Fetal endocrinology and development – manipulation and adaptation to long-term nutritional and environmental challenges

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This article reviews the fetal endocrine system in sheep, a species that has a long gestation and primarily produces a singleton fetus. Attention is focused on information that is applicable to humans. The endocrinology of metabolic homeostasis in sheep fetuses is well adapted to respond to a range of metabolic challenges, including placental restriction and maternal undernutrition. A small placenta results in hypoxaemia, hypoglycaemia, reduced abundance of anabolic hormones, and fetal growth restriction. Fetuses with restricted growth are characterized by tissue-specific reductions in hormone receptor mRNA, for example mRNA for the long form of prolactin receptor is reduced in adipose tissue. In contrast, the adipose tissue of fetuses with accelerated growth, stimulated by increasing maternal nutrition in the second half of gestation, has more protein for the long form of the prolactin receptor and more uncoupling protein 1, by which large amounts of heat are generated at birth. Maternal undernutrition in early gestation, coinciding with the period of rapid placental growth, initially restricts placental growth, but when mothers are fed to requirements, a longer fetus results with a disproportionately large placenta. This nutritional manipulation replicates, in part, epidemiological findings from the Dutch famine of 1944–1945, for which the offspring are at increased risk of adult obesity.

Effect of altered environment linked to seasonality and environmental temperature on fetal endocrinology

There are marked seasonal effects on placental and fetal mass: ewes giving birth in Spring have larger placentae and offspring than those that give birth in Autumn (McCoard et al., 1997). Whether these outcomes are a direct response to environmental conditions, or are linked to differences in...
both the quality and quantity of the maternal diet, has not been determined. In both adult and fetal sheep, plasma prolactin concentrations respond directly to daylength (Bassett et al., 1989). However, daylength has no effect on liver mass near to term, or on the abundance of hepatic prolactin receptor mRNA (Phillips et al., 1999). The abundance of prolactin receptor in fetal adipose tissue is more responsive than that in the liver to an altered nutritional environment (Budge et al., 2000b). It is, therefore, hypothesized that the development of fetal adipose tissue may be more sensitive than that of the liver and other tissues to changes in photoperiod and concomitant changes in plasma prolactin concentrations (Fig. 2).

Maternal nutrition is a critical factor influencing the interaction between photoperiod, fetal metabolic environment and tissue development. Lambs born to twin-bearing ewes that were underfed (that is, consuming 60% of calculated metabolizable energy requirements necessary to produce twin lambs each weighing 4.5 kg in an unshorn ewe) during the final 4 weeks of gestation had reduced adipose tissue deposits at birth (Symonds et al., 1992). As a result, they had an increased dependence on shivering rather than non-shivering thermogenesis to maintain body temperature. This fetal response to maternal underfeeding could be overcome by winter shearing, which results in chronic cold exposure (Symonds et al., 1992). Lambs born to cold-exposed ewes did not show increased non-shivering thermogenesis when the mother was fed adequately (that is, to requirements; Gate et al., 2000). The extent to which these differential responses are mediated by the more established fetal metabolic hormones, such as insulin (Fowden, 1995), as opposed to lactogenic hormones, is unknown. Given the increasing amount of evidence indicating that prolactin and its receptor may have a critical role in fetal development (McMillen et al., in press), it is possible that changes in prolactin secretion could provide a unifying mechanism by which fetal growth and tissue maturation is modified in response to changes in the environment of the mother (Fig. 2).

Alterations in maternal and fetal metabolism, particularly glucose supply, have the potential to determine fetal prolactin secretion and adipose tissue deposition (Symonds et al., in press). The extent to which this may be protective in terms of preventing postnatal mortality but may subsequently contribute to adult morbidity remains to be established.

**Resetting of fetal endocrine homeostasis after placental insufficiency and concomitant fetal growth restriction**

A model of intrauterine growth restriction has resulted from using carunclectomized sheep (Owens et al., 1995). This model involves the removal of nearly all of the visible endometrial caruncles (placental implantation sites) in non-pregnant sheep. The resulting pregnancy usually produces a fetus of restricted growth with a small placenta. The outcome is dependent on the extent to which the growth of individual placentomes compensates for the surgical removal of most of the caruncles. Over the large range of fetal and placental masses resulting from carunclectomy, the values are positively correlated both with each other and with the oxygen content of the fetal blood (Owens et al., 1995). Fetal plasma concentrations of anabolic hormones (that is, insulin-like growth factor I (IGF-I), insulin, prolactin and thyroid hormones) near term are normally decreased (with the exception of IGF-II), in accordance with the degree of fetal hypoxaemia or hypoglycaemia (Table 1). An altered endocrine environment ensues after carunclectomy, even when there are marginal changes in fetal mass compared with controls (Table 1) (Harding et al., 1985). These endocrine adaptations increase with gestational age and a greater effect is observed on IGF-I than on IGF-II, and for thyroid hormones and prolactin compared with insulin concentrations (Table 1). Similarly, there is little effect of placental restriction on the abundance of IGF-II mRNA in fetal tissue, whereas IGF-I mRNA is lower in most fetal tissues studied to date (Kind et al., 1995). These adaptations are likely to reflect a decrease in hormonal synthetic capacity, as, for example, abundance of prolactin mRNA in the fetal pituitary is decreased in placentaly restricted fetuses (Phillips et al., 2001). Fetal pituitary prolactin mRNA is positively correlated with both fetal and placental masses in placentaly restricted but not control fetuses, and decreases in proportion to the magnitude of hypoxaemia and hypoglycaemia. Whether the suppression of prolactin synthesis in the fetal pituitary is a direct nutritional effect or is related to changes in other anabolic hormones, such as IGF-I, remains to be established.

**The prolactin receptor and fetal development**

The extent to which the compromised endocrine environment of placentaly restricted fetuses may be accompanied by alterations in the abundance and function...
of hormone receptors has not been studied extensively. The expression of mRNA for the long but not the short form of the prolactin receptor is reduced in perirenal adipose tissue in placentally restricted fetuses (Symonds et al., 1998). Prolactin binds to the long form of the prolactin receptor, which is associated with functional significance as a result of activating the JAK/STAT cell signalling pathways (Bole-Feysot et al., 1998). The biological importance of this observation remains to be established, although when maternal nutrition is enhanced, greater abundance of the long form of the prolactin receptor is associated with a higher concentration of uncoupling protein 1 (UCP-1) (Budge et al., 2000b). The ability of brown adipose tissue to generate heat is due to the unique presence of UCP-1 on the inner mitochondrial membrane (Cannon and Nedergaard, 1985), activation of which results in proton flow across mitochondria without the need to produce ATP. Thereby, all chemical energy liberated can be used for heat production. It is possible that the reduced abundance of prolactin receptor in the brown adipose tissue of placentally restricted fetuses during late gestation may impact on thermoregulation after birth. The effect of a compromised fetal substrate supply on the abundance of prolactin receptors may be specific to adipose tissue, as carunclectomy has no effect on the abundance of mRNA for either form of prolactin receptor in fetal liver or kidney near to term (Phillips et al., 2001).

The postnatal consequences of these adaptations have yet to be studied, although it should be noted that, in twins, which grow and develop in a very different placental environment from singletons, the abundance of both forms of the prolactin receptor in perirenal adipose tissue is actually enhanced immediately after birth (Budge et al., 2000a). It is hypothesized that increased prolactin receptor and UCP-1 may facilitate the fetal metabolic response at birth and minimize risk of hypothermia when volume: surface area is greater than in singleton births.

**Fetal catabolic response to placental insufficiency**

Plasma concentrations of catabolic hormones, including cortisol, in placentally restricted fetuses are higher than in non-restricted controls only after day 130 of gestation (Phillips et al., 1996). The trigger for this developmental change may be mediated through a resetting of the fetal hypothalamic–pituitary–adrenal axis which operates at a new central set point. Although mRNA for the adrenocorticotropic hormone (ACTH) precursor, pro-opiomelanocortin (POMC), is reduced close to term in the pituitary of placentally restricted fetuses, fetal plasma concentrations of bioactive ACTH 1-39 and immunoreactive ACTH are not different from controls (Phillips et al., 1996). In addition, the absolute and relative adrenal mass is increased in placentally restricted fetuses at term. Although the growth and function of the fetal adrenal cortex is increased during late gestation, the capacity of the fetal adrenal medulla to synthesize and secrete catecholamines is potentially impaired from at least mid-gestation in placentally restricted fetuses (Coulter et al., 1998). The mRNA content for the adrenaline-synthesizing enzyme phenylethanolamine-N methyltransferase (PNMT) is suppressed in the adrenal medulla of placentally restricted fetuses, and expression of PNMT mRNA in the adrenal is directly correlated with the mean gestational fetal arterial pO2 (Adams et al., 1998). Although the adrenaline-synthesizing capacity of the fetal adrenal may be reduced, plasma noradrenaline concentrations in late gestation are indicated by the following symbols: ↑, increase; ↓, decrease; =, no change. UCP-1: uncoupling protein 1.
of specific fetal tissues may be enhanced in late gestation, resulting in a greater ‘overspill’ of catecholamines into the fetal circulation (Fig. 3).

The role of altered plasma concentrations of each anabolic and catabolic hormone accompanying placental restriction remains a matter of debate. For most organs and tissues, with the exception of the brain and spleen, absolute mass is normally in proportion to the reduction in total fetal mass, both at mid- and late gestation (Owens et al., 1995; Coulter et al., 1998). The maintenance of these proportional growth relationships is indicative of compromised fetal growth throughout pregnancy (since carunclectomy is undertaken before conception) as opposed to nutritional challenges imposed at specific stages of gestation, which can alter fetal length, for example (Heasman et al., 1998).

The fetal endocrine adaptations to nutritional restriction at different stages of gestation may result in altered fetal phenotypes in the absence of overt effects on fetal body weight. With the increased clinical and scientific interest in the long-term consequences of fetal growth restriction, studies on placentaly restricted fetuses have now been extended into neonatal and later life. These studies have shown that survival immediately after birth is compromised and preliminary evidence indicates that smaller lambs have a higher arterial blood pressure at about 1 year of age (Robinson et al., 2001). In contrast, intrauterine growth retardation, associated with placental insufficiency over the final month of gestation, results in hypotension over the first 60 days of postnatal life (Louey et al., 2000). Intervention strategies aimed at overcoming these complications will be extremely difficult to introduce.

### Table 1. Comparison of metabolic and endocrine differences, as measured in late gestation, between ‘normal-sized’ and ‘growth-restricted’ placentaly restricted sheep fetuses

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Normal-sized placentaly restricted fetuses</th>
<th>Growth-restricted placentaly restricted fetuses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative fetal size</td>
<td>100</td>
<td>95 ± 4</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>pO2 (mm Hg)</td>
<td>17.7 ± 0.6</td>
<td>16.8 ± 0.4</td>
<td>12.0 ± 0.2</td>
</tr>
<tr>
<td>Glucose (mmol 1⁻¹)</td>
<td>0.75 ± 0.03</td>
<td>0.74 ± 0.05</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>IGF-I (ng ml⁻¹)</td>
<td>140</td>
<td>95 ± 7</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>IGF-II (ng ml⁻¹)</td>
<td>709 ± 206</td>
<td>1023 ± 269</td>
<td>744 ± 157</td>
</tr>
<tr>
<td>Insulin (µIU ml⁻¹)</td>
<td>18.8 ± 1.0</td>
<td>8.2 ± 0.7</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>Tri-iodothyronine (ng ml⁻¹)</td>
<td>0.27 ± 0.02</td>
<td>0.10 ± 0.01</td>
<td>0.05 ± 0.02</td>
</tr>
<tr>
<td>Thyroxine (ng ml⁻¹)</td>
<td>63.3 ± 3.6</td>
<td>19.6 ± 1.4</td>
<td>8.1 ± 1.0</td>
</tr>
<tr>
<td>Prolactin (ng ml⁻¹)</td>
<td>52.9 ± 1.2</td>
<td>20.4 ± 3.1</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>Growth hormone (ng ml⁻¹)</td>
<td>55.2 ± 5.8</td>
<td>39.1 ± 7.5</td>
<td>34.3 ± 5.0</td>
</tr>
<tr>
<td>Cortisol (ng ml⁻¹)</td>
<td>22.4 ± 5.1</td>
<td>34.0 ± 10.6</td>
<td>63.8 ± 12.7</td>
</tr>
<tr>
<td>ACTH (pg ml⁻¹)</td>
<td>495 ± 136</td>
<td>397 ± 90</td>
<td>532 ± 118</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
ACTH: adrenocorticotrophic hormone; IGF-I and -II: insulin-like growth factor I and II.
Adapted from Harding et al. (1985), Owens et al. (1994) and Robinson et al. (1980).

### Fetal endocrine responses to manipulation of maternal nutrition

The potential advantage of examining the fetal response to maternal nutrient restriction is that this intervention can be targeted at specific stages of gestation. It is clear that fetal responses to maternal nutrient restriction are dependent on both the magnitude and timing of undernutrition (Fig. 4, Table 2). Fetal endocrine responses to maternal nutrient restriction occur in the absence of fetal hypoxaemia, which usually accompanies experimentally induced or pathological placental insufficiency. The opportunity to enhance understanding of the physiologically relevant fetal adaptations to altered maternal nutrition are confounded, in part, by the different criteria or descriptions used to quantify maternal diet (Table 2). For sheep, it is not necessarily informative to include details of diet in terms of set reference data published by accredited bodies (for example Ministry of Agriculture Fisheries and Food, 1976; Agricultural Research Council, 1980; Agricultural and Food Research Council, 1992) as these are based on limited data from dissection of carcasses of a few breeds with the ‘target’ outcome of a 4.5 kg lamb. In addition, when more modest alterations in diet are studied, ideally information on maternal food intake, actual body weight and calculated metabolizable energy intake should be included. It is now apparent that feeding ewes to 100% of metabolizable energy intake (taking into account additional energy requirements for conceptus growth) does not actually provide sufficient food compared with allowing ewes to feed to appetite (Brameld et al., 2000).
Long-term consequences of maternal nutrient restriction

The fetus is adapted to respond to apparently quite severe maternal nutrient deprivation, that is, as low as 50% of the total metabolizable energy requirements predicted to produce a 4.5 kg lamb (Heasman et al., 1998; Brameld et al., 2000). Studies in which the nutrition of the mother has been restricted in this way have shown little effect on fetal body, organ or tissue masses, but have shown an altered placental mass (Table 2). These fetal and placental characteristics are in accordance with those shown, in epidemiological studies, to be associated with a greater risk of adult disease. Longitudinal studies have been performed on people born during the Dutch famine of 1944–1945. During a 5 month period of the famine, mean energy intake was 3.2 MJ day⁻¹ compared with 6.3 MJ day⁻¹ immediately afterwards (Roseboom, 2000). This cohort has been used to follow-up babies born to mothers subjected to undernutrition at different stages of gestation. Dietary restriction during early gestation had the greatest effect on the ratio of placental size: birth weight and resulted in the lowest perceived health (Table 3). Infants born after dietary restriction
<table>
<thead>
<tr>
<th>Gestational period of NR (days)</th>
<th>Diet composition</th>
<th>Metabolizable energy intake in NR sheep (MJ day⁻¹)</th>
<th>Metabolizable energy intake during remainder of pregnancy (MJ day⁻¹)</th>
<th>Maternal body weight at start of study (kg)</th>
<th>Change (%) in maternal body weight over period of NR</th>
<th>Day of gestation tissue masses measured</th>
<th>Placental mass difference NR versus control (%)</th>
<th>Fetal mass difference NR versus control (%)</th>
<th>Fetal endocrine adaptation to NR in late gestation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>28–80</td>
<td>Concentrate/hay (1:3)</td>
<td>3.03 (50%)</td>
<td>6.64 (120%)</td>
<td>7.20 (85%)</td>
<td>38</td>
<td>10</td>
<td>145</td>
<td>20</td>
<td>7</td>
<td>Heasman et al., 1998, in press</td>
</tr>
<tr>
<td>28–80</td>
<td>Concentrate/hay (1:3)</td>
<td>3.42 (60%)</td>
<td>9.31 (150%)</td>
<td>9.25 (110%)</td>
<td>36</td>
<td>0</td>
<td>140</td>
<td>1</td>
<td>0</td>
<td>Brameld et al., 2000</td>
</tr>
<tr>
<td>0–70</td>
<td>Concentrate only</td>
<td>ND (85%)</td>
<td>ND (100%)</td>
<td>ND</td>
<td>2</td>
<td>129</td>
<td>ND</td>
<td>2/3</td>
<td></td>
<td>Hawkins et al., 2000</td>
</tr>
<tr>
<td>105–115</td>
<td>Concentrate/hay</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>125</td>
<td>ND</td>
<td>ND</td>
<td>4</td>
<td></td>
<td>Gallaher et al., 1998</td>
</tr>
</tbody>
</table>

*Values in brackets indicate percentage of total metabolizable energy intake according to calculated requirements predicted to produce a 4.5 kg lamb at term.

*Sufficient to lower maternal plasma glucose from 2.5 to 1.4–1.6 mmol l⁻¹.

GHR: growth hormone receptor; IGF: insulin-like growth factor; ND, data not published.
in early gestation were longer and heavier, as well as having larger placentae and, as adults, these individuals were at much greater risk of obesity and coronary heart disease, but not hypertension (Table 4). It is likely that the ascribed reduction in maternal nutrition resulted in marked changes in fetal endocrine development. In studies of maternal nutrient restriction in sheep, changes in fetal endocrine status are apparent both at the end of the period of nutrient restriction as well as after a period of nutritional rehabilitation (Clarke et al., 1998; Brameld et al., 2000).

**Fetal endocrine adaptation to maternal nutrient restriction**

A nutritional model replicating, in part, the circumstances of the Dutch famine is the imposition of maternal
nutrient restriction between early and mid-gestation, that is, between day 28 and day 77 or 80 of gestation (Heasman et al., 1998). At term, lambs born to previously nutrient-restricted ewes were longer and heavier compared with controls. This nutritional manipulation initially restricted placental growth (Clarke et al., 1998), but resulted in a larger placenta at term (Heasman et al., 1998). The nutritional manipulation is associated with a range of diverse fetal endocrine responses, in the absence of effects on fetal organ or tissue mass. These responses include an accelerated increase in IGF-II mRNA content in skeletal muscle, as measured at day 80 of gestation, resulting in a decline in IGF-II mRNA between mid- and late gestation, which was not present in ewes that were fed well throughout pregnancy (Brameld et al., 2000). In contrast, IGF-I and IGF-II mRNA as well as growth hormone receptor mRNA are not present in ewes that were fed well throughout pregnancy. These responses may result in changes in hepatic glucose production in postnatal life.

The precise mechanisms mediating fetal adaptations to altered maternal nutrition remain to be elucidated, but are likely to be a consequence of alterations in the structural and functional development of the placenta. It is well documented that a period of maternal undernutrition of ≥ 1 month in mid- or late gestation is associated with an enlarged placenta at term (Faichney and White, 1987; Symonds et al., 1997). The extent to which this effect may be due to inhibition of the normal decline in placental mass up to term remains to be established. This finding is important when considering the consequences for cardiovascular disease as infants with either large or small placentae are at increased risk of disease in adulthood (Godfrey and Robinson, 1997).

**Maternal nutrition and fetal cortisol exposure**

One common theme currently being explored is that excess, or inappropriate, fetal exposure to glucocorticoids reprogrammes fetal development (Phillips et al., 1998). Support for this proposal comes from the finding that dexamethasone treatment of pregnant sheep results in repeatable hypertension, the magnitude of which increases with juvenile age (Dodic et al., 1998). Such an effect is observed only when the ewe is treated with dexamethasone between day 22 and day 29 of gestation, but not between day 59 and day 66. It remains to be determined whether the ascribed fetal responses to nutritional deprivation are similarly mediated through changes in maternal or fetal cortisol production and exposure. Moreover, the extent to which maternal dexamethasone treatment may alter the nutritional environment of the fetus or maternal food intake remain to be studied.

**Can the fetus sense its metabolic environment?**

An increasing range of fetal neuroendocrine responses to maternal nutrient restriction have been reported, including an increase in the abundance of neuropeptide Y mRNA in the fetal hypothalamus (Warnes et al., 1998) and a decreased cortisol response to hypoxia (Hawkins et al., 2000), as well as end organ adaptations. These responses appear to extend across the range of nutrient intakes, as allowing ewes to feed to appetite (that is, ad libitum, when they consume up to 150% of calculated metabolizable energy requirements necessary to produce a 4.5 kg lamb) not only results in larger lambs but the abundance of UCP-1 is also enhanced in perirenal adipose tissue (which constitutes 80% of fetal adipose tissue in lambs) (Budge et al., 2000b). In contrast to fetal growth restriction, when body fat deposition around the kidneys, as a fraction of fetal body weight, is unaltered (Symonds et al., 1998), the amount of perirenal adipose tissue per kg of body weight actually decreases in fetuses of ewes fed to appetite. Taken together, these findings indicate that the fetus is able to make both central and peripheral adaptations to a relative decrease or increase in nutrient supply.

**Future perspectives**

The next decade promises to be an exciting period for fetal researchers, particularly those using large animal models. It
is now possible to produce both cloned and transgenic sheep, although the number of live births remains very low at 1% (Campbell et al., 1996; McCread et al., 2000). This potential provides the dual challenge of determining why so many cloned fetuses fail to survive to term as well as understanding the biological significance of these experimental procedures. Use of functional genomics and the ability to determine a large range of molecular responses within fetal organs and tissues after hormonal stimulation should greatly facilitate understanding of the consequences of fetal endocrine adaptation to perturbations of the intrauterine environment. It is further predicted that the consequences for cell signalling pathways at critical periods of development will enable understanding of the precise role of hormone–receptor interactions of specific fetal tissues. Ultimately, this may enable the use of intervention strategies through which changes in fetal organ growth and development, both in isolation and in conjunction with overall growth restriction, can be achieved. Some of these interventions may eventually involve single or multiple manipulations of the fetal endocrine environment at specific stages of gestation.

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