ACTA PHYSIOLOGICA

Acta Physiol 2014, **210**, 288–295

REVIEW

Foetal and placental 11β-HSD2: a hub for developmental programming

E. C. Cottrell,[1] J. R. Seckl,[2] M. C. Holmes[2] and C. S. Wyrwoll[3]

1 Maternal and Fetal Health Research Centre, Institute of Human Development, University of Manchester, Manchester, UK

2 University/BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK

3 School of Anatomy, Physiology and Human Biology, University of Western Australia, Crawley, WA, Australia

Received 15 July 2013,
revision requested 31 July 2013,
revision received 8 October 2013,
accepted 23 October 2013
Correspondence: C. S. Wyrwoll, School of Anatomy, Physiology and Human Biology, The University of Western Australia (M309), 35 Stirling Highway, Crawley, WA 6009, Australia.
E-mail: caitlin.wyrwoll@uwa.edu.au

Abstract

Foetal growth restriction (FGR), reflective of an adverse intrauterine environment, confers a significantly increased risk of perinatal mortality and morbidity. In addition, low birthweight associates with adult diseases including hypertension, metabolic dysfunction and behavioural disorders. A key mechanism underlying FGR is exposure of the foetus to glucocorticoids which, while critical for foetal development, in excess can reduce foetal growth and permanently alter organ structure and function, predisposing to disease in later life. Foetal glucocorticoid exposure is regulated, at least in part, by the enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which catalyses the intracellular inactivation of glucocorticoids. This enzyme is highly expressed within the placenta at the maternal–foetal interface, limiting the passage of glucocorticoids to the foetus. Expression of 11β-HSD2 is also high in foetal tissues, particularly within the developing central nervous system. Down-regulation or genetic deficiency of placental 11β-HSD2 is associated with significant reductions in foetal growth and birth weight, and programmed outcomes in adulthood. To unravel the direct significance of 11β-HSD2 for developmental programming, placental function, neurodevelopment and adult behaviour have been extensively investigated in a mouse knockout of 11β-HSD2. This review highlights the evidence obtained from this mouse model for a critical role of feto-placental 11β-HSD2 in determining the adverse programming outcomes.

Keywords 11β-hydroxysteroid dehydrogenase type 2, anxiety, developmental programming, glucocorticoids, hypothalamic-pituitary-adrenal axis.

Maternal health during pregnancy is a key determinant of both foetal outcome and long-term susceptibility to disease. Foetal growth restriction (FGR), reflective of an adverse intrauterine environment and defined as the inability of a foetus to achieve its genetic growth potential, is associated with increased risk of perinatal morbidity and mortality (Bernstein *et al.* 2000). In addition to these more immediate complications, individuals of low birthweight are significantly more likely to develop disease in adult life, including hypertension, type 2 diabetes and neuropsychiatric disorders such as anxiety and depression (Cottrell & Seckl 2009).

In response to adverse intrauterine conditions, the foetus is able to adapt its physiology to promote survival. However, these adaptations can result in permanent changes in tissue structure and function that directly 'programme' predisposition to disease. The exact nature of the signal(s) that triggers these foetal adaptations is not known, but foetal malnutrition and

 doi: 10.1111/apha.12187

in utero overexposure to glucocorticoids are the two main hypotheses put forward to explain the associations between adverse intrauterine conditions and developmental programming of adult disease (Reynolds 2013). The placental enzyme, 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), inactivates glucocorticoids and thus protects the developing foetus from the deleterious effects of excess glucocorticoids (Cottrell & Seckl 2009). In addition to the placenta, this enzyme is highly expressed in foetal tissues including kidney, gut, lung and notably the brain, where it may regulate the effects of glucocorticoids on neurodevelopment (Wyrwoll *et al.* 2011). Inhibition of 11β-HSD2 is associated with reduced foetal growth and programmed outcomes in adult life, and recent studies in both humans and experimental animal models have highlighted in particular a range of behavioural and cognitive impairments arising from inhibition of 11β-HSD2 and glucocorticoid overexposure during prenatal development.

In this review, we will primarily address the evidence for an association between elevated glucocorticoid exposure of feto-placental tissues during prenatal development in relation to long-term metabolic and behavioural outcomes, with a focus on the role of 11β-HSD2, as highlighted by studies on genetically modified mice.

FGR in humans: association with adult cardiometabolic disease and neuropsychiatric impairments

A large body of epidemiological evidence has demonstrated that low birthweight (an indicator of an adverse prenatal environment) is associated with the increased risk of subsequent metabolic disease in the later life, including glucose intolerance, insulin resistance, cardiovascular disease and obesity (reviewed elsewhere in detail, Godfrey & Barker 2001). These associations are thought to reflect adaptations of the foetus *in utero* to an adverse environment, which, although beneficial in the short-term to promote survival, lead to permanent alterations in tissue function, which subsequently predispose to disease. Importantly, the relationships between low birthweight and increased risk of adult metabolic disease exist across the normal birthweight range, are independent of potentially confounding lifestyle factors and have been replicated within multiple different populations (Newsome *et al.* 2003, Whincup *et al.* 2008).

In terms of mechanisms, two main hypotheses have been proposed to explain the associations between low birthweight and subsequent postnatal disease – foetal malnutrition (Barker *et al.* 1989) and overexposure to glucocorticoids (Edwards *et al.* 1993). Importantly, these hypotheses are not mutually exclusive, as malnutrition is associated with elevation of glucocorticoid levels in both mother and foetus, and glucocorticoids themselves can regulate maternal food intake, as well as affect placental function and nutrient delivery. Recent evidence, including a growing number of studies in humans, has focused on the role of glucocorticoids as mediators of developmental programming (Reynolds 2013), and increasingly, the effects of maternal stress on offspring outcomes have been recognized.

Increased foetal cortisol levels are found in FGR pregnancies (Goland *et al.* 1993), implicating raised endogenous glucocorticoids as mediators of growth restriction. Similarly, high maternal cortisol levels (measured in either serum or saliva) and maternal stress during pregnancy are associated with reduced birthweight (Goedhart *et al.* 2010, Bolten *et al.* 2011). Low birthweight in humans is furthermore associated with increased hypothalamic-pituitary-adrenal (HPA) axis activity and raised plasma cortisol levels in adult life, which in turn are strongly related to cardiovascular risk factors (Phillips *et al.* 1998, 2000). Thus, increased glucocorticoids during foetal development may contribute not only to FGR, but in many cases, the resetting of HPA axis function associated with low birthweight likely contributes significantly to the observed increase in metabolic disease risk in later life (Reynolds 2013).

In addition to programming of metabolic disorders, maternal stress/increased foetal glucocorticoid exposure has significant consequences for the development of the central nervous system (CNS). Excess foetal glucocorticoid exposure is associated with altered neonatal neurodevelopment, neuroendocrine and cognitive function, and affective disorders including anxiety and depression. Behavioural and emotional problems, as well as decreased grey matter density in children, are linked with high maternal anxiety in mid-gestation (Buss *et al.* 2010). Severe maternal stress is associated with lower childhood cognitive and language abilities (Laplante *et al.* 2008), and in some cases, prenatal stress has also been associated with alterations in offspring HPA axis function in humans. Thus, maternal anxiety and depression seem to elevate cortisol in the child (O'Connor *et al.* 2005). In contrast, children of mothers present at or near to the World Trade Centre atrocity on 9/11, who themselves developed symptoms of post-traumatic stress disorder (PTSD), had lower cortisol levels (Yehuda *et al.* 2005). The discrepancy in cortisol response in PTSD in comparison with anxiety is likely a consequence of increased cell sensitivity to glucocorticoids in addition to persistent lowering of glucocorticoid catabolism (for a review see Yehuda & Seckl 2011). Dysregulated glucocorticoid signalling in

the offspring therefore appears to be a common outcome in response to different prenatal stressors, although the impairments likely occur at different levels, depending on the maternal condition.

Glucocorticoid exposure during development: balance is essential

In normal pregnancy, both maternal and foetal glucocorticoid levels rise as pregnancy progresses, the latter is in association with the activation of the foetal HPA axis (Challis *et al.* 2001). For the foetus, this rise in glucocorticoid levels near term is important for organ maturation, notably the lungs, to prepare for extrauterine life (Challis *et al.* 2001). However, overexposure to glucocorticoids can result in premature cessation of foetal cell proliferation, leading to FGR (Reynolds 2013).

In addition to adrenal sources, glucocorticoid levels within foetal tissues are regulated by the enzyme 11β-hydroxysteroid dehydrogenase type 1 and type 2 (11β-HSD1 and 11β-HSD2). Active glucocorticoids (cortisol in humans, corticosterone in rodents) are oxidized by 11β-HSD2 to inactive steroids (cortisone, 11-dehydrocorticosterone respectively), to reduce intracellular glucocorticoid levels. Conversely, regeneration of active steroids can occur by 11β-HSD1, although the degree to which this enzyme regulates glucocorticoid levels within foetal tissues remains almost entirely unaddressed.

During development, 11β-HSD2 is highly expressed in both foetal and placental tissues (Blasco *et al.* 1986, Stewart *et al.* 1994, Brown *et al.* 1996), and is thought to provide a barrier function, protecting foetal tissues from glucocorticoid excess. Maternal glucocorticoid levels are approx. 5- to 10-fold higher compared with those in the foetus, and this is largely attributed to the activity of placental 11β-HSD2, as placental transfer studies have indicated that approx. 80–90% of maternally administered glucocorticoids are metabolized across the placenta (Murphy *et al.* 1974, Benediktsson *et al.* 1993). However, it is also important to note that differences in corticosteroid binding globulin concentration (and affinity for glucocorticoids) within the maternal and foetal circulations add an additional degree of complexity, regulating free cortisol levels and hence glucocorticoid actions during pregnancy (Challis *et al.* 1995, Benassayag *et al.* 2001, Jung *et al.* 2011). Implicating placental 11β-HSD2 as a key factor in determining foetal growth, foetal weight is positively correlated with placental enzyme activity at term in humans (Stewart *et al.* 1995, McTernan *et al.* 2001) and rats (Benediktsson *et al.* 1993, Edwards *et al.* 1993), and 11β-HSD2 expression levels (McTernan *et al.* 2001) and activity (Shams *et al.* 1998) are reduced in FGR pregnancies. Furthermore, in humans harbouring 11β-HSD2 mutations and in 11β-HSD2-deficient mice, birth weight is markedly reduced (Dave-Sharma *et al.* 1998, Holmes *et al.* 2006a).

Within foetal tissues, 11β-HSD2 is broadly expressed, including the kidney, gut and lung and particularly within the brain (Brown *et al.* 1996). 11β-HSD2 is abundant in neuroepithelium throughout mid-gestation and then strikingly and rapidly declines, coinciding with the terminal stage of neurogenesis (Brown *et al.* 1996, Diaz *et al.* 1998). Similar patterns of expression occur in the human foetal brain with 11β-HSD2 silenced between gestational weeks 19–26 (Stewart *et al.* 1994). Thus, this abundance and expression pattern of 11β-HSD2 suggest that 11β-HSD2 acts to protect immature mitotically active brain cells from premature exposure to the maturational effects of glucocorticoids. An important point to note is that 11β-HSD2 is also abundantly expressed in the kidney postnatally, where it has a key role to play in fluid retention and therefore blood pressure regulation (for a review see Draper & Stewart 2005). Within the kidney, 11β-HSD2 inactivates glucocorticoids to prevent the illicit activation of mineralocorticoid receptors (MR). Without this inactivation, excessive activation of MR by glucocorticoids leads to the syndrome of apparent mineralocorticoid excess (AME), as occurs in patients with mutations in 11β-HSD2 (Dave-Sharma *et al.* 1998).

Experimentally, pharmacological inhibition of 11β-HSD2 enzyme function during pregnancy (Lindsay *et al.* 1996, Welberg *et al.* 2000) or the administration of synthetic glucocorticoids (dexamethasone or betamethasone, which are poorly metabolized by 11β-HSD2) leads to a reduction in birth weight and programmed outcomes in adult offspring (Nyirenda *et al.* 1998, Tang *et al.* 2011). Placental 11β-HSD2 activity is also downregulated in late gestation by environmental manipulations, including maternal stressors (such as chronic restraint stress, Mairesse *et al.* 2007) or malnutrition (Langley-Evans *et al.* 1996, Vieau *et al.* 2007), manipulations which have been shown to reduce foetal growth/birth weight and lead to cardiometabolic and neurobehavioural abnormalities in the offspring. These studies have been carried out in a range of species (as reviewed in Cottrell & Seckl 2009), highlighting an apparent central role for 11β-HSD2 in mediating the effects of various environmental manipulations on programming outcomes.

Fascinatingly, the commonplace high maternal consumption of liquorice (which contains glycyrrhizin, an inhibitor of 11β-HSD enzymes) in Finland is associated with a reduction in gestation length (Strandberg *et al.* 2001), altered HPA axis activity (Raikkonen

  doi: 10.1111/apha.12187

et al. 2010) and detrimental cognitive and psychiatric outcomes (Raikkonen *et al.* 2009) in the offspring. Moreover, recent data have shown that placental 11β-HSD2 expression is reduced in association with maternal anxiety during pregnancy (O'Donnell *et al.* 2012). Taken together, there is strong epidemiological and experimental evidence for altered placental 11β-HSD2 enzyme function in mediating the programmed outcomes of an adverse maternal environment during pregnancy.

Effects of 11β-HSD2 deficiency on adult cardiometabolic and behavioural outcomes

As outlined above, modelling developmental programming has frequently highlighted changes in feto-placental 11β-HSD2 as a mediator for the effects of an adverse prenatal environment, among numerous species. To definitively assess this notion, genetically modified mouse models have proven invaluable in unpicking the role of 11β-HSD2 in programming. In the first instance, a mouse model of targeted 11β-HSD2 disruption on an outbred 129/MF1 background revealed mice with an apparently normal phenotype at birth but within 48 h, a severe phenotype is apparent; 50% exhibit motor deficiencies, perhaps due to hypokalaemia, and die (Kotelevtsev *et al.* 1999). Survivors are fertile, but exhibit severe hypertension, hypokalaemia and polyuria, all typical characteristics of AME associated with 11β-HSD2 deficiency. These mice did not, however, exhibit reduced foetal weight, and given the severe postnatal phenotype were not suitable for assessing the significance of 11β-HSD2 for developmental programming as they did not accurately reflect human 11β-HSD2 deficiency. Subsequent breeding shifted the 11β-HSD2 knockout model onto a C57BL/6J background, largely obviating the neonatal phenotype. On this background, low birth weight was clearly apparent (Holmes *et al.* 2006a), signifying that absence of 11β-HSD2 during the development was retarding foetal growth as would be predicted in the face of glucocorticoid excess. Intriguingly, these studies also highlight the possibility of gene interaction effects of 11β-HSD2 on foetal programming, although it is currently unknown what these gene interactions are, and demonstrate the importance of background strain when considering which strain to use or comparing experimental outcomes across the literature. Symptoms of AME are still apparent in these mice postnatally, due to the absence of renal 11β-HSD2, and as such, to assess the relevance of 11β-HSD2 in developmental programming, two separate breeding approaches have been taken. In homozygous breeding experiments, male and female mice null or wild-type for 11β-HSD2 are mated; however, this experimental model is complicated by the potential effects that life-long loss of 11β-HSD2 has on either maternal physiology during gestation or on maternal care of offspring, as well as the fact that transgenic and control mice will come from different litters. To eliminate this, a heterozygous mating approach has been taken, having the added advantage of all three possible genotypes for 11β-HSD2 being represented within the one pregnancy. However, AME is still a confounder in the HSD2 knockout offspring, which renders them unsuitable for studying possible programmed cardiometabolic outcomes. As such, the focus of research using 11β-HSD2 knockout mice has concentrated on two key areas: neurodevelopment and subsequent affective behaviours and placental function.

11β-HSD2 knockout mice have altered affective behaviours

Naturally, given the epidemiological studies linking maternal stress with offspring neuropsychiatric disorders, a key area of research interest is whether 11β-HSD2 knockout mice exhibit similar affective behaviours. This is especially pertinent as the adult mouse forebrain lacks 11β-HSD2, so any phenotype is likely to reflect developmental effects. Using the elevated plus maze test, we have shown that adult 11β-HSD2$^{-/-}$ offspring generated from either a homozygous or heterozygous mating approach exhibit reduced exploration of the anxiogenic open arm of the elevated plus maze in comparison with wild-type littermates (Holmes *et al.* 2006a). Open field exploration is altered in the homozygous-bred 11β-HSD2$^{-/-}$ offspring, with them being more reluctant to explore the anxiogenic central field. Interestingly, open field exploration is unaltered in heterozygous-bred 11β-HSD2$^{-/-}$ offspring, which implies that aspects of adult behaviour are influenced by maternal factors in this model (Holmes *et al.* 2006a). Behavioural phenotyping of the heterozygous model has been recently extended to include depressive-like behaviours, and we have shown that 11β-HSD2$^{-/-}$ offspring spend a greater percentage of total time immobile for the tail suspension test and the forced swim test, both indicating increased depressive-like behaviour (Wyrwoll & Holmes 2011).

How might 11β-HSD2 programme affective behaviour?

Given that 11β-HSD2$^{-/-}$ offspring appear to exhibit affective-like behaviours analogous to anxiety and depression in humans, what are the underlying mechanisms of this altered behaviour? Interestingly, despite

increased anxiety, corticosterone levels, as the final outcome of HPA axis activity, of 11β-HSD2$^{-/-}$ offspring appears unaffected. However, adrenal size is reduced, perhaps a reflection of the reduced HPA axis drive required to maintain glucocorticoid levels in the face of reduced glucocorticoid clearance with absence of renal 11β-HSD2 (Holmes & Seckl 2006). Additionally, there may be HPA axis resetting by 11β-HSD2 deficiency during development. Outside of the HPA axis, we have noted alterations in serotonin (5-HT) and catecholamine pathways in 11β-HSD2$^{-/-}$ adult brains, which may be responsible, at least in part, for the altered behaviours (Wyrwoll & Holmes 2011).

With regard to the neurodevelopment, cerebellar size is reduced in homozygous-bred 11β-HSD2$^{-/-}$ mice in early postnatal life due to a decrease in the molecular and internal granule layers (Holmes *et al.* 2006b). This associates with a delay in attainment of neurodevelopmental landmarks such as negative geotaxis and eye opening (Holmes *et al.* 2006b). These layers are unusually sensitive to remodelling by glucocorticoids in the early postnatal period, a process that is largely prenatal in other brain regions. Thus, the timing of exposure of the developing brain to glucocorticoids seems to be tightly regulated by the presence of local 11β-HSD2 and the cell-specific patterns of its down-regulation during maturation. However, the cerebellar morphology of heterozygous-bred 11β-HSD2$^{-/-}$ mice has yet to be characterized. Furthermore, gross morphology of the adult 11β-HSD2$^{-/-}$ brains has never been investigated, and this may provide further insight into the underlying reasons behind altered affective-like behaviour with feto-placental 11β-HSD2 deficiency.

The many roles of placental 11β-HSD2

Absence of 11β-HSD2 within the placenta not only has ramifications for transplacental glucocorticoid transfer but also for placental development and function, essential for optimal foetal growth and development. Using the heterozygous mating strategy described above, examination of placental function in 11β-HSD2$^{-/-}$ mice identified significant effects of feto-placental 11β-HSD2 deficiency compared with 11β-HSD2$^{+/+}$ littermates (Wyrwoll *et al.* 2009). At embryonic day 15 (E15), 11β-HSD2$^{-/-}$ placentas were significantly smaller than 11β-HSD2$^{+/+}$ littermates, yet foetal growth was similar, indicating increased placental efficiency. Indeed, enhanced placental transport of the non-metabolisable amino acid ^{14}C-MeAIB at this age was seen in 11β-HSD2$^{-/-}$ placentas, in association with an upregulation of gene expression for the amino acid transporters *Slc38a2* and *Slc38a4*. However, by late gestation (E18), foetal growth was decreased, associated with reduced glucose transport by the placenta and impaired vascularization within the labyrinthine (foetally-derived) zone of the placenta. It is interesting to note that in the 11β-HSD2$^{-/-}$ model, the placenta seems remarkably robust given the complete absence of 11β-HSD2 as a protective glucocorticoid barrier until late gestation when it apparently 'exhausts' and placental vascularity, haemodynamics, and nutrient transfer are compromised, resulting in FGR (Wyrwoll *et al.* 2009).

Although disruption of placental 11β-HSD2 activity is clearly associated with foetal development, until recently, the postulation that reduction in 11β-HSD2 enzyme activity is the crucial link between an adverse maternal environment and subsequent programming outcomes had not been tested experimentally. The availability of the 11β-HSD2$^{-/-}$ mouse allowed this question to be addressed directly. Using an established model of FGR (maternal low protein diet; LPD) previously shown to downregulate placental 11β-HSD2 and lead to postnatal hypertension in rats (Langley-Evans *et al.* 1996), we found that 11β-HSD2$^{-/-}$ foetuses of LPD-fed dams exhibited a reduction in foetal weight compared with 11β-HSD2$^{-/-}$ foetuses of control dams (Cottrell *et al.* 2012). These data clearly indicate that the growth-restricting effects of LPD act in addition to any reduction of the 11β-HSD2 activity, refuting previous notions that maternal malnutrition reduces foetal weight solely through a reduction in 11β-HSD2. Intriguingly, in these same studies, we also identified that although maternal LPD reduced placental 11β-HSD2 activity in late gestation (in line with previous results in rats), at earlier gestational ages, placental 11β-HSD2 activity was unexpectedly upregulated, suggesting that the placental response to malnutrition is dynamic and developmentally regulated.

An emerging area of research is the notion that the placenta is a common mediator between the maternal milieu and the developing CNS of the foetus. Activity of the foetal HPA axis increases in late gestation and is thought to play a role in determining gestation length and the timing of parturition (Challis *et al.* 2001). Of note, premature activation of the HPA axis is associated with adverse intrauterine conditions, including maternal malnutrition (Bloomfield *et al.* 2003, Cottrell *et al.* 2012). We found that foetuses of dams fed an LPD exhibited an upregulated foetal HPA axis, coinciding with the increased placental 11β-HSD2 activity, perhaps suggesting a cross-talk between placental glucocorticoid metabolism and the regulation of foetal HPA function. How these foetal adaptations may shape development (and also potentially influence placental function, by a foetal→placental signalling mechanism) is not clear, but will be the focus of future studies. Overall, the possibility remains

  doi: 10.1111/apha.12187

that while maternal glucocorticoids could play a direct role in programming the foetus, and notably its CNS, placental development and function additionally play a key role in mediating foetal development and modulating the effects of an adverse maternal environment.

An area yet to be definitively explored in the 11β-HSD2-deficient mouse model is the possibility for sex differences in placental function and foetal outcomes. Indeed, it is emerging that programmed outcomes are more evident in male than female offspring (for a review see Aiken & Ozanne 2013), including programmed outcomes due to excess *in utero* glucocorticoid exposure (Clifton 2005, Cuffe *et al.* 2011) Furthermore, behavioural outcomes have been solely measured in male offspring due to the complicating effects of oestrous cycle on female behaviour.

Future directions: refining the models by tissue-specific 11β-HSD2 manipulation

Clearly, the absence of 11β-HSD2 in mice alters placental development and function, retards foetal growth, impairs neurodevelopment and leads to affective-like behaviours. There is a caveat to this work, however, as 11β-HSD2 is not just absent from feto-placental tissues but also from the kidney in prenatal and postnatal life. So it could well be that the effects we have described on neurodevelopment and behaviour are a consequence of life-long loss of renal 11β-HSD2 as opposed to outcomes specific to the developmental absence of placental and neural 11β-HSD2. Furthermore, while the heterozygous mating approach eliminates maternal factors as influences, the significance of placental vs. foetal brain deficiency of 11β-HSD2 for programming behaviour has yet to be determined. Further refinement of the global knockout of 11β-HSD2 is clearly required to specifically address these issues.

The development of a placenta-specific knockout of 11β-HSD2 will be the defining piece of work: once and for all determining whether or not placental 11β-HSD2 lies at the centre of developmental programming effects, not only in terms of neuropsychiatry but also cardiometabolic disease.

Summary

The level of foetal glucocorticoid exposure is dependent not only on the regulation of placental glucocorticoid passage by 11β-HSD2, but also on foetal HPA axis function, both of which are affected by a perturbed intrauterine environment. In addition, exposure of the placenta to increased levels of glucocorticoid, whether by a local deficiency of 11β-HSD2 or from an upregulation of either maternal or foetal HPA axis, markedly affects the function of this key tissue. Although the 11β-HSD2 mouse models do not reproduce a natural human state, they have proven to be a useful tool with which to unpick the role of 11β-HSD2 in developmental programming. The next few years of research in this field will provide some definitive answers as to whether feto-placental 11β-HSD2 is indeed a hub for eliciting developmental programming outcomes.

Conflict of interest

The authors declare no conflict of interests.

This work was supported by a Wellcome Trust project grant (WT079009; M.C.H., J.R.S.).

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