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Review

Fetal growth restriction: adaptations and consequences*

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A range of pathophysiological factors can result in a perturbation or restriction of fetal growth, and the cardiovascular, neuroendocrine and metabolic adaptations of the fetus to these stimuli will depend on their nature, timing and intensity. The critical importance of these physiological adaptations for both immediate survival and long-term health outcomes has provided an impetus for experimental studies of the nature and consequences of specific fetal adaptations to a poor intrauterine environment. This review summarizes data from recent studies that have focused on the responses of the fetal cardiovascular, sympathoadrenal, hypothalamo–pituitary–adrenal and renin–angiotensin systems to experimental restriction of placental function in the sheep and discusses the consequences of these adaptations for fetal, neonatal and adult health.

Intrauterine growth restriction is a relatively common condition that results in disproportionately high perinatal morbidity and mortality (Knutzen and Sher, 1982; Newton *et al.*, 1987). Intrauterine growth restriction may be classified clinically on the basis of a birth weight below the tenth percentile for gestational age or, in experimental studies, as a fetal body weight below two standard deviations of the mean of the relevant study population. A range of pathophysiological factors can result in a perturbation or restriction of fetal growth, including gene defects, chromosomal abnormalities, poor placental function, maternal smoking, maternal alcohol or drug abuse and altered maternal substrate concentrations (Robinson *et al.*, 1994). The specific physiological adaptations of the fetus to an adverse intrauterine environment, including restriction of its growth rate, may depend on the nature, timing and intensity of such extra- and intrauterine challenges. It is clear that the physiological adaptations of the fetus to its suboptimal intrauterine environment are of critical importance in determining the health and survival of the fetus and newborn. Furthermore, a series of worldwide epidemiological studies (Barker *et al.*, 1990; Barker, 1992, 1998; Huxley *et al.*, 2000) has highlighted the potential importance of fetal adaptations to a poor intrauterine environment for longer term health outcomes. These studies have demonstrated that there are significant relationships between birth weight or birth phenotype and the relative risk of onset of ischaemic heart disease, hypertension and non-insulin-dependent diabetes. These associations are independent of adult lifestyle or adult size and are summarized in the hypothesis known as the 'fetal origins of adult disease' (Barker, 1998, 1999), in which it is proposed that the physiological, neuroendocrine or metabolic adaptations that enable the fetus to survive a period of intrauterine deprivation result in a permanent reprogramming of the developmental pattern of proliferation and differentiation events within key tissue and organ systems and pathological consequences in adult life (Barker, 1999). The critical importance of fetal adaptations for both immediate survival and long-term health outcomes has provided an impetus for experimental studies of the nature and consequences of specific fetal adaptations to a poor intrauterine environment. This review summarizes data from recent studies that have focused on the range of cardiovascular, neuroendocrine and metabolic adaptations the fetus makes in response to experimental restriction of placental function and discusses the consequences of these adaptations for fetal, neonatal and adult health.

Experimental models of restriction of placental function

The major substrates for mammalian fetal growth and development are oxygen, glucose, lactate and amino acids. Placental dysfunction resulting in a restriction of fetal substrate supply is a major cause of altered or reduced fetal growth (Robinson *et al.*, 1994). Several different experimental

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approaches have been used to produce placental insufficiency with resultant fetal growth restriction in small and large animal models. The primary methods include a reduction in uterine blood flow by vascular occlusion or ligation in rats, guinea-pigs and sheep (Lafeber *et al.*, 1985; Boyle *et al.*, 1996), placental infarction by repetitive embolization in pregnant ewes (Clapp *et al.*, 1981; Block *et al.*, 1990; Murotsuki *et al.*, 1997), or limitation of placental growth through surgical removal of most of the endometrial caruncles from the uterus of non-pregnant ewes before conception (Robinson *et al.*, 1979). This procedure restricts the number of placental cotyledons formed, and subsequently limits placental and hence fetal growth. Several comprehensive reviews have summarized the effects of this experimental method of restriction of placental growth on oxygen and glucose delivery and consumption by the placenta and fetus (Owens *et al.*, 1989; Robinson *et al.*, 1994). Placentally restricted fetuses are chronically hypoxaemic, hypoglycaemic and have increased blood lactate concentrations and, usually, no change in fetal arterial pH (Owens *et al.*, 1989; Robinson *et al.*, 1994). The changes in fetal blood gas status and nutrient supply in placentally restricted sheep fetuses are similar to those measured in cordocentesis studies of human infants who are small for their gestational age (Economides *et al.*, 1991).

Placental restriction and fetal organ growth

As is also the case in human intrauterine growth restriction (IUGR), experimental restriction of placental growth results in an asymmetrical pattern of fetal growth restriction whereby body weight is reduced to a greater extent than crown–rump length or girth (Robinson *et al.*, 1994). We have analysed the pattern of relative fetal organ masses in a large cohort of placentally restricted sheep fetuses and control sheep fetuses between day 137 and day 147 of gestation. The placentally restricted cohort ($n = 71$) included animals that were or were not growth-restricted, depending on the extent of compensatory growth of the placenta, whereas the control cohort ($n = 270$) also included animals that were either normally grown or spontaneously growth restricted, for example twins. The analysis of fetal organ masses in such cohorts spanning a wide range of fetal body weights allowed comparisons to be made between the effects of spontaneous or experimental growth restriction on relative organ growth. It was also possible to determine from such analyses whether changes emerge in the growth patterns of specific organs at particular levels or thresholds of fetal growth restriction. We have found a significant inverse relationship between the relative mass of the fetal brain and fetal body weight, which is present across the full spectrum of fetal body weights from 1 to 6 kg (Fig. 1). The strength of this relationship indicates that brain growth is maintained through compensatory mechanisms across the entire weight range of both normally grown and growth-restricted sheep fetuses. The maintenance of brain mass appears to be of primary importance for all fetuses whether they are normally grown or growth-restricted, indicating

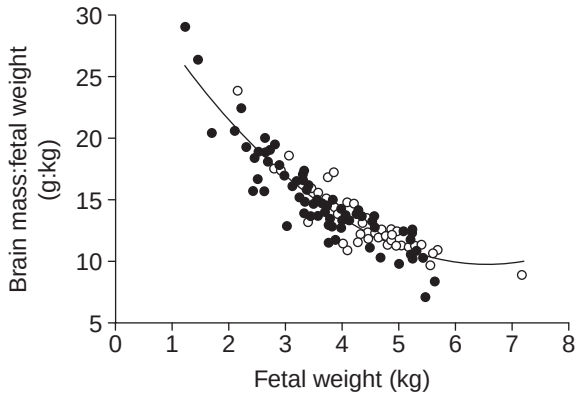

Fig. 1. Relationship between relative brain mass and fetal body weight in a cohort of normally grown (○; $n = 50$) and placentally restricted (●; $n = 74$) sheep fetuses between day 137 and day 147 of gestation. The relative brain mass increased with decreasing fetal weight according to the equation: brain mass:fetal weight = 0.91 (fetal weight)^2 – 10.1 (fetal weight) + 39.2 ($r^2 = 0.85$, $P < 0.0001$).

that, whereas compensatory mechanisms may maintain disproportionate brain growth in growth-restricted fetal lambs, similar physiological mechanisms must operate, albeit to a lesser extent, to ensure brain mass is maintained within an optimal range even in normoxaemic, apparently well grown animals. This pattern of fetal organ growth is dissimilar to that of other organs such as the kidney (Fig. 2). Fetal kidney growth occurs in proportion to body weight until the fetal body weight decreases below about 2 kg, at which point fetal kidney mass is then maintained disproportionate to fetal body weight. In contrast to the fetal brain and kidney, there is a direct relationship between the relative mass of the fetal liver and fetal body weight, and the variation in the relative mass of the liver is less related to fetal body weight than is fetal brain or kidney mass (Fig. 3). Furthermore, there is only a decrease in relative liver mass once the fetal body weight decreases below about 3 kg.

Thus, variations in the timing, intensity and duration of placental restriction of fetal substrate supply result in different fetal cardiovascular, neuroendocrine and metabolic adaptations and differential patterns of the relative growth of key fetal organs such as the brain and the liver as fetal body weight decreases. This variation in the fetal responses to a decreasing substrate supply may underpin the differences in the pattern of the associations between specific adult disease outcomes, such as high blood pressure or non-insulin-dependent diabetes mellitus (NIDDMS), and birth weight. Adult systolic blood pressure is reported to be inversely related to birth weight across the full birth weight ranges of normally grown and growth-restricted babies (Huxley *et al.*, 2000). This finding is similar to the relationship found between the relative mass of the fetal brain and fetal body weight in our cohort of normally grown and growth-restricted sheep fetuses. In a systematic review of the association between systolic blood pressure and birth measurements other than birth weight, the most

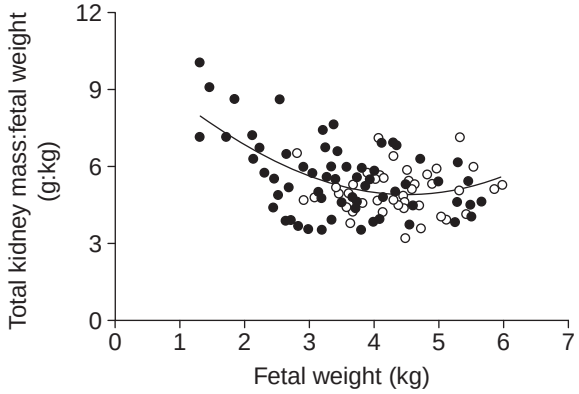

Fig. 2. Relationship between relative kidney mass and fetal body weight in a cohort of normally grown (○; $n = 49$) and placentally restricted (●; $n = 65$) sheep fetuses between day 137 and day 147 of gestation. The relative kidney mass increased with decreasing fetal weight according to the equation: kidney mass:fetal weight = 0.31 (fetal weight)^2 – 2.77 (fetal weight) + 11.13 ($r^2 = 0.27$, $P < 0.0001$).

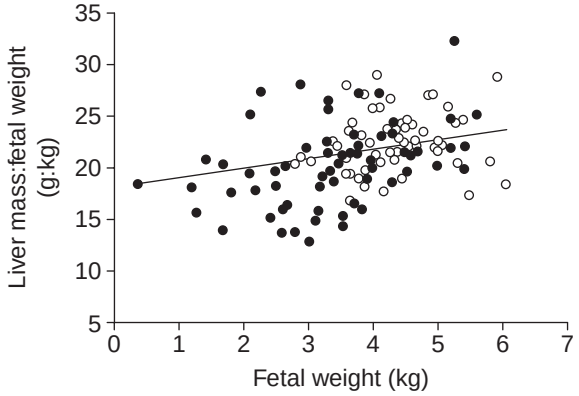

Fig. 3. Relationship between relative liver mass and fetal body weight in a cohort of normally grown (○; $n = 57$) and placentally restricted (●; $n = 65$) sheep fetuses between day 137 and day 147 of gestation. The relative liver mass decreased with decreasing fetal weight according to the equation: liver mass:fetal weight = –0.18 (fetal weight)^2 + 2.7 (fetal weight) + 14.1 ($r^2 = 0.12$, $P < 0.0001$).

consistently reported finding was an inverse association between head circumference at birth and systolic blood pressure in later life (Huxley *et al.*, 2000). One possibility is that the fetal cardiovascular and neuroendocrine adaptations that ensure that substrate delivery to the brain and hence brain growth are maintained at any given fetal weight underlie the emergence of the relationship between birth weight and systolic blood pressure in adult life.

Fetal cardiovascular adaptations to placental restriction

Arterial blood pressure

Although the fetal cardiovascular responses to acute episodes of hypoxaemia lasting several hours or fewer have been well documented (Giussani *et al.*, 1994), there are fewer studies on the fetal cardiovascular responses to the imposition of chronic hypoxaemia, that is, periods of hypoxaemia extending across weeks or months. Murotsuki *et al.* (1997) reported a significant increase in fetal blood pressure in response to a 21 day period of embolization of the fetal placental circulation. However, there are no differences in mean arterial blood pressure between normally grown sheep fetuses and sheep fetuses chronically hypoxaemic and growth restricted after either restriction of placental growth or embolization of the uteroplacental vascular bed in late gestation (Llanos *et al.*, 1980; Robinson *et al.*, 1983; Walker *et al.*, 1990). Edwards *et al.* (1999) found that although there was no difference in the mean arterial blood pressure between normally grown and placentally restricted sheep fetuses, there was a direct relationship between blood pressure and the mean gestational pO_2 in control animals, which was not present in the placentally restricted group. Given that the sheep fetuses with higher mean gestational arterial pO_2 values are also larger, it is possible that the higher mean blood pressure values in the normally grown sheep fetuses reflect an increased cardiac output. Alternatively, it may be that, as the fetus grows in late gestation, fetal vascularity does not increase in parallel with fetal size, resulting in increased fetal peripheral vascular resistance and fetal arterial blood pressure. Daniel *et al.* (1996a) also found a positive relationship between arterial blood pressure and the fetal:maternal weight ratio in a combined group of control sheep fetuses and sheep fetuses in which mild growth restriction and hypoxaemia was produced by withdrawal of 25 ml maternal blood per day throughout the second half of pregnancy. Thus, fetal hypotension may be a good indicator of mild but not moderate or severe growth restriction.

The loss of the relationship between arterial blood pressure and pO_2 in moderately or severely growth-restricted sheep fetuses may be a consequence of a reduction in arteriolar branching in key fetal circulatory regions due to an adverse effect of chronic hypoxaemia on angiogenesis. Alternatively, the loss of this relationship may be a consequence of an increase in circulating vasoactive hormones secreted in response to a decrease in fetal oxygenation and nutrient status, for example noradrenaline, angiotensin II and cortisol. During acute hypoxaemia, blood flow to the brain, heart and adrenal glands is increased and blood flow to the gastrointestinal, renal and peripheral vascular beds decreases (Jensen *et al.*, 1987a,b; Yaffe *et al.*, 1987; Jansen *et al.*, 1989; Giussani *et al.*, 1993). This redistribution of fetal cardiac output is also maintained with prolonged hypoxaemia in pregnancy, presumably as a consequence of the action of vasoactive hormones (Bocking *et al.*, 1988; Rurak *et al.*, 1990).

Clearly, the redistribution of the fetal cardiac output as an adaptation to chronic placental restriction is critically important for the maintenance of the relative growth and optimal function of key fetal organs such as the heart and brain. However, redistribution of cardiac output away from

particular regional circulations in late gestation may have some negative consequences in the immediate newborn period. Doppler ultrasound studies have shown that on the first day of postnatal life, both superior mesenteric artery and coeliac axis blood flow velocity are reduced in growth-restricted infants, when compared with appropriately grown, weight matched and gestation matched control infants (Kempley *et al.*, 1991). Reduced blood flow velocity was found only in those growth-restricted infants who showed evidence in Doppler ultrasound studies of fetal hypoxia as indicated by absence of end-diastolic flow in the fetal aorta. This difference in blood flow velocity persisted for about a week of postnatal life and, after this age, the superior mesenteric blood flow velocity was the same in IUGR infants as that found in appropriately grown infants on the first day of life. The differences in blood flow velocity during the first week of life could not be explained by differences in blood pressure or arterial oxygen tension at the time of measurement. Therefore, Kempley *et al.* (1991) suggested that the differences in visceral blood flow velocity were due to a persistently increased intestinal vascular resistance that was 'programmed' during fetal life. Therefore, the increased risk of necrotizing enterocolitis in those growth-restricted infants with absent end-diastolic flow in the aorta in fetal life may be a result of hypoxaemic–ischaemic tissue damage occurring *in utero*, or a consequence of increased vascular resistance persisting after delivery.

We have found that systolic blood pressure in a cohort ($n = 35$) of normally grown and placentally restricted lambs at 1 year of age is inversely related to the weight or ponderal index of the lamb at birth in an analysis that adjusts for current shoulder height. Maternal undernutrition during the first 30 days of pregnancy in ewes results in lower blood pressure in the fetus during later gestation but an increased blood pressure in postnatal life (Hoet and Hanson, 1999). Thus, it appears that the timing, type and duration of fetal substrate restriction are each important in determining the specific nature of the fetal neuroendocrine and cardiovascular adaptive responses and their interactions in determining long-term consequences. The following sections review the impact of placental restriction on the fetal sympathoadrenal, pituitary–adrenal and the renin–angiotensin systems and the importance of these fetal neuroendocrine adaptations for short-term survival and longer-term adverse health outcomes such as hypertension.

Placental restriction and the fetal sympathoadrenal system

Adrenaline and the fetal adrenal medulla

Although plasma adrenaline concentrations are higher in placentally restricted sheep fetuses, the relationship between plasma adrenaline and arterial pO_2 is different in control and in placentally restricted animals. In control animals, there is an inverse relationship between adrenaline and arterial pO_2, such that a decrease in arterial pO_2 results in an increase in circulating adrenaline concentrations (Simonetta *et al.*, 1997). In contrast, in the placentally restricted group, a decrease in arterial pO_2 is associated with a relative suppression, rather than a stimulation, of plasma adrenaline concentrations. Thus, factors other than arterial pO_2 are important for maintaining high basal adrenaline concentrations in the circulation of the placentally restricted sheep fetuses. Although the fetal adrenal gland is the source of increased circulating catecholamines during acute fetal hypoxaemia (Jones *et al.*, 1988), the impact of chronic hypoxaemia on the fetal adrenomedullary chromaffin cells has been less well defined. Immunostaining for the catecholamine synthetic enzymes dopamine β-hydroxylase and phenylethanolamine *N*-methyltransferase (PNMT) is significantly suppressed in the adrenal medulla of the placentally restricted fetus at as early as day 90 of gestation (Coulter *et al.*, 1998). Furthermore, placental restriction is associated with a decrease in the adrenal concentrations of PNMT mRNA of approximately 70% and a decrease in the adrenomedullary area stained positive with anti-PNMT of approximately 50% at about day 140 of gestation (Adams *et al.*, 1998). There is also a positive correlation between mean arterial pO_2 and the amount of adrenal PNMT mRNA during late gestation (Fig. 4). The direct relationship between PNMT mRNA expression and mean gestational arterial pO_2 in individual sheep fetuses indicates that low arterial pO_2 acts by either neurogenic or non-neurogenic mechanisms to suppress PNMT synthesis. Placental restriction had no effect on tyrosine hydroxylase mRNA concentrations in the fetal adrenal. Thus, in contrast to acute hypoxaemia, placental restriction and the associated chronic hypoxaemia result in a suppression of the adrenaline synthetic capacity of the fetal adrenal medulla. Fetal growth restriction induced by single umbilical artery ligation in sheep (Oyama *et al.*, 1992) results in a diminished fetal adrenaline response to delivery, and growth-restricted rat pups have impaired adrenaline secretory responses to acute hypoxia after birth (Shaul *et al.*, 1989). Therefore, the impact of intrauterine hypoxaemia on adrenaline synthesis and secretion may have significant physiological consequences before, during and immediately after birth.

Noradrenaline and the sympathetic nervous system

Acute episodes of intrauterine hypoxia or asphyxia stimulate an increase in the plasma concentrations of noradrenaline and adrenaline in sheep fetuses during late gestation. The increase in circulating catecholamines is important in the initiation and co-ordination of a range of fetal physiological responses to hypoxic stress (Giussani *et al.*, 1994). Plasma noradrenaline concentrations were significantly higher in chronically hypoxaemic, growth-restricted sheep fetuses than in control sheep fetuses between day 110 and day 140 of gestation (Simonetta *et al.*, 1997). Covariate analysis demonstrated that at any given arterial pH value, plasma noradrenaline concentrations

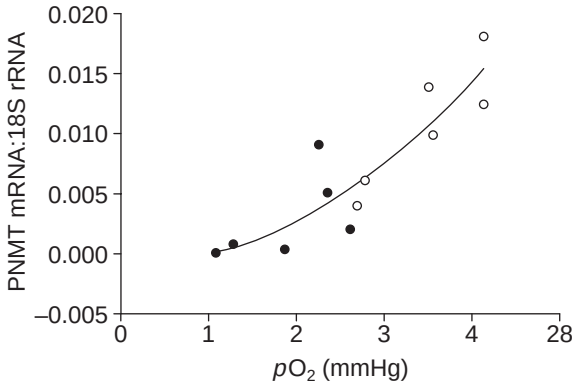

Fig. 4. Relationship ($r = 0.88$, $P < 0.0005$) between adrenal phenylethanolamine *N*-methyltransferase (PNMT) mRNA:18S rRNA expression with mean gestational arterial pO_2 in control (○; $n = 6$) and placentally restricted (●; $n = 6$) sheep fetuses at days 140–141 of gestation. (Redrawn from Adams *et al.,* 1998.)

were about 2.9 pmol ml^{-1} higher in the placentally restricted than in the control group. For every 1 mmHg decrease in arterial pO_2, noradrenaline increased by 0.4 pmol ml^{-1} during basal conditions in both the placentally restricted and control groups. Throughout late gestation, the prevailing mean arterial pO_2 was approximately 8 mmHg lower in the placentally restricted group than in the control group and, therefore, would account for the difference in noradrenaline concentrations of about 3.2 pmol ml^{-1} between the groups (Simonetta *et al.*, 1997). Therefore, it appears that chronic hypoxaemia is the major factor contributing to the increase in circulating noradrenaline concentrations in the placentally restricted group in late gestation. Plasma noradrenaline concentrations were also doubled in sheep fetuses made chronically hypoxaemic after a 10 day period of fetal placental embolization using repeated injections of non-radioactive microspheres (Gagnon *et al.*, 1994). The source of the increase in circulating noradrenaline concentrations during chronic or intermittent restriction of placental function may be either increased secretion from the fetal adrenal medulla, extra adrenal chromaffin tissue or sympathetic neurones. Intrafetal infusion of tyramine, which acts to displace noradrenaline from catecholamine-containing vesicles within postganglionic sympathetic neurones, results in a significantly greater increase in plasma noradrenaline in placentally restricted sheep than it does in control sheep fetuses (Simonetta *et al.*, 1997). However, when these noradrenaline responses were expressed in relation to basal circulating concentrations, there was no difference in the fold changes in noradrenaline in response to tyramine between the placentally restricted and control animals. The proportional relationship between basal and stimulated noradrenaline concentrations in the two groups indicates that the increased basal noradrenaline concentration is derived from sympathetic nerve terminals in the placentally restricted group. One possibility is that placental restriction and the presence of chronic hypoxaemia throughout late gestation is a stimulus for hyperinnervation of fetal vessels and tissues by sympathetic, postganglionic neurones. Alternatively, low pO_2, or other factors associated with placental restriction, reflexly stimulate catecholamine synthesis and secretion in developing sympathetic neurones. Although the vasoconstrictor responses to acute hypoxaemia and asphyxia are reduced by sympathectomy and α-adrenergic blockade (Jones *et al.*, 1988; Giussani *et al.*, 1994), it is not yet clear whether the redistribution of fetal cardiac output during chronic hypoxaemia is dependent on the increase in fetal sympathetic activity. One possibility is that cerebral blood flow, and therefore brain growth, is maintained by the precise relationship between the prevailing arterial pO_2 and plasma noradrenaline concentrations in both normally grown and growth-restricted fetuses.

Postnatal consequences of adaptations of the fetal sympathoadrenal system to placental restriction

Placental restriction results in an increase in the functional capacity of the sympathetic nervous system with an apparent concurrent suppression in the functional capacity of the adrenal medulla. Although basal adrenaline concentrations are maintained, adrenaline responses to acute stressors, such as neonatal hypoglycaemia, may be impaired in the growth-restricted fetus and newborn. It is also unknown whether changes in sympathetic 'tone' within particular regional circulations are maintained into adult life after a period of intrauterine growth restriction. In a study of 449 men and women born in Preston, UK, a direct relationship was found between adult pulse rate and birth weight (Flanagan *et al.*, 1999). Pulse rate decreased progressively from 76 beats min^{-1} in subjects who weighed 2.5 kg or less at birth to 71 beats min^{-1} in those who weighed 3.3 kg or more (a 2.7 beat min^{-1} decline in pulse rate per kg increase in birth weight). This association was independent of current body mass index, waist:hip ratio and of potential confounding variables including smoking, alcohol consumption and social class. The authors of this study concluded that, although resting pulse rate is an imperfect index of activity of the sympathetic nervous system (SNS), these findings were consistent with the hypothesis that increased SNS activity established *in utero* is one mechanism linking small size at birth with other adverse outcomes such as high blood pressure or insulin resistance syndrome in adult life.

Placental restriction and the fetal hypothalamo–pituitary–adrenal (HPA) axis

There is growing interest in the consequences of premature exposure of the fetus to excess glucocorticoids, occurring after either therapeutic administration of synthetic glucocorticoids to women in threatened preterm labour, as a consequence of acute maternal stress, or activation of

the fetal hypothalamo–pituitary–adrenal (HPA) axis by intrauterine substrate deprivation (Seckl *et al.*, 1999, 2000). Maternal undernutrition during pregnancy in rats results in high blood pressure in the offspring and this effect is prevented by the inhibition of maternal corticosterone biosynthesis during pregnancy (Langley-Evans *et al.*, 1996). Treatment of pregnant rats with the synthetic glucocorticoid dexamethasone (Levitt *et al.*, 1996) or with carbenoxolone, an inhibitor of the placental enzyme that metabolizes corticosterone to the inert 11-dehydrocorticosterone (Langley-Evans, 1997), results in a lower mean birth weight, persistent increased arterial blood pressure and fasting hyperglycaemia in the adult offspring. Therefore, overexposure of the fetus to excess glucocorticoids may be implicated in the association between fetal growth restriction and the programming of adult cardiovascular and metabolic diseases. There has been less experimental evidence on the impact of placental restriction or maternal undernutrition on the fetal HPA axis in a longer gestation species, such as sheep or humans, or on the role that the endogenous fetal cortisol response to intrauterine substrate deprivation plays in postnatal programming in these species.

The relative growth of the fetal adrenal is increased (Fig. 5) and fetal plasma concentrations of cortisol are higher in placentally restricted animals than in their normally grown counterparts after day 127 of gestation (Phillips *et al.*, 1996). The late gestation increase in fetal cortisol occurs in the absence of any change in fetal adrenocorticotrophic hormone (ACTH) concentrations and there is a significant decrease in the pituitary mRNA concentrations of the ACTH precursor pro-opiomelanocortin (POMC) in placentally restricted fetuses when compared with controls (Phillips *et al.*, 1996). An increase in fetal cortisol is a consistent response to prolonged or repeated fetal hypoxaemia in late gestation and this increase is not always associated with an increase in fetal ACTH. In experiments in which hypoxaemia was induced in sheep fetuses by a reduction in utero–placental blood flow for 24 h, fetal ACTH concentrations were increased at only 2 h after the onset of the hypoxaemia and then returned to baseline values (Sug Tang *et al.*, 1992). In contrast, fetal cortisol concentrations were increased by 2 h after the onset of hypoxaemia and remained high throughout the 24 h period. Cordocentesis studies have also found that plasma cortisol concentrations were higher and plasma ACTH concentrations lower in IUGR human fetuses than in normally grown fetuses at weeks 18–38 of gestation (Economides *et al.*, 1988). In contrast to these studies, fetal ACTH concentrations remained high during a 20 day period in which the fetal placental circulation was repeatedly embolized (Murotsuki *et al.*, 1996). Thus, the fetal HPA axis may adapt to the effects of prolonged or sustained hypoxaemia, whereas repeated hypoxaemic episodes, such as those experienced during repeated placental embolization, may maintain stimulation of the fetal HPA axis.

It is possible that the HPA axis in the growth-restricted fetus is operating at a new central set point, which results in maintained ACTH concentrations, increased adrenal sensitivity to ACTH and increased adrenal mass and corticosteroid output. However, there is a decrease, rather than an increase, in the adrenal expression of ACTH receptor mRNA in growth-restricted sheep fetuses (Ross *et al.*, 2000). Another possibility is that there are factors other than ACTH in the placentally restricted group that stimulate adrenal cortisol synthesis and secretion. This contention is supported by the observation that placental restriction resulted in a relative increase in adrenal cytochrome P450 side chain cleavage (CYP11A1) mRNA in the absence of changes in the adrenal mRNA concentrations of steroidogenic enzymes known to be responsive to ACTH stimulation, that is, cytochrome P450 17α-hydroxylase (CYP17), cytochrome P450 21-hydroxylase (CYP21A1) and 3β-hydroxysteroid dehydrogenase/Δ^5,Δ^4-isomerase (3β-HSD) (Ross *et al.*, 2000). Braems *et al.* (1998) reported an increase in the adrenal mRNA concentrations of CYP11A1, 3βHSD and CYP21A1, but not CYP17, after 48 h of fetal hypoxaemia. These data indicate that factors other than ACTH, for example, placental prostaglandin E_2 or angiotensin II, may stimulate an increase in adrenal steroidogenesis in the chronically hypoxaemic fetus.

Increases in circulating cortisol may play an important role in the adaptation of the fetal cardiovascular system to intrauterine growth restriction (Phillips *et al.*, 1996; Langley-Evans, 1997; Edwards *et al.*, 1999; Hoet and Hanson, 1999). Intrafetal infusion of either cortisol or the synthetic glucocorticoids betamethasone and dexamethasone for periods of up to 48 h at about days 120–130 of gestation results in an increase in fetal femoral vascular resistance (Derks *et al.*, 1997) and in arterial blood pressure (Wood *et al.*, 1987; Tangalakis *et al.*, 1992; Anwar *et al.*, 1999). Infusion of cortisol for 6 days after bilateral fetal adrenalectomy also restored fetal arterial blood pressure to

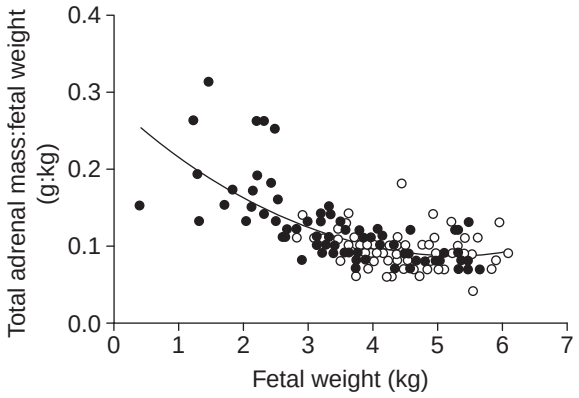

Fig. 5. Relationship between relative adrenal mass and fetal body weight in a cohort of normally grown (○; $n = 108$) and placentally restricted (●; $n = 74$) sheep fetuses between day 137 and day 147 of gestation. The relative adrenal mass increased with decreasing fetal weight according to the equation: adrenal mass:fetal weight = 10.3 (fetal weight)^2 – 101 (fetal weight) + 335 ($r^2 = 0.50$, $P < 0.0001$).

values measured in intact fetuses at about day 125 of gestation (Unno *et al.*, 1999). Furthermore, blood pressure responses to increasing doses of angiotensin II, but not noradrenaline, were increased in sheep fetuses after infusion of cortisol for 48 h at about day 125 of gestation (Tangalakis *et al.*, 1992). Intrafetal infusion of cortisol also results in an increased expression of angiotensin II type I (AT1) receptor mRNA within the fetal heart (right and left atrium and right ventricle) (Segar *et al.*, 1995) and there is a greater hypotensive effect after blockade of AT1 receptors in sheep fetuses that have been infused with cortisol (Forhead *et al.*, 2000). Thus, there is evidence that increased exposure to cortisol during fetal life results in an increased sensitivity to the vasoconstrictor actions of angiotensin II through either an increase in the expression of the AT1 receptor or changes in the post-receptor-mediated events within the vascular smooth muscle.

Placental restriction and the renin–angiotensin system

Intrafetal infusion of an angiotensin-converting enzyme inhibitor, captopril, decreased arterial blood pressure in growth restricted, but not normally grown, sheep fetuses after day 135 of gestation (Fig. 6) (Edwards *et al.*, 1999). This finding indicates that the renin–angiotensin system (RAS) plays a greater role in the regulation of arterial blood pressure in placentally restricted than in normally grown sheep fetuses in late gestation. Captopril infusion did not alter the mean arterial blood pressure and peripheral blood flow changes during a 60 min period of fetal hypoxaemia (Green *et al.*, 1998), but it did blunt the hypertensive response to hypoxia in sheep fetuses in which the carotid sinus nerves were cut (Green *et al.*, 1998). Thus, once the carotid chemoreflex mechanisms were removed, there was a significant role for angiotensin II in the regulation of the blood pressure and peripheral blood flow responses to hypoxaemia. The functioning of the carotid chemoreflex mechanisms may be downregulated by the presence of a chronically low arterial pO_2 in the placentally restricted fetus, and a role for circulating angiotensin II in the long-term regulation of mean arterial blood pressure may then emerge. It is also possible that prolonged hypoxaemia stimulates an increase in circulating angiotensin II. Alternatively, the increased circulating cortisol concentrations in the placentally restricted fetus may act to increase expression of the vasoactive angiotensin II receptor subtypes within the vascular smooth muscle and hence increase vascular responsiveness to angiotensin II. Therefore, the enhanced hypotensive response to captopril in the placentally restricted sheep fetuses during late gestation may be a consequence of an interaction between cortisol and the RAS. It is not known whether the enhanced hypotensive response to captopril persists after birth in this model. Treatment of the offspring of protein-restricted pregnant rats for 3 weeks with captopril abolished the increase in blood pressure normally present in this model in the postnatal period. Thus, interactions between the effects of excess glucocorticoids and the RAS during the prenatal period may result in an increase in blood pressure in postnatal life that is independent of the source (maternal, fetal or species) of the increased fetal glucocorticoids.

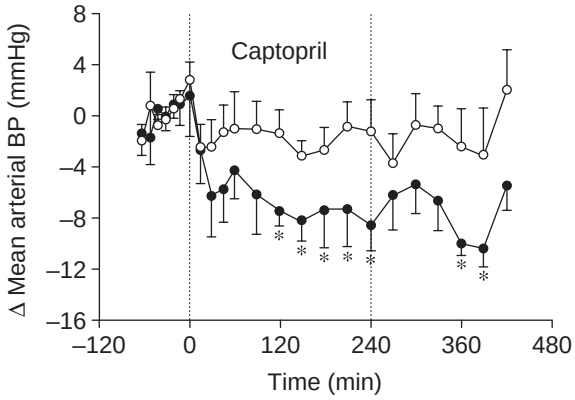

Fig. 6. Effect of an intravenous captopril infusion (15 μg min^{-1}) on mean arterial blood pressure (BP) in control (○; $n = 6$) and placentally restricted (●; $n = 7$) sheep fetuses between day 135 and day 145 of gestation. *Values significantly different from preinfusion values. (Redrawn from Edwards *et al.*, 1999.)

Glucocorticoids and the metabolic consequences of intrauterine growth restriction

The actions of the increased plasma concentrations of glucocorticoids in fetal tissues in the growth-restricted fetus may be significantly modulated by the presence of the enzyme 11β-hydroxysteroid dehydrogenase (11βHSD). There are two distinct isoforms of 11βHSD present in sheep and human fetal tissue during late gestation (Langlois *et al.*, 1995; Yang *et al.*, 1997). In sheep, 11βHSD-2 acts as a dehydrogenase to convert cortisol to cortisone in the fetal kidney. In addition, the NADP(H)-dependent isoform, 11βHSD-1, is expressed in fetal liver, where it acts as a reductase to convert cortisone to cortisol (Funder *et al.*, 1988). Although there are tissue-specific changes in the expression of 11βHSD-2 mRNA in the adrenal gland and kidney of sheep fetuses during late gestation, there is no additional impact of placental and fetal growth restriction on 11βHSD-2 mRNA expression in these tissues. In contrast, restriction of placental growth resulted in a twofold increase in 11βHSD-1 mRNA expression in the liver of the growth-restricted sheep fetus (Fig. 7) (McMillen *et al.*, 2000). Given the established relationship between 11βHSD-1 mRNA expression and isoenzyme activity, it appears likely that there is an increase in 11βHSD-1 reductase activity in the liver of the placentally restricted fetus. This finding indicates that there is increased hepatic exposure to cortisol in the growth-restricted fetus during late gestation, and this may have important pre- and postnatal consequences for the growth-restricted animal. There is a

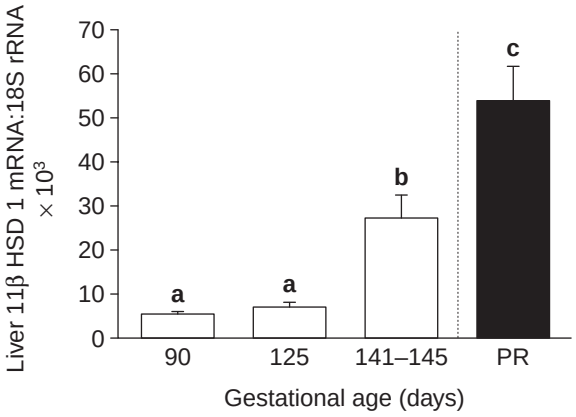

Fig. 7. Mean (± SEM) expression of 3β-hydroxysteroid dehydrogenase/Δ^5,Δ^4-isomerase (3β-HSD) type 1 mRNA:18S rRNA in the liver of normally grown sheep fetuses (□) at days 90, 125 and 141–145 of gestation and in placentally restricted sheep fetuses (■, PR) at days 141–145 of gestation. Values with different letters differ significantly ($P < 0.05$). (Redrawn from McMillen *et al.*, 2000.)

progressive increase in glycogen deposition and gluconeogenesis in the liver of sheep fetuses during late gestation, and circulating cortisol concentrations correlate positively with the activity of the hepatic gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (PEPCK), in late gestation (Fowden *et al.*, 1993). Furthermore, the induction of gluconeogenic enzyme expression is reduced in mice with a targeted disruption of the 11βHSD-1 gene (Kotelevtsev *et al.*, 1997). Thus, the increase in intrahepatic 11βHSD-1 expression in sheep fetuses in late gestation and in growth-restricted fetuses may play a role in glucocorticoid-mediated increases in glycogen deposition and gluconeogenesis in the liver that occur immediately before birth and which may be important for fetal survival after a period of intrauterine substrate deprivation. In growth-restricted fetuses, intrahepatic exposure to excess glucocorticoids may also be important in the context of epidemiological evidence of an association between growth restriction *in utero* and non-insulin-dependent diabetes (Barker, 1998). Treatment of pregnant rats with dexamethasone in late pregnancy resulted in an increased hepatic expression of glucocorticoid receptor (GR) and PEPCK mRNAs, an associated increase in hepatic PEPCK activity and fasting hyperglycaemia in the adult offspring (Nyirenda *et al.*, 1998). Since PEPCK is the rate-limiting enzyme of gluconeogenesis, the increased hepatic PEPCK expression may result in an increased hepatic glucose production and impaired glucose tolerance (Nyirenda *et al.*, 1998). However, in these studies, dexamethasone treatment of the pregnant rat did not alter hepatic 11βHSD-1 mRNA expression in the newborn or adult offspring, in contrast to the increase in hepatic 11βHSD-1 mRNA concentrations in sheep fetuses placentally restricted in late gestation. Thus, although the mechanisms by which excess glucocorticoids act on the immature liver may differ depending on the nature of the glucocorticoid (synthetic versus endogenous) and species (rats versus sheep or humans), the outcomes in later life, including a persistent increase in hepatic gluconeogenic enzyme expression and glucose production, may be similar.

Conclusion

Further clarification of the molecular and cellular mechanisms underpinning the physiological adaptations of the fetus to a reduced substrate supply is required. The relative contributions of hypoxaemia and hypoglycaemia in the stimulation of the sympathoadrenal and hypothalamo–pituitary–adrenal axes when placental growth and function are restricted are not understood and there are limited data on the relative roles of the peripheral and central mechanisms that sense and respond to alterations in fetal substrate supply. For instance, it is unclear whether the carotid chemoreflex is up- or downregulated during placental restriction. The impact of hypoxaemia on angiogenesis and vascular branching during the development of fetal and placental circulations needs to be defined. It remains to be determined whether chronic hypoxaemia is associated with a hyperinnervation of the vasculature in placentally restricted fetuses. Finally, a detailed investigation of the interaction among the endogenously derived glucocorticoids, the renin–angiotensin system and vasoactive compounds, including noradrenaline and nitric oxide, within different regional circulations of the placentally restricted fetus remain to be carried out. Such mechanistic studies are required to determine how physiological adaptations promote the short-term survival of the fetus and newborn while predisposing the individual to adult hypertension, cardiovascular and metabolic disease.

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