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.

Fetal endocrine development, in terms of functional capacity, commences early in gestation and continues to term to prepare the fetus for life after birth. Throughout this period, the fetal endocrine system is able to adapt to a range of challenges including hypoxaemia and hypoglycaemia, the magnitude and duration of which elicit different responses (Owens *et al.*, 1995). As a consequence of exposure to such challenges, the subsequent growth trajectory and endocrine organ function of the fetus can be permanently reset. These developmental adaptations may not result in clinically apparent sequelae until much later in life, and may explain, in part, why altered fetal growth and body composition are strongly associated with a range of adult diseases (Barker, 1998). These diseases include infertility (Ibnaez *et al.*, 2000), hypertension (Barker *et al.*, 1992), obesity (Ravelli *et al.*, 1999) and insulin insensitivity (Ravelli *et al.*, 1998). It is not only the relative size of the fetus that determines longevity. A primary factor is the ratio of placental:fetal size. Fetuses with either a disproportionately small or large placenta have increased standardized mortality ratios (Godfrey and Robinson, 1997).

In reviewing current understanding of the fetal endocrine system, this article will focus on sheep, which, like humans, have a long gestation and primarily produce a singleton fetus. This focus reflects our interest in relating knowledge of fetal endocrine development to clinically important situations (for example premature deliveries, small for gestational age infants). The critical importance of fetal endocrine development for subsequent health is demonstrated by reviewing experimental manipulations designed to perturb critical stages of gestation, namely placentation and maximum placental growth after uterine attachment, as well as subsequent fetal growth (Fig. 1). Particular attention is given to the impact of experimental restriction of placental growth using the carunclectomized sheep model.

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

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Fig. 1. Relative placental and fetal growth curves in sheep bearing singletons.

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 depots 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 post-natal 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 placentally restricted fetuses (Phillips *et al.*, 2001). Fetal pituitary prolactin mRNA is positively correlated with both fetal and placental masses in placentally 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 placentally 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 adrenocorticotrophic 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

Fig. 2. Proposed interaction between the maternal metabolic environment, that is nutrition, ambient temperature, photoperiod and placental size, on plasma prolactin and glucose concentrations with respect to prolactin receptor (PRLR) abundance in the fetal liver and adipose tissue. Putative effects on the regulation of growth and development of these tissues in fetuses with high or low plasma prolactin concentrations in late gestation are indicated by the following symbols: ↑, increase; ↓, decrease; =, no change. UCP-1: uncoupling protein 1.

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 pO_2 (Adams *et al.*, 1998). Although the adrenaline-synthesizing capacity of the fetal adrenal may be reduced, plasma noradrenaline concentrations are significantly increased in placentally restricted fetuses and there is an inverse relationship between prevailing arterial pO_2 and plasma noradrenaline in both placentally restricted and control fetuses (Simonetta *et al.*, 1997). It is, therefore, possible that sympathetic innervation

Table 1. Comparison of metabolic and endocrine differences, as measured in late gestation, between 'normal-sized' and 'growth-restricted' placentally restricted sheep fetuses

Characteristic	Control	Normal-sized placentally restricted fetuses	Growth-restricted placentally restricted fetuses
Relative fetal size	100	95 ± 4	61 ± 2
pO ₂ (mm Hg)	17.7 ± 0.6	16.8 ± 0.4	12.0 ± 0.2
Glucose (mmol l ⁻¹)	0.75 ± 0.03	0.74 ± 0.05	0.38 ± 0.02
IGF-I (ng ml ⁻¹)	140 ± 9	95 ± 7	38 ± 7
IGF-II (ng ml ⁻¹)	709 ± 206	1023 ± 269	744 ± 157
Insulin (μiu ml ⁻¹)	18.8 ± 1.0	8.2 ± 0.7	3.9 ± 0.7
Tri-iodothyronine (ng ml ⁻¹)	0.27 ± 0.02	0.10 ± 0.01	0.05 ± 0.02
Thyroxine (ng ml ⁻¹)	63.3 ± 3.6	19.6 ± 1.4	8.1 ± 1.0
Prolactin (ng ml ⁻¹)	52.9 ± 1.2	20.4 ± 3.1	2.9 ± 0.3
Growth hormone (ng ml ⁻¹)	55.2 ± 5.8	39.1 ± 7.5	34.3 ± 5.0
Cortisol (ng ml ⁻¹)	22.4 ± 5.1	34.0 ± 10.6	63.8 ± 12.7
ACTH (pg ml ⁻¹)	495 ± 136	397 ± 90	532 ± 118

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).

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 placentally 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.

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).

Fig. 3. Developmental effect of placental restriction and resulting hypoxia on chronic adaptations in the fetal hypothalamic-pituitary-adrenal axis in the regulation of cortisol and catecholamine secretion in mid- to late gestation. ACTH: adrenocorticotrophic hormone; PNMT: phenylethanolamine-*N* methyltransferase; POMC: pro-opiomelanocortin. Based on Adams *et al.* (1998), Phillips *et al.* (1996) and Simonetta *et al.* (1997).

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 epidemio-

logical 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 2. Comparison of the effect of timing and duration of maternal nutrient restriction (NR) in sheep on placento–fetal mass differences and endocrine status near to term

Gestational period of NR (days)	Diet composition	Metabolizable energy intake			Maternal body weight at start of study (kg)	Change (%) in maternal body weight over period of NR	Day of gestation tissue masses measured	Placental mass difference NR versus control (%)	Fetal mass difference NR versus control (%)	Fetal endocrine adaptation to NR in late gestation	Reference
		Metabolizable energy intake in NR sheep (MJ day ⁻¹) ^a	Metabolizable energy intake in controls (MJ day ⁻¹) ^a	Metabolizable energy intake during remainder of pregnancy (MJ day ⁻¹) ^a							
28–80	Concentrate/hay (1:3)	3.03 (50%)	6.64 (120%)	7.20 (85%)	38	10	145	20	7	Plasma IGF-I concentration no longer related to body size	Heasman <i>et al.</i> , 1998, in press
28–80	Concentrate/hay (1:3)	3.42 (60%)	9.31 (150%)	9.25 (110%)	36	0	140	1	0	Increased fetal hepatic IGF-I, IGF-II and GHR mRNA	Brameld <i>et al.</i> , 2000
0–70	Concentrate only	ND (85%)	ND (100%)	ND (100%)	ND	2	129	ND	–3	Lower basal and stimulated plasma cortisol concentration	Hawkins <i>et al.</i> , 2000
105–115	Concentrate/hay	ND ^b	ND	–	ND	ND	125	ND	4	Lower plasma IGF-binding protein 3 concentration	Gallaher <i>et al.</i> , 1998

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

Fig. 4. Effect of maternal nutrient restriction in early gestation on the relationship between placental and fetal growth throughout gestation with respect to adaptations in the fetal metabolic and hormonal environment that could contribute to an increased predisposition to adult disease in the resulting offspring. IGF-I: insulin-like growth factor I.

Table 3. Comparison of the effect of stage of gestation during exposure to the Dutch famine of 1944–1945 on size at birth, placental mass and adult body mass index (BMI)

Characteristic	Time of maternal exposure to famine					Reference
	Before conception	Early gestation	Mid-gestation	Late gestation	After birth	
Birth weight (g)	3383	3450	3217	3166	3444	Roseboom <i>et al.</i> , 1999
Placental area (cm)	298	278	260	270	275	Roseboom <i>et al.</i> , 1999
Placental:birth weight ratio (cm g ⁻¹)	6.2	6.4	6.2	6.1	5.9	Roseboom <i>et al.</i> , 2000a
Adult BMI (kg m ⁻²) ^a	26.6	27.9	26.5	26.7	27.2	Roseboom <i>et al.</i> , 1999
Perceived health poor (%)	5.3	10.3	3.7	6.4	5.3	Roseboom, 2000

Values are means.

^aGeometric means.

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

Table 4. Differences in adult disease markers, adjusted for sex, between adults exposed prenatally at specific stages of gestation to the Dutch famine of 1944–1945 and those born to non-exposed mothers (that is born before or conceived after the famine)

	Early gestation	Mid-gestation	Late gestation	Reference
LDL:HDL ratio (%) ^a	13.9 (2.6 to 26.3)	–5.3 (–13.1 to 3.3)	–2.5 (–10.1 to 5.6)	Roseboom <i>et al.</i> , 2000c
Odds ratio for CHD	3.0 (1.1 to 8.1)	0.3 (0.0 to 2.2)	0.8 (0.2 to 2.8)	Roseboom <i>et al.</i> , 2000b
Adult blood pressure ^b	–3.1 (–6.7 to 0.6)	–0.1 (–3.2 to 2.9)	1.6 (–1.4 to 4.5)	Roseboom <i>et al.</i> , 1999

Values in parentheses are 95% confidence intervals.

^aRelative differences in cholesterol, expressed as percentages of the means in non-exposed people.

^bDifferences in systolic blood pressure when adjusted for sex, age and body mass index.

CHD: coronary heart disease; HDL: high density lipid; LDL: low density lipid.

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 enhanced in the livers of previously nutrient-restricted fetuses when the mother is subsequently re-fed to appetite to term. These adaptations 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 an 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; McCreath *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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