of the cortisol in the fetal circulation is of maternal origin before the fetal

adrenal begins cortisol production close to term. However, once the fetal adrenal cortex is activated in late gestation, the fetus becomes the primary source of circulating glucocorticoids and there is a progressive increase in both the basal cortisol levels and the adrenocortical responsiveness to adverse conditions (Challis *et al.* 2001). Increased fetal glucocorticoid exposure can, therefore, occur due to increased maternal cortisol levels, decreased placental 11βHSD2 activity or increased cortisol output by the fetal adrenal. The importance and relative contribution of each of these sources to changes in the fetal glucocorticoid level varies with gestational age and in response to the prevailing nutritional and endocrine conditions.

During the stage of gestation when fetal glucocorticoid levels are low, glucocorticoids appear to have a relatively minor role in controlling tissue accretion compared with other hormones (Fowden 1995). However, when concentrations are raised either endogenously or exogenously in fetal sheep, the growth rate of the fetus declines (Fowden *et al.* 1996, Jensen *et al.* 2002). Fetal overexposure to glucocorticoids by maternal administration of synthetic glucocorticoids has also been shown to retard fetal growth in rats, rabbits, sheep, monkeys and man (Seckl 2001). Glucocorticoids, therefore, inhibit tissue accretion when their concentrations are elevated. They also have major effects on the differentiation of a wide range of tissues including the lungs, liver, kidneys, muscle, fat and gut (see Fowden *et al.* 1998). They stimulate morphological and functional changes in these tissues and activate many of the biochemical processes which have little or no function *in utero* but which are essential for survival postnatally (Fowden *et al.* 1998). Glucocorticoids, therefore, signal adverse intrauterine conditions and adapt fetal development to ensure the maximum chances of survival both *in utero* and at birth.

Hormones and intrauterine programming

Sexually dimorphic programming of tissues is well known to be hormone dependent. Exposure to androgens early in life alters expression of steroid metabolising enzymes in the liver, neuronal structure in the hypothalamus, central feedback sensitivity to peripheral hormones and sexual behaviour in the adult (see Austin *et al.* 1981). These effects can only be induced by androgen exposure at a critical window of perinatal development but then persist throughout life, independently of subsequent sex steroid levels. Of the hormones known to regulate fetal development, it is the glucocorticoids that are most likely to cause tissue programming *in utero*. They are growth inhibitory and affect development of all the tissues and organ systems that are at increased risk of adult pathophysiology when fetal growth is impaired (Fowden *et al.* 1998). Their concentrations *in utero* are also elevated in IUGR and in response to most of the nutritional and other challenges known to have programming effects, including maternal undernutrition, placental insufficiency and restriction of

Table 2 Species differences in fetal and maternal cortisol concentrations and in placental 11*β*HSD type 2 activity during late gestation.

Species	Plasma cortisol concentration (ng/ml)			Placental 11βHSD2 activity (pmol/min per mg protein)
	Mother	Fetus	Materno-fetal gradient	
Sheep	12	10	2	2.0
Pig	30	15	15	2.5
Horse	40	7	33	2.5
Monkey	300	150	150	3.5
Human	200	20	180	5.0
Guinea pig	1000	200	800	9.0

Data from Seckl (2001) and Clarke *et al.* (2002).

placental blood flow (Challis *et al.* 2001, Fowden & Forhead 2001).

Fetal overexposure to glucocorticoids either via maternal administration or by inhibition of placental 11βHSD2 leads to hypertension, glucose intolerance and abnormalities in HPA function after birth (Table 1). The specific postnatal effects of these treatments depend on the gestational age at onset and on the duration of exposure. In sheep, maternal glucocorticoid treatment early in gestation leads to hypertension but not glucose intolerance in the adult offspring while glucocorticoid exposure late in gestation has the opposite effects (Gatford *et al.* 2000, Moss *et al.* 2001). With treatment late in gestation, post-natal glucose intolerance is magnified with repeated ante-natal glucocorticoid administration (Moss *et al.* 2001). When maternal glucocorticoid concentrations are raised endogenously in rats during pregnancy by stress or adreno-corticotrophic hormone (ACTH) administration, there are permanent changes in HPA function, behaviour and neuroendocrine responsiveness in the adult offspring (Welberg & Seckl 2001). Furthermore, in rats, the programming effects of undernutrition and 11βHSD2 inhibition can be prevented by abolishing maternal glucocorticoid synthesis by adrenalectomy or metyrapone treatment (Langley-Evans 1997). Glucocorticoids can, therefore, programme tissues *in utero* and may also mediate the programming effects of nutritional and other environmental challenges during pregnancy.

Cellular and molecular mechanisms of glucocorticoid programming

Glucocorticoids act at cellular and molecular levels to induce changes in tissue accretion and differentiation by direct and indirect mechanisms. At a cellular level, gluco-corticoid exposure *in utero* alters receptors, enzymes, ion channels and transporters in a wide range of different cell types during late gestation (Table 3). They also change the expression of various growth factors, cytoarchitectural pro-teins, binding proteins and components of the intracellular signalling pathways (Breed *et al.* 1997, Chinoy *et al.* 1998, Hai *et al.* 2002, Antonow-Schlorke *et al.* 2003). These changes will influence the basal functioning of the cell and its responses to endocrine, metabolic and other stimuli with consequences for its size, proliferation rate and terminal differentiation. In addition to these direct effects, glucocorticoids can act indirectly on tissue pro-liferation and differentiation through changes in the cellu-lar secretion of proteins, hormones, growth factors and metabolites. Even when the effects of glucocorticoids are confined to a single tissue or gestational age, they may have more widespread effects on fetal development. For instance, the cortisol-induced changes in placental GLUT gene expression may permanently alter transplacental glucose transport to the fetus with implications for fetal metabolism and growth more generally (Hahn *et al.* 1999, Langdown & Sugden 2001).

One important factor linking the glucocorticoid-induced changes in cell function, proliferation and differentiation is the fetal IGF status. Glucocorticoids affect tissue expression of both *Igf* genes in the fetus (Fowden 2003). In fetal sheep, cortisol suppresses IGF-II mRNA abundance in liver, skeletal muscle and adrenal, and has tissue-specific effects on IGF-I gene expression (Fig. 2; see Fowden 2003). These changes are observed in response to both the endogenous rise in plasma cortisol close to term and when cortisol is infused exogenously earlier in ges-tation (Fig. 2). They also depend on the gestational age of the fetus. Cortisol suppresses muscle IGF-I gene expression at 130 days of gestation but not earlier in gestation, whereas it up-regulates hepatic IGF-I gene expression at both gestational ages in the sheep fetus (Fig. 3). Tissue- and age-specific effects of the glucocorticoids are also seen with other genes. Cortisol increases hepatic but not muscle GH receptor (GHR) mRNA abundance and induces pulmonary but not renal angiotensin-converting enzyme activity in fetal sheep during late gestation (Li *et al.* 1999, 2002, Forhead *et al.* 2000*b*). Similarly, in the gastro-intestinal tract of fetal pigs, exogenous cortisol activates some but not all of the digestive enzymes and is effective only in the period of gestation just before fetal cortisol levels rise endogenously (Trahair & Sangild 1997). The cellular effects of the glucocorticoids are, therefore, tissue specific and dependent on gestational age.

At a molecular level, glucocorticoids affect a number of different processes. They may act on transcription, mRNA stability, translation and/or the post-translational proces-sing of the protein products. Several of the genes known to be regulated by glucocorticoids (e.g. *Igf2*, angiotensino-gen, tropoelastin) have the necessary glucocortiocoid response elements (GRE) in their promoter regions to allow direct transcriptional control of the gene by cortisol. Certainly, cortisol acts directly on the *Igf2* gene in fetal liver to decrease transcription (Li *et al.* 1998). However, other genes which appear to be glucocorticoid sensitive (e.g. *Igf1*) do not have recognisable GRE consensus sequences. In these instances, the effects of cortisol must be mediated indirectly via changes in GHR gene expression or via other transcription factors or cortisol-dependent hormones. Glucocorticoids have been shown to affect the expression of several transcription factors including *cfos*, AP-1 and C/EBPδ in fetal tissues (Breed *et al.* 1997, Slotkin *et al.* 1998). They also raise fetal plasma T_3, which is known to affect expression of the *Igf* genes in fetal ovine liver and skeletal muscle (see Fowden 2003). In genes which have multiple mRNA transcripts derived from alternate exon slicing and promotor usage, the effects of the glucocorticoids may be specific to certain leader exons in the genes. Indeed, differential pro-motor usage has been observed in response to glucocorti-coids in the *GHR*, *Igf* and glucocorticoid receptor (*GR*) genes in fetal liver during late gestation (Li *et al.* 1996, 1999, McCormick *et al.* 2000). Glucocorticoids may, therefore, initiate use of specific promoters which, in turn,

Table 3 Cell functions affected by glucocorticoids *in utero*.

Function	Specific change	Tissue	Reference
Receptors	Glucocorticoid	Lungs, brain, anterior pituitary, liver	Erdeljan *et al.* (2001), Holloway *et al.* (2001)
	Mineralocorticoid	Brain	McCabe *et al.* (2001)
	ACTH	Adrenal	Leavitt *et al.* (1997)
	Vasopressin	Anterior pituitary	Young *et al.* (2003)
	Noradrenaline and adrenaline	Liver, lung	Cheng *et al.* (1980), Fowden *et al.* (1995)
	GH	Liver	Li *et al.* (1996)
	IGF	Liver	Price *et al.* (1992)
	Prolactin	Liver	Phillips *et al.* (1999)
	Dopamine	Brain	Labaune *et al.* (2002)
	Leptin	Placenta	Sugden *et al.* (2001), Smith & Wadell (2002)
	Angiotensin II	Liver, kidney, heart, brain	Segar *et al.* (1995), Dodic *et al.* (2002)
Enzymes	11βHSD types 1 and 2	Liver, placenta, adrenal	Ross *et al.* (2000), Clarke *et al.* (2002), Gupta *et al.* (2003)
	3β-Hyroxysteroid dehydrogenase	Adrenal	Leavitt *et al.* (1997)
	Prostaglandin G/H synthetase	Placenta	Wu *et al.* (2001)
	17α-hydroxylase	Placenta	Anderson *et al.* (1975)
	17,20-lyase	Placenta	Anderson *et al.* (1975)
	Aromatase	Placenta	France *et al.* (1988)
	Angiotensin-converting enzyme	Lungs	Forhead *et al.* (2000*b*)
	Endothelial nitric oxide synthetase	Lungs	Grover *et al.* (2000)
	Fatty acid synthetase	Lungs	Xu & Rooney (1997)
	Argininosuccinate synthetase	Liver	Bourgeois *et al.* (1997)
	Argininosuccinate lyase	Liver	Renouf *et al.* (1995)
	Type I 5′-monodeiodinase	Liver	Wu *et al.* (1978)
	Pyruvate carboxylase	Liver	Fowden *et al.* (1993)
	Glucose-6-phosphatase	Liver, kidney	Fowden *et al.* (1993)
	Fructose diphosphatase	Liver, kidney	Fowden *et al.* (1993)
	Phosphoenolpyruvate carboxykinase	Liver, kidney	Fowden *et al.* (1993)
	Aspartate transaminase	Liver, kidney	Fowden *et al.* (1993)
	Renin	Kidney	Segar *et al.* (1995)
	Chymosin	Stomach	Sangild *et al.* (1994)
	Amylase	Pancreas	Sangild *et al.* (1994)
	Lactase	Small intestine	Sangild *et al.* (1995)
	Aminopeptidase	Small intestine	Sangild *et al.* (1995)
	Phenylethanolamine N-methyltransferase	Heart, adrenal	Kennedy & Ziegler (2000)
	Collagenase	Bone	Delany *et al.* (1995)
Ion channels	Epithelial Na^+ channel	Lungs, kidney	Venkatesh & Katzberg (1997), Nakamura *et al.* (2002)
	Voltage-gated Na^+ channel	Heart	Fahmi *et al.* (2003)
Transporters	GLUT 1 and 3	Placenta	Hahn *et al.* (1999), Langdown & Sugden (2001)
	Na^+/K^+ ATPase	Lungs, kidney	Chalaka *et al.* (1999), Petershack *et al.* (1999)
	Na^+/H^+ exchanger	Kidney	Guillery *et al.* (1995)

GLUT, glucose transporter.

could alter the relative abundance of particular mRNA slice variants with consequences for protein translation. In genes which are imprinted and expressed from only one parental allele (e.g. *Igf2*), the effects of the glucocorticoids may also be mediated through changes in imprint status. Imprinting of *Igf2* is controlled by the *H19* gene which is itself imprinted and nutritionally regulated in a tissue-specific manner (see Reik *et al.* 2003). Certainly, in sheep, there is a perinatal transition from monoallelic to biallelic *Igf2* gene expression in the liver, which closely parallels the prepartum cortisol surge in the fetus (McLaren & Montmonery 1999).

The cellular and molecular changes induced by glucocorticoids in individual tissues combine to produce integrated changes in function at a systems level. In fetal sheep, the hypertensive effect of cortisol may be due to functional changes in the brain, heart, vasculature and kidneys induced by altered expression of hormone receptors, enzymes, ion channels, transporters and cytoskeletal proteins in these tissues (Table 3). It also depends on local
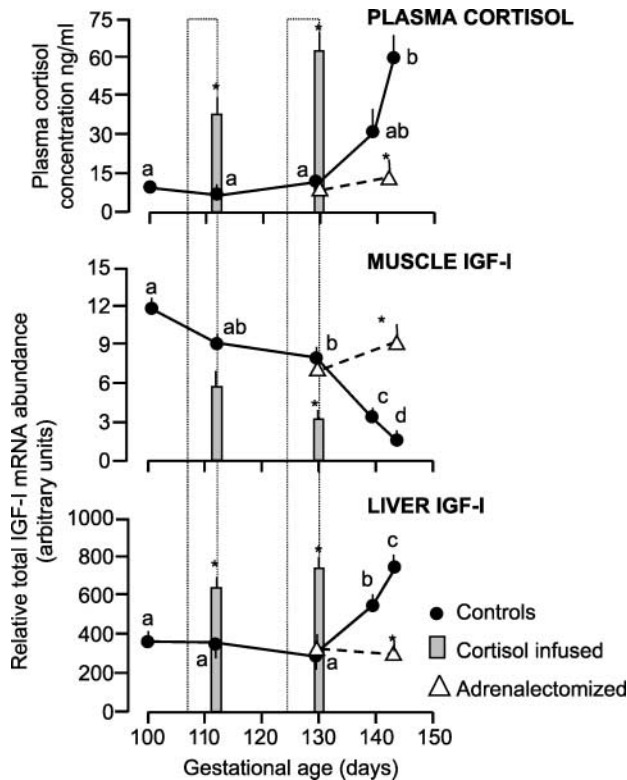
Figure 2 Cortisol and tissue IGF gene expression. Mean (± S.E.) values of plasma cortisol and IGF-I mRNA abundance in fetal ovine liver and skeletal muscle with respect to gestational age in normal intact ($n > 5$) and adrenalectomised sheep fetuses ($n > 4$) and in fetuses infused with cortisol for 5 days before delivery ($n > 4$). Values with different letters are significantly different from each other ($P < 0.05$, ANOVA). *$P < 0.05$ compared with normal, intact fetuses at the same gestational age (t-test). Data from Li *et al.* (1996, 2002).

and systemic changes in the secretion of vasoactive agents, such as angiotensin II (AII), adrenaline, nitric oxide and vasopressin by several different tissues (Dodic *et al.* 2002). Glucocorticoid programming of physiological systems is, therefore, multifactorial and involves co-ordinated and interdependent changes in many different tissues.
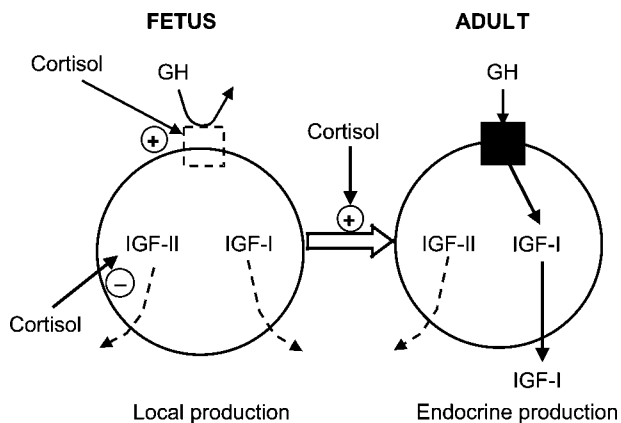

Figure 3 Schematic diagram showing the effects of cortisol on the activation of the GH–IGF axis in ovine hepatocytes. Data from Li *et al.* (1996, 1998, 1999).

Endocrine mechanisms of glucocorticoid programming

One of the major mechanisms by which glucocorticoids act on physiological systems is via changes in hormone bioavailability. Glucocorticoids are known to alter the production and secretion of a number of hormones by the placenta and fetal endocrine glands (Table 4). They also regulate hormone receptor densities and the activities of several enzymes involved in activating and inactivating hormones at the fetal tissues (Table 4). For instance, cortisol activates T_3 production by inducing 5′ monodeiodinase in fetal liver and has tissue-specific effects on its own availability by regulating activity of both 11βHSD isoforms (Table 3). In addition, by altering the concentration of hormone-binding proteins, such as corticosteroid-binding globulin and IGF-binding proteins (Price *et al.* 1992, Sloboda *et al.* 2002b), glucocorticoids control the availability of free hormone for receptor binding in the fetus.

Some of the endocrine changes induced by glucocorticoids *in utero* are transient while others persist after glucocorticoid levels have returned to normal values (Fletcher *et al.* 2000, Forhead *et al.* 2002). Even transient endocrine changes may have permanent effects by altering tissue development. In fetal sheep, cortisol up-regulates activity of the renin–angiotensin system (RAS) by increasing fetal plasma AII concentrations and altering AII type 1 receptor expression in the heart and kidneys (Table 3). These changes may cause cardiac hyperplasia and reduce the number and size of the glomeruli in the kidney (Woods & Rasch 1998, Sundgren *et al.* 2003). Even if the enhanced RAS activity does not persist after birth, the alterations in cardiac and renal morphology may predispose these tissues to pathophysiology later in life.

Glucocorticoid-stimulated changes in hormone production, particularly in the placenta, may have their programming effects via the mother. Placental hormones, such as progesterone and placental lactogen, influence maternal metabolism in favour of glucose delivery to the fetus. Changes in these hormone levels will, therefore, affect the partitioning of nutrients between the maternal and fetal tissues, and alter the availability of substrates for tissue accretion by the fetus. In fetal sheep, the cortisol-induced reduction in the number of placental binucleate cells producing placental lactogen may also compromise mammary development and cause a lactational constraint on nutrition after birth (Ward *et al.* 2002). Certainly, in human populations, the risk of adult onset cardiovascular disease is greatest in individuals who were growth retarded *in utero*, grew slowly during the first year of postnatal life and then showed rapid catch-up growth during later childhood to become obese as adults (Eriksson *et al.* 2001). Changes in lactation induced by prenatal glucocorticoid exposure may, therefore, provide a mechanism linking pre- and immediate postnatal growth, and lead to postnatal programming of tissues that were unaffected by glucocorticoids *in utero*.

Table 4 The effects of natural or synthetic glucocorticoids on circulating hormone concentrations in fetal sheep at the time of exposure.

Hormone class	Specific hormone	Change in concentration	Reference
Steroids	Oestrogen	increase	Sloboda *et al.* (2000)
	Cortisol	decrease	Bennet *et al.* (1999)
Eicosanoids	PGF_2	increase	Challis *et al.* (2002)
	$PGF_{2\alpha}$	increase	Challis *et al.* (2001)
	PGI_2	increase	Ibe *et al.* (1996)
Proteins	Insulin	decrease	Sloboda *et al.* (2000)
	Leptin	increase	Forhead *et al.* (2002)
	Erythropoietin	decrease	Lim *et al.* (1996)
	Gastrin	increase	Trahair & Sangild (1997)
	ACTH	decrease	Derks *et al.* (1997)
Peptides	Neuropeptide Y	increase	Fletcher *et al.* (2000)
	Angiotensin II	increase	Forhead *et al.* (2000*a*)
Iodothyronines	T_3	increase	Thomas *et al.* (1978)
	rT_3	decrease	Thomas *et al.* (1978)
Amines	Noradrenaline	decrease	Derks *et al.* (1997)
	Adrenaline	decrease	Derks *et al.* (1997)

PG, prostaglandin.

In the long term, prenatal glucocorticoid exposure may permanently reset the endocrine axes. In fetal sheep, cortisol alters the growth-regulatory mechanisms by initiating the transition from the fetal to the adult mode of IGF expression in the liver and other tissues (Fig. 3). The cortisol-induced rise in hepatic *Igf1* gene expression is probably mediated through an increase in hepatic *GHR* gene expression as the GH-sensitive transcript of IGF-I mRNA is specifically up-regulated in response to cortisol (Li *et al.* 1996, 1999). In turn, up-regulation of GHR mRNA abundance depends on the cortisol-induced increase in plasma T_3 (see Fowden 2003). Cortisol, therefore, initiates a switch in the somatotrophic axis from GH-independent, local production of IGFs *in utero* to GH-dependent hepatic production of endocrine IGF-I in the adult hepatocyte (Fig. 3). It is also responsible for the perinatal transition from IGF-II to IGF-I as the predominate growth-regulatory IGF (Fig. 3). Premature activation of these switches by early exposure to cortisol may, therefore, alter the growth trajectory both before and after birth. Certainly, in rats, there are permanent changes in the GH–IGF-I axis after prenatal undernutrition, which persist into old age (Woodall *et al.* 1996*b*). Precocious onset of the mechanisms for GH-dependent growth may also explain, in part, the rapid catch-up growth seen in growth-retarded fetuses with placental insufficiency once the nutrient restriction is lifted after birth (Kind *et al.* 2003, Poore & Fowden 2003). However, in human populations, there is no evidence of a link between low birth weight and the function of the somatotrophic axis in old age (Kajantie *et al.* 2003).

In other endocrine axes, glucocorticoids may change the set point and sensitivity of the feedback mechanisms (Bertram & Hanson 2002). This leads to permanent changes in basal hormone levels and in the endocrine responses to stimuli. Basal and stimulated glucocorticoid concentrations are known to be high in adult sheep, rats and guinea pigs over-exposed to glucocorticoids *in utero* (Langley-Evans *et al.* 1996, Matthews *et al.* 2002, Sloboda

et al. 2002*a*). Similarly, in man, basal hypercortisolaemia and greater adrenocortical responsiveness to ACTH are observed in adults who were small at birth (Phillips *et al.* 1998, Reynolds *et al.* 2001). Postnatal adrenocortical responsiveness is also exaggerated in experimental animals after natural and experimentally induced IUGR (Bloomfield *et al.* 2003, Poore & Fowden 2003). The postnatal changes in HPA function associated with IUGR and prenatal glucocorticoid exposure are sex linked in some species and, generally, become more pronounced with increasing postnatal age (Bertram & Hanson 2002, Matthews *et al.* 2002). Persistently enhanced HPA function in the adult may itself contribute to the pathogenesis of cardiovascular and metabolic diseases, independently of any other programming events, as high glucocorticoid levels are known to cause diabetes and hypertension in the adult (Benediktsson *et al.* 1993).

Intrauterine resetting of the HPA and other endocrine axes may occur at a central or peripheral level through permanent changes in receptors, enzymes and/or binding proteins (Table 3). Prenatal glucocorticoid exposure has been shown to alter GR gene expression in peripheral (liver and kidney) and central (hippocampus, hypothalamus and amygdala) tissues in adult rats, guinea pigs and sheep (see Welberg & Seckl 2001, Dodic *et al.* 2002, Matthews *et al.* 2002). These changes are tissue specific and dependent on gestational age at the time of glucocorticoid exposure (Welberg & Seckl 2001). Similar tissue-specific changes in GR gene expression have been observed in adult rats that were undernourished before birth (Langley-Evans *et al.* 1996). In addition, prenatal glucocorticoids permanently alter the monoaminergic and other transmitter systems involved in regulating GR expression in the brain (Muneoka *et al.* 1997). The central changes in GR expression will alter the functioning of the HPA axis while the peripheral changes in GR mRNA abundance may explain the tissue-specific nature of glucocorticoid programming. Central changes in receptor density for the

gonadal and adrenal steroids may also explain, in part, the altered behaviour and abnormalities in hypothalamic–pituitary–gonad function seen in adults after IUGR and prenatal exposure to undernutrition or excess glucocorticoids (Rhind *et al.* 2001, Welberg & Seckl 2001).

Conclusions

Hormones have a central role in regulating fetal growth and development. They act as maturational and nutritional signals *in utero*, and control tissue accretion and differentiation in relation to the prevailing environmental conditions in the fetus. The glucocorticoids, in particular, have a key role in intrauterine programming. They induce permanent changes in physiological systems by altering hormone bioavailability and the cellular expression of receptors, enzymes, ion channels, transporters and various cytoarchitectural proteins in the fetal tissues. Glucocorticoids act directly on genes and indirectly via other hormones and growth factors. Endocrine changes are, therefore, both the cause and the consequence of intrauterine programming.

Acknowledgements

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