Mechanisms underlying the role of glucocorticoids in the early life programming of adult disease

Amanda J. DRAKE, Justin I. TANG and Moffat J. NYIRENDA
Endocrinology Unit, Centre for Cardiovascular Science, Queen’s Medical Research Institute, University of Edinburgh, 47 Little France Crescent, Edinburgh EH16 4TJ, Scotland, U.K.

ABSTRACT
Compelling epidemiological evidence suggests that exposure to an adverse intrauterine environment, manifested by low-birth weight, is associated with cardiometabolic and behavioural disorders in adulthood. These observations have led to the concept of ‘fetal programming’. The molecular mechanisms that underlie this relationship remain unclear, but are being extensively investigated using a number of experimental models. One major hypothesis for early life physiological programming implicates fetal overexposure to stress (glucocorticoid) hormones. Several animal studies have shown that prenatal glucocorticoid excess, either from endogenous overproduction with maternal stress or through exogenous administration to the mother or fetus, reduces birth weight and causes lifelong hypertension, hyperglycaemia and behavioural abnormality in the offspring. Intriguingly, these effects are transmitted across generations without further exposure to glucocorticoids, which suggests an epigenetic mechanism. These animal observations could have huge implications if extrapolated to humans, where glucocorticoids have extensive therapeutic use in obstetric and neonatal practice.

EARLY LIFE PROGRAMMING
There is now a well-established link between low birth weight and the subsequent development of hypertension, insulin resistance, Type 2 diabetes and hyperlipidaemia, a cluster of cardiovascular risk factors that are termed the metabolic syndrome [1]. This relationship between birth weight and adult disease is largely independent of classical adult lifestyle risk factors, such as smoking, adult weight, social class, excess alcohol intake and sedentariness, which are additive to the effects of birth weight [2,3] and holds for the full range of birth weights, including those within the normal range rather than severely undersized, multiple or premature babies [2,4]. To explain this association between low birth weight and subsequent disease, the concept of early life programming has been advanced, which proposes that a stimulus or insult acting during critical periods of growth and development may permanently alter tissue structure and function, producing effects which may persist throughout life.

The effects of programming are determined by the timing of the exposure and on the developmental stage of individual organ systems. This concept is not without precedence; more than a century ago, Weisman [5]...
demonstrated that the early environmental temperature plays a critical role in phenotypic development in butterflies. Thus butterflies that hatched in summer were coloured differently from those that hatched in winter, and this season-dependent colouration could be mimicked by incubating larvae at different temperatures [5]. It is now known that many agents regulate the development of mammalian systems and, therefore, influence later pathophysiology, including homeotic genes, transcription factors, growth factors, hormones and nutrients.

Evidence from both human and animal studies [1,6–8] suggests that many diseases of adult life can be induced by manipulating the environment of the fetus; however, the molecular mechanisms underlying the association between low birth weight and later disease are unknown. Two major hypotheses have been proposed to explain this relationship: fetal undernutrition [2,9] and overexposure of the fetus to glucocorticoids [10]. These hypotheses are not mutually exclusive, but this review will focus on the programming effects of glucocorticoids.

**ROLE OF GLUCOCORTICOIDS**

The programming effects of steroid hormones were initially best characterized in androgens. Neonatal exposure to androgens permanently programmes the expression of hepatic steroid-metabolizing enzymes and the development of sexually dimorphic structures in the anterior hypothalamus, leading to lifelong changes in sexual behaviour [11,12]. These effects persist, irrespective of subsequent hormonal manipulation or the genetic sex of the animal. More recently, the programming effects of glucocorticoids have been extensively investigated. Secretion of glucocorticoids from the adrenal cortex is controlled by the HPA (hypothalamic–pituitary–adrenal) axis in a classical endocrine negative-feedback loop (Figure 1). Glucocorticoids exert their effects by binding intracellular GRs (glucocorticoid receptors), members of the nuclear hormone superfamily of ligand-activated transcription factors. Additionally, in some tissues, glucocorticoids bind with high affinity to MRs (mineralocorticoid receptors). GRs and MRs are activated upon ligand binding and the receptor–ligand complex translocates to the nucleus, binding to GREs (glucocorticoid-response elements) in the promoter region of target genes to influence gene transcription [13] (see Figure 2). In addition, evidence suggests that rapid non-genomic effects of these steroids may be mediated via novel cell-membrane receptors [14] (see Figure 2). The GR is found in the cells of almost all vertebrate tissues and, in adult mammals, glucocorticoids regulate a variety of important cardiovascular, metabolic, immunological and other homoeostatic functions, although, at least in mice, glucocorticoids appear to be more important in fetal development than in adult life [15]. Excessive glucocorticoid levels, resulting from either exogenous administration or endogenous overproduction (e.g. in Cushing’s syndrome), have effects on many systems including well-characterized diabetogenic and hypertensive effects [16].

**Glucocorticoids and fetal development**

GRs are expressed in most fetal tissues from midgestation onwards [17] and in the placenta [18]. The expression of MRs has a more limited tissue distribution and is present only at later gestational stages, at least in rodents [19]. Steroid hormones are typically associated with organ development and maturation and with long-term organizational effects. Many of the significant
maturational changes in organ systems, such as the lungs, heart, liver, gut and kidneys [20–22], are glucocorticoid-dependent and can be induced prematurely by exogenous glucocorticoid administration [23,24], underpinning their wide-spread therapeutic use in threatened preterm labour and in the perinatal period, particularly to accelerate the rate of lung maturation [25]. Furthermore, GR-null mice die within the first few hours after birth of respiratory failure, due to severe lung atelectasis, and have severely retarded maturation of the adrenergic chromaffin cells and hepatic gluconeogenic enzymes [26].

Glucocorticoid treatment during pregnancy reduces birth weight in animals and in humans [7,27–31]. Furthermore, cortisol levels are increased in human fetuses with intrauterine growth retardation or in pregnancies complicated by pre-eclampsia, which may indicate a role for endogenous cortisol in fetal growth retardation [32,33]. However, there may be significant differences between the effects of fetal exposure to endogenous glucocorticoids and those of synthetic glucocorticoids. For example, although endogenous glucocorticoids can bind both GRs and MRs, and the effects in tissues such as brain may be mediated by both of these receptors, synthetic glucocorticoids are more selective for GRs. Similarly, there may be differences in local concentrations in tissues, which may be governed by differences in transport (e.g. across the blood–brain barrier) and metabolism of endogenous and synthetic glucocorticoids [34,35].

However, it is also apparent that programming effects may be seen in the absence of changes in birth weight and, indeed, the programming effects of prenatal glucocorticoid excess are not dependent on alterations in fetal growth. In rats, short-term prenatal exposure (2 days) to dexamethasone or corticosterone is associated with programmed effects on BP (blood pressure) and renal development [36–38] and, in sheep, short-term exposure to either dexamethasone or cortisol resulted in hypertension in the absence of changes in birth weight [39,40]. Such studies suggest that birth weight is a crude measure of exposure to an adverse environment in utero and that disease risk may be increased in the absence of changes in weight at birth.

11β-HSD2 (type 2 isoform of 11β-hydroxysteroid dehydrogenase): the feto-placental glucocorticoid barrier

Although glucocorticoids are highly lipophilic molecules and should readily cross biological barriers, such as the placenta, fetal glucocorticoid levels are normally much lower than maternal levels due to the presence of an enzyme called 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) in the fetal compartment. This enzyme inactivates cortisol in the fetus, thereby preventing excessive glucocorticoid exposure and maintaining fetal homeostasis.
Figure 3  Feto–placental barrier to glucocorticoids

Placental 11β-HSD2 rapidly inactivates physiological glucocorticoids (red circles) to inert 11-keto forms (green circles). The enzyme thus ensures that high maternal glucocorticoid levels are largely excluded from the fetus.

lower than the levels in the maternal circulation [41]. This is thought to be mediated by placental 11β-HSD2, which catalyses the conversion of active glucocorticoids (cortisol in humans, and corticosterone in rats) into their inactive 11-keto metabolites (cortisone and 11-dehydrocorticosterone respectively; see Figure 3) [42].

The 11β-HSD2 enzyme is expressed in the placenta of humans and other mammalian species. It is localized to the syncytiotrophoblast, the site of maternal–fetal exchange, where it is well-positioned to serve as the barrier for glucocorticoid transfer. Given that fetal exposure to excessive amounts of glucocorticoids leads to intrauterine growth retardation, it has been hypothesized that the physiological significance of this placental 11β-HSD barrier is to protect the fetus from adverse effects of maternal glucocorticoids. The enzyme is not a complete barrier to maternal glucocorticoids, and studies in rats and humans indicate that the efficiency of placental 11β-HSD2 near term varies considerably [43,44]. The lowest placental 11β-HSD2 activity, and presumably the highest fetal exposure to maternal glucocorticoids, is seen in babies with the smallest birth weights [44]. Likewise, in humans, mutations of the gene encoding 11β-HSD2 (HSD17B2) are associated with significant reductions in birth weight [45]. Although the mechanisms involved in acute regulation of placental 11β-HSD2 are largely unknown, evidence suggests that epigenetic mechanisms may modify expression of the gene encoding 11β-HSD2 [46] and, in vitro, human placental 11β-HSD2 is inhibited by progesterone, oestrogen and NO and stimulated by activators of the cAMP pathway [47]. In the baboon, oestrogen synthesized locally in the placenta from fetal adrenal androgens are thought to promote 11β-HSD2 activity [47]. Intriguingly, very recent studies in rats have demonstrated that exogenously administered corticosterone, resulting in circulating maternal corticosterone levels similar to those seen as a result of restraint stress, are associated with programming of hypertension in the absence of changes in birth weight [38], suggesting that levels of glucocorticoids associated with physiological stress may overcome the placental 11β-HSD2 barrier, leading to effects on the developing fetus.

Intriguingly, maternal protein restriction in rodents reduces the activity of 11β-HSD2 in the placenta [6], suggesting that 11β-HSD2 activity is influenced by maternal environmental factors and that other environmental insults, such as maternal malnutrition, may operate through glucocorticoids in exerting their programming effects (Figure 4). In support of this notion, the programming of hypertension by maternal protein restriction during pregnancy in the rat can be prevented by the inhibition of maternal corticosterone biosynthesis during pregnancy [48]. Conversely, glucocorticoid treatment in rodents may be associated with reduced food intake and impaired weight gain [49,50], providing further support for an inter-relationship between these hypotheses.

Fetal glucocorticoid load can be artificially increased through a number of ways. A number of synthetic glucocorticoids, such as dexamethasone and betamethasone, are poor substrates for 11β-HSD2 and will readily cross the placenta following maternal administration. Fetal glucocorticoid exposure can also be increased by inhibiting feto–placental 11β-HSD2 by liquorice or its derivatives, such as carbenoxolone [42]. These strategies have been employed in animal models to study the effects of prenatal glucocorticoid overexposure on long-term pathophysiology. We have shown that rats exposed to dexamethasone during the last third of pregnancy are of low birth weight and develop hypertension and glucose intolerance in adulthood [7,43].

PROGRAMMING OF CARDIOVASCULAR AND METABOLIC SYSTEMS

BP

Cortisol elevates BP in fetal sheep when infused directly in utero [51] and at birth in humans [52] and in sheep [53]. The administration of betamethasone to pregnant baboons elevates fetal BP [54]. In rats, antenatal glucocorticoid overexposure is associated with a modest reduction in birth weight. Although this deficit in body weight is almost reversed by the time of weaning, both male and female offspring show a persistent elevation in arterial BP in adulthood [43,49,50,55], as do sheep exposed to excess glucocorticoid in utero either as maternally administered dexamethasone or as a maternal cortisol infusion [39,40,56,57]. The timing of glucocorticoid exposure appears to be important, as exposure to glucocorticoids during the final week of pregnancy is sufficient to produce permanent adult hypertension in the rat [55]; indeed, recent studies suggest that exposure to excess glucocorticoid for just 2 days at this time is sufficient to programme renal altered renal development and hypertension in the absence of altered fetal growth [38]. In contrast, in sheep, such effects are seen after glucocorticoid exposure earlier in gestation [58–60]. The reason for such differences are unclear, but may reflect the complex species-specific
Excessive fetal glucocorticoid exposure might result from inhibition of fetoplacental 11β-HSD2 activity or through exogenous administration of synthetic glucocorticoids. Additionally, maternal environmental insults increase circulating glucocorticoid concentrations and/or reduce placental 11β-HSD2 activity, which might increase delivery of glucocorticoids to the fetus. Increased fetal glucocorticoid load influences development of various physiological systems and programmes aberrant tissue responses, leading to later disease. Possible interactions with genetic and postnatal environmental factors are shown.

Patterns of expression of GRs and the isoenzymes of 11β-HSD [42], which regulate maternal glucocorticoid transfer to the fetus and modulate glucocorticoid action in individual tissues. In rats, maternal administration of carbenoxolone, a potent inhibitor of 11β-HSD, also leads to reduced birth weight and elevated BP in the adult offspring [61]. The programming effects of carbenoxolone require the presence of maternal glucocorticoids, as the offspring of adrenalectomized pregnant rats are protected from carbenoxolone actions upon birth weight and adult hypertension [61].

Glucocorticoid programming of BP is likely to involve a number of processes. Prenatal glucocorticoid overexposure is associated with an irreversible reduction in nephron number in rodents [36,37,62] and sheep [63]. Rats exposed to glucocorticoids prenatally had altered activity of the RAAS (renin–angiotensin–aldosterone system) and altered vascular function in a region-specific manner [64,65], and have increased expression of the AT₁ and AT₂ receptors (angiotensin II type 1 and 2 receptors respectively) and ACE (angiotensin-converting enzyme) [66,67]. Vascular responsiveness to other vasoconstrictors may also be altered, with enhancement of endothelin-induced vasoconstriction and attenuation of endothelium-dependent vasorelaxation reported from studies in sheep [68]. Prenatal glucocorticoid exposure alters reactivity of the coronary arteries in the newborn lamb prior to the development of systemic hypertension [69]. Additionally, the offspring have altered cardiac noradrenergic innervation and sympathetic activity of
baroreceptor response [20,66]. Changes in expression of several other genes in the heart have been reported. These include glucose transporter 1, Akt/PKB (protein kinase B), specific uncoupling proteins, PPARγ (peroxisome-proliferator-activated receptor γ) and calreticulin [70,71]. Overexpression of cardiac calreticulin is known to cause cardiac dysfunction and death [71]. Thus increased coronary heart disease deaths in individuals born with low birth weight may reflect programmed primary cardiac dysfunction as well as the increased prevalence of cardiovascular risk factors such as hypertension.

**Glucose and insulin homoeostasis**

Prenatal glucocorticoid overexposure in rats as a result of 11β-HSD2 inhibition or maternal dexamethasone administration programmes permanent hyperglycaemia and hyperinsulinaemia in adult rats [7,72]. As with programming of BP, week 3 of gestation appears to be an important ‘window’ during which glucocorticoid exposure results in programming effects: exposure in early gestation or during the neonatal period does not appear to produce the same long-lasting changes in blood glucose and insulin levels [7,73]. Additionally, the effects of carbamazepine on birth weight and adult glucose metabolism can be prevented by maternal adrenalectomy, confirming that they are dependent on maternal glucocorticoids [72]. Increasing endogenous glucocorticoid production through prenatal stress (which presumably overcomes the activity of 11β-HSD2) is thought to have similar persisting effects [74,75].

Maternal glucocorticoid administration has an effect on cord glucose and insulin levels in the ovine fetus [76] and on glucose homoeostasis in the adult offspring. Intriguingly, in sheep, antenatal glucocorticoid exposure with or without fetal growth restriction altered glucose metabolism [60]; maternal, but not fetal, injections of betamethasone restricted fetal growth [29], although the offspring of both groups had altered glucose metabolism postnatally [60], suggesting that programming relates to fetal exposure to excess glucocorticoids in utero, rather than intrauterine growth retardation itself. The molecular mechanisms through which prenatal supraphysiological levels of glucocorticoids programme hyperglycaemia have not been fully determined, but may involve derangements in several target organs. In particular, these effects may relate to altered structure or function of the endocrine pancreas and/or insulin-sensitive target tissues.

Glucocorticoids regulate expression of critical hepatic metabolic enzymes, notably PEPCK (phosphoenolpyruvate carboxykinase), which develops in late gestation and catalyses a rate-limiting step in gluconeogenesis. In rats, in utero exposure to dexamethasone induces lifelong elevations in PEPCK mRNA and enzyme activity, selectively within the portal region of the hepatic acinus, the site of gluconeogenesis [7]. PEPCK is the rate-limiting enzyme of gluconeogenesis [77], and transgenic mice with overexpression of hepatic PEPCK have impaired glucose tolerance [78]. In addition, overexpression of PEPCK in a rat hepatoma cell line impairs suppression of gluconeogenesis by insulin [79]. It has therefore been proposed that the increase in PEPCK in programmed animals may therefore be of functional significance in the pathogenesis of hyperglycaemia following prenatal exposure to glucocorticoids [7]. PEPCK expression is normally regulated at the level of gene expression by distinct hepatocyte-enriched nuclear transcription activators that bind their cognate DNA motifs in the PEPCK gene promoter [80]. These include family members of HNF (hepatocyte nuclear factor) 1, HNF3, HNF4 and HNF6, members of the C/EBP (CCAAT/enhancer-binding protein) family and the GR itself. Intriguingly, livers of dexamethasone-programmed rats have increased expression some of these key transcription factors, notably GRs [7,81] and HNF4α [82] in the liver, suggesting that the increase in hepatic PEPCK expression may be secondary to the alterations in these transcription factors. Increased hepatic GR expression is also seen in other models of in utero programming of hyperglycaemia, such as maternal protein restriction or uterine artery ligation [83]. This suggests that changes in transcription factors, such as GRs, may provide a common mechanism through which intrauterine environmental insults might lead to persistent derangements in metabolic control.

**Programming of the pancreas**

Relatively little is known about the effects of prenatal exposure to glucocorticoids on the development of the endocrine pancreas. Glucocorticoid signalling is important in pancreatic development [84], and glucocorticoids have been shown to impair β-cell development in rats [85]. Prenatal undernutrition also causes marked restriction of fetal β-cell growth and impairs glucose-stimulated insulin secretion in the adult rat offspring. In vivo, the isolated pancreatic islets have decreased insulin content and have impaired secretory response to glucose and arginine [86]. Again, this effect appears to be dependent on maternal/fetal corticosterone, as preventing the increase in corticosterone in food-restricted dams restores β-cell mass. Indeed, fetal pancreatic insulin content correlates inversely with fetal corticosterone levels [85]. The mechanisms by which glucocorticoids modulate pancreatic development are not fully understood, but in vitro data suggest that these may involve interaction with other transcription factors that control proliferation and/or differentiation of the pancreas [87]. Additionally, in the adult pancreas, glucocorticoids are known to directly inhibit insulin secretion. The elevated circulating glucocorticoid levels in animals exposed to dexamethasone in utero may therefore also impair β-cell function and contribute to hyperglycaemia. Finally, glucocorticoids influence the expression of IGF (insulin-like growth factor) 2, a key peptide growth factor in pancreatic
have shown that prenatal glucocorticoid excess affects activity of this axis at several levels. For example, prenatal dexamethasone exposure or 11β-HSD2 inhibition permanently increases basal plasma corticosterone levels or responsiveness of the HPA axis in adult rats [55,107], sheep [108], guinea pigs [109,110] and primates [111]. These effects are dependent on timing of exposure [108] and may be sex-specific [112]. Intriguingly, maternal undernutrition in rodents [113] or sheep [114] also affects adult HPA axis function, suggesting that HPA axis programming may be a common outcome of prenatal environmental challenge.

In utero exposure to glucocorticoids during the last third of pregnancy reduces MR and GR levels in the hippocampus. It is thought that the decrease in hippocampal GR expression reduces the sensitivity of feedback and, thus, permanently alters the ‘set point’ of the HPA axis. [115]. In contrast, in the ‘neonatal handling’ paradigm [93], where the neonatal environment is enriched by short (15 min daily) handling of rat pups during the first 2 weeks of life, there is a permanent increase in hippocampal GR levels with potentiation of HPA axis sensitivity to glucocorticoid negative feedback. This results in lower plasma glucocorticoid levels and a better response to stress throughout life [98].

Programming offspring behaviour

Overexposure to glucocorticoids in utero, as a result of prenatal dexamethasone administration or 11β-HSD inhibition, leads to alterations in adult behaviour and may programme ‘behavioural inhibition’ and reduced coping under conditions of stress. For example, adult animals exposed to dexamethasone in utero have reduced ambulation and show anxious behaviour with impaired exploration in a number of analyses [116]. These effects are influenced by the timing of treatment, and late gestation is thought to be the critical window. Intriguingly, maternal prenatal stress, which presumably increases fetal glucocorticoid load, is also associated with increased anxiety in the adult offspring in rodents. The offspring have increased ‘emotionality’ in response to behavioural tests and novel environments [98].

The molecular mechanisms through which adverse prenatal environment or glucocorticoids might mediate behavioural changes are largely unknown. However, several molecules that are involved in regulation of neuronal survival, HPA function, other higher centre functions and behaviour have been identified as possible targets of prenatal glucocorticoid effects. These include hippocampal GRs, BDNF (brain-derived neurotrophic factor), CRH (corticotropin-releasing hormone), and neuropeptide Y [75,117]. Prenatal glucocorticoid treatment may permanently reprogramme expression of these molecules. For example, prenatal dexamethasone or 11β-HSD inhibition increases CRH mRNA levels in the central nucleus of the amygdala, a key locus for the effects.
of the neuropeptide on the expression of fear and anxiety [107,116]. Intra-amygdala administration of CRH has an anxiogenic effect [118]. The increase in CRH expression in the amygdala is associated with changes in GR or MR expression [55,116], and corticosteroids facilitate CRH mRNA expression in this nucleus [119] and increase GR and/or MR in the amygdala [107,116]. A direct relationship between brain corticosteroid receptor levels and anxiety-like behaviour is supported by the phenotype of transgenic mice with disrupted GR expression in the brain, which have markedly reduced anxiety behaviours [120]. NMDARs (N-methyl-D-aspartate receptors) have also been implicated in the programming of behaviour; in guinea pigs, exposure to repeated doses of betamethasone prenatally is associated with altered expression of the hippocampal NMDAR subunits and increased locomotor activity in females [121].

Prenatal glucocorticoid exposure also affects the developing dopaminergic system [122–126], and this may have implications for proposed developmental contributions to schizo-affective, attention-deficit hyperactivity, extrapyramidal disorders and drug addiction. There is also evidence suggesting that prenatal treatment with dexamethasone renders cholinergic neurons more vulnerable to challenges later in life [123]. In humans, stressful events in the second trimester of pregnancy are associated with an increased incidence of schizophrenia in the offspring [127] and, similarly, postnatal maternal deprivation or separation increases anxiety-related behaviours in rodents and humans [128,129], suggesting that the window of sensitivity in programming behaviour extends into the early postnatal period. These postnatal environmental insults are also associated with increased CRH content in limbic structures, such as the amygdala, and overactivity of the HPA axis, at least in the rat [115].

EVIDENCE FOR GLUCOCORTICOID PROGRAMMING IN HUMANS

The effects of prenatal glucocorticoid exposure observed in animal models could have huge implications if extrapolated to the human fetus. Glucocorticoids are used as immunosuppressants to control various maternal conditions, such as connective tissue disorders [130], and are used extensively in obstetric practice, primarily to accelerate lung maturation in cases of threatened preterm labour [131], which may occur in up to 10% of pregnancies. There is no doubt that such synthetic glucocorticoids enhance lung maturation and reduce mortality in preterm infants [132]. Additionally, a single course of prenatal corticosteroid is associated with a significant reduction in the incidence of intraventricular haemorrhage and a trend toward less neurodevelopmental disability [132]. However, 98% of British obstetric departments have prescribed repeated courses of antenatal glucocorticoids [133], although there is little evidence for the safety and efficacy of such a regime [134]. In addition, women at risk of bearing fetuses at risk of CAH (congenital adrenal hyperplasia) often receive low-dose dexamethasone from the first trimester to suppress fetal adrenal androgen overproduction. Birth weight in such infants has been reported as normal [135,136]; however, it must be remembered that programming effects of antenatal glucocorticoids are seen in animal models in the absence of any reduction in birth weight [60].

The long-term effects of fetal glucocorticoid exposure in humans have been poorly investigated, mainly because these studies have been small and the duration of follow-up short [137]. In one randomized controlled trial in which BP was recorded in 81 individuals aged 20 years, mean systolic BP was lower in those exposed to antenatal glucocorticoid treatment than in controls [138]. In contrast, in a non-randomized cohort of 177 adolescents aged 14 years, those exposed to glucocorticoid treatment had higher mean systolic and diastolic BP than did those exposed to placebo [8]. In a recent double-blind placebo-controlled randomized trial of antenatal betamethasone for the prevention of neonatal respiratory distress syndrome, 534 individuals were followed up over 30 years; antenatal exposure to betamethasone did not significantly affect lipid profile, prevalence of diabetes or cardiovascular disease [139]. However, betamethasone-exposed participants in this study had evidence of increased insulin resistance, which might signify an increased risk of diabetes and cardiovascular disease as this cohort ages [139].

Studies aimed at establishing the long-term neurological and developmental effects of antenatal glucocorticoid exposure have been complicated by the fact that most of the children studied were born before term and were therefore already at risk of delayed neurological development. In a group of 6-year-old children, antenatal glucocorticoid exposure was associated with subtle effects on neurological function, including reduced visual closure and visual memory [140]. Children exposed to dexamethasone in early pregnancy, because they were at risk of CAH, and who were born at term, had increased emotionality, unsociability, avoidance and behavioural problems [141], in addition to long-term effects on verbal working memory [142]. Furthermore, multiple doses of antenatal glucocorticoids given to women at risk of preterm delivery have been associated with reduced head circumference in the offspring [30] and significant effects on behaviour; three or more courses of glucocorticoids were associated with an increased risk of externalizing behaviour problems, distractibility and inattention [143]. Children born preterm exposed to repeated doses of prenatal glucocorticoids had reduced risk of cerebral palsy, but did have an increased risk of postnatal aggressive/destructive behaviour, increased distractibility and hyperactivity [144] and behavioural problems later in childhood [145]. Likewise, antenatal stress/anxiety has
a programming effect on the fetus, which lasts at least until middle childhood [146,147], and the offspring of mothers who developed post-traumatic stress disorder following exposure to the World Trade Center attacks of 9/11 also had altered cortisol levels and temperament in the first year of life [148,149].

On the basis of findings in the prenatal dexamethasone-exposed rat model of low birth weight and adult hypercortisolemia, studies have examined the relationship between birth weight and HPA axis function in adult humans. As in other animals, the human HPA axis appears to be programmed by the early life environment. Programming of the HPA axis appears to occur in disparate populations [150] and may precede overt adult disease [151], at least in a socially disadvantaged South African population. Higher plasma and urinary glucocorticoid levels are found in children and adults who were of lower birth weight [152,153]. In children, low birth weight is associated with altered adrenocortical responses to stress in boys and altered basal adrenocortical activity in girls [154] and, in adulthood, HPA axis responses to corticotropin [ACTH (adrenocorticotropic hormone)] stimulation are exaggerated in those of low birth weight [151,155], reflecting the stress axis biology elucidated in animal models. Furthermore, this HPA axis activation is associated with higher BP, insulin resistance, glucose intolerance and hyperlipidaemia [155].

INTERGENERATIONAL CONSEQUENCES OF PROGRAMMING

There is evidence that programming effects might not be limited to the first generation and that effects may persist in subsequent generations. Epidemiological studies in humans suggest that there may be intergenerational effects on birth weight, cardiovascular risk factors and Type 2 diabetes (reviewed in [156]). In rats, the effects of prenatal dexamethasone treatment are not only observed in the immediate offspring as adults, but are transmitted to the second generation offspring, which also have elevated PEPCK and insulin levels, without further dexamethasone exposure [157]. The mechanisms for this transgenerational ‘inheritance’ of the programming phenotype are unknown; however, the transmission of the phenotype through either maternal or paternal lines indicates the potential involvement of epigenetic processes, such as changes in DNA methylation [156,157].

EPIGENETIC MECHANISMS IN FETAL PROGRAMMING

The potential role of epigenetic changes in the early life origins of disease is being increasingly recognized [156]; indeed, in a number of animal models, environmental manipulations have been shown to alter methylation at specific genes. Thus altered maternal diet during pregnancy can increase methylation of the agouti gene and the mouse AxinREF gene, which alters the phenotype of offspring [158,159]. Unbalanced prenatal nutrition can induce changes in DNA methylation which affects gene transcription, notably the expression of GRs in the liver [160,161]. Similarly, early postnatal behavioural programming in the rat can stably alter the epigenetic state of the GR gene in the hippocampus, with long-term effects on offspring behaviour [162]. Adult offspring of mothers that exhibit increased postnatal licking and grooming of pups have increased expression of hippocampal GR expression in association with demethylation at a specific CpG dinucleotide in exon 1, of the GR within the binding site of a key transcription factor NGFI-A (nerve growth factor-inducible protein A). This demethylation is persistent and is associated with altered histone acetylation and NGFI-A binding to the GR promoter. Additionally, NGFI-A appears to participate in this epigenetic programming of GR expression [163].

The GR itself may play a direct role in mediating epigenetic changes that may underlie glucocorticoid programming. Thus, in cultured fetal hepatocytes, glucocorticoid treatment causes differential demethylation of target gene promoters, and this demethylation persists after steroid withdrawal [164]. During development, such target promoter demethylation occurs before birth and may fine-tune the promoter to ‘remember’ regulatory events occurring during development.

There is increasing recognition that epigenetic modifications at some alleles might be transmissible to offspring, resulting in the intergenerational inheritance of the epigenetic state [165,166], which may underpin the transgenerational effects described in the glucocorticoid-programmed rat [157].

CONCLUSIONS

Thus there is accumulating data in humans, as in rodents, suggesting that prenatal glucocorticoid overexposure programmes an adverse adult cardiovascular, metabolic, neuroendocrine and behavioural phenotype, and these effects appear to be transmitted across generations. Whether this is an unusual or a common mechanism to explain the link between low birth weight/size at birth and adult disorders is the subject of ongoing studies. Taken as a whole, these data support the hypothesis that fetal overexposure to glucocorticoids mediates, at least in part, the relationship between the prenatal environment and adult disorders (see Figure 4). However, despite recent progress in our understanding of fetal programming, a number of questions remain unanswered. These include understanding the fundamental molecular mechanisms involved in glucocorticoid programming and intergeneration inheritance to establish the role of
glucocorticoids in programming for human disease, to
determine the importance of genetic variation, as well as
to explore how adverse programming can be overcribed.

ACKNOWLEDGMENTS
We are supported by the Medical Research Council and
Medical Research Scotland.

REFERENCES
1 Barker, D. (1998) In utero programming of chronic
gastrointestinal disease. Clin. Sci. 95, 115–128
2 Barker, D. J., Gluckman, P. D., Godfrey, K. M., Harding,
nutrition and cardiovascular disease in adult life. Lancet
341, 938–940
3 Barker, D. J., Osmond, C., Golding, J., Kuh, D. and
Wadsworth, M. E. (1989) Growth in utero, blood pressure
in childhood and adult life, and mortality from
growth and glucose and insulin metabolism in
four-year-old Indian children. Diabetic Med. 12, 330–336
5 Weismann, A. (1892) Essays on Heredity and Kindred
Biological Problems, Clarendon, Oxford
6 Langley-Evans, S. C., Phillips, G. J., Benediktsson, R.
et al. (1996) Protein intake in pregnancy, placental
glucocorticoid metabolism and the programming of
hypertension in the rat. Placenta 17, 169–172
7 Nyirenda, M. J., Lindsay, R. S., Kenyon, C. J.,
exposure in late gestation permanently programs rat
hepatic phosphoenolpyruvate carboxykinase and
glucocorticoid receptor expression and causes glucose
intolerance in adult offspring. J. Clin. Invest. 101,
2174–2181
8 Doyle, L. W., Ford, G. W., Davis, N. M. and Callanan, C.
(2000) Antenatal corticosteroid therapy and blood
98, 137–142
9 Hales, C. N. and Barker, D. J. P. (1992) Type 2
(non-insulin-dependent) diabetes mellitus: the thrifty
phenotype hypothesis. Diabetologia 35, 595–601
10 Edwards, C. R., Benediktsson, R., Lindsay, R. S. and
barrier: a link between the fetal environment and adult
hypertension? Lancet 341, 355–357
for androgenization of the developing hypothalamus in
the female rat. Endocrinology 82, 1010–1014
12 Gustafsson, J. A., Mode, A., Norstedt, G. and Skett, P.
(1983) Sex steroid induced changes in hepatic enzymes.
Annu. Rev. Physiol. 45, 51–60
transcription of specific genes and gene networks.
Annu. Rev. Genet. 19, 209–252
hydroxysteroid dehydrogenase and the heart.
Vitam. Horm. 66, 77–112
15 Muglia, L., Jacobson, L., Dikkes, P. and Majzoub, J. A.
(1998) Corticotropin-releasing hormone deficiency
reveals major fetal but not adult glucocorticoid need.
Nature 393, 427–432
16 Howlett, T. A., Rees, L. H. and Besser, G. M. (1985)
Cushing's syndrome. Clin. Endocrinol. Metab. 14,
911–945
17 Cole, T. J., Blendy, J. A., Monaghan, A. P., Schmidt, W.,
analysis of glucocorticoid signalling during mouse
development. Steroids 60, 93–96
18 Sun, K., Yang, K. and Chiu, J. B. (1997) Differential
expression of 11β-hydroxysteroid dehydrogenase types 1
and 2 in human placenta and fetal membranes.
J. Clin. Endocrinol. Metab. 82, 300–305
19 Brown, R. W., Diaz, R., Robson, A. C. et al. (1996) The
ontogeny of 11β-hydroxysteroid dehydrogenase type 2
and mineralocorticoid receptor gene expression reveal
intricate control of glucocorticoid action in development.
Endocrinology 137, 794–797
dexamethasone exposure interferes with establishment of
cardiac noradrenergic innervation and sympathetic
activity. Teratology 47, 109–117
Promotional role for glucocorticoids in the development of
intracellular signalling: enhanced cardiac and renal
adenylate cyclase reactivity to β-adrenergic and
non-adrenergic stimuli after low-dose fetal
dexamethasone exposure. J. Dev. Physiol. 17, 289–297
22 Celi, G., Kistner, A., Ecklof, A. C., Cecchelli, S.,
growth by prenatal dexamethasone and the programming of
blood pressure in the offspring. J. Am. Soc. Nephrol.
8, A1360–A1360
Monogr. Endocrinol. 12, 493–515
Targeted disruption of the glucocorticoid receptor gene
blocks adrenergic chromaffin cell development and
severely retards lung maturation. Genes Dev. 9, 1608–1621
27 Reischl, J. M., Simon, N. G., Karow, W. G. and
Gandelman, R. (1978) Prenatal exposure to prednisone in
humans and animals retards intrauterine growth. Science
202, 436–438
28 Ikegami, M., Jobe, A. H., Newnham, J., Polk, D. H.,
glucocorticoids improve lung function and decrease
156, 178–184
29 Newnham, J. P., Evans, S. F., Godfrey, M., Huang, W.,
Ikegami, M. and Jobe, A. (1999) Maternal, but not fetal,
administration of corticosteroids restricts fetal growth.
J. Matern. Fetal Med. 8, 81–87
30 French, N. P., Hagan, R., Evans, S. F., Godfrey, M. and
size at birth and subsequent development. Am. J.
Obstet. Gynecol. 180, 114–121
31 Bloom, S. L., Sheffield, J. S., McIntire, D. D. and Leveno,
K. J. (2001) Antenatal dexamethasone and decreased birth
32 Goland, R. S., Jozak, S., Warren, W. B., Conwell, I. M.,
Stark, R. I. and Tropper, P. J. (1993) Elevated levels of
umbilical cord plasma corticotropin-releasing hormone in
growth-retarded fetuses. J. Clin. Endocrinol. Metab. 77,
1174–1179
33 Goland, R. S., Tropper, P. J., Warren, W. B., Stark, R. I.,
corticotropin-releasing hormone in the umbilical-cord
blood of pregnancies complicated by pre-eclampsia.
Reprod. Fertil. Dev. 7, 1227–1230
34 McCabe, L., Marash, D., Li, A. and Matthews, S. G.
(2001) Repeated antenatal glucocorticoid treatment decreases
hypothalamic corticotropin-releasing hormone mRNA but not corticosteroid receptor mRNA
13, 425–431
differential glucocorticoid and mineralocorticoid action in
the brain and peripheral tissues. Clin. Biochem. 38,
401–409
36 Ortiz, L. A., Quan, A., Weinberg, A. and Baum, M.
(2001) Effect of prenatal dexamethasone on rat renal
development. Kidney Int. 59, 1663–1669
37 Ortiz, L. A., Quan, A., Zarrar, F., Weinberg, A. and
hypertension and renal injury in the rat. Hypertension
41, 328–334


Levitt, N. S., Lindsay, R. S., Holmes, M. C. and Seckl, J. R. (1996) Dexamethasone in the last week of pregnancy attenuates hypocalcemic glucocorticoid receptor gene expression and elevates blood pressure in the adult offspring in the rat. Neuroendocrinology 64, 412–418


Lindsay, R. S., Lindsay, R. M., Waddell, B. J. and Seckl, J. R. (1996) Inhibition of 11β-hydroxysteroid dehydrogenase in pregnant rats and the programming of blood pressure in the offspring. Hypertension 27, 1250–1254


Dodic, M., Samuel, C., Moritz, K. et al. (2003) Glucocorticoids and fetal programming 229

Glucocorticoids and fetal programming 229

The Authors Journal compilation © 2007 Biochemical Society


Received 28 March 2007/20 April 2007; accepted 27 April 2007
Published on the Internet 1 August 2007, doi:10.1042/CS20070107