Cytokines and Cytokine Receptors in Fetal Growth and Development

Abstract
The cytokine receptors for growth hormone (GH), prolactin and leptin have a critical role in regulating embryo, placental and/or fetal development, which is dependent on stage of gestation and species. GH and prolactin receptors are detectable from conception, and alterations in the maternal hormonal environment may impact on placental growth from this early stage of gestation. Leptin is critical for conception, but its role in fetal growth remains elusive. During late gestation, when fetal growth accelerates and organ maturation occurs, prolactin and insulin-like growth factor-I may have interactive roles in regulating the growth of specific tissues, including adipose tissue. Prolactin, leptin and GH all have specific effects on fetal and neonatal energy balance, which are mediated in part through promoting lipolysis and/or enhancing the expression of uncoupling proteins. An increased understanding of these interactions is likely to have important implications for a number of potentially pathological conditions, including infection, obesity and hypertension.

Introduction
The impact of alterations in the maternal, placental and fetal metabolic environment with respect to a range of cytokine hormones and members of the class 1 cytokine-receptor superfamily are only now being fully recognized. This is due to the development of appropriate molecular tools that are able to describe receptor abundance, in conjunction with relevant mouse-knockout and large-animal nutritional models. In the present review, emphasis will be placed on the functional role of growth hormone (GH), prolactin and leptin, as well as the interactions between these hormones from the time of conception through to adulthood. Each hormone has a primary role in the regulation of both energy balance and tissue development. Perturbations in nutrient supply in conjunction with reduced cytokine receptor abundance can therefore have a profound effect on fetal development, which may determine survival after birth and subsequent morbidity and mortality.

Conception
GH is widely recognized to regulate postnatal growth, but there is an increasing amount of evidence demonstrating that GH can enhance embryonic growth. Studies in mice have shown GH and prolactin receptors to be present in mouse zygotes, cleaving embryos and blastocysts [1,2]. Although both receptor types are present in the inner cell mass and trophectoderm of blastocysts, receptor abundance is much greater in the trophectoderm, indicating a specific role in early placental development. This contrasts with insulin and insulin-like growth factor (IGF)-I, which only have an anabolic effect on the inner cell mass, an effect proposed to be mediated by inhibition of apoptosis [3].
In vitro studies have shown that both GH and prolactin can have an anabolic effect, producing blastocysts with increased cell numbers [2]. It is therefore likely that alterations in maternal GH and/or prolactin secretion will significantly impact on placental growth. Prolactin receptor deficiency is also associated with reproductive failure [4]. This can be partially overcome by treatment with progesterone, although the number of pups surviving decreases from mid pregnancy and those surviving to term is 3-fold lower than for wild-type controls. Whether this response may be mediated through impaired function of the placenta, in which prolactin receptors are highly abundant, is unknown.

The extent to which leptin modulates placental or fetal growth remains a matter of debate. Leptin may only have an indirect effect on reproduction, acting through enhancement of GH secretion [5]. Leptin is critical for the onset of reproductive activity, but not for implantation or subsequent maintenance of pregnancy, in ob/ob mice at least [6]. Direct effects on placental or fetal growth have yet to be documented, despite the finding that leptin is present in the placenta and is influenced by nutritional state [7].

Placental development and subsequent adult health

It is not only gross changes in placental size that contribute to a large or small fetus. Longitudinal studies from the Dutch famine have shown that, when maternal energy intake was reduced to close to 50% of pre- or post-famine levels, marked changes in placental and fetal weight ensued [8]. Surprisingly, if mothers were only subjected to the famine in early gestation, at term they had a larger placenta and longer infants compared with mothers who were nutrient-restricted in mid or late gestation [9,10]. As adults, these infants have been shown to exhibit a more atherogenic lipid profile, to be more obese and to be at increased risk of coronary heart disease. It is likely that the apparent increase in placental growth is programmed by the altered maternal endocrine environment in early gestation. GH secretion has been established to increase in response to under-nutrition [11]. It is hypothesized that increased exposure of the developing embryo to GH could have a direct effect on placental growth, as indicated by the mouse studies described above. Although GH does not appear to have a direct role in fetal growth, when its secretion is markedly enhanced and adult-type GH receptors are not present [12] GH may be able to influence subsequent fetal growth by initial effects on placentation.

Cytokines and fetal growth

A large number of clinical studies have produced a range of relationships between plasma leptin concentration and the growth parameters fetal weight and size and placental weight (see [13]). Although in human fetuses the plasma leptin concentration increases with gestational age, there is a substantial increase, both within and between studies, in the range of values obtained as term approaches [14,15]. The reason for this remains in doubt, but it may be partly explained by the relative insensitivity of some of the commercially available assay kits that have been used. There is also an appreciable decrease in plasma leptin concentration within the first few hours of birth, which may commence in utero, thereby contributing to the wide variations in published results. Furthermore, as the rise in plasma leptin secretion with gestation may be of fetal (i.e. adipose tissue) rather than placental origin, differences in adipose tissue weight at birth may contribute to differences between groups [16]. It is likely, however, that a close relationship exists between birth weight and plasma leptin concentration, since in sheep (a species which has very limited adipose tissue stores at birth) a strong correlation between plasma leptin and birth weight was shown when samples were taken hourly over the first 6 h of neonatal life ($r^2 = 0.94$ [17]).

Prolactin has been shown to be present in the fetal circulation in several species, but it does not cross the placenta from ewe to fetus [18,19]. In the fetal sheep, prolactin synthesis and secretion are both highly regulated prior to birth [20]. The role of prolactin in the growth and development of the fetus, however, remains poorly understood. Prolactin mRNA levels in the fetal pituitary gland and the plasma prolactin concentration are maintained by a tonic stimulatory drive from the hypothalamus, and rise gradually during late gestation [21]. Prolactin receptors are present in a large number of fetal tissues in all species studied to date. For example, mRNAs for both the long and short forms of the prolactin receptor are present in the fetal sheep liver, and a gestational-age-related increase in the level of receptor mRNA occurs in parallel with the increase in plasma prolactin concentrations [22]. The stimulus for this increase
Cytokines and Cytokine Receptors in Fetal Growth and Development

does not appear to be mediated directly by cortisol, but may be an indirect response acting via an increase in IGF-I secretion [23]. Support for this proposal comes from the finding that, in fish, which like the fetus exhibit a low endogenous rate of prolactin release, IGF-I has a marked stimulatory effect on prolactin secretion [24]. The extent to which prolactin may be acting directly or via placental lactogen receptors remains an area of debate. It should be noted, however, that immunoneutralization against placental lactogen has no detectable effects on maternal or fetal metabolism [25]. Moreover, in vivo effects on bone metabolism of 50 ng/ml circulating prolactin have been demonstrated in developing rats in the face of μg/ml quantities of placental lactogen [26].

It is not only prolactin that can have biological effects, as phosphorylation of prolactin has been shown to be physiologically important at different stages of the reproductive cycle [27]. When prolactin promotes cell proliferation, phosphorylated prolactin antagonizes this effect. In contrast, when the function of prolactin is not proliferative, phosphorylated prolactin may be an equal or greater receptor agonist than prolactin. Studies in pregnant rats have demonstrated profound and differential effects of maternal administration with prolactin or phosphorylated prolactin on a large range of fetal tissues, confirming that it is not the absolute level of prolactin that is important, but the ratio of unmodified to phosphorylated prolactin [28]. For example, fetal thymus size was decreased by maternal prolactin administration, but increased by phosphorylated prolactin. The extent to which these effects may contribute to clinically significant effects in response to low plasma prolactin concentrations, including prematurity, remains to be explored.

**Prolactin and fetal energy balance**
Prolactin has also been suggested to regulate energy balance. The demonstration of high expression of uncoupling protein 2 (UCP2) throughout the non-human primate adult brain, including the hypothalamus and pituitary, could be linked to prolactin secretion [29]. In the anterior pituitary, cell expression of UCP2 is widespread, although it is not co-localized with GH-producing cells. It is therefore hypothesized that UCP2 is co-expressed by prolactin-secreting cells, thereby providing a direct link between prolactin and brain metabolism. In the late-gestation fetus, a central indicator of nutrient supply is the abundance of neuropeptide Y [30]. In the adult, neuropeptide Y is a potent appetite stimulant. Interestingly, the peak in prolactin mRNA abundance in the fetal anterior pituitary precedes the peak in hypothalamic neuropeptide Y (Figure 1). Maternal nutrient restriction has been shown to result in a precocious peak in the fetal plasma prolactin concentration [31]. This may be a critical stimulus for the higher levels of fetal hypothalamic neuropeptide Y mRNA observed following maternal undernutrition, thereby leading to parturition.

**UCPs and energy balance**
Prolactin, leptin and GH have been shown to have direct or indirect effects on fetal and neonatal energy balance, as summarized in Table 1. These include effects on intermediary metabolism and on UCP abundance. Not only the amount of UCP1, but also that of voltage-dependent anion channel (VDAC), peaks at birth [32] (Figure 2). Importantly our antibody does not appear to be cross-reacting with UCP1, and the loss of VDAC in parallel with UCP1 may be indicative of pronounced apoptosis [33] within brown adipocytes. We are unable to detect UCP1 in adipose tissue at 30 days of postnatal age, whereas VDAC is clearly detectable. The role of VDAC in the both the young and adult mammal is the subject of speculation. It should be further noted that lambs, sheep and humans are all able to maintain normo-
Table I

Summary of the effects of prolactin, GH and leptin on UCP1 actions during fetal and neonatal development

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Stage of development</th>
<th>Relationship with UCP1 actions</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prolactin</td>
<td>Late gestation</td>
<td>Increase in prolactin receptor mRNA coincides with initial appearance of UCP1</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td>Neonatal</td>
<td>Prolactin can promote a thermogenic effect up to 2 days after birth</td>
<td>[40]</td>
</tr>
<tr>
<td>GH</td>
<td>Birth</td>
<td>Rapid decline in plasma GH at the time of birth may be one signal for the initiation of lipolysis</td>
<td>[41]</td>
</tr>
<tr>
<td>Leptin</td>
<td>Late gestation</td>
<td>Increase in mRNA abundance in perirenal adipose tissue in late gestation coincident with the rise in UCP1 abundance</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>Neonatal</td>
<td>Leptin promotes loss of UCP1</td>
<td>[13]</td>
</tr>
</tbody>
</table>

The extent to which the hormonal regulation of each UCP differs remains to be established. Interestingly, VDAC is detectable in adipose tissue at 1 month of age, when UCP1 is no longer present. This coincides with the large increase in fat mass, and may represent a transition in mitochondrial function from heat generation to regulation of the production of reactive oxygen species [35]. Rapid loss of UCPs in a variety of tissues may have a beneficial role, by enabling the postnatal mammal to cope effectively with infection by allowing effective production of reactive oxygen species. The role of UCP3, which is present in both adipose tissue and skeletal muscle, also remains to be established; overexpression of UCP3 in skeletal muscle is associated with growth retardation, despite an increase in food intake [37]. The extent to which this is a direct response to excess UCP3 abundance in skeletal muscle, as opposed to alterations in fetal development, possibly associated with impaired mitochondrial development, has not been addressed.

Conclusions

In conclusion, GH, prolactin and leptin all have a critical and potentially interactive role in regulating placental and fetal development. Plasma concentrations of each may be significantly compromised throughout pregnancy, thereby contributing to altered placental function and/or fetal growth and development. Each hormone may be critical in the regulation of fetal energy balance mediated through changes in UCP abundance. An increased understanding of these interactions is likely to have important implications for a number of potentially pathological conditions, including infection, obesity and hypertension.
References


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