Ontogeny and nutritional manipulation of the hepatic prolactin–growth hormone–insulin-like growth factor axis in the ovine fetus and in neonate and juvenile sheep

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The somatotrophic axis is the main endocrine system regulating postnatal growth; however, prenatal growth is independent of growth hormone (GH). Fetal development relies on the coordinated actions of a range of hormones, including insulin-like growth factors (IGF), and prolactin (PRL), in the control of differentiation, growth and maturation. In the sheep the abundance peaks for liver IGF-II and PRL receptors occur during late gestation while that for IGF-I receptor occurs at birth. All receptors, with the exception of GH receptor subsequently decrease by age 6 months. It has been proposed that maternal undernutrition during gestation regulates the maturation of the fetal hypothalmic–pituitary–adrenal axis and endocrine sensitivity. Critically, the timing of the nutritional insult may affect the magnitude of reprogramming. Maternal malnutrition during early to mid-gestation (3·2–3·8 MJ/d (60 % total metabolisable energy requirements) *v.* 8·7–9·9 MJ/d (150 % total metabolisable energy requirements) between 28 and 80 d of gestation) had no effect on body or liver weight. Nutrient-restricted (NR) fetuses sampled at 80 d (mid-gestation) showed up-regulation of hepatic PRL receptor, but following refeeding the normal gestational rise in PRL and GH receptors did not occur. Hepatic IGF-II receptor was down regulated in NR fetuses at both mid- and late gestation. Conversely, 6-month-old offspring showed no difference in the abundance of either GH receptor or PRL receptor, while IGF-II mRNA was increased. Offspring of ewes malnourished during late gestation (9·1 MJ/d (60 % total metabolisable energy requirements) *v.* 12·7 MJ/d (100 % total metabolisable energy requirements) from 110 d of gestation to term) showed reduced abundance of hepatic GH and PRL receptor mRNA. In conclusion, maternal undernutrition during the various stages of gestation reprogrammed the PRL–GH–IGF axis. Nutritional regulation of cytokine receptors may contribute to altered liver function following the onset of GH-dependent growth, which may be important in regulating endocrine adaptations during subsequent periods of nutritional deprivation.

Maternal nutrition: PRL–GH–IGF axis: Liver: Fetal and neonatal development

Growth hormone–insulin-like growth factor axis

Growth hormone (GH) and prolactin (PRL) receptors are members of the class I cytokine receptor superfamily characterised as tyrosine kinase-associated receptors (Bazan, 1990), whereas insulin-like growth factor (IGF)-I receptor (IGF-IR) is a receptor tyrosine kinase and IGF-II receptor (IGF-IIR) is a mannose-6-phosphate receptor (Rother & Accili, 2000). Each receptor within the PRL–GH–GF axis has a substantial role to play in the regulation of fetal growth and development. The physiological properties of the GH–IGF system have been extensively reviewed elsewhere (Owens, 1991). It is not the aim of the present paper to detail the physiological properties of this system but to review its role in fetal and neonatal development and how this process responds to an *in utero* nutritional manipulation.

Abbreviations: GH, growth hormone; GHR, growth hormone receptor; HPA, hypothalamic–pituitary–adrenal; IGF, insulin-like growth factor; IGF-IR, IGF-IIR, IGF-I and IGF-II receptors respectively; NR, nutrient restricted; PRL, prolactin; PRLR, prolactin receptor.
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Growth hormone and prolactin

GH and PRL are proteins produced from many cell types under both physiological and pathological conditions. They function at either a local or on a systemic level, exerting their biological effects by binding to specific cell-surface receptors and inducing an intracellular signalling cascade that ultimately modulates gene expression (Ihle, 1994). During fetal development many cytokines and growth factors act as cellular survival and maturation factors by promoting cell proliferation and inhibiting apoptosis. As cytokines, GH and PRL are important mediators of embryogenesis, organ development and postnatal growth (Symonds *et al.* 2001).

GH is synthesised and secreted by somatotrophic cells in the anterior pituitary in a sexual dimorphic pattern (Jaffe *et al.* 2002). In addition to the pituitary gland, GH can also be produced in the brain, placenta, mammary tissue, lymphocytes and the pineal gland (Gluckman *et al.* 1983*a*). Prolactin, like GH, is primarily secreted from the lactotroph cells of the anterior pituitary. Extrapituitary PRL can also be produced by the placenta, uterus, myometrium, brain, immune system and mammary and adrenal glands (Gellersen *et al.* 1991), functioning in an autocrine–paracrine manner and thus adding to the physiological effects of this multifaceted hormone. PRL, traditionally thought to be a lactogenic hormone, is reported to have in excess of 300 physiological roles across the species (Boyle-Feysot *et al.* 1998).

GH receptor (GHR), the first of the class I cytokine receptors to be cloned, shares a single transmembrane domain structure with other family members, including the PRL receptor (PRLR; Cosman, 1993). Class 1 cytokine receptors are tyrosine kinase-associated receptors that bind ligands with their extracellular domain (Bazan, 1990), thus causing receptor dimerisation and induction of associated tyrosine kinases (Janus kinase) and signal transducers and activators of transcription (Fig. 1(a)). GH-induced phosphorylation of signal transducers and activators of transcription types1, 3 and 5 has been shown to increase their specific DNA binding in promotor regions of GH-regulated genes (Meyer *et al.* 1993), resulting in the activation of a variety of genes, including IGF-I. As various Janus kinases and signal transducers and activators of transcription are common to many cytokine and hormone receptors, it is not known how the specificity of such signalling mechanisms is maintained. Consequentially, ligands are able to exert their biological effects through more than just their own cytokine receptor.

PRL and the various forms of its receptors have been shown to be present in many tissues, including liver and adipose tissue, from early gestation (Budge *et al.* 2000). Circulating plasma PRL levels increase during gestation, reaching peak levels about the time of birth (Phillips *et al.* 1996). Conversely, plasma GH is only detectable in the sheep fetus during late gestation and is thought to increase from approximately 130 d of gestation, coincident with the prepartum surge in cortisol and the maturation of the hypothalamic–pituitary–adrenal (HPA) axis. The exact role of GH and PRL receptors in the growth and development of the fetus remains unclear (Lacroix *et al.* 1999).
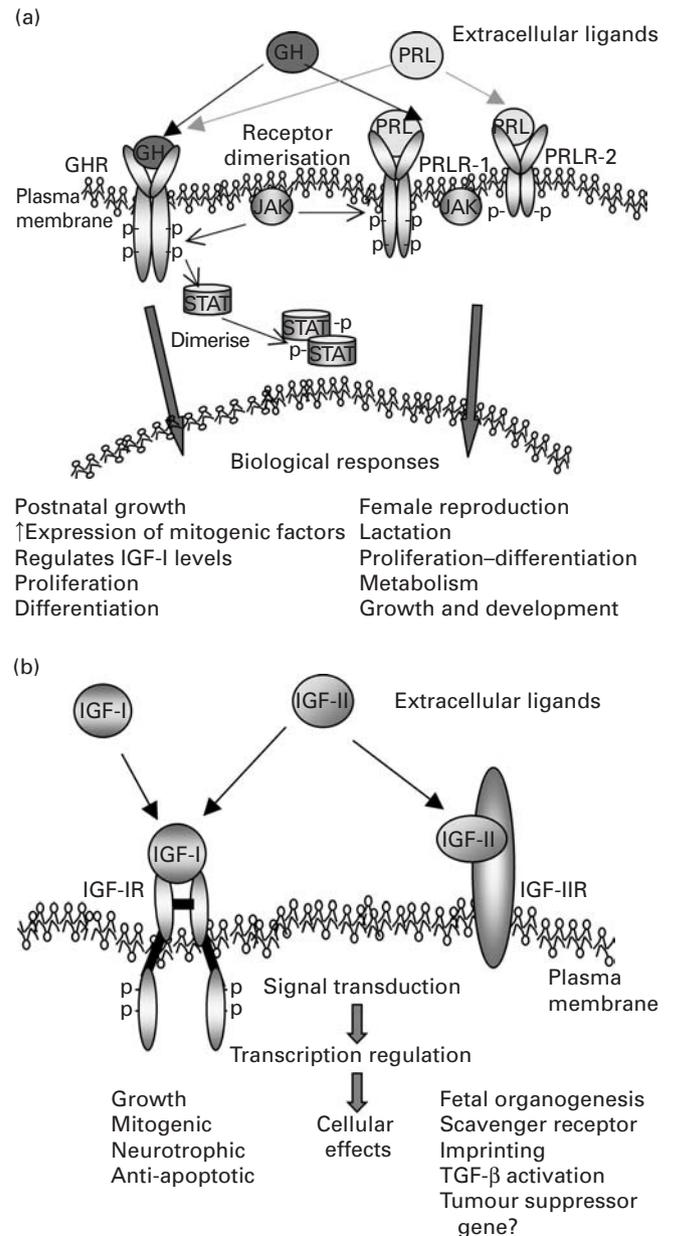

Fig. 1. Schematic illustration of (a) class 1 cytokine receptors; growth hormone (GH) receptor (GHR) and prolactin receptor (PRLR); (b) insulin-like growth factor-I receptor (IGF-IR), insulin-like growth factor-II receptor (IGF-IIR). JAK, Janus kinase; STAT, signal transducers and activators of transcription; TGF-β, transforming growth factor β; p, sites of tyrosine kinase phosphorylation.

Insulin-like growth factors

In addition to the cytokines, growth factors are paramount in fetal and postnatal growth and development. Of particular importance are the insulin-like growth factors (IGF) and their receptors. The IGF axis consists of IGF-I, IGF-II, IGF-IR, IGF-IIR and six known IGF-binding proteins (1–6; Cohick & Clemmons, 1993). IGF are important mitogenic factors involved in cell proliferation and metabolism (Rother & Accili, 2000). Insulin and IGF-II are the most important mitogens and determinants

of fetal development, while IGF-I, under the control of GH, is the most potent postnatal mitogen (Jones & Clemmons, 1995).

In sheep, as in man, IGF-I and -II are synthesised in numerous fetal and adult tissues, including liver, kidney, heart, lung and adipose tissue (Owens, 1991). Recent studies have shown that liver-derived IGF-I knock-out mice have substantially reduced circulating IGF-I levels; however, normal growth and metabolism was sustained by IGF-I produced by the kidney, heart, lung, chondroblasts, fibroblasts and adipose tissue, which acts in an autocrine–paracrine fashion (Sjogren et al. 1999). IGF-I is a growth factor for astrocytes and skeletal muscle, and promotes the differentiation of oligodendrocytes, neurones and myoblast during fetal development (Gluckman et al. 1993). The IGF are also autocrine modulators of growth for human rhabdomyosarcomas (El-Brady, 1990) and neuroblastoma cells (Kiess et al. 1997).

The biological functions of the IGF system are achieved through ligands binding to and activating membrane-bound receptors (Fig. 1(b)). Activation of IGF-IR regulates gene expression of proteins actively involved in cellular proliferation and differentiation. IGF-IIR functions primarily as a scavenger receptor, regulating circulating plasma IGF-II levels (O'Dell & Day, 1998). The removal of extracellular IGF-II through lysosomal degradation regulates circulating levels of IGF-II and thus the mitogenic potential of IGF-II acting through IGF-IR. Thus, binding of IGF-II to its receptor has an overall negative effect on growth.

The importance of the IGF receptors in fetal growth and survival were emphasised by the generation of knock-out mice. IGF-IIR knock-out mice are not viable (Wang et al. 1994), while inactivation of IGF-I (Liu et al. 1993) and/or IGF-II (DeChiara et al. 1990) genes results in a 45 and 60 % reduction in fetal birth weight respectively. IGF-IR null mice are 55 % smaller at birth when compared with control littermates. Deletion of IGF-IR is fatal at birth due to respiratory failure (Liu et al. 1993); however, heterozygous IGF-IR knock-out mice, i.e. IGF-IR +/− mice, live on average 26 % longer than their wild-type littermates (Holzenberge et al. 2003).

Role of liver during fetal development

The liver is an important endocrine organ during fetal development and postnatal growth through its ability to regulate the synthesis and secretion of potent cytokines and growth factors, as previously discussed. The transition from fetus to neonate represents a time of major physiological and endocrine change. Expression of functional GHR are essential for normal postnatal growth and metabolic homeostasis (Zhou et al. 1997). However, it is not known whether GH is secreted from the fetal anterior pituitary throughout gestation.

Onset of growth hormone-dependent growth

Over the first few months of life infants undergo a major endocrine transition, that of the onset of GH-dependent growth (Li et al. 1999). This process represents a switch in the relative importance of growth factors and hormones. The precise timing of this transition is unclear, but has usually occurred by 6 months of age, with its exact time-course probably unique to the maturation of the individual's endocrine system. The onset of GH-dependent growth is simply understood to be the conversion from autocrine–paracrine control of growth, in which insulin and IGF-II are the major mitogens, to endocrine control, in which systemic growth is primarily under the influence of GH (Forhead et al. 2000). The establishment of a GH-responsive endocrine system through an intact IGF network is thought to be crucial for successful adaptation from fetal to postnatal life (Li et al. 1996).

GHR mRNA is present in all fetal tissues during the first trimester of human pregnancies, with immunoblotting detecting GHR protein in the liver from 8·5 weeks of gestation (Zogopoulos et al. 1996). However, no developmental or physiological role has been determined for the presence of GHR in the fetus (Gluckman & Harding, 1997). GHR is transcribed from two promoters within the ovine liver; promoter, P1, is a developmentally-regulated liver-specific transcript that alternatively splices exon 1A onto the GHR mRNA, while the second promoter, P2, is constitutively active in many tissues and alternatively splices exon 1B onto the GHR mRNA (Adams, 1995).

Ontogeny of hepatic somatotrophic (prolactin–growth hormone–insulin-like growth factor) axis

The normal ontogenic change in the expression patterns of hepatic cytokine and IGF receptors was elucidated in order to determine accurately the effect of maternal nutrition on liver development and maturation of the somatotrophic axis.

Growth hormone receptor ontogeny

In the fetal sheep GHR are present in the ovine liver from 51 d of gestation (Klempt et al. 1993). However, ligand-binding studies reveal little binding of GH to its receptor in the fetal liver; GHR binding only becomes apparent at 24 h postpartum, reaching maximal levels by 8 d of age (Gluckman et al. 1983). Semi-quantitative reverse transcriptase–PCR studies reveal that ovine GHR mRNA abundance steadily increases from mid- to late gestation, reaching a peak level at 1 d of age, followed by a modest decrease by 6 months of postnatal age (Fig. 2(a)). Although GHR abundance is consistently high about the time of birth, it was not found to be significantly different from that at any other time point studied (Hyatt et al. 2003a). These findings differ from recent reports in which GHR significantly increased throughout late gestation ($P < 0.05$), reaching peak levels in adulthood (Li et al. 1999). Such discrepancies can be accounted for when consideration is given to the nature of oligonucleotide primers utilised. Li et al. (1999) used riboprobes in RNase protection assays designed to detect the ontogeny of individual GHR transcripts, i.e. exon 1A, 1B, 2 and 3. However, our study (Hyatt et al. 2003a) was designed to determine the ontogenic change in all forms of GHR, using oligonucleotide primers designed to amplify all functional forms of the GHR.
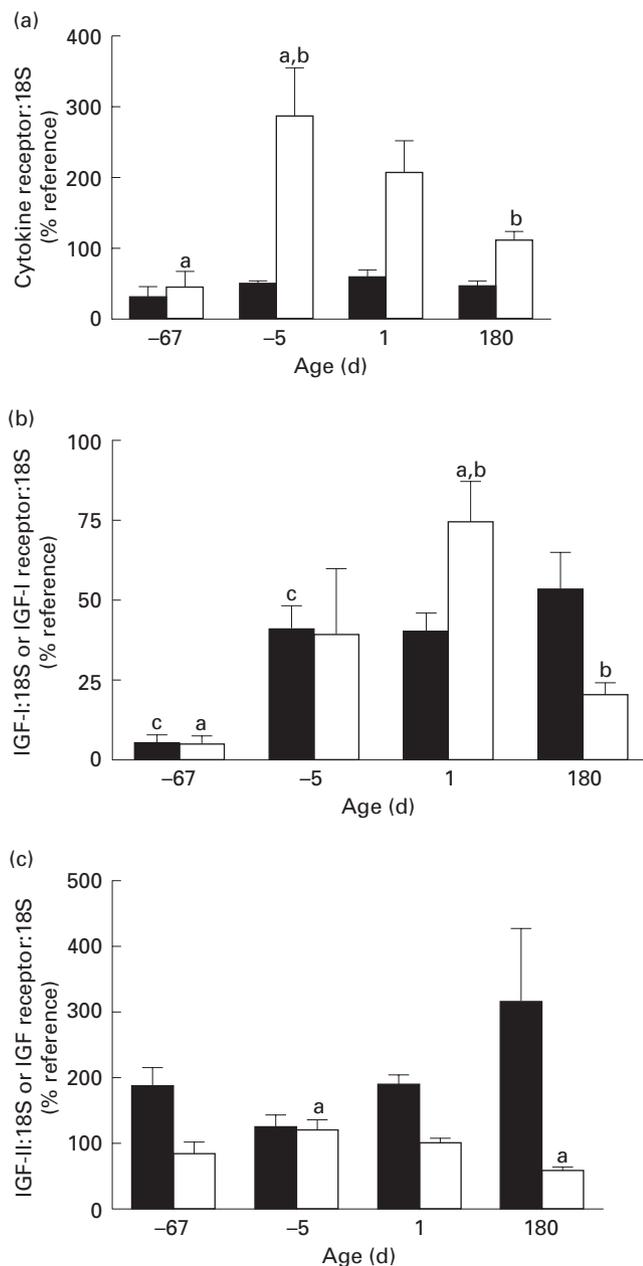
Fig. 2. Gestational and postnatal ontogeny of hepatic (a) class 1 cytokine receptors; growth hormone receptor (■) and prolactin receptor (□); (b) insulin-like growth factor (IGF)-I (■) and IGF-I receptor (□); (c) IGF-II (■) and IGF-II receptor (□). Ontogenic change in receptor and ligand mRNA abundance was determined in liver tissue sampled from -67 d (mid-) and -5 d (late) gestation fetuses and lambs at 1 d and 1 and 6 months of age. Results, given as target gene: 18S (arbitrary units) and expressed as a percentage of a reference sample present on all gels, are means with their standard errors represented by vertical bars for four to nine samples per group. [a,b,c]Mean values with the same superscript letter were significantly different: (a) [a]$P < 0.01$, [b]$P < 0.05$; (b) [a]$P < 0.001$, [b]$P < 0.01$, [c]$P < 0.05$; (c) [a]$P < 0.01$. (Adapted from Hyatt *et al.* 2003*a*; Hyatt *et al.* 2004*c*.)

Prolactin receptor ontogeny

Hepatic PRLR isoforms are barely detectable at 80 d mid-gestation. The levels increase significantly during gestation ($P < 0.01$), reaching a peak in the liver at 140 d (late-gestation), just before birth. The hepatic PRLR levels steadily decrease postnatally, the decrease reaching significance at 6 months of age ($P < 0.05$; Fig. 2(a); Hyatt *et al.* 2003*a*).

Insulin-like growth factor-I ligand and receptor ontogeny

Components of the IGF system are readily detectable in many human fetal tissues from as early as the first trimester, while fetal plasma IGF concentrations increase during gestation (Reynolds *et al.* 1997). In the sheep fetus, hepatic IGF-I transcript levels were found to increase significantly from mid- to late gestation ($P < 0.05$). Following birth, hepatic IGF-I expression remained steady up to 1 month of age. A further elevation in IGF-I in the juvenile liver was seen at 6 months postpartum, possibly indicative of the onset of GH-dependent growth (Fig. 2(b); Hyatt *et al.* 2004*c*). Recent reports have suggested that there is a more sizeable increase in IGF-I abundance postnatally (Li *et al.* 1996); the conflict in the data could be related to a variation in IGF-I transcripts detected. In our study, as a starting point, all IGF-I mRNA transcripts were amplified. There are two forms of IGF-I proteins, encoded for by differing promoters as a result of alternative splicing. Class 1 IGF-I (exons 1, 3, 4) is found in most tissues, whereas class 2 (exons 2, 3, 4) IGF-I is almost exclusively expressed in the liver and has been shown to be GH-responsive (O'Sullivan *et al.* 2002); it therefore increases considerably during development, especially over the first few months of life, coincident with the onset of GH-dependent growth. Despite recent advances in the detection of IGF-I transcripts, the nature of the underlying control mechanisms for the switch from class 1 to class 2 IGF-I and whether this switch is caused by, or as a result of, GH-dependent growth remains unclear.

In the fetal liver IGF-IR mRNA significantly ($P < 0.001$) increases from day 80 of gestation (mid-gestation) to reach a peak level about the time of birth. Down-regulation of hepatic IGF-IR occurs after birth, with IGF-IR mRNA levels being significantly reduced at 6 months postpartum ($P < 0.01$; Fig. 2(b); Hyatt *et al.* 2003*a*). These findings are consistent with previous reports that indicate that peak IGF-IR mRNA levels occur during fetal development and in the early postnatal period, before subsequent down-regulation (Khandwala *et al.* 2000).

Insulin-like growth factor-II ligand and receptor ontogeny

The ontogenic change in IGF-II mRNA abundance in the developing fetal liver is more variable than that of any other gene tested. Hepatic IGF-II levels are high at mid-gestation, decreasing during late gestation (in accordance with the prepartum cortisol surge), before increasing on the first day of life to reach peak levels between 1 and 6 months postpartum (Fig. 2(c); Hyatt *et al.* 2004*c*). In contrast, Owens *et al.* (1994) found no changes in IGF-II mRNA expression levels in fetal lung, kidney and skeletal muscle throughout gestation. Unlike that of IGF-IR, fetal hepatic IGF-IIR mRNA was found to be consistently high throughout gestation, before steadily decreasing up to 6 months of age (Fig. 2(c); Hyatt *et al.* 2003*a*).

In summary, the normal ontogeny of cytokine and growth factor receptors of the PRL–GH–IGF axis were determined over an extensive age-range, from mid-gestation to juvenile life. In our studies there was no appreciable increase in GHR postnatally, coincident with the onset of GH-dependent growth (Li *et al*. 1999). However, there was a clear loss of hepatic PRLR between 1 and 6 months of age. These findings indicate that the transition from insulin to GH dependency may in fact be more responsive to the abundance of GHR relative to that of other cytokine receptors such as PRLR than to alterations in GHR *per se*. Moreover, the abundance of IGF receptors may also play a direct role in the transition to GH dependency.

Nutritional and endocrine regulation of fetal development

Epidemiological, clinical and animal studies have demonstrated that a suboptimal intrauterine environment during pregnancy caused by maternal undernutrition reprogrammes the setting of the fetal HPA axis (Hawkins *et al*. 2001). In some, but not all, cases this condition can result in low birth weight, enhanced placental growth (as a result of increased glucose transport), modified organogenesis (e.g. a smaller liver), distorted tissue conformation (e.g. kidney) and altered homeostatic functions (Harding, 2001). Resultant offspring are often at a greater risk of developing adult CVD and metabolic diseases in later life (Barker, 2002). It has since become apparent that the timing of maternal nutrient manipulation is a major determining factor of compromised adult health (Harding, 2001); the resultant endocrine adaptations to maternal undernutrition during gestation are perhaps more important in determining fetal outcome than low birth weight alone. It is the effect of an adverse intrauterine environment on the reprogramming of the HPA axis (i.e. early maturation of the HPA axis), in terms of setting fetal plasma cortisol levels and endocrine sensitivity (Edwards & McMillen, 2001), that is now believed to be a key factor in the development of diseases such as CVD (Barker, 2002), insulin resistance (Newsome *et al*. 2003), type 2 diabetes mellitus (Ozanne & Hales, 2002) and hypertension (Symonds *et al*. 2003) in adult life.

In addition, low birth weight infants have an increased risk of developing hepatoblastoma, a paediatric liver tumour, affecting infants and children between the ages of 6 months and 3 years (Herzog *et al*. 2000). In later life low-birth-weight offspring are at a greater risk of developing ovarian (Barker *et al*. 1995) and breast cancer (Vatten *et al*. 2002) as a consequence of catch-up growth and/or alterations in circulating plasma hormone levels. A recent prospective study (Hankinson *et al*. 1999) has shown a significant positive association between increased circulating PRL and the risk of breast cancer in post-menopausal women (highest *v*. lowest quartile, multivariate relative risk 2·03 (95 % CI 1·24, 3·31), $P = 0.01$). Components of the PRL–GH–IGF axis are susceptible to nutritional programming during pregnancy; thus, this axis provides plausible candidates for the initiation and/or propagation of tumorigenesis.

Timing of maternal nutrient manipulation and liver development

In sheep the growth and maturation of the liver continues throughout gestation, with liver function commencing during the last 2 weeks of pregnancy, coincident with the prepartum cortisol surge (Fowden *et al*. 1998). Fetal nutrient restriction as a result of severe maternal undernutrition (i.e. 14 d at 25 % metabolisable energy requirements; Bauer *et al*. 1995) or placental restriction results in smaller livers (Kind *et al*. 1995) and reduced plasma and tissue IGF-I levels, while IGF-II levels are unchanged (Owens *et al*. 1994). To determine the effect of a more modest maternal undernutrition on fetal development and postnatal liver function a set of nutritional studies were undertaken.

Effect of maternal nutrient restriction during early to mid-gestation on fetal liver growth and prolactin–growth hormone–insulin-like growth factor axis

Nutrient-restricted (NR) ewes were fed 3·2–3·8 MJ/d (60 % total metabolisable energy requirements) between 28 and 80 d of gestation, whilst control ewes consumed 8·7–9·9 MJ/d (150 % the total metabolisable energy requirements). Previous studies have shown that this NR diet produces a longer fetus with a disproportionately larger placenta at term (Heasman *et al*. 1998). There were no differences in body or liver weights between control and NR fetuses sampled at either mid- or late gestation (Whorwood *et al*. 2001). Following birth and adequate postnatal nutrition, no difference in offspring body or liver weights was found in 6-month-old juvenile lambs. NR fetuses sampled at 80 d gestation showed up-regulation of hepatic PRLR; however, following refeeding the normal gestational rise in PRLR and GHR failed to occur (Fig. 3 (a,b)). Consequently, GHR and PRLR were significantly down regulated ($P < 0.05$) in livers of late-gestational (140 d) NR fetuses (Hyatt *et al*. 2002*a*). Similarly, hepatic IGF-IIR was nutritionally down regulated in NR fetuses at both sampling dates (Hyatt *et al*. 2004*a*). In contrast, 6-month-old offspring showed no difference in either GHR or PRLR levels, while IGF-II mRNA was significantly up regulated in NR livers ($P < 0.05$; Hyatt *et al*. 2002*c*).

These findings suggest that following 52 d of maternal undernutrition during early to mid-gestation cytokine receptor levels are susceptible to programming in the developing fetal liver. Consequently, GHR is nutritionally down regulated in late gestation, a time when hepatic GHR normally peaks in accordance with HPA maturity. Conversely, hepatic PRLR is prematurely up regulated in mid-gestation; thus, maternal undernutrition limited its gestational rise and ontogenic peak. IGF-IIR mRNA levels were also decreased in NR fetuses in conjunction with an increase in hepatic IGF-II mRNA expression (Brameld *et al*. 2000). The long-term metabolic and endocrine consequences of compensatory molecular adaptations remain unclear. As juveniles, NR offspring have comparable GHR and PRLR levels; therefore, nutritional down-regulation of cytokine receptors was transient. Interestingly, NR juvenile
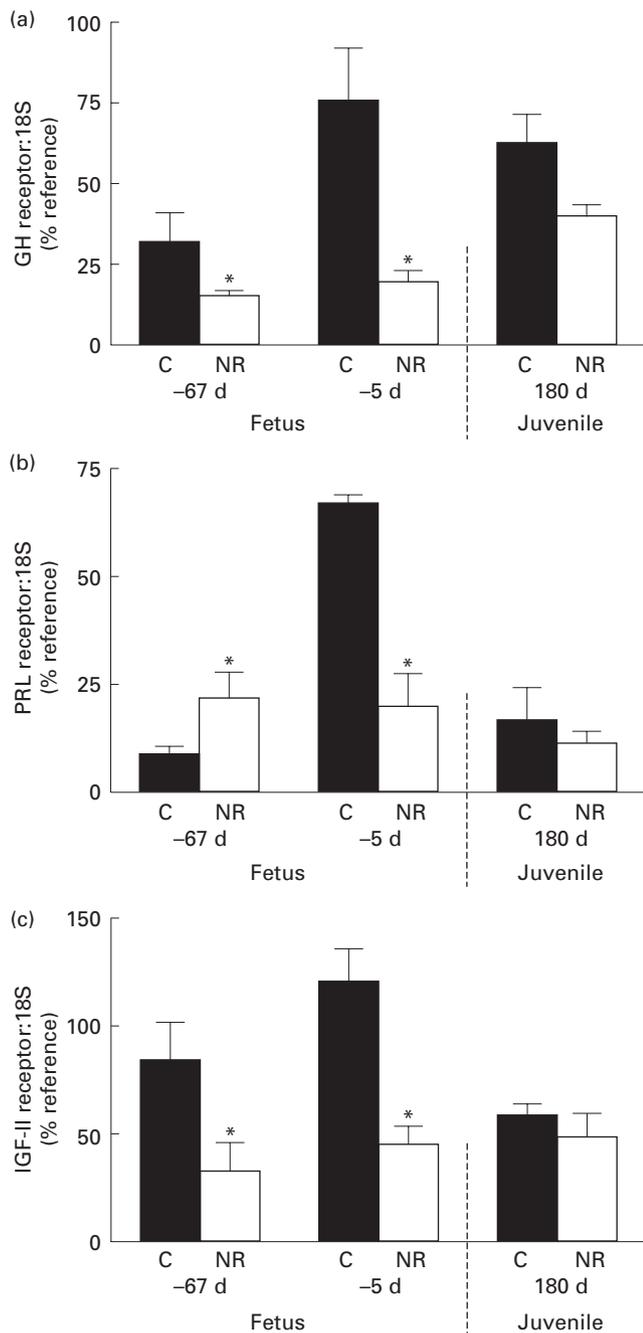
(a)

(b)

(c)

Fig. 3. Effect of maternal nutrient restriction during early to mid-gestation on hepatic (a) growth hormone (GH) receptor; (b) prolactin (PRL) receptor; (c) insulin-like growth factor (IGF)-II receptor. (■), Control (C) 8·7–9·9 MJ/d (*n* 15); (□), nutrient restricted (NR) 3·2–3·8 MJ/d (*n* 15). Results, given as mean receptor:18S (arbitrary units) and expressed as a percentage of a reference sample present on all gels, are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the C group: *$P < 0.05$. (Adapted from Hyatt *et al.* 2002*a,c*; Hyatt *et al.* 2004*a*.)

Effect of maternal nutrient restriction during late gestation, coincident with hypothalamic–pituitary–adrenal maturation, on fetal growth and hepatic prolactin–growth hormone–insulin-like growth factor axis

In the sheep maternal nutrient restriction over the final month of pregnancy can influence fetal liver growth by accelerating maturation of the hypothalamus. Maternal malnutrition during late gestation increases fetal plasma GH levels, whereas circulating IGF-I (Bauer *et al.* 1995), glucose and insulin levels are decreased (Edwards & McMillen, 2001). This present study investigated the effects of maternal undernutrition during late gestation (from 110 d of gestation to term) on the hepatic PRL–GH–IGF axis in the resultant offspring at 1 d and 1 month of age. Control ewes were fed 12·7 MJ/d (100 % total metabolisable energy requirements) while NR ewes consumed 9·1 MJ/d (60 % total metabolisable energy requirements). No nutritional differences were seen in lamb body or liver weight at either age. However, NR offspring sampled at 30 d had a significantly higher liver weight:body weight when compared with control lambs ($P = 0.03$; Hyatt *et al.* 2002*b*). Hepatic GHR and PRLR mRNA transcript levels were significantly down regulated at both 1 and 30 d of age ($P < 0.05$ in all cases; Fig. 4(a)). IGF-IR and IGF-IIR mRNA abundance were significantly decreased in NR offspring at 1 d but not at 30 d of age ($P < 0.05$ in each case; Fig. 4(b); Hyatt *et al.* 2003*b*). No differences in hepatic IGF-I or -II ligand levels were detected at either age.

In summary, restricting ewes to 60 % of their total metabolisable energy requirements from 110 d of gestation to term results in normal-sized offspring, although the NR lambs at 30 d of age had a significantly higher liver weight:body weight ($P < 0.05$). Livers sampled from 1-d-old neonates showed a nutritional down-regulation of all receptors analysed; however, at 30 d of age only GHR and PRLR levels were decreased. Decreased cytokine and IGF receptor levels, especially IGF-IIR mRNA abundance at 1 d of age, may have contributed to the increased liver size relative to whole-body weight observed in NR offspring at 1 month of age. The functional importance of such a compensatory mechanism to maternal malnutrition in late gestation remains unclear, but may prove adaptive during further life stresses following the onset of GH-dependent growth. Maternal malnutrition over the final month of pregnancy, coincident with an increase in glycogen and gluconeogenic enzymes in the liver, may contribute to altered gluconeogenesis following birth and glucose intolerance in later life. Decreased cytokine and IGF receptor levels in response to fetal undernutrition may act to delay the conversion from insulin to GH dependency; hepatic GHR are reduced and consequently liver GH sensitivity is decreased. On the other hand, however, the onset of GH-dependent growth may be brought forward in accordance with the early maturation of the HPA axis to ensure postnatal growth at the expense of continued tissue development.

No obvious phenotypic change was observed up to 1 month of age; therefore, NR offspring have adjusted to maternal undernutrition after birth. However, long-term consequences of maternal undernutrition during early to
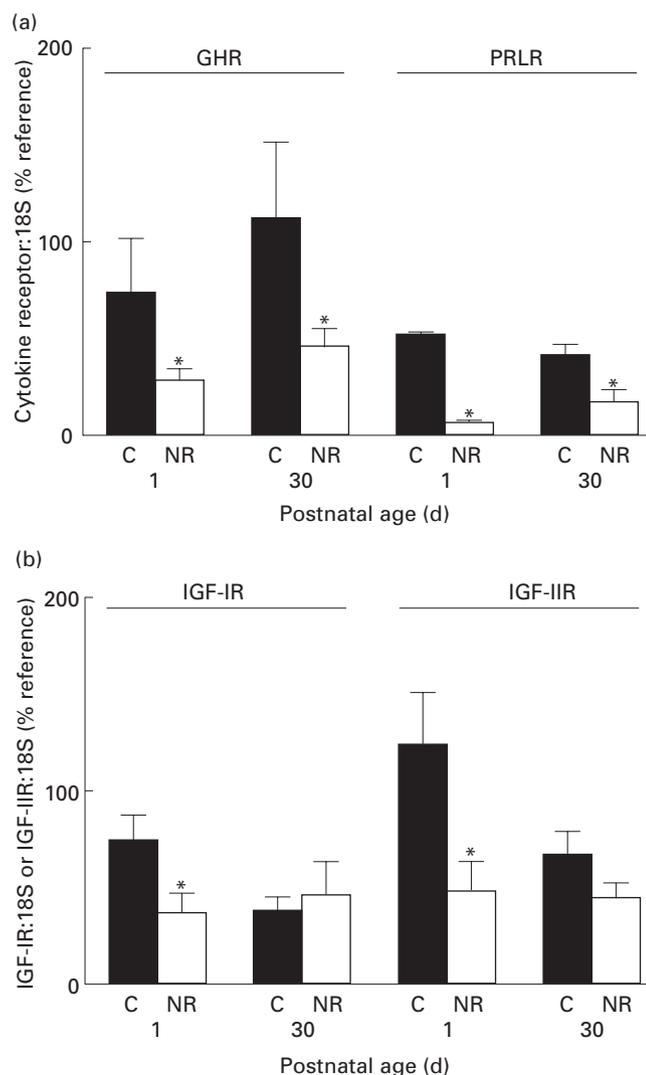
offspring have enhanced hepatic IGF-II levels, which may place the offspring at a higher risk of developing proliferative disorders such as liver cancer.

Fig. 4. Effect of maternal nutrient restriction over the final month of gestation on hepatic (a) cytokine receptors; growth hormone receptor (GHR) and prolactin receptor (PRLR) (b) insulin-like growth factor (IGF)-I receptor (IGF-IR) and IGF-II receptor (IGF-IIR) mRNA abundance in the postnatal lambs. (■), Control (C) 12·7 MJ/d (n 12) (□), nutrient restricted (NR) 9·1 MJ/d (n 16). Results, given as mean receptor:18S (arbitary units) and expressed as a percentage of a reference sample present on all gels, are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the C group: *$P < 0.05$. (Adapted from Hyatt et al. 2002b; Hyatt et al. 2003b.)

mid-gestation remain to be determined. A study currently underway has looked at the effects of maternal undernutrition from 0 to 95 d of gestation on adult offspring sampled at 2 years of age. Preliminary results reveal NR offspring to be smaller at birth, with significantly smaller livers at 2 years of age ($P < 0.05$). At 2 years of age NR offspring have correspondingly lower hepatic GHR and PRLR mRNA expression levels (Hyatt et al. 2004b).

Summary

Maternal undernutrition during gestation acts to reprogramme the fetal HPA axis. Nutritional adaptation occurs regardless of the timing of maternal nutritional manipulation; however, the down-regulation of PRL–GH receptor axis is more prominent when ewes are NR during late gestation, coincident with maximal fetal growth and maturation of the HPA–GH–IGF axis and liver function. However, this finding could be related to the age at which the offspring were sampled, i.e. on day 1 and 30 of neonatal life, when endocrine systems are functioning maximally. Maternal undernutrition did not affect the size of juvenile sheep; however, the observed down-regulation of hepatic cytokine receptors may be important in regulating endocrine adaptations during subsequent periods of nutritional deprivation.

Conclusion

The effects of nutritional manipulation of hepatic PRL–GH–IGF receptors in fetal, neonatal and juvenile NR offspring suggest that the PRL–GH–IGF axis may be important in regulating liver development and function in later life. Further research is needed to determine whether such adaptations are liver-specific or whether they extend to other tissues. Future research that is aimed at further understanding the role of the PRL–GH–IGF axis in liver development and its role in endocrine and metabolic function during subsequent periods of nutritional deprivation after the onset of GH-dependent growth has the potential to illustrate candidate genes involved in the pathogenesis of associated adult diseases, including glucose intolerance, diabetes and cancer.

Acknowledgement

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