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# Placental 11β-Hydroxysteroid Dehydrogenase and the Programming of Hypertension

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**Excessive foetal exposure to glucocorticoids retards growth and "programmes" adult hypertension in rats. Placental 11β-hydroxysteroid dehydrogenase (11β-HSD), which catalyses the conversion of corticosterone and cortisol to inert 11 keto-products, normally protects the foetus from excess maternal glucocorticoids. In both rats and humans there is considerable natural variation in placental 11β-HSD, and enzyme activity correlates with birth weight. Moreover, inhibition of placental 11β-HSD in the rat reduces birth weight and produces hypertensive adult offspring, many months after prenatal treatment with enzyme inhibitors; these effects are dependent upon maternal adrenal products. These data suggest that placental 11β-HSD, by regulating foetal exposure to maternal glucocorticoids, crucially determines foeto–placental growth and the programming of hypertension. Maternal protein restriction during pregnancy also produces hypertensive offspring and selectively attenuates placental 11β-HSD activity. Thus, deficiency of the placental barrier to maternal glucocorticoids may represent a common pathway between the maternal environment and foeto–placental programming of later disease. These data may, at least in part, explain the human epidemiological observations linking early life events to the risk of subsequent hypertension. The recent characterization, purification and cDNA cloning of a distinct human placental 11β-HSD (type 2) will aid the further study of these intriguing findings.**

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## INTRODUCTION

Much evidence and popular opinion suggest that common cardiovascular and metabolic diseases of middle-age (including hypertension, ischaemic heart disease and non-insulin-dependent diabetes mellitus) are caused by specific adult "lifestyle" factors, particularly smoking, dietary indiscretions, lack of exercise, stress and excessive alcohol consumption. These act both directly and via amplification of other risk factors, such as hyperlipidaemia and hyperfibrinogenaemia. Taken together with the documented familial clustering of these disorders, this engenders the accepted idea that environmental risk factors act in adult life upon a genetic background to determine disease occurrence.

Recently however, a burgeoning series of epidemiological studies, initiated by Professor David Barker and colleagues in Southampton, and since confirmed by other workers, has produced persuasive evidence, in several distinct populations in the U.K., elsewhere in Europe and Asia, to show that low birth weight predicts subsequent hypertension, hyperlipidaemia, insulin resistance, non-insulin-dependent diabetes mellitus and ischaemic heart disease deaths in adult life [1–3]. For hypertension, low birth weight has been shown to predict higher blood pressures in young children, adolescents and middle aged men and women [1, 2, 4, 5]. Indeed the most striking predictor of high blood pressure in middle life, in the important Preston study, was the otherwise unusual combination of low birth weight and a large placenta [2, 6]. A second group of small infants, who were long and thin at birth but had a small placenta, also has higher blood pressures in middle age. The differing patterns are likely to reflect variations in the nature, degree or timing of growth

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retarding influences [2]. Placental size *per se* appears to be of secondary importance to birth weight and neonatal morphology, and presumably reflects whether compensatory placental changes occur following a particular insult to foetal growth. Importantly, the relationship between birth weight and adult blood pressure is continuous and represents birth weights within the normal range, rather than severely undersized, multiple or premature babies [7]. These data should perhaps be less surprising, since it has long been recognized that blood pressure levels track and amplify from infancy to adulthood, clearly implicating early events in the determination of blood pressure throughout life [8].

These associations appear to be important predictors of later blood pressure—a small baby with a large placenta has a relative risk of adult hypertension approx. 3 times that of a large baby with a normal placenta [2, 6]. In contrast, the angiotensinogen M235T genotype, one of the better molecular markers of adult hypertension, is associated with a relative risk of only 1.6 [9]. Moreover, the documented adult "lifestyle" factors (smoking, exercise, nutrition and obesity, social class) are additive to the influence of early life [1, 2]. The presence of prominent postnatal "catch-up" growth may also be a risk factor for subsequent hypertension [1, 4], suggesting that smallness at birth due to environmental influences restraining intrauterine growth are of particular importance. However, not all aspects of infant and adult blood pressure are environmentally determined, and the roles of genetic [10, 11] and epigenetic [12, 13] factors in have also been emphasized.

## PROGRAMMING

What mechanism(s) might link between early life environmental influences and disease several decades later? The key concept suggested here is that an adverse intrauterine environment may "programme" or "imprint" the development of foetal tissues and organs, permanently determining responses, producing later dysfunction and disease [2, 3, 14]. Such programming has been shown in a variety of systems and reflects the ability of a factor, acting during a defined developmental stage or "window", to exert organizational effects that persist throughout life.

Maternal malnutrition, particularly deficiency of specific dietary components such as protein or iron deficiency, has been proposed to determine foetal growth and programming [2, 3]. Data in a variety of experimental species show that maternal dietary restriction during pregnancy restricts foetal growth [2, 3], although any links with later disease are not so clear. In rats, however, relatively modest dietary protein restriction in pregnancy causes hypertensive offspring [15], effects apparently exerted directly upon the foetus [16]. Nevertheless, the importance of maternal nutrition has not yet been established within the range of dietary variation (which might be anticipated to vary with social class) in the human populations reported [1] and, in humans and animals, maternal dietary deprivation may need to be extreme to affect birth weight appreciably [14].

Of course, maternal dietary manipulations also do not specify the biochemical or molecular pathogenic mechanisms, in the foetus, placenta and/or mother, which programme later disease. In this regard, although many agents (homeotic genes, transcription factors, growth factors, hormones) may exert programming actions, these are typically found with steroids. One of the best-defined examples is the action of endogenous androgens, in many vertebrate species, to programme androgen metabolizing enzyme expression in the liver [17], the development of sexually-dimorphic structures in the brain and sexual behaviour [17–20]. These effects can only be exerted during a specific perinatal period, but then persist throughout life, largely irrespective of any subsequent sex steroid manipulations [18, 20].

## GLUCOCORTICOID EXPOSURE *IN UTERO*

Several features of glucocorticoid excess suggest that adrenal steroids may play a role in determining foetal development and blood pressure. Thus, (i) it has been clearly shown that foetal exposure to excessive glucocorticoid levels retards foetal growth, both in animal models and humans [21–24]. Moreover, endogenous foetal cortisol levels are elevated in human intrauterine growth retardation [25]. (ii) Cortisol also affects placental size, with the direction and magnitude of change dependent upon the dose and timing of exposure [26]. (iii) Glucocorticoids act directly upon vascular tissues [27] and indirectly (for example upon hepatic angiotensinogen production) to increase blood pressure in adult animals and humans [28]. Moreover, cortisol increases foetal blood pressure directly *in utero*, at least in sheep, an action perhaps mediated by increased vascular responsivity to angiotensin II [29]. (iv) Perinatal stress and glucocorticoids exert programming actions upon the brain, particularly affecting the responses of the hypothalamic–pituitary–adrenal "stress" axis [30–33] and immune systems [34].

Recent data suggest that prenatal glucocorticoid exposure may programme offspring blood pressure levels in later life. Treatment of pregnant rats with the synthetic glucocorticoid dexamethasone, in a modest dose which reduces average birth weight by only 14% and does not affect foetal viability or gestation length, causes elevated blood pressures in the adult offspring, many months after the last exposure to the glucocorticoid *in utero* [35].

*The materno–foetal barrier: placental 11β-HSD*

Dexamethasone is a synthetic glucocorticoid which crosses the placenta fairly readily, but normally the foetus has much lower levels of physiological glucocorticoids (cortisol in humans, corticosterone in rats) than the mother [36, 37]. Glucocorticoids are highly lipophilic and readily cross biological membranes and barriers such as the placenta. However maternal cortisol is clearly substantially excluded from the foetus. This is achieved by placental 11β-HSD, which catalyses the rapid metabolism of cortisol and corticosterone to the inert 11-keto products (cortisone, 11-dehydrocorticosterone) [38]. This protective placental enzymic barrier is very efficient, so that most (but not all) maternal cortisol in inactivated on crossing to the foetus, ensuring that about three-quarters of active cortisol in the human foetal circulation at term is derived from the foetal adrenals [36]. A key example of the importance of 11β-HSD is provided by its regulation of ligand access to mineralocorticoid receptors in the distal nephron of the kidney [39, 40]. Purified mineralocorticoid receptors are non-selective for corticosteroid ligands, and bind cortisol (corticosterone) and aldosterone with high and similar affinity *in vitro* [41, 42]. However, it is clear that *in vivo* only aldosterone exerts mineralocorticoid actions in the kidney, despite a 100–1000-fold molar excess of circulating cortisol. This selective access of aldosterone *in vivo* is ensured by the action of 11β-HSD, which rapidly inactivates glucocorticoids. In contrast, aldosterone, because it forms an 11–18 hemi-ketal bridge [43], is not a substrate for 11β-HSD, and thus gains access to the receptors. When the enzyme is congenitally absent (the rare hypertensive "syndrome of apparent mineralocorticoid excess") or is inhibited by liquorice or its derivatives (glycyrrhetinic acid, carbenoxolone), then cortisol illicitly occupies and activates renal mineralocorticoid receptors, causing sodium retention, hypokalaemia and hypertension [44, 45]. At the cellular level this mechanism has been difficult to demonstrate, since most mammalian cell lines poorly express 11β-HSD activity. Indeed, a recent report has suggested that aldosterone-selectivity may reflect more an intrinsic property of mineralocorticoid receptors [46]. However, very recently clonal cells derived from mammalian kidney have been described in which 11β-HSD is highly expressed and clearly determines glucocorticoid access to mineralocorticoid receptors, thus regulating which ligands can control target gene transcription [47].

A very similar or identical enzyme in the placenta has been proposed to protect the foetus from maternal cortisol [48, 49]. It has been suggested that relative deficiency of placental 11β-HSD, by allowing increased access of maternal glucocorticoids to the foetus, may produce retarded growth and programme responses leading to later disease [14]; some data have recently been produced in support of this hypothesis:

(i) The efficiency of the placental enzymic barrier to maternal glucocorticoids varies considerably, at least in the rat [35]. Intriguingly, the lowest placental 11β-HSD activity, and presumably the highest foetal exposure to maternal glucocorticoids, is seen in the smallest term foetuses with the largest placentae, which, by extrapolation from human studies, are predicted to show the highest adult blood pressures. Moreover, because maternal glucocorticoid levels are much higher than foetal, a relative deficiency of placental 11β-HSD has far greater potential consequences, in terms of the glucocorticoid load upon the foetus, than any alterations in foetal adrenal production.

(ii) Inhibition of 11β-HSD by carbenoxolone treatment of pregnant rats exerts similar effects to administration of the synthetic steroid dexamethasone (which is a poorer substrate for placental 11β-HSD than physiological glucocorticoids [50, 51]). Thus maternal carbenoxolone reduces birth weight in rats [52]. The effect of carbenoxolone on birth weight is independent of changes in maternal blood pressure or electrolytes, but requires *maternal* glucocorticoids, suggesting that it is mediated via inhibition of placental 11β-HSD, rather than by less specific actions upon maternal physiology or via direct effects upon the foetus. Importantly, the male and female offspring of carbenoxolone-treated pregnancies are hypertensive when adult.

(iii) Over-exposure of foetal rats to maternal glucocorticoids may also model the other epidemiological associations in humans. The adult male offspring of carbenoxolone-treated pregnant rats show significant basal hyperglycaemia and appear to be insulin-resistant, at least as assessed by markedly increased plasma insulin responses to a standardized oral glucose load [53].

Indeed, exposure to excessive maternal glucocorticoids may be a common rather than exceptional mechanism whereby maternal environmental influences act upon foeto-placental growth. Thus, dietary protein restriction during rat pregnancy, which also reduces birth weight and produces hypertensive offspring [15], attenuates placental 11β-HSD activity [54].

*Placental 11β-HSD in humans*

Do any of these results in animal models apply to humans? Measurement of glucocorticoid levels in foetal or cord vessels is technically demanding and complicated by the potential response of plasma cortisol (but not cortisone) to the stresses of delivery. In adults, plasma osteocalcin, a protein derived from osteoblasts,

is very sensitive to longer-term glucocorticoid excess. The foetus also produces osteocalcin which does not appear to cross the placenta and might be a useful and convenient marker of chronic glucocorticoid exposure. Recent data suggest that placental 11β-HSD activity *in vivo*, as assessed by its ability to inactivate cortisol to cortisone across the cord artery to the cord vein (blood going to the foetus), closely correlates with foetal osteocalcin levels [55]. These data suggest that the efficiency of the placental enzymic barrier to maternal glucocorticoids is an important level of control of foetal glucocorticoid exposure. As for birth weight, this also correlates with placental 11β-HSD. In particular, use of an immediate *ex vivo* perfusion system that closely reflects the situation at term *in vivo*, shows that total placental 11β-HSD efficiency in humans correlates closely with birth weight [56]. Similar data have been reported in homogenates of placental tissues [57], although the direct relationship between birth weight and placental 11β-HSD activity is less strong than seen with *ex vivo* perfusion of fresh intact placenta, perhaps reflecting the moderate instability of 11β-HSD in stored placental samples (R. Benediktsson, unpublished data) or the measurement of activity in both "barrier" and "non-barrier" regions of the placenta. It remains to be determined whether the lower birth weight neonates with reduced placental 11β-HSD activity subsequently develop higher blood pressures or other disorders.

*Molecular biology of placental 11β-HSD*

From the above it is clear that placental 11β-HSD and its regulation are of considerable interest. An NADP(H)-dependent 11β-HSD has been purified from rat liver [58], antisera raised [59] and an encoding cDNA isolated [60]. The human, monkey, sheep and mouse cDNA homologues have also been cloned [61–64]. However, several lines of evidence indicate that this is not the enzyme responsible for protecting the foetus from maternal glucocorticoids [38]. In particular, (i) the micromolar affinity of this "liver-type" 11β-HSD (11β-HSD1) is too low to effectively exclude glucocorticoids from the foetus; (ii) there is a similar "protective" 11β-HSD in the distal nephron and yet this tissue is devoid of 11β-HSD1 immunostaining [39, 65]. Little NADP-associated 11β-HSD1 activity [57, 66] or immunoreactivity is found in the placenta; (iii) transcription of the 11β-HSD1 gene can be almost completely inhibited by high dose oestrogen and yet placental and renal enzyme activities are increased by oestrogens [67, 68]; (iv) transfection of 11β-HSD1 cDNA into mammalian cells usually produces predominantly 11β-reductase activity in intact cells, which, far from inactivating glucocorticoids, regenerates corticosterone from otherwise inert 11-dehydrocorticosterone [69, 70]; (v) there is no detectable defect of the 11β-HSD1 gene in the syndrome of apparent mineralocorticoid excess [71].

Thus, it seemed likely that there were one or more additional higher-affinity 11β-HSDs in placenta, kidney and other aldosterone-target tissues. Recently, a novel enzyme (11β-HSD2) has been characterized in human placenta [66]. This is NAD-dependent, and also differs in pH-optima, detergent solubility, latency and immunoreactivity to liver-type 11β-HSD1. Importantly, the placental enzyme has low nanomolar affinities for corticosterone and cortisol, making it better suited to metabolize the majority of maternal glucocorticoids entering the placenta and thus protect the foetus. A similar NAD-associated high affinity 11β-HSD activity has been found in the distal nephron [72].

Using subcellular fractionation, solubilization in the bile acid-related detergent CHAPS, 5′-AMP-affinity chromatography and 2-dimensional gel electrophoresis, human placental 11β-HSD2 has been purified 16,000-fold to homogeneity [73]. The purified protein has an apparent molecular weight of 40 kDa, in contrast to the 34 kDa 11β-HSD1 [59]. Extensive microsequencing of peptides from proteolytic digests of the purified protein reveal the placental enzyme to be a member of the short chain alcohol dehydrogenase superfamily of enzymes. Degenerate oligonucleotide primers based on the peptide sequence were used in RT–PCR on human placental RNA, allowing the amplification and cloning of an 11β-HSD2 cDNA fragment. This was then used to screen human placental libraries and a full-length clone (1919 bp), containing an open-reading frame encoding a protein encompassing all of the sequenced peptides, was isolated. Expression of the cDNA clone in CHO cells produces an NAD-dependent 11β-HSD with exclusive 11β-dehydrogenase activity and affinities for glucocorticoid substrates essentially identical to the purified placental enzyme; $K_m$ for corticosterone 12 nM, cortisol 45 nM and dexamethasone 140 nM, whereas aldosterone is not metabolized [51]. The enzyme is potently inhibited by the active liquorice component, glycyrrhetinic acid, and carbenoxolone. Northern analysis reveals 1.9 kb hybridizing transcripts in RNA prepared from human placenta, kidney, parotid, colon, skin and pancreas, but not in liver, adrenal or various brain subregions. *In situ* hybridization and immunohistochemical studies confirmed abundant 11β-HSD2 in the placental trophoblast and kidney, the latter localized to the distal convoluted tubule and collecting ducts. Sequence comparison with the recently reported human renal 11β-HSD2 cDNA reveal considerable similarity [50]. Whether the differences between the two clones represent polymorphisms in a single gene or perhaps microheterogeneity, as appears to occur in 3α-HSD and 3β-HSD isoforms [74–77], remains to be determined. The gene for 11β-HSD2 will be an important candidate locus for the syndrome of apparent mineralocorticoid excess and for studies of genetic linkage with blood pressure and birth weight.

*Regulation of placental 11β-HSD*

In the baboon, oestrogens, synthesized in the placenta from foetal adrenal androgens, maintain placental 11β-HSD activity [67]. The control of adrenal androgen release in the foetus is poorly understood. However, intrauterine growth retardation in humans is also associated with reduced foetal adrenal androgen production [25] which may then attenuate placental 11β-HSD activity, increasing materno-foetal glucocorticoid transfer. Maternal and/or foetal stress also stimulates placental secretion of corticotrophin-releasing hormone, which is elevated in the neonatal circulation in association with intrauterine growth retardation [25]. This may stimulate the foetal pituitary–adrenal axis to secrete glucocorticoids, amplifying the foetal glucocorticoid excess. Thus a cascade of effects may increase glucocorticoid levels in the growth retarded foetus [14, 24]. Such glucocorticoid excess may achieve a short-term benefit, perhaps by increasing the availability of glucose and other metabolic fuels. The consequences of such survival measures may be the programming of elevated glucocorticoid levels, higher blood pressure and hyperglycaemia, responses geared to coping with predicted increased levels of environmental stress, but at the expense of disease in later life. In contrast, progesterone inhibits placental 11β-HSD, at least in high concentrations [51, 67]. This may be of relevance near term, when progesterone levels are very high, but the biological importance of this remains to be determined.

## GLUCOCORTICOIDS AND PROGRAMMING

By what mechanism(s) might glucocorticoid exposure *in utero* cause later pathology? Neonatal stress or glucocorticoids permanently programme the pattern of hypothalamic–pituitary–adrenal axis responses, effects largely mediated via altered expression of glucocorticoid receptor genes in the brain regions responsible for glucocorticoid feedback [78]. However, the relationships between the early environment and programming, at least of the hypothalamic–pituitary–adrenal axis, are very complicated indeed and relatively subtle differences between stimuli may exert widely differing effects. Thus relatively minor stress in the neonatal period (15 min of handling of rat pups every day for the first 2 weeks of life) permanently increases glucocorticoid receptors in the brain, increasing sensitivity to feedback and thus keeping glucocorticoid levels low, a state compatible with good adjustment to environmental stress [30]. In contrast, more severe neonatal stress (180 min of maternal separation per day, immune challenge or higher dose glucocorticoid administration) has the opposite effect, programming reduced brain sensitivity to glucocorticoid feedback and hence greater responses to stress throughout life [79, 80]. This may represent a state of poor adjustment of the hypothalamic–pituitary–adrenal axis and associates with adverse consequences for glucocorticoid target organs. Moreover, the long-term manifestations of some prenatal programming stimuli may be substantially modified by the immediate postnatal environment [81], suggesting the existence of distinct "programming windows" which sometimes determine contrasting effects.

These data illustrate an important point, that apparently similar early life events may programme completely different responses, depending upon the degree, duration, developmental timing or number of stimuli. Hence, the long-term outcome in very severe intrauterine growth retarded neonates or the very large (macrosomic) babies of diabetic pregnancies may be entirely unrelated to the more subtle programming effects associated with birth weights varying within the normal range, as illuminated by the epidemiological studies of Barker and colleagues, discussed above.

*Tissue mechanisms*

Glucocorticoids, like other steroid and thyroid hormones, act by binding to and activating intracellular receptors, which then attach to specific pentadecamer palindromic DNA sequences (glucocorticoid response elements) in the regulatory regions of target genes, affecting transcription [82]. There are two receptor subtypes: type I or mineralocorticoid receptors, and type II, or glucocorticoid receptors, [82, 83]. Most cells express many glucocorticoid-regulated genes and the precise targets for glucocorticoid programming of hypertension are obscure. However, several pieces of data may help guide further study.

*Cardiovascular development.* In rats, prenatal low-dose dexamethasone affects the development and maturation of specific organs including the lungs, heart, vasculature, kidney and brain. Important biochemical effects include permanent induction of the pattern of adrenergic (α and β) receptor expression and potentiation of adenylate cyclase [84, 85]; both might alter subsequent vascular responsivity to vasoconstrictors. In this light, it is interesting that foetal sheep directly infused with cortisol become hypertensive and show increased pressor responses to angiotensin II [29] which is also an important vasoconstrictor in humans. Cardiac development is also programmed by dexamethasone *in utero* [86], as is the pattern of development of the sympathetic innervation of many target organs [87].

*CNS effects.* Stress and glucocorticoids, acting prenatally or in the immediate postnatal period, can programme glucocorticoid receptor expression in regions of the brain (particularly the hippocampus) responsible for mediating glucocorticoid negative feedback on the hypothalamic–pituitary–adrenal axis [30, 88]. These effects appear to be mediated via actions upon glucocorticoid receptor gene expression [89] and glucocorticoid-sensitive transcriptional control regions of this

gene have been described [90]. Some preliminary data suggest that prenatal dexamethasone permanently attenuates glucocorticoid receptor expression in the adult brain, reducing sensitivity to feedback and thus programming increased plasma glucocorticoid levels in later life [91]. Such chronically elevated glucocorticoid levels might indeed contribute to the hypertension observed in this model [35].

*Growth factors.* Considerable evidence points to a central role for the insulin-like growth factors, IGF-1 and IGF-2, in the determination of foetal and placental growth [92]. Both IGFs, their receptors and several IGF-binding proteins are regulated by glucocorticoids in foetal tissues [93, 94]. The IGF system, which is also affected by maternal nutrition [2], thus provides a reasonable final common pathway, through which a range of maternal and foetal (or placental) genetic and environmental factors (including 11$\beta$-HSD and glucocorticoids) might affect foeto-placental development and growth.

*Glucose–insulin relationships.* Glucocorticoids regulate several important hepatic processes, including many enzymes controlling the production and fate of metabolic fuels. A notable example is phosphoenolpyruvate carboxykinase, the rate-limiting step in gluconeogenesis, and an important target gene/protein in non-insulin-dependent diabetes mellitus [95]. Glucocorticoids also attenuate insulin sensitivity, and regulate adipocyte distribution and function. Indeed, there may be more than coincidence in the morphological similarities between the adult centripetal obesity that associates with cardiovascular disease, non-insulin-dependent diabetes mellitus and particularly Syndrome X and the truncal adiposity of Cushing's syndromes. Prenatal exposure to excess glucocorticoids might programme these systems leading to subsequent abnormalities in insulin–glucose and fat homeostasis (which in humans associate with low birth weight [96]).

## GLUCOCORTICOIDS IN HUMAN PREGNANCY

In humans, very severe maternal malnutrition has been associated with foetal growth retardation, as in the Dutch famine in 1944–1945 [97]. Interestingly, it has been known for some time that chronic dietary deficiency also increases hypothalamic–pituitary–adrenal axis activity, and hence glucocorticoid levels, in humans [98, 99]. Whether this effect also occurs in pregnancy is unknown. Although placental 11$\beta$-HSD metabolizes most maternal cortisol, some transplacental passage occurs. However, it is unclear if elevated maternal cortisol levels cross the placenta sufficiently to increase foetal exposure.

High-dose exogenous glucocorticoid treatment during human pregnancy reduces birth weight [21, 22], but might have little relevance to physiological or modern therapeutic situations. In non-human primates, short-term prenatal dexamethasone administration in the last trimester programmes offspring's hypothalamic–pituitary–adrenal axis function and neuronal density, but again the doses employed have been high [100, 101]. There is scanty clinical evidence concerning the effects of low-dose glucocorticoids *in utero*. 11$\beta$-HSD substrates, such as cortisol and prednisolone, would be anticipated to have little effect once placental 11$\beta$-HSD2 is active. Other glucocorticoids are rarely administered for long, although women at risk of bearing female foetuses affected by congenital adrenal hyperplasia (mostly 21-hydroxylase deficiency) often receive dexamethasone from the first trimester in an attempt to suppress foetal adrenal androgen overproduction and consequent virilization. No teratogenic effects of low-dose dexamethasone (10–30 $\mu$g/kg/day) have been found [102] and birth weight has been reported as normal, although this has not been rigorously addressed and in one report 3 of 3 cases had 25th centile birth weights [103] (it is important to recognize that the increased incidence of adult disease occurs within the normal birth weight range). No data have been reported on the effects of such therapy on offspring blood pressure or other cardiovascular risk factors. Moreover, affected patients have markedly abnormal adrenal steroid (e.g. androgen) levels throughout life, complicating the interpretation of any findings. The effects of such therapeutic manoeuvres upon treated, but otherwise normal offspring (i.e. those without congenital adrenal hyperplasia) have barely been reported, although some persistent behavioural and affective changes have been noted [104]! 11$\beta$-HSD inhibition (liquorice abuse, carbenoxolone therapy) has not been associated with teratogenesis, but subtle reductions in birth weight and the incidence of subsequent disease have not been sought.

The intriguing animal data suggest that the effects of prenatal corticosteroids should be examined in some detail in humans. In particular, detailed observations of cardiovascular and metabolic risk factors are needed in the children of women receiving synthetic glucocorticoids during pregnancy, either for congenital adrenal hyperplasia or for threatened premature labour, for which shorter courses of higher doses of dexamethasone are often employed. Understanding of the biochemical and molecular processes which underlie prenatal programming of hypertension (and other disorders) may eventually lead to the development of preventive strategies, which will have considerable clinical importance.

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