Endocrine Regulation of Fetal Growth

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Abstract. Hormones have an important role in the control of fetal growth. They act on both tissue accretion and differentiation and enable a precise and orderly pattern of growth to occur during late gestation. In part, their actions on growth may be mediated by other growth factors such as the insulin-like growth factors (IGFs). Insulin stimulates fetal growth by increasing the mitotic drive and nutrient availability for tissue accretion. It has little effect on tissue differentiation. In contrast, the main effects of cortisol in utero are on tissue differentiation and maturation. Cortisol appears to act directly on the cells to alter gene transcription or post-translational processing of the gene products. Cortisol may also initiate the transition from the fetal to the adult modes of growth regulation by inducing the switch from IGF-II to IGF-I gene expression in the fetal liver. Thyroxine affects both tissue accretion and differentiation in the fetus by a combination of metabolic and non-metabolic mechanisms. Pituitary growth hormone, on the other hand, appears to have little part in the control of fetal growth, unlike its role postnatally. Fetal hormones, therefore, promote growth and development in utero by altering both the metabolism and gene expression of the fetal tissues. These hormonal actions ensure that fetal growth rate is commensurate with the nutrient supply and that prepartum maturation occurs in preparation for extrauterine life.

Extra keywords: hormones, insulin, cortisol thyroid hormones, insulin-like growth factors.

Introduction

Fetal growth involves both the accretion and differentiation of tissue and requires tight coordination between these two processes if development is to proceed normally. Abnormalities in either process or in their coordination will alter the pattern of intrauterine growth and may have adverse effects on neonatal viability as a consequence (see Silver 1992). Poor growth in utero is also associated with a failure to thrive after birth in domestic animals and with an increased risk of cardiovascular and other diseases in later life in man (Barker 1992). The factors affecting tissue accretion and differentiation in utero, therefore, have an important role in determining the subsequent health and life expectancy of the animal.

Postnatally, a wide variety of hormones and growth factors are known to be involved in regulating growth. In particular, the pituitary, adrenal and pancreatic hormones have been shown to affect postnatal growth and their mechanisms of action on tissue accretion and differentiation have been widely investigated (Gluckman 1986; Hill 1989). Much less is known about the endocrine regulation of fetal growth, particularly at a tissue or cellular level. Intrauterine removal of the key hormones known to influence postnatal growth indicates that these hormones are also critical for normal fetal development (Fowden 1989). Ablation of the fetal pancreas, thyroid, pituitary and adrenal have all been shown to affect fetal development, although the extent and precise nature of the abnormalities in growth depend on the particular endocrine deficiency, its severity and duration in utero (Table 1). The aims of this review are, therefore, two-fold: firstly, to consider the effects that individual fetal hormones have on fetal growth during late gestation; and secondly, to discuss how these hormones may affect tissue accretion and differentiation in utero.

Insulin

Insulin has a major role in promoting tissue accretion in the fetus and is required throughout late gestation for normal fetal growth. Its deficiency in utero leads to fetal growth retardation whereas, conversely, excessive insulin secretion can lead to enhanced body weight at term (Fowden 1989). Experimental manipulation of insulin concentrations in the sheep fetus has shown that the fetal growth rate, measured as crown rump length (CRL) increment, is directly related to insulin concentrations in utero (Fig. 1a). Even in the conditions normally observed in utero, fetal bodyweight has been shown to be positively correlated with the insulin concentration in fetal sheep, rats, rabbits, guinea-pigs and human infants (Fowden 1989).

Fetal insulin deficiency has been induced experimentally by surgical ablation of the fetal pancreas and by administration of diabetogenic drugs (Fowden 1987).
A. L. Fowden

Plasma insulin concentration (pU mL\(^{-1}\))
Before and after abdominal surgery (days)

Fig. 1. Relationship between growth and plasma insulin concentrations in the sheep fetus. (a) Mean daily crown rump length (CRL) increment with respect to plasma insulin concentration in individual sheep fetuses (○, sham-operated; ●, pancreatectomized; ■, pancreatectomized and given insulin treatment). (b) Daily increment in CRL with respect to time before and after abdominal surgery in fetuses that were: sham-operated (○, \(n = 5\)); pancreatectomized (●, \(n = 6\)); and pancreatectomized and given insulin treatment to restore normal insulin concentrations (■, \(n = 4\)). Data from Fowden et al. (1989) with permission.

Table 1. Effects of specific endocrine deficiencies on bodyweight and crown rump length (CRL), and individual tissues adversely affected by treatment in sheep fetuses delivered near term (>95% gestation)

<table>
<thead>
<tr>
<th>Endocrine deficiency</th>
<th>Procedure</th>
<th>Gestational age at onset (days)</th>
<th>Bodyweight</th>
<th>CRL</th>
<th>Tissues with specific developmental abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Streptozotocin</td>
<td>70-85</td>
<td>↓ 50%</td>
<td>↓ 20%</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Pancreatectomy</td>
<td>115-120</td>
<td>↓ 30%</td>
<td>↓ 15%</td>
<td>None</td>
</tr>
<tr>
<td>Thyroid hormones</td>
<td>Thyroidectomy</td>
<td>80-96</td>
<td>↓ 30%</td>
<td>↓ 10%</td>
<td>Skeleton, skin, lungs, nervous system</td>
</tr>
<tr>
<td></td>
<td></td>
<td>105-115</td>
<td>↓ 20%</td>
<td>↓ 10%</td>
<td>Skeleton, nervous system</td>
</tr>
<tr>
<td>Adrenal hormones</td>
<td>Adrenalectomy</td>
<td>110-120</td>
<td>↑ 10-15%</td>
<td>No change</td>
<td>Liver, lungs, gut, pituitary(^A)</td>
</tr>
<tr>
<td>Pituitary hormones</td>
<td>Hypophysectomy</td>
<td>70-79</td>
<td>↓ 30%</td>
<td>↓ 8%</td>
<td>Bones, liver, lungs, placenta</td>
</tr>
<tr>
<td></td>
<td></td>
<td>105-110</td>
<td>↓ 20%</td>
<td>↓ 10%</td>
<td>Bones, liver, lungs, placenta, adrenal, gonads</td>
</tr>
<tr>
<td></td>
<td></td>
<td>110-125</td>
<td>No change</td>
<td>No change</td>
<td>Bones, gonads, adrenal, liver</td>
</tr>
<tr>
<td>Pituitary stalk section</td>
<td>108-112</td>
<td>↓ 15%</td>
<td>No change</td>
<td>No change</td>
<td>Adrenals, other tissues?</td>
</tr>
</tbody>
</table>

\(^A\) For description of developmental abnormalities, see Table 2.

Both procedures reduce fetal bodyweight near term by between 10% and 40% depending on the species and gestational age at the onset of hypoinsulinemia (Fowden 1993). In fetal sheep, bodyweight and CRL at term were reduced by 30-40% and 10-20%, respectively, when insulin deficiency was induced in mid-to-late gestation by either pancreatectomy or streptozotocin treatment (Table 1). The daily increment in CRL fell by 40-50% immediately after fetal pancreatectomy and remained uniformly low throughout the remaining 20-30 days of gestation (Fig. 1b). These changes were accompanied by reductions in limb lengths and in the weights of most of the individual fetal organs (Fowden et al. 1986b, 1989). However, when tissue weights were expressed as a percentage of bodyweight, only the spleen and thymus were significantly lighter in pancreatectomized fetuses than in sham-operated fetuses (Fowden et al. 1986b). When insulin replacement treatment was given to the pancreatectomized fetuses, the daily increment in CRL and body size at term were restored to their normal
values (Fig. 1b). Fetal insulin deficiency therefore affects the growth of both bone and soft tissues in the fetus and leads to a symmetrical type of intrauterine growth retardation.

Fetal hyperinsulinaemia, on the other hand, appears to produce a more disproportionate pattern of fetal growth. It enhances bodyweight but not body length in fetal sheep, rats, monkeys and human infants (Fowden 1993). This weight gain is due primarily to increased fat deposition and is greatest in the human which normally has a high fat content at term. Macrosoma is therefore more pronounced in the hyperinsulinaemic infant of the diabetic woman after fetal fat accumulation begins at 28 weeks of gestation (Milner 1988; Fowden 1993). In species such as the pig which have little fat at birth, there is no increase in fetal bodyweight in response to fetal hyperinsulinaemia although the fat content of the fetus rises in these circumstances (Garssen et al. 1983). Since the period of prepartum fat deposition coincides with the proliferation of pancreatic β cells and a rise in the concentration of circulating insulin in the fetal pig (Fowden et al. 1986a), fetal lipogenesis may be determined by insulin even at physiological concentrations in this species. Thus, insulin stimulates not only a general increase in tissue accretion but also a specific accumulation of adipose tissue in the fetus.

Insulin stimulates cell growth primarily by stimulating cell proliferation. In hypoinsulinaemic fetuses, tissue DNA and protein levels are reduced but there is little, if any, change in the tissue protein:DNA ratio (Fowden 1989). Fetal tissues therefore appear to contain a smaller number of relatively normal-sized cells during insulin deficiency. In hyperinsulinaemic fetuses, the changes in tissue DNA and protein:DNA ratio are more variable although tissue weight is generally increased (Fowden 1989, 1993). Insulin, therefore, acts on cell growth mainly by increasing cell number although hyperplasia and hypertrophy can occur simultaneously under certain circumstances.

By contrast, insulin appears to have little effect on the differentiation and prepartum maturation of the fetal tissues. Fetuses with abnormal insulin concentrations deliver normally at term and are viable at birth (Fowden 1987). However, hyperinsulinaemic infants of diabetic mothers are more prone to respiratory distress syndrome after birth which suggests that excessive insulin in utero may adversely affect pulmonary maturation and surfactant production (Milner 1988). Insulin also affects the development of the hepatic glucogenic capacity during late gestation. Fetal hyperinsulinaemia increases the concentration of glycogen and lowers the concentration of gluconeogenic enzyme in fetal liver whereas insulin deficiency has the reverse effect in fetal sheep and monkeys (Fowden 1988; Fowden et al. 1990). However, these actions of insulin are relatively minor compared with those of glucocorticoids (described below).

Insulin promotes fetal growth mainly through its anabolic actions on fetal metabolism. In addition to increasing fat deposition, exogenous infusion of insulin into the fetus increases the uptake, utilization and oxidation of glucose by fetal tissues (see Hay 1991). In fetal sheep glucose utilization and oxidation increased by 50–60% in response to fetal infusion of insulin alone and increased by 100% when extra glucose was supplied exogenously with the insulin. Conversely, glucose utilization and oxidation were reduced by 30–40% when the fetus was made hypoinsulinaemic by pancreatectomy or streptozotocin treatment (Fig. 2; Fowden 1993). Infusion of insulin into these two groups of hypoinsulinaemic fetuses restored the normal rates of glucose metabolism although higher than normal insulin concentrations may be required to achieve this (Fowden 1993). Once insulin has stimulated glucose uptake into the cell, it has no effect on the metabolic fate of the glucose carbons as the distribution of glucose between the oxidative and non-oxidative metabolic pathways is unaffected by the fetal insulin concentration (see Hay 1991). Variations in the insulin concentration will, therefore, produce parallel changes in the availability of glucose carbon for tissue accretion.

On a weight-specific basis, insulin has little effect on the rate of fetal O2 consumption. Small increases (10%) in fetal O2 consumption have been observed in hyperinsulinaemic fetuses but no reduction in umbilical O2 uptake is seen during fetal hypoinsulinaemia (Fig. 2). Since the amount of O2 used to oxidize glucose varies with the fetal insulin concentration, there must be a reciprocal relationship between the use of glucose and other substrates for oxidative metabolism in utero. Amino acids are the most likely alternative fuel. Their concentrations decrease in response to fetal insulin infusion and increase in insulin-deficient fetuses (Fowden 1993). Similarly, the transplacental gradient in urea, the main deamination product of amino acid catabolism, falls during fetal insulin infusion and rises in hypoinsulinaemic sheep fetuses (Fowden 1993). Thus, insulin reduces amino acid catabolism, stimulates glucose utilization and leads to the preferential use of glucose for oxidative metabolism in the fetal tissues. The net result of these changes would be to increase the rate of carbon and nitrogen accumulation in the fetus thereby enhancing the fetal growth rate. Insulin therefore alters the balance between the anabolic and catabolic processes of fetal metabolism in favour of anabolism.

However, this is unlikely to be the sole explanation for the growth-promoting effects of insulin. Insulin could also act on growth by altering the concentrations of other growth-promoting factors such as the insulin-like growth factors (IGFs) which regulate cell division.
Glucose utilization

Glucose oxidation

and differentiation and have anabolic actions similar to insulin (described below). Circulating concentrations of IGF-I are increased during fetal hyperinsulinaemia and are reduced in fetuses made hypoinsulinaemic by pancreatectomy, streptozotocin treatment, diabetes or maternal hypoglycaemia (Fowden 1993). Insulin has also been shown to increase IGF secretion and gene expression in cultured fetal hepatocytes (Townsend et al. 1991a). Insulin is also a potent mitogen in several embryonic cell lines and may directly stimulate cell division early in gestation (Milner and Hill 1985; Hill 1989). Insulin may therefore stimulate cell proliferation in utero either directly or more indirectly through changes in fetal metabolism and IGF production.

Thyroid Hormones

Thyroid hormones have an important role in fetal growth and development. They have been shown to affect both tissue accretion and differentiation in the fetus but their specific actions in utero differ between species and with gestational age. In all species studied so far, intrauterine deficiency of thyroid hormones is associated with developmental abnormalities in certain individual tissues (Browne and Thorburn 1989). In some animals these changes are accompanied by a more general retardation of fetal growth (Thorburn 1974). For instance, in species such as the sheep in which the placenta is impermeable to thyroid hormones, surgical ablation of the fetal thyroid at mid gestation leads to reduced bodyweight and CRL at term and delayed maturation of the skin, skeleton and pulmonary and neuromuscular systems (Table 1).

The reduction in body growth observed after thyroidec- tomy in fetal sheep is not uniform (Table 1). Bodyweight, CRL and limb lengths at term were reduced by 30%, 9% and 25%, respectively, after fetal thyroidec- tomy and were restored to normal values when thyroxine (T₄) replacement treatment was given from the day of thyroid ablation (Hopkins and Thorburn 1972; Mesiano et al. 1987; Fowden and Silver 1995). With the exception of
the fetal lungs which decreased in weight, there were no significant changes in the weight of individual organs after fetal thyroidectomy (Erenberg et al. 1974). Nor was there any apparent alteration in the subcutaneous or total body fat content of the thyroidectomized sheep fetus (Erenberg et al. 1974; Thorburn 1974). The reduced bodyweight of these fetuses is, therefore, due primarily to the decrease in weight of the fetal carcass which is mainly skin, bone and muscle (Erenberg et al. 1974). Hence, in contrast to fetal insulin deficiency, fetal hypothyroidism produces an asymmetrical type of growth retardation in the sheep fetus with a relative reduction in muscle mass and a disproportionate shortening of the long bones.

At a cellular level, hypothyroidism causes growth retardation by hypoplasia and hypotrophy in different fetal tissues. In muscle, thyroidectomy reduces both the protein content and DNA content but has no effect on the protein:DNA ratio which suggests that thyroid hormones stimulate muscle growth by increasing cell number during late gestation (Erenberg et al. 1974). However, in the lung, hypothyroidism causes hypotrophy with reductions in the protein content and protein:DNA ratio (Erenberg et al. 1974; Liggins and Schellenberg 1988). These changes in cell growth are also accompanied by structural and functional alterations in cell maturation. For instance, the lungs of the thyroidectomized fetus have a lower functional residual capacity and surfactant content at term and fail to inflate properly after delivery at term (Liggins and Schellenberg 1988). Similarly, there is retarded development of the ossification centres in the fetal long bones and of several structures in the fetal skin (wool follicles, sweat and sebaceous glands) after thyroidectomy (Browne and Thorburn 1989). In addition, thyroidectomized sheep fetuses show abnormal sympatho-adrenal responses to hypoxia which indicates that the development of the nervous system is also adversely affected by hypothyroidism in utero (Walker and Schuijers 1989).

The specific effects that thyroid hormones have on cell differentiation appear to depend on the stage of development of the fetus. In sheep fetuses, thyroidectomy only prevents wool growth when it is performed before 90 days of gestation (Thorburn 1974). Thereafter, it has little effect on the primary wool follicles although body size and bodyweight are still reduced at term (Table 1). Similarly, in fetal rats, hypothyroidism has little effect on body growth or central nervous system (CNS) development before birth but significantly reduces bodyweight gain and arborization of cerebellar neurones in the immediate neonatal period (Timiras and Nzekwe 1989). Hence, there appear to be critical periods during early life when the thyroid hormones are required for the normal sequence of development.

Thyroid hormones stimulate fetal growth by both metabolic and non-metabolic mechanisms. The main metabolic action of T4 which is important in controlling fetal growth is the stimulation of O2 utilization by the fetal tissues. On a weight-specific basis, umbilical O2 uptake is reduced by 20–30% after fetal thyroidectomy and is restored to normal values when fetal T4 concentrations are maintained after thyroidectomy by exogenous T4 administration (Fig. 2). Conversely, increasing fetal T4 concentrations above normal levels, raised O2 consumption by the sheep fetus (Fig. 3). When the data from all fetuses were combined irrespective of treatment, there was a significant positive correlation between fetal O2 consumption and the plasma T4 concentration in utero in individual sheep fetuses (Fig. 3). T4 is therefore a physiological regulator of O2 consumption by fetal tissues.

![Fig. 3. Relationship between fetal O2 consumption and plasma T4 concentrations in individual sheep fetuses; intact fetuses (●, n = 8); untreated, thyroidectomized fetuses (○, n = 5); T4-treated thyroidectomized fetuses (△, n = 7); untreated hypophysectomized fetuses (□, n = 7); and T4-treated hypophysectomized fetuses (▲, n = 5). y = 0.61x + 209; n = 32; r = 0.842; P < 0.01. Data from Fowden and Silver (1995) with permission.](image)
kg\(^{-1}\) day\(^{-1}\) in the intact fetus. Availability of glucose carbon does not appear to be a limiting factor in the growth of these fetuses as fetal and maternal glucose concentrations and the distribution of glucose between the oxidative and non-oxidative metabolic pathways were not altered by fetal thyroidectomy (Fig. 2; Fowden and Silver 1995). However, normal fetal growth also depends on the availability of nitrogen and other non-glucose carbon sources and little is known about their supply in the thyroideectomized fetus.

Thyroid hormones probably also affect fetal growth by altering tissue production and circulating concentrations of various fetal growth factors and their binding proteins (see Sara and Hall 1990; Han and Fowden 1994). Certainly, the specific developmental abnormalities observed in the skin of the thyroidectomized sheep fetus have been attributed to a reduction in epidermal growth factor (Thorburn et al. 1981). In addition, circulating and tissue concentrations of IGF-I, but not IGF-II, are known to be reduced in sheep and pig fetuses made thyroidectomized by fetal hypophysectomy in mid gestation (Mesiano et al. 1989; Latimer et al. 1993). Replacement of T\(_4\) in these fetuses increased the plasma and tissue IGF-I concentrations although not to normal levels in the sheep fetus (Mesiano et al. 1989). In the fetal pig, these changes in IGF production were accompanied by alterations in the serum concentrations of the major IGF binding proteins (Latimer et al., 1993). Changes in IGF status after fetal thyroidectomy are, therefore, likely to contribute to the growth retardation observed in these circumstances.

In adult animals, virtually all the physiological effects of the thyroid hormones are believed to be due to triiodothyronine (T\(_3\)) and its activation of gene transcription (Brent et al. 1991). Plasma T\(_3\) concentrations are low in the fetus and no correlation was observed between plasma T\(_3\) and O\(_2\) consumption in the sheep fetus during late gestation (Fowden and Silver 1995). Although there might be some local production of T\(_3\) from T\(_4\) in the fetal tissues, these observations indicate that T\(_4\) may be the more effective thyroid hormone in utero and suggest that response elements for T\(_4\) may exist on the fetal genome in mammals as occurs in amphibian embryos.

**Glucocorticoids**

Glucocorticoids are known to inhibit postnatal growth but appear to have a more equivocal role in tissue accretion in utero. They do, however, have major effects on the differentiation and prepartum maturation of many fetal tissues in the period immediately before birth (see Silver 1990). In fetal sheep, cortisol concentrations are low for most of gestation and rise gradually from 10 to 15 days before term with a final rapid increase in the last 3–5 days before birth (Silver and Fowden 1988). The cortisol present in the fetal circulation before concentrations begin to increase is mainly of maternal origin whereas, thereafter, it is derived principally from the fetal adrenal (Wintour et al. 1985). Hence, removal of the fetal adrenal is likely to produce only minor changes in body growth near term compared with the effects of removing other endocrine glands. Certainly, adrenalectomized fetuses delivered 7 or more days before full term are of normal bodyweight whereas those delivered closer to term are 10–15% heavier than intact fetuses of a similar gestational age in which the final cortisol surge has begun (Table 1; Silver 1990 and unpublished observations). Hence, the prepartum rise in the concentration of fetal cortisol may inhibit fetal growth and account for the decline in growth rate that normally occurs in sheep fetuses during the last 10–15 days before birth (Mellor 1983). Premature increases in fetal adrenocortical secretion caused by adverse conditions such as hypoxia or undernutrition may, therefore, contribute to the intrauterine growth retardation observed in these circumstances.

Cortisol also influences the structural and functional development of a wide variety of individual fetal tissues and is essential for the prepartum maturation of organs vital for neonatal survival (Table 2). In the lung, it increases compliance and surfactant release which ensures that spontaneous breathing can occur at birth (Table 2). In the fetal liver, cortisol induces \(\beta\) receptors, glycogen deposition and gluconeogenic enzyme activities thereby helping to maintain a glucose supply to the neonate immediately after birth (Table 2). In the gut, the fetal cortisol surge is responsible for villi proliferation and for the induction of digestive enzymes which enable the neonate to switch to enteral feeding after birth (Table 2). All these maturational changes are prevented when the prepartum increase in cortisol concentration is abolished by fetal adrenalectomy and can be induced prematurely by infusing cortisol into immature fetuses (see Silver 1990, 1992).

In sheep, the prepartum increase in the concentration of fetal cortisol also affects the placenta (Table 1) and, by inducing changes in placental steroidogenesis, triggers the sequence of endocrine and other events that eventually leads to parturition (Liggins and Thorburn 1993). Cortisol therefore links prepartum maturation with the onset of labour which means that the delivery of non-viable young is rare in sheep. In other species, this link is more tenuous and the incidence of prematurity is higher because, although cortisol stimulates the maturation of fetal tissues in all animals studied so far, its effects on the placenta are species-specific and do not always lead to delivery (Silver 1990, 1992).

In postnatal animals, cortisol inhibits growth partially by antagonizing the actions of insulin and partially by down-regulating IGF gene expression. However, relatively little is known about its mechanism of action in utero. In sheep fetuses made hypoinsulinaemic
Table 2. Maturational effects of the surge in the prepartum cortisol concentration on various tissues in the sheep fetus

Data from Barnes et al. 1978; Wintour et al. 1985; Liggins and Schellenberg 1988; Fowden et al. 1990; Silver 1990; Challis et al. 1993; Liggins 1994; Sangild et al. 1995

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>deposition of glycogen; induction of enzymes for gluconeogenesis and fatty acid oxidation; suppression of IGF-II gene expression; induction of β adrenergic receptors; induction of T4 deiodinase enzymes; induction of production of corticosteroid-binding globulin</td>
</tr>
<tr>
<td>Lung</td>
<td>induction of surfactant production and release; structural maturation of alveoli; induction of β adrenergic receptors</td>
</tr>
<tr>
<td>Gut</td>
<td>structural maturation of gastrointestinal tract; induction of digestive enzymes</td>
</tr>
<tr>
<td>Pituitary</td>
<td>induction of switch from fetal to adult corticotrophs; decrease in propiomelanocortin mRNA levels</td>
</tr>
<tr>
<td>Adrenal</td>
<td>induction of phenylethanolamine N-methyl transferase enzyme; cytoarchitectural maturation of zona fasiculata; induction of receptors for adrenocorticotropic hormone; induction of 11β-hydroxylase and P450c17 enzymes</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>induction of switch from fetal to adult haemoglobin synthesis; increase in blood neutrophil:lymphocyte ratio</td>
</tr>
<tr>
<td>Placenta</td>
<td>induction of 17α-hydroxylase and aromatase enzymes; separation of fetal and maternal cotyledonary tissue</td>
</tr>
</tbody>
</table>

by fetal pancreatectomy, there is no decrease in growth rate towards term despite a normal prepartum surge in the cortisol concentration (Fig. 1b). Hence, insulin may be involved, in some way, in mediating the growth-inhibitory effects of cortisol during late gestation. However, it is more likely that cortisol acts directly on gene transcription or post-translational processing to switch fetal tissues from proliferation to differentiation in the prepartum period. Glucocorticoid receptors have been found in a number of fetal tissues and putative binding sites for the activated glucocorticoid receptor have been identified in the promoter sequences of several different genes (Gilmour et al. 1992, 1994; Challis et al. 1993). In part, the effects of cortisol on cell differentiation and maturation may be mediated through changes in tissue IGF production. In sheep, cortisol has been shown to suppress IGF-II mRNA levels and to enhance IGF-I gene expression in the fetal liver at a time in late gestation when major biochemical changes are occurring in the hepatocytes (Li et al. 1993; Gilmour et al. 1994). Similarly, there are reductions in adrenal IGF-II gene expression towards term, which appear to be cortisol dependent, and which closely parallel the normal prepartum changes in cytoarchitecture and steroidogenesis of the zona fasiculata (Table 2; Lü et al. 1994).

Changes in fetal cortisol concentrations during late gestation appear to have little effect on fetal metabolite concentrations or on the rates of glucose and oxygen utilization by the sheep fetus (Barnes et al. 1978; Townsend et al. 1991b). The cortisol-dependent increases in hepatic glycogen and gluconeogenic enzyme activities enhance the gluconeogenic capacity of the fetus during late gestation and may contribute to the endogenous glucose production observed in normal fetuses just before term (Fowden et al. 1991, 1993). Certainly, raising fetal cortisol concentrations to prepartum levels by exogenous infusion stimulates hepatic glucose output in sheep fetuses close to term (Townsend et al. 1991b). But since fetal glucose and oxygen utilization are unaffected by cortisol treatment even in late gestation, changes in fetal metabolism are unlikely to have a significant role in mediating the effects of cortisol on growth and development in the prepartum period.

Pituitary Hormones

Pituitary growth hormone (GH) is essential for normal linear growth in postnatal animals but appears to have little part in the control of growth before birth despite the presence of GH receptors and relatively high GH concentrations in utero (Thorburn et al. 1988; Breier et al. 1992).
Congenital absence of GH in the human infant and specific manipulation of fetal GH concentrations in experimental animals (rat, sheep, monkey, pig) are not associated with major changes in fetal growth (Gluckman 1986; Millner 1988). However, total deficiency of all pituitary hormones can lead to intrauterine growth retardation although the deficits in growth appear to relate primarily to the lack of thyroid-stimulating hormone (TSH) and adrenocorticotrophic hormone (ACTH) and not to GH deficiency *per se* (Table 1). Certainly, GH treatment of the hypophysectomized sheep fetus has little, if any, effect on bodyweight at term (Stevens and Alexander 1986).

The pattern of body growth observed after hypophysectomy of the sheep fetus is asymmetrical and closely resembles that seen in thyroidectomized fetuses with disproportionate shortening of the long bones and a greater reduction in bodyweight than CRL (Table 1). The maturation of individual fetal tissues is also adversely affected by fetal hypophysectomy in a manner similar to that seen in thyroidectomized and adrenalectomized sheep fetuses (Table 1). With the exception of the fetal gonads, most of these abnormalities in tissue development can be corrected by treating the hypophysectomized fetus with T₄, ACTH or cortisol (Barnes et al. 1978; Mesiano et al. 1987; Fowden et al. 1990; Silver 1990). Treatment with T₄ also restores normal bodyweight but not CRL or limb lengths in the hypophysectomized sheep fetus (Table 1). With the exception of the fetal gonads, most of these abnormalities in tissue development can be corrected by treating the hypophysectomized fetus with T₄, ACTH or cortisol (Barnes et al. 1978; Mesiano et al. 1987; Fowden et al. 1990; Silver 1990). Treatment with T₄ also restores normal bodyweight but not CRL or limb lengths in the hypophysectomized sheep fetus at term (Stevens and Alexander 1986; Mesiano et al. 1987; Fowden and Silver 1995). Certain aspects of fetal growth, such as bone elongation, therefore appear to require the presence of more than one pituitary secretion.

Fetal hypophysectomy also has specific effects on fetal body composition. It leads to increased subcutaneous fat deposits which are not found in either thyroidectomized or adrenalectomized fetuses (Hopkins and Thorburn 1972; Barnes et al. 1978; Stevens and Alexander 1986). Similar increases in lipogenesis are observed in fetal pigs after hypophysectomy (Latimer et al. 1993). In fetal sheep, the increase in fat accumulation after hypophysectomy is prevented by GH treatment but not by T₃ or ACTH treatment (Stevens and Alexander 1986) which suggests that GH may be involved in fetal lipid metabolism. But since GH antagonizes the actions of insulin in utero (Parkes and Bassett 1985), its lipolytic effect in the fetus may be indirect and mediated through changes in the effective insulin concentration.

In other respects, the metabolic status of the hypophysectomized fetus is similar to that of the thyroidectomized fetus in the fed state. Fetal O₂ consumption is low after hypophysectomy, even on a weight-specific basis (Fig. 3) and there are no changes in glucose metabolism or its distribution between the oxidative and non-oxidative metabolic pathways in the hypophysectomized sheep fetus (Fig. 2; Fowden and Silver 1995). Replacement of T₄ after hypophysectomy restores the normal rate of umbilical O₂ uptake and increases the rate of fetal glucose oxidation as occurs in T₄-treated thyroidectomized fetuses (Fig. 2). However, in contrast to thyroidectomy, there is no decrease in glucose oxidation in untreated hypophysectomized fetuses (Fig. 2). Since insulin is known to stimulate fetal glucose oxidation (see Hay 1991), these observations suggest that the enhanced action of insulin in the hypophysectomized fetus counterbalances the hypothyroidism and prevents any significant reduction in glucose oxidation after fetal hypophysectomy.

During maternal fasting, fetal hypophysectomy abolishes the normal increase in fetal glucogenesis observed in these circumstances (Fowden and Silver 1992). This is due, in part, to the low concentrations of hepatic glycogen and gluconeogenic enzyme in the hypophysectomized fetus near term but it may also reflect the reduced catecholamine release observed in response to adverse stimuli after fetal hypophysectomy (Barnes et al. 1978; Fowden et al. 1990; Coulter et al. 1993). Lack of pituitary hormones, therefore, affects the metabolic adaptation of the fetus to stressful situations and may thereby alter the availability of nutrients for growth in the compromised fetus. However, in normal nutritional conditions, it is the rate of oxidative metabolism rather than the availability of glucose carbon which is the main metabolic limitation on growth in the hypophysectomized fetus.

Lack of pituitary hormones has no effect on IGF concentrations in fetal rats and rabbits but reduces IGF-I, although not IGF-II, concentrations in fetal sheep and pigs (Thorburn et al. 1988; Mesiano et al. 1989; Latimer et al. 1993). In fetal pigs, there are also reductions in the IGF-I content of the liver, heart and skeletal muscle after hypophysectomy (Latimer et al. 1993). These changes in IGF-I production do not appear to be due to GH deficiency *per se* as T₄ replacement in the hypophysectomized pig fetus completely restored the concentrations of circulating and tissue IGF-I (Latimer et al. 1993). In hypophysectomized sheep fetuses, increasing T₄ concentrations by 50% produced a similar increment in plasma IGF-I concentrations (Mesiano et al. 1989). Although large increases in plasma GH concentrations have been shown to elevate IGF-I concentrations in the sheep fetus (Bauer et al. 1994), the current evidence suggests that thyroid hormones have a more important role than that of GH in regulating fetal IGF-I production (Thorburn et al. 1988). The growth retardation observed after fetal hypophysectomy is therefore probably due, in part, to GH-independent changes in local and circulating IGF concentrations. The only effect that GH appears to have on fetal growth is to alter body fat content (Stevens and Alexander 1986).
Insulin-like Growth Factors

The IGFs have metabolic, mitogenic and differentiative activities all of which play a major role in promoting growth both before and after birth (Sara and Hall 1990). However, IGF production and its control differ in pre- and postnatal animals. In contrast to adult animals, the IGF-II gene is expressed in a wide variety of tissues in the fetus (Gilmour et al. 1992). Tissue abundance of IGF-II mRNA is therefore high in the fetus compared with postnatal values although down-regulation of the gene does occur in certain fetal tissues in the period immediately before birth (Delhanty and Han 1993; Li et al. 1993). Circulating IGF-II concentrations in the fetus mirror tissue mRNA abundance and are 5-6-fold greater than postnatal values in most species. There is also a prepartum decrease in plasma IGF-II concentration in the sheep fetus which closely parallels the decrease in tissue IGF-II abundance observed in the fetal liver and adrenal (Delhanty and Han 1993; Li et al. 1993). By contrast, circulating concentrations of IGF-I and its tissue expression are low in utero but rise rapidly after birth (Sara and Hall 1990). The actions of the IGFs in the fetus may, therefore, be more paracrine than endocrine, although changes in circulating IGF concentrations do occur in response to physiological stimuli and with increasing gestational age (Han and Fowden 1994).

Fetal IGF production is controlled by both hormonal and nutritional factors. Circulating concentrations of IGF-I and IGF-II closely parallel the fetal glucose concentration during manipulations of the maternal nutritional state (Gluckman 1986; Oliver et al. 1993). In sheep, mean fetal IGF-I concentrations are reduced by maternal fasting and are restored to normal values by fetal or maternal glucose infusion but not by fetal amino acid administration (Oliver et al. 1993). In part, these changes in IGF-I concentrations may be due to the concomitant changes in fetal plasma insulin concentrations as insulin can alter IGF-I concentrations independently of the fetal glucose concentration (Fowden 1989). Fetal IGF-I concentrations are therefore directly related to plasma insulin concentrations over the range of concentrations observed in utero (Gluckman et al. 1987). These observations suggest that it is the cellular availability of glucose, which is controlled by insulin, which is the critical factor in regulating fetal IGF-I production. Fetal IGF-II concentrations, on the other hand, are more closely correlated with the concentration of fetal glucose than fetal insulin throughout the various endocrine and nutritional manipulations (Fowden 1989). Changes in fetal cortisol concentration may account, in part, for this relationship as cortisol is known to affect IGF-II gene expression in several fetal tissues and its concentration rises in response to fetal hypoglycaemia during late gestation (Silver 1990; Li et al. 1993; Lü et al. 1994).

Other hormones such as T4 are also known to affect fetal IGF-I production and may also influence fetal IGF-II gene expression in certain species (Mesiano et al. 1989; Latimer et al. 1993). Cortisol and T4 may act by altering IGF gene transcription but the mechanisms whereby nutrients such as glucose regulate the IGF genes remain unknown. But whatever the molecular processes involved, hormonal and nutritional regulation of IGF production in the fetus does not appear to involve GH as occurs in postnatal animals.

The IGFs are anabolic and stimulate fetal growth by both metabolic and non-metabolic mechanisms. Both IGFs increase protein and glycogen synthesis in fetal tissues and cell lines maintained in culture (Sara and Carlsson-Skwirut 1988). They also act as progression factors in the cell cycle and increase DNA synthesis and cell differentiation in a variety of different fetal cells in vitro (Hill et al. 1987). However, the effects and relative importance of the two IGFs in stimulating cell growth in vivo remain unclear. In rodents, IGF-II is essential for cell proliferation. Deletion of IGF-II gene in fetal mice retards fetal growth by 40% whereas IGF-II administration to 10-day-old rat embryos accelerates their growth (Liu et al. 1989; De Chiara et al. 1990). However, in other species (human, sheep, guinea-pig, pig), fetal bodyweight is more closely correlated with the concentration of serum IGF-I than serum IGF-II (Hill et al. 1987; Sara and Hall 1990). In addition, conditions which lead to fetal growth retardation, such as fasting, hypoxia and placental insufficiency, have more pronounced effects on tissue IGF-I mRNA abundance than on IGF-II mRNA abundance, even in the rat (Owens 1991; Han and Fowden 1994). Fetal IGF-I may therefore have a more prominent role than fetal IGF-II in modulating cell proliferation in relation to the specific endocrine and nutritional conditions prevailing in utero. Fetal IGF-II, on the other hand, appears to provide a general stimulus for cell growth in utero and may also be responsible for developmental and tissue-specific changes in cell differentiation. Certainly, in the sheep fetus, changes in the concentration of circulating and tissue IGF-II have only been observed close to term and in extreme cases of growth retardation when changes in tissue differentiation are known to occur in association with a fall in the fetal growth rate (McLellan et al. 1992; Li et al. 1993).

The differential effects of the two IGFs on growth observed between species and during different intrauterine conditions may be due, in part, to changes in the production of the IGF-binding proteins (IGFBPs). At least six different IGFBPs have been identified and their absolute concentrations and relative abundance in utero vary with species and gestational age (Han and Fowden 1994). They are a major determinant of IGF concentrations in the blood and substantially modify the biological
activities of the IGFs (Sara and Carlsson-Skwirut 1988). Their production is regulated by both endocrine and nutritional factors but not necessarily in the same manner as IGF production itself (Owens 1991; Han and Fowden 1994). In hypophysectomized pig fetuses, the fall in plasma and tissue IGF-I concentrations is accompanied by decreased concentrations of IGFBP-1 and IGFBP-2 and increased IGFBP-4 concentrations (Latimer et al. 1993). Although T₄ replacement in these animals restored the normal concentrations of IGF-I, IGFBP-1 and IGFBP-2, it increased IGFBP-4 concentrations further (Latimer et al. 1993). Similarly, in hypoxic sheep fetuses, the reductions in hepatic IGF-I and IGF-II gene expression were accompanied by an increase in IGFBP-1 mRNA levels and a decrease in IGFBP-2 mRNA abundance in the fetal liver (McLellan et al. 1992). The IGFBPs can, therefore, either amplify or inhibit the effects of the fetal IGFs and may provide an important mechanism whereby IGF actions can be controlled in the developing fetus (Han and Fowden 1994).

Other Hormones

There are a number of other hormones which may have effects on specific aspects of fetal growth and development but which are not classical growth hormones or factors. For instance, the GH homologue, placental lactogen (PL), can bind to fetal GH receptors and has been shown to stimulate glycogen synthesis and IGF production in fetal tissues in vitro (Hill 1989; Breier et al. 1994). It may also stimulate fetal growth indirectly by redirecting maternal metabolism in favour of transplacental glucose transport. In addition, hormones which regulate fetal blood flow can affect the supply of nutrients and growth factors to the fetal tissues and may alter the pattern of tissue growth and development as a consequence. Similarly, hormones such as PTH and the Vitamin D derivatives which control the fetal Ca²⁺ balance may affect ossification and remodelling of the fetal bones. Certainly, reducing 1,25-dihydroxycholecalciferol concentrations in fetal sheep by nephrectomy retards bone age at term in a manner similar to that seen in thyroidectomized fetuses (Thorburn 1974).

Interactions between the Hormones and with the IGFs

In normal conditions in vivo, the fetal growth rate and pattern of tissue development will be affected by a number of different hormones acting simultaneously. Multiple hormone changes also occur in response to adverse conditions in utero. During maternal undernutrition, there are decreases in fetal insulin and IGF-I concentrations which are accompanied by increases in plasma cortisol and catecholamine concentrations if the stimulus is prolonged (Fowden 1989). When hypoxia accompanies nutrient restriction, fetal hypothyroidism also occurs (Owens 1991). There may therefore be synergistic and antagonistic interactions between the various hormones and growth factors in the control of fetal growth which would not necessarily be observed when the concentration of a single hormone is manipulated experimentally.

Hormone interactions have been observed in the control of both cell proliferation and cell differentiation in fetal tissues in experiments in vitro and in vivo (Milner and Hill 1985; Hill et al. 1987; Fowden 1989). In the sheep fetus, the growth-promoting action of T₄ on the appendicular skeleton requires the presence of pituitary secretions as T₄ treatment restores normal limb lengths in thyreoectomized and not hypophysectomized fetuses (Fowden and Silver 1995). Similarly, in some tissues, the maturational effects of cortisol depend on other fetal hormones (see Silver 1990). For example, T₄ enhances and insulin antagonizes the actions of cortisol on pulmonary development and surfactant production (Liggins and Schellenberg 1988). Likewise, insulin modulates the effects of cortisol on hepatic glycogen deposition and gluconeogenic enzyme induction in the sheep fetus (Fowden 1988; Fowden et al. 1990). These interactions probably occur at both the metabolic and molecular level and will ensure that the rate and pattern of growth is appropriate for the specific conditions prevailing in utero.

The effects of the classical hormones on fetal growth may be mediated, in part, via the IGFs or their receptors (Fig. 4). Insulin, T₄ and cortisol have all been shown to affect circulating or tissue IGF concentrations and their effects on tissue accretion and differentiation are similar to those of the IGFs (Fig. 4). In addition, the structural homology between the insulin and Type 1 IGF receptors means that insulin may interact directly with the Type 1 receptor and vice versa (Sara and Hall 1990). Experiments in vivo and in vitro have shown that insulin, T₄ and IGF-I all enhance protein synthesis and nitrogen accumulation by fetal and neonatal tissues (see Hill et al. 1987; Sara and Carlsson-Skwirut 1988; Fowden 1989). Since insulin and T₄ enhance fetal IGF-I production, these effects may all be mediated via the Type 1 IGF receptor which preferentially binds IGF-I (Han and Fowden 1994). By contrast, the actions of insulin and IGF-I on fetal glucose metabolism appear to be more distinct. In the sheep fetus, infusion of IGF increases placental in preference to fetal glucose consumption whereas insulin infusion increases fetal but not placental glucose utilization (Hay 1991; Owens 1991). Although IGFs may therefore provide a final common pathway for some hormonal interactions, they do not account for all of the growth regulatory actions of the classical hormones.

Interactions are also likely to occur between the hormones, IGFs and the placenta in the control of
growth and development; known pathways; possible pathway; stimulatory effects; inhibitory effects. GH, growth hormone; ACTH, adrenocorticotrophic hormone; PGE, prostaglandin E; IGFs, insulin-like growth factors.

fetal growth. Fetal IGF-I administration increases placental glucose extraction and alters the distribution of glucose between the fetal and placental compartments in fetal sheep and guinea-pigs (see Owens 1991). Analysis of placental glucose transfer with respect to the transplacental glucose concentration gradient in pancreatectomized fetuses suggests that the glucose transport capacity of the ovine placenta is also reduced by chronic fetal hypoinsulinaemia even though the cotyledons weigh more than normal in about 20% of these animals (Fowden et al. 1986b and Fowden, unpublished observations). In addition, there are changes in the structure and endocrine function of the ovine placenta in adrenalectomized and hypophysectomized fetuses (Stevens et al. 1978; Deayton et al. 1993) which are consistent with the alterations in fetal bodyweight observed in these circumstances (Table 1). Hormones therefore interact at the level of the placenta and may exert some of their effects on fetal growth by altering placental function.

Conclusions

Fetal hormones are essential for normal fetal growth and development. They affect both tissue accretion and differentiation and, with the IGFs, coordinate a precise and orderly increase in growth throughout late gestation. The hormones act through both metabolic and non-metabolic mechanisms to ensure that the growth rate is commensurate with the nutrient supply. At a cellular level, the hormonal effects on growth may be mediated, in part, by IGFs or other growth factors but the extent to which specific hormones act alone or through local IGF or IGFBP production remains to be determined.

Tissue accretion and differentiation appear to be regulated by different fetal hormones (Fig. 4). Insulin primarily stimulates tissue accretion and has little effect on tissue differentiation whereas cortisol induces tissue differentiation but has only minor inhibitory effects on tissue accretion (Fig. 4). Insulin also has major metabolic effects in utero whereas cortisol does not. Tissue accretion therefore appears to be closely related to fetal metabolism and to be dependent on an adequate nutrient supply to the tissues (Fig. 4). Tissue differentiation, on the other hand, appears to be controlled by hormones that can enter the cells and alter gene transcription and/or protein processing (Fig. 4). Hence, hormones such as T4 which affect both tissue accretion and differentiation, may stimulate cell proliferation through their metabolic actions and activate tissue differentiation by changing gene expression for the IGFs or other growth factors.

Since insulin and T4 are regulated by nutrition, they act as signals of nutrient availability and match fetal nutrient utilization to its umbilical supply. They are therefore required throughout late gestation to ensure appropriate growth in both normal and adverse nutritional circumstances. T4 is also required at specific times during development to initiate the differentiation of particular fetal tissues. In contrast, cortisol appears to modulate growth only during late gestation and in adverse conditions when the exogenous nutrient supply is restricted. In these circumstances, it induces key maturational processes which ensures that the fetus survives in utero and is ready for extraterine life should delivery occur. It may also contribute to the reduced rate of tissue accretion observed in these circumstances.

The role of cortisol as a maturational signal has important implications for the somatotrophic axis and the regulation of growth in the perinatal period. By inducing the switch from IGF-II to IGF-I gene expression in the fetal liver (and possibly in other tissues), cortisol may initiate the transition from the fetal mode (GH-independent IGF-I and IGF-II) to the adult mode (GH-dependent IGF-I) of growth regulation. Further studies are required to identify which specific processes in the switch from local to endocrine IGF production are cortisol dependent but there are increases in hepatic GH receptor mRNA abundance towards term which closely parallel the increase in the prepartum cortisol concentration observed in the sheep fetus (Adams et al. 1990). It is also the GH-sensitive transcript of the hepatic IGF-I gene which is increased by cortisol treatment in the sheep fetus (Gilmore et al. 1994). Any abnormalities in the magnitude or timing of the surge in the fetal cortisol concentration may have inappropriate effects on cell growth for the stage of development and may trigger changes in tissue accretion
and differentiation which may have long-term adverse sequelae.

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