Abstract

During late gestation, the maturation of fetal adipose tissue is geared towards the synthesis of high levels of uncoupling protein 1 (UCP1), which is unique to brown adipose tissue. At birth, rapid activation of UCP1 ensures a large increase in heat production. These adaptations are nutritionally sensitive, and may be mediated in part by rapid changes in prolactin and leptin secretion after birth. Restriction of maternal nutrition reduces adipose tissue deposition, with no effect on UCP1. Increased maternal food intake results in increases in levels of UCP1 and the short form of the prolactin receptor, but in a decrease in adipose tissue content per kg of fetus. The ontology of the 

Key words: adipose tissue, fetus, leptin, prolactin receptor, uncoupling protein.

Abbreviations used: BAT, brown adipose tissue; UCP, uncoupling protein.

Introduction

Adipose tissue development commences in utero, with the adipocyte lineage being derived from stem cell precursors which have the potential to become brown adipose tissue (BAT) or white...
adipose tissue [1]. Both forms of adipose tissue have critical functions, which are dependent on the stage of the life cycle. BAT can rapidly generate large amounts of heat [2], whereas white adipose tissue represents an endogenous energy store that secretes leptin, a cytokine hormone with a range of biological functions, including appetite regulation [3]. The ability of BAT to generate heat is due to the expression of a unique uncoupling protein (UCP), UCP1, on the mitochondrial inner membrane [2]. Activation of UCP1 results in proton flow across mitochondria without the need to produce ATP. Thereby, all chemical energy liberated can be used directly for heat production. It is now apparent that another UCP, UCP3, which is present in both adipose tissue and skeletal muscle and shares 57% identity with UCP1 [4], has the potential to influence metabolic rate [5], although its relevance to thermoregulatory adaptation after birth is unknown. In contrast, UCP2 has been detected in a large range of tissues, including white adipose tissue and liver [6]. The extent to which mRNA abundance or protein expression for UCP2 or UCP3 are regulated in utero remains to be determined.

In precocial species, including human infants and lambs, born with a mature hypothalamic-pituitary axis at birth, the rapid initiation of non-shivering thermogenesis is vital in enabling the newborn to maintain body temperature following cold exposure in the extra-uterine environment [7]. There is rapid loss of UCP1 mRNA soon after birth [8], which is followed by a gradual loss of UCP1 protein [9]. Subsequently, there is an appreciable deposition of white adipose tissue. Rat pups are, however, born immature and are altricial, i.e. rely on huddling with littersmates in a nest to maintain body temperature. mRNAs for UCP1, UCP2 and UCP3 all increase post-natally [10,11], in conjunction with maturation of the hypothalamic-pituitary axis. The ability to thermoregulate independently at birth is, therefore, more critical in lambs and human infants. In these species, this ability can be compromised by a range of factors, including premature birth, caesarean section delivery and maternal under-nutrition [12,13]. An increased understanding of the precise role of specific cytokine receptors, particularly the prolactin and leptin receptors (both members of the class 1 cytokine receptor superfamily), in fetal and neonatal maturation may allow the development of strategies that lead to decreases in morbidity and mortality during post-natal and later life.

**Figure 1**

Ontogeny of the fetal hormonal environment with respect to primary indices of fetal adipose tissue development during late gestation

Ontogeny of fetal adipose tissue development

The fetus has a low metabolic rate, which ensures that available energy can be partitioned towards growth rather than energy expenditure. The growth and development of adipose tissue in late gestation is primarily in preparation for life after birth. During this period, the plasma concentrations of prolactin and leptin increase up to term [14,15] (Figure 1), in parallel with an increase in leptin mRNA abundance [16] in perirenal adipose tissue, which is the most abundant adipose tissue store in the fetal lamb. In the adequately nourished ovine fetus, adipose tissue deposition continues up to term, in conjunction with a parallel increase in the thermogenic potential of UCP1 [8,16]. Both UCP1 mRNA and protein peak within a few hours of birth [8,17], after which UCP1 mRNA disappears rapidly, in conjunction with gradual loss of UCP1 protein [9], which has a half-life of 7 days. The extent to which leptin may have a direct influence on fetal growth or adiposity remains to be established. Indirect evidence for such a role includes the finding that, in the late-gestation sheep fetus, the abundance of leptin mRNA in perirenal adipose tissue is positively correlated with fetal weight [16]. In addition, the plasma leptin concentration in cord blood at birth is positively correlated with the degree of adiposity [18].

The primary hormones necessary for maximizing UCP1 expression are noradrenaline and thyroid hormones [19–21], but those regulating the initial expression of UCP1 as well as adipose tissue deposition over the final few weeks of gestation remain to be fully determined (Figure 2). We have indirect evidence that prolactin has a role in fetal BAT development. In the ovine fetus there are marked increases in mRNA abundance for both the long and short forms of the prolactin receptor between 90 and 125 days of gestation [22]. The stage of gestation at which prolactin receptor mRNA in fetal adipose tissue peaks coincides with the developmental age at which UCP1 is first detected [17] and, as discussed below, alterations in fetal plasma prolactin concentration, prolactin receptor abundance and fetal adipose tissue deposition are closely associated.

Nutritional manipulation of fetal adipose tissue development and prolactin receptor abundance

Increasing the amount of feed provided to the mother during the second half of gestation enhances both fetal weight and BAT maturation (i.e. more UCP1 with a higher thermogenic activity), as measured near to term [23]. This is associated with a greater abundance of each isoform of the long form of the prolactin receptor in perirenal adipose tissue and higher plasma prolactin concentrations in cord blood (well fed, 49 ± 3 ng/ml (n = 7); control, 41 ± 1 ng/ml (n = 6); P = 0.02). No effect of maternal nutrition has been observed on the abundance of the short form of the prolactin...
receptor in perirenal adipose tissue, or for either form of the prolactin receptor in hepatic tissue, which does not possess UCPL. Interestingly, we were unable to detect the 15 kDa isoform of the long form of the prolactin receptor in hepatic tissue, which may be indicative of an adipose-tissue-specific role. With the finding of a low-molecular-mass isoform of the long form of the prolactin receptor has come the proposition that it represents a separate extracellular domain of the receptor [24], the formation of which may be dependent on receptor glycosylation status [25]. These 15 kDa isoforms of the long form of the prolactin receptor could then form homodimers with each other or heterodimers with full-length receptor proteins, thereby regulating receptor function.

Growth-restricted fetuses may be studied by use of the carunclectomized sheep model, which involves the removal of nearly all visible endometrial caruncles in non-pregnant sheep. Subsequent pregnancy normally produces a hypoxic and hypoglycaemic fetus, with a small placenta [26]. The weights of the majority of fetal organs, including perirenal adipose tissue, are decreased in proportion to body weight [22]. In these fetuses, plasma prolactin concentrations are chronically depressed and the level of mRNA for the long form, but not the short form, of the prolactin receptor is reduced in perirenal adipose tissue, suggesting that only the long form of the receptor is sensitive to low plasma prolactin concentrations [22]. Interestingly, the effect of compromised nutrient supply on the relationship between prolactin receptor abundance and tissue development may be specific to adipose tissue, as carunclectomy has no effect on mRNA abundance for either form of the prolactin receptor in fetal liver or kidney near to term [27].

The extent to which either enhanced or compromised fetal adipose tissue development may subsequently alter postnatal adipose tissue development has not been studied directly. It should be noted, however, that an increasing number of epidemiological studies are demonstrating a link between nutrition during pregnancy, size at birth and subsequent predisposition to adult obesity [28]. The metabolic environment in which adipose tissue deposition is initiated, during fetal life, may determine adipose tissue function throughout the life cycle. This is particularly important, as it is now recognized that adipose tissue has the potential to influence an increasingly wide range of biological functions, including reproduction, due to its ability to secrete leptin [3].

**Postnatal ontogeny of prolactin receptor abundance in active metabolic tissues**

Prolactin receptors are not only highly abundant in adipose and hepatic tissue [29], but are also present in skeletal muscle [30]. These tissues all contribute to the rapid increase in metabolic rate that occurs immediately after birth, coincident with a peak in abundance of both forms of the prolactin receptor. A marked divergence in ontogeny between tissues then occurs postnatally (Figure 2). Within adipose tissue, a rapid loss of the short form of the prolactin receptor occurs over the first month of life, coincident with the loss of UCPL [31], whereas the long form of the prolactin receptor remains abundant although at a reduced concentration [32], presumably in white adipocytes. The relative conservation of the long form of the prolactin receptor has the following functional consequences. (1) It may be important in explaining why prolactin is able to stimulate leptin secretion, as demonstrated in adult rats [33]. In both liver and skeletal muscle, however, prolactin receptor abundance remains relatively constant after birth. (2) This may indicate completely different functions for the prolactin receptor and/or prolactin in these tissues. It is possible that prolactin, acting through its receptor, may regulate the expression of UCPL in the liver and UCP3 in skeletal muscle. (3) The loss of prolactin receptors in conjunction with a parallel loss of UCPL could explain why suppression of prolactin secretion by the use of melatonin in 3-week-old lambs, which no longer possess BAT, has no detectable effect on colonic temperature [34].

**Postnatal manipulation of prolactin and leptin status**

There is very little information relating prolactin status after birth with postnatal development. We have shown recently that once-daily administration of prolactin to neonatal lambs delays the rate of decline in colonic temperature over the first 2 days after birth [35] (Figure 3, upper panel). This response is observed in the absence of any visible signs of shivering and is, therefore, indicative of enhanced heat production by the BAT. This effect is not, however, maintained, and is not associated with any chronic changes in prolactin receptor abundance in adipose tissue. By the end
of a 6-day period of prolactin treatment, sufficient to increase plasma prolactin concentrations 5-fold (Figure 3, lower panel), the abundance of the short form of the prolactin receptor is increased in the liver, but not other tissues. Again, this is indicative of tissue-specific effects of prolactin that may be different with respect to developmental age. The extent to which stimulation of both prolactin and leptin receptors may further alter adipose tissue function and development is not known. During late gestation, a much greater increase occurs in fetal plasma prolactin than in leptin (Figure 1). Furthermore, leptin appears to have a markedly different effect on postnatal adipose tissue from that of prolactin.

Although plasma leptin concentrations have been shown to decrease very soon after birth [18], the role of leptin in regulating energy balance in the newborn period remains a subject of debate. Studies in rats to which very high doses of exogenous leptin have been administered reflect pharmacological effects. In contrast, when a dose of leptin is used to produce a physiological increase in plasma leptin [36], surprising effects on energy balance have been observed. Chronic administration to neonatal lambs has been shown to result in accelerated loss of UCP1, in the absence of any detrimental effects on temperature control or growth rate [37]. Leptin may, therefore, act to promote the loss of UCP1 at a time when the neonate is able to maintain body temperature through dietary-induced thermogenesis and/or increased recruitment of other UCPs. At the same time, this is associated with increased deposition of white adipose tissue both around the central body organs and subcutaneously, thereby increasing insulation and the surface area to body weight ratio [9]. These adaptations occur in conjunction with behavioural adaptations, including sleeping close to the mother and having ad libitum access to maternal milk production, thereby maximizing the lamb’s or infant’s growth potential.

Future perspectives

The ability to understand the precise roles of prolactin and leptin in adipose tissue development will require the complementary use of animal models with a mature hypothalamic–pituitary axis at birth (i.e. lambs and infants) together with those in which maturation occurs well after birth (i.e. mice and rats). It is likely that such information will contribute substantially to our understanding of how fetal development subsequently programmes postnatal, juvenile and adult development. This is particularly important when considering the developmental control of energy balance. The use of gene knockouts which avoid the deleterious effects on reproduction described in prolactin receptor knockout mice may allow some progress [38]. Results from such studies must be interpreted with caution, however. It has been shown, for example, that mice in which neuropeptide Y has been knocked show no discernable changes in food intake [39]. In contrast, direct manipulation of neuropeptide Y gene expression in growing rats has shown significant and physiologically important effects on food intake and energy balance [40]. In order to gain a better insight into many of the current medical and basic science problems pertaining to premature birth, metabolic complications in the newborn and programming of adult disease, large-animal models, which have proven to be so beneficial in the past, should continue to be used. This has the benefit of improving our understanding of complementary
biological control mechanisms in the whole animal in a more physiological context than the complete knockout of the gene in a small laboratory animal.

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References


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