Regulation of leptin synthesis and secretion before birth: implications for the early programming of adult obesity

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Abstract

A series of epidemiological, clinical and experimental studies have shown that there are associations between the fetal and neonatal nutritional environment and the amount and distribution of adipose tissue in adult life. This review considers the evidence for these relationships and discusses the potential impact of the prenatal nutritional experience on the development of the endocrine and neuroendocrine systems that regulate energy balance, with a particular emphasis on the role of the adipocyte-derived hormone, leptin. In the rodent, leptin derived from the mother may exert an important influence on the development of the appetite regulatory neural network and on the subsequent regulation of leptin synthesis and the risk for obesity in the offspring. In species such as the human and sheep, there is also recent evidence that the synthesis and secretion of adipocyte-derived hormones, such as leptin, are regulated in fetal life. Furthermore, the hypothalamic neuropeptides that regulate energy intake and expenditure in adult life are also present within the fetal brain and may be regulated by the prevailing level of maternal and hence fetal nutrient and hormonal signals, including leptin. This work is important in determining those initiating mechanisms within the 'fat-brain' axis in early life that precede the development of adult obesity.

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Introduction

Currently more than half of all adults in the United States and the United Kingdom are either overweight (i.e. have a body mass index (BMI) over 25 kg/m²) or obese (i.e. have a BMI over 30 kg/m²) (James 1996, Campfield *et al.* 1998, Flegal *et al.* 2002, Ogden *et al.* 2002). Adult obesity is associated with the co-morbidities of type 2 diabetes, high blood pressure and ischaemic heart disease, and consequently incurs significant health-care costs (James 1996). Abdominal or visceral obesity in particular is associated with the metabolic or 'insulin-resistance' syndrome, i.e. the presence of insulin resistance (with or without glucose intolerance), raised blood pressure, atherogenic dyslipidaemia, and prothrombotic and proinflammatory states (Reaven 1988). The difficulty of effective intervention in obesity is well established and this difficulty relates in part to the complexities of an energy balance regulating system that has evolved features reflecting the requirements, present for most of human history, to protect and maintain energy stores in order to survive long periods of famine, rather than feasting.

In this context, it is of interest that epidemiological, clinical and experimental studies have shown that there are associations between the early nutritional environment,

patterns of postnatal growth and the amount and distribution of adipose tissue in adult life (Fall *et al.* 1995b, Sorensen *et al.* 1997, Loos *et al.* 2001b, Yajnik 2003). This review considers the evidence for these relationships and discusses the potential impact of the prenatal nutritional experience on the development of the endocrine and neuroendocrine systems that regulate energy balance, with a particular emphasis on the role of the adipocyte-derived hormone, leptin.

Birth weight and adult obesity

The relationship between birth weight and fatness, measured in childhood or adulthood, is generally positive, although a number of studies have reported that there is a J-shaped or U-shaped relationship between birth weight and adult fat mass, with a higher prevalence of obesity occurring at both low and high birth weights (Maffeis *et al.* 1994, Curhan *et al.* 1996a, 1996b, Parsons *et al.* 2001). A study in a large British cohort found a weak but positive relationship between birth weight and BMI at 33 years of age and determined that this relationship was largely accounted for by maternal weight, i.e. heavier mothers had heavier babies and these babies went on to have a

high BMI in adult life (Parsons *et al.* 2001). It has been suggested that the influence of maternal weight on the relationship between birth weight and subsequent BMI may operate through an impact of a high maternal and hence fetal nutrient supply. In diabetic or glucose-intolerant mothers, maternal and fetal blood glucose levels are higher and this results in offspring who are heavier at birth and who are at risk of developing obesity and glucose intolerance in later life (Silverman *et al.* 1991, Dorner & Plagemann 1994). Thus an increase in fetal nutrient supply, as a consequence of an increase in maternal energy intake or resulting from maternal glucose intolerance, may program childhood and later obesity.

While people who were small babies tend to have a lower BMI in adult life than people who were larger at birth, these individuals have a more central distribution of obesity, a significantly reduced muscle mass and a high body fat content in adolescent and adult life (Law *et al.* 1992, Fall *et al.* 1995a, Malina *et al.* 1996, Okosun *et al.* 2000, Loos *et al.* 2001a, 2002, Singhal *et al.* 2003). Exposure to a reduced nutrient supply in the first trimester, as occurred in the Dutch Winter Famine in 1944–1945, also resulted in increased fat mass in later life (Ravelli *et al.* 1976). Parsons and colleagues (2001) have shown that those low birth weight babies who were most vulnerable to developing obesity were men who had been light and thin at birth and had experienced a period of rapid childhood growth. A range of studies have also shown that people who were thin at birth but who developed obesity in childhood or adulthood have the highest risk of insulin resistance and cardiovascular disease (Bavdekar *et al.* 1999, Eriksson *et al.* 2002).

While there are clear associations between birth weight, early childhood growth and the pattern of adult obesity, the mechanisms underlying these associations are unknown. The two primary targets for the 'programming' of adult obesity are: (a) the developing fat cell and the synthesis and secretion of adipokines, such as leptin; and (b) the neuroendocrine network that regulates appetite and energy balance in adult life.

Early nutrition and programming of adipose tissue and leptin synthesis and secretion in the human

In the human infant, there is a positive relationship between cord blood concentrations of leptin at delivery and birth weight or neonatal adiposity. In pregnancies complicated by maternal diabetes, the fetus is hyperglycaemic, hyperinsulinaemic and hyperleptinaemic (Koistinen *et al.* 1997, Matsuda *et al.* 1997, Jaquet *et al.* 1998, Shekawat *et al.* 1998, Cetin *et al.* 2000, Tapanainen *et al.* 2001). Newborn infants who are small for their gestational age (SGA) also show a marked reduction in body fat mass at birth, which mainly reflects a decreased accumulation of lipid in the adipocytes (Levy-Marchal *et al.* 2004). Children born SGA who grow rapidly in infancy have an increased

body fat mass but they have a more central distribution of adipose tissue when compared with children born at normal birth weight (Levy-Marchal *et al.* 2004). While plasma leptin concentrations are low in growth-restricted infants at birth, they increase to become higher in these infants at 1 year of age when compared with their normal birth weight counterparts (Jaquet *et al.* 1999). People with a low birth weight also go on to have higher leptin concentrations in adult life when compared with individuals with the same adult BMI but who had a higher birth weight (Phillips *et al.* 1999). There is also evidence that nutrition in the early postnatal period may be an important determinant of the future leptin synthetic capacity of adipose cells. One study reported that the ratio of leptin to fat mass in adolescents was significantly greater in those who had received a nutrient-enriched formula than in those who received a standard formula or banked breast milk after pre-term delivery (Singhal *et al.* 2002). Human milk intake was also associated with lower leptin concentrations relative to fat mass in adolescence (Singhal *et al.* 2002). It has therefore been suggested that programming of leptin synthesis or secretion in adipocytes by early nutritional exposure may be one mechanism that links early nutrition with later obesity (Singhal *et al.* 2002). Thus a programmed alteration in the synthesis, secretion or actions of leptin may play a role in the early origins of later obesity following exposure to either relative over- or undernutrition in early life (Figs 1 and 2).

The role of leptin in adult obesity

The adipocyte-derived hormone, leptin, a polypeptide (~16 kDa), is synthesised and secreted by adipose tissue and acts as a circulating signal of fat mass through binding to specific receptors at a number of central and peripheral sites to decrease food intake and increase energy utilisation (Friedman & Halaas 1998, Ahima & Flier 2000, Schwartz 2001). In the adult, appetite and energy balance homeostasis are primarily regulated by a neuronal network within the hypothalamus which receives nutrient, hormonal and neural signals from a range of sources including adipocytes, the pancreas, the gastrointestinal tract and other brain regions. There are a range of appetite regulatory neuropeptides, which either stimulate appetite (e.g. neuropeptide Y (NPY) and agouti-related protein (AgRP)) or inhibit it (e.g. pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART)) (Schwartz 2001). The blood–brain barrier is effectively reduced within the area of the arcuate nucleus and NPY neurones are therefore able to sense and respond to a range of peripheral metabolic signals including leptin, insulin and glucose. The leptin receptor (OB-Rb) is highly expressed on cell bodies in the arcuate (ARC) nucleus of the hypothalamus and increases in circulating leptin concentrations during periods of increased food intake result in a decrease in hypothalamic NPY mRNA and a

Figure 1 Potential pathways explaining the relationship between high birth weight and adult obesity.

subsequent fall in energy intake (Schwartz 2001). AgRP is an appetite stimulatory peptide that is coexpressed with NPY in the ARC nucleus and is an endogenous antagonist of the anorexigenic melanocortin receptors, MC3 and MC4R, in the paraventricular nucleus (PVN) and other hypothalamic regions. The POMC-derived peptide, α -melanocyte-stimulating hormone (MSH), is an endogenous appetite inhibitor which acts at these melanocortin receptors to suppress food intake. Leptin increases POMC expression within the ARC nucleus which results in a decrease in energy intake (Schwartz 2001).

Resistance to the actions of leptin would result in a relative inability of high circulating leptin concentrations to suppress appetite and increase energy utilisation (Ahima

& Flier 2000). Adult obesity is associated with relatively high circulating leptin concentrations and the tendency to gain weight in some non-obese populations that have relatively high basal leptin concentrations may indicate an underlying role for leptin resistance in obesity (Chessler *et al.* 1998, Lindroos *et al.* 1998, Lissner *et al.* 1999). It has been suggested that elevated circulating leptin concentrations result in an uncoupling of the action of leptin at its receptors in the hypothalamus, thereby disrupting signal transduction pathways that are required for appetite suppression (Kieffer *et al.* 1996, Ahima & Flier 2000). The presence of functional leptin receptors on pancreatic β cells and the observation that leptin directly inhibits insulin secretion also led to the concept of an 'adipoinular

Figure 2 Potential pathways explaining the relationship between low birth weight, visceral adiposity and insulin resistance.

axis' (Kieffer & Habener 2000) whereby insulin stimulates adipogenesis and the synthesis of leptin, and leptin in turn inhibits the production of insulin in the pancreas. It has been proposed that pancreatic leptin resistance may be a mechanism underlying the hyperinsulinism frequently associated with obesity and that it may contribute to the subsequent development of diabetes in obese individuals (Seufert *et al.* 1999).

Leptin synthesis and secretion before birth

In rodents, the capacity of fetal adipocytes to synthesise leptin is relatively limited until late in gestation and, while the placenta synthesises little if any leptin (Kawai *et al.* 1997, Amico *et al.* 1998), there is significant transplacental transfer of maternal leptin to the fetus. This transfer increases during late pregnancy in parallel with an up-regulation of expression of the shorter isoforms of the leptin receptor in the placenta (Smith & Waddell 2002, 2003).

In species such as the sheep and pig, in which fat is deposited before birth, leptin is synthesised in fetal

adipose tissue and is present in the fetal circulation through late gestation (Yuen *et al.* 1999, Chen *et al.* 2000, Devaskar *et al.* 2002, Ehrhardt *et al.* 2002, Muhlhausler *et al.* 2002, 2003, Yuen *et al.* 2002). Leptin is also expressed in the fetal sheep brain and liver but, in contrast to the human, leptin is not expressed in the sheep placenta (Thomas *et al.* 2001, Ehrhardt *et al.* 2002). Leptin is present in the circulation of the sheep fetus from as early as 40 days gestation, which is before the development of visible adipose tissue depots, and fetal plasma leptin may therefore originate from either the maternal circulation or from fetal tissues other than adipose tissue at this early stage of pregnancy (Ehrhardt *et al.* 2002). Circulating leptin concentrations are lower in the fetus than in the pregnant ewe throughout late gestation (Ehrhardt *et al.* 2002, Muhlhausler *et al.* 2002, Yuen *et al.* 2002). It has been reported that the sheep placenta expresses the leptin receptor gene (Thomas *et al.* 2001) and, as maternal and fetal plasma leptin concentrations are positively correlated throughout late gestation (Yuen *et al.* 2002), it is possible

that in the sheep the placental leptin receptor may mediate the uptake of leptin from the maternal into the fetal circulation. There has been a recent report, however, that the leptin receptor is not expressed in the sheep placenta (Bispham *et al.* 2003) and maternal body composition or fatness either at the beginning or during pregnancy may therefore determine the leptin synthetic and secretory capacity of both maternal and fetal adipose tissue. Importantly, there is also a positive relationship between the relative abundance of leptin mRNA in fetal perirenal adipose tissue (which comprises >80% of the fetal fat mass) and fetal plasma leptin concentrations (Yuen *et al.* 2002). It has been reported in the South Australian Merino ewe that fetal plasma leptin concentrations do not vary significantly during late gestation or during the period immediately before birth (Yuen *et al.* 2004); whereas in the Welsh Mountain ewe there is an increase in circulating leptin concentrations in the fetus at 130–140 days gestation (Forhead *et al.* 2002). Interpretation of differences in baseline plasma leptin concentrations in ruminants is also complicated to some extent by the use of different assays, which appear to provide different values for leptin in animals in the same body weight and body condition range (Chilliard *et al.* 2001).

Ultrastructural studies of adipose tissue in the sheep fetus have demonstrated that fetal adipocytes contain multiple lipid locules and an abundance of mitochondria, and express uncoupling protein 1 (UCP1): characteristic features of thermogenic or brown adipose tissue (Gemmell *et al.* 1972, Gemmell & Alexander 1978, Symonds & Stephenson 1999, Yuen *et al.* 2003). Fetal adipocytes also contain larger or dominant lipid locules and there is a direct relationship between the relative mass of the 'unilocular' component of perirenal and interscapular fat, and circulating leptin concentrations in a cohort of fetuses in well-nourished pregnant ewes (Muhlhausler *et al.* 2002). This suggests that circulating leptin concentrations may be a signal of the unilocular component of fetal fat, rather than total fat mass in fetal life, as it is in the neonate and adult.

Maternal undernutrition and the regulation of maternal and fetal plasma leptin concentrations

In sheep, the impact of maternal undernutrition on plasma leptin concentrations appears to be dependent on the breed and age of the ewe and on the level of nutrient restriction. In growing adolescent ewes (Suffolk or Dorset Horn × Greyface; body weight 40–45 kg) pregnant as a result of embryo transfer, increases or decreases in maternal nutrition during the first 100 days of gestation were associated with parallel increases or decreases in maternal plasma leptin concentrations (Thomas *et al.* 2001). In this study, when data were pooled across nutritional treatments, circulating leptin concentrations at 104 days gestation were positively correlated with maternal weight and body condition score. A 40% restriction of

metabolisable energy (ME) requirements between 28 and 80 days gestation also resulted in a decrease in maternal leptin concentrations in Welsh Mountain ewes (body weight 35–40 kg) (Bispham *et al.* 2003); whereas a 50% restriction from 115 days gestation resulted in a fall in plasma glucose, but not leptin, concentrations in Merino ewes (body weight 50–60 kg) during late gestation (Yuen *et al.* 2002). Similarly, in a study in which Merino ewes were undernourished from day 8 of pregnancy, plasma glucose, but not leptin, concentrations were lower in singleton-bearing ewes (Edwards *et al.* 2005). Thus in large adult ewes carrying singleton fetuses, maternal leptin concentrations are not as sensitive a measure of the level of prevailing nutrition as maternal glucose concentrations. In the latter study, however, there was a significant relationship between maternal plasma leptin and glucose concentrations during late gestation when all nutritional groups (including ewes exposed to restricted nutrition from before pregnancy until 7 days after mating only, from 8 to 145 days gestation inclusive and from before pregnancy until 145 days gestation) were combined. Interestingly in ewes carrying twin pregnancies, while maternal plasma leptin concentrations were not lower in ewes exposed to undernutrition during late gestation, they were directly related to the amount of weight loss experienced before pregnancy when nutrition treatment groups were combined. As there is a greater energy demand on the mother during a twin pregnancy, it is not surprising that relationships between the level of maternal leptin during late gestation and changes in pre-pregnancy weight emerge in ewes carrying twins. In contrast to ewes carrying singletons, there was no direct relationship between maternal plasma leptin and glucose concentrations in ewes carrying twins during late gestation – presumably reflecting the greater dependence of maternal leptin concentrations in late gestation on the body fat stores at the start of pregnancy in these animals. These results highlight the interaction between the amount of body fat stores at the start of pregnancy, the number of fetuses and the level of nutrient intake throughout pregnancy in determining circulating leptin concentrations in the pregnant ewe.

It has been reported that there is no impact of maternal undernutrition, imposed during either the periconceptional period or throughout gestation, on fetal plasma leptin concentrations during late gestation (Edwards *et al.* 2005). Previous studies also reported that an ~50% decrease in maternal nutrient intake in late gestation did not decrease fetal plasma concentrations of leptin or the relative abundance of leptin mRNA in perirenal adipose tissue in the fetal sheep (Ehrhardt *et al.* 2002, Yuen *et al.* 2002). Thus the synthesis and secretion of leptin in the sheep fetus appears relatively resistant to the changes in fetal glucose and insulin concentrations associated with moderate maternal undernutrition. Interestingly, while there was no difference in fetal plasma leptin concentrations in ewes undernourished at different windows of early and late gestation, there was a relative increase in adiposity in twin

fetuses of ewes exposed to maintenance nutrition before pregnancy and for 1 week after conception followed by a decrease in nutrition for the remainder of pregnancy (Edwards *et al.* 2005). Fetal adiposity was not increased in those ewes that had been undernourished from before and throughout pregnancy. This suggests that adiposity may be programmed in twin fetal sheep as a consequence of changes in the plane of maternal nutrition during early pregnancy. The altered fetal fat deposition is unlikely to reflect the increased metabolic demand of twin fetuses, as the change in the level of nutrition occurred at a time when the metabolic demands of the embryos were minimal. It has been shown, however, that manipulation of the early nutritional environment of the embryo, either *in vivo* or *in vitro*, can alter the allocation of cells within the inner cell mass and trophoctoderm, and subsequent fetal somatic and organ growth (Kwong *et al.* 2000). Maternal hormonal or metabolic responses to the change in the level of nutrition at the end of the first week of pregnancy may act to alter the expression of key genes within the developing blastocyst to result in enhanced adiposity in late gestation. Bispham and colleagues (2003) reported that there was an increase in relative fetal adiposity in fetuses in ewes that had been nutrient restricted between 28 and 80 days gestation and then fed to appetite (i.e. 150% ME requirements) until 140 days gestation when compared with fetuses in ewes that had been fed to appetite between 28 and 140 days gestation. In this latter study, fetuses of ewes that had been nutrient restricted between 28 and 80 days gestation and then fed to energy requirements (100% ME) did not have an increase in relative adiposity when compared with their control counterparts, although there was an increase in insulin-like growth factor (IGF)-1R and IGF2-R mRNA levels in fetal adipose tissue at 140 days (Bispham *et al.* 2003). It appears therefore that there are different critical windows during which the imposition of maternal undernutrition (~8 days gestation) or maternal overnutrition (~80 days gestation) can result in an increased fetal adiposity. While nutrient restriction between 28 and 80 days gestation did not result in a change in leptin mRNA expression in fetal adipose tissue, there was a decrease in leptin mRNA abundance in fetal adipose tissue at 140 days gestation in ewes that were fed on a high nutritional plane from 80 days gestation (Bispham *et al.* 2003). Future studies are clearly required to determine the interactions between maternal nutrition, fetal adiposity, the IGFs, leptin and other adipocyte-derived hormones in the programming of postnatal obesity in animals in which fat is deposited and leptin is expressed within fat depots before birth.

Maternal overnutrition and the regulation of maternal and fetal plasma leptin concentrations

In the sheep, a moderate increase (~50%) in maternal nutrient intake above maintenance requirements increases maternal plasma glucose and leptin concentrations during

late gestation in the absence of a concomitant increase in either total fetal fat mass or fetal plasma leptin concentrations (Muhlhausler *et al.* 2002). In these well-nourished ewes there was, however, a significant relationship between fetal glucose and the relative mass of fetal unilocular fat, and a positive relationship between fetal insulin concentrations and the relative abundance of leptin mRNA in fetal perirenal adipose tissue (Muhlhausler *et al.* 2003). Insulin-responsive elements have been identified within the promoter of the leptin gene in the adult mouse and insulin upregulates leptin gene transcription in adipocytes of adult rodents (Saladin *et al.* 1995). A moderate increase in maternal nutrition also resulted in a strong reciprocal relationship between leptin and UCP1 expression in fetal perirenal adipose tissue in late gestation.

In a model that mimics the effects of a diabetic pregnancy and the associated fetal hyperglycaemia, infusion of glucose directly to the sheep fetus for 10 days in late gestation resulted in an increase in the size of the dominant lipid locules and in the total mass of unilocular perirenal adipose tissue in the fetus (Muhlhausler *et al.* 2005). The mean lipid locule size was also directly related to fetal glucose, but not insulin, concentrations during the infusion period in the saline- and the glucose-infused fetuses. This is consistent with the finding of a similar relationship between glucose concentrations and lipid locule size in the fetal adipose tissue of the well-nourished ewe (Muhlhausler *et al.* 2003), and suggests that glucose, rather than insulin, is the principal determinant of lipid storage in fetal adipocytes during late gestation. This is supported by previous reports that the abundance of the insulin-independent glucose transporter (GLUT1) is greater than that of the insulin-dependent glucose transporter (GLUT4) in the adipose tissue of the sheep fetus in late gestation (Das *et al.* 1998).

While a 10-day glucose infusion resulted in an increase in lipid locule size, it did not result in a significant increase in relative adiposity in the fetal sheep. It has previously been reported that a 30-day infusion of glucose in fetal sheep increased total fetal fat mass (Stevens *et al.* 1990). It may be that the fetus is able to adapt to 10 days of glucose infusion, but not to a more prolonged period of hyperglycaemia, through mechanisms that limit the impact of an increase in fetal glucose on fetal adiposity. Prolonged fetal hyperglycaemia is associated with a decline in adipose GLUT-1 levels in the sheep fetus (Das *et al.* 1999); this may be one mechanism that acts to counter a chronic increase in fetal nutrient supply. As in the well-nourished ewe, while leptin expression in fetal adipose tissue was not increased in glucose-infused fetuses, there was a strong positive relationship between either plasma insulin or glucose concentrations and leptin mRNA expression in fetal adipose tissue in these fetuses. Infusions of glucose resulting in prolonged periods (14–20 days) of hyperglycaemia and hyperinsulinaemia, however, do increase fetal fat mass (Stevens *et al.* 1990) and leptin mRNA abundance in fetal perirenal adipose tissue (Devaskar *et al.* 2002). Thus, while leptin synthesis is not

upregulated in the presence of moderate maternal overnutrition or a 10-day intrafetal infusion of glucose, there is evidence of a positive relationship between circulating insulin concentrations and the level of leptin expression in these fetuses. This suggests that within a 'moderate' range of fetal plasma glucose and insulin concentrations, there may be active counter-regulatory mechanisms present within the fetus to limit the potential impact of fetal overnutrition on leptin synthesis and secretion, and these mechanisms may be overwhelmed in the presence of excessive glucose or insulin exposure.

Actions of leptin in the fetus

There is evidence that leptin may have a range of neuroendocrine and endocrine actions in the sheep fetus. Intrafetal leptin infusion, resulting in a 4- to 5-fold increase in circulating leptin concentrations suppressed the normal prepartum increase in fetal cortisol concentrations for an extended period from 90 to 42 h before delivery but did not alter the timing of parturition in these animals (Yuen *et al.* 2004). Intrafetal leptin infusion in the presence of normoglycaemia and normoinsulinaemia also results in an increase in the proportion of multilocular tissue and a decrease in the proportion and relative mass of unilocular tissue in the perirenal adipose depot; the relative abundance of leptin mRNA in perirenal adipose tissue was also

lower in leptin-infused fetuses and, in addition, there was a strong correlation between the proportion of unilocular fat and leptin mRNA expression in this tissue (Yuen *et al.* 2003). These findings suggest that leptin may act as a signal of energy supply and have a 'lipostatic' role before birth (Fig. 3). The extent to which leptin can act on the central neural network which normally regulates appetite in adult life, is clearly dependent on the extent to which this network is present before birth.

Development of the appetite regulatory system before birth

In the rat brain, neurones expressing the appetite regulatory peptides develop in the hypothalamus at around 14.5 days gestation (term ≈ 21 days) and NPY mRNA expression rapidly increases during the first 2 weeks after birth (Grove & Smith 2003). POMC, AgRP and MC4R mRNA are also all present within the rat hypothalamus during this neonatal period. While NPY is present in the ARC nucleus from late gestation, connections between this nucleus and other hypothalamic nuclei that act to regulate appetite are not complete until 2 weeks of age (Grove & Smith 2003).

In contrast to the rodent, NPY has been found to be present from as early as 21 weeks gestation in the human hypothalamus and at this stage there are already projec-

Figure 3 Schematic diagram representing a summary of the potential effects of an increase in circulating leptin concentrations on the structural and functional characteristics of fetal adipose tissue (reprinted from Yuen *et al.* (2003)). It is speculated that leptin secreted by the unilocular component of fetal adipose tissue activates the long form of the leptin receptor (OB-Rb) in the fetal hypothalamus. This results in a stimulation of the sympathetic nervous system, an increase in lipolysis and an associated decrease in the size of the lipid locules within the unilocular component of the fetal adipose tissue. The decrease in the proportion of the unilocular adipose tissue results in a decrease in the relative abundance of leptin mRNA in this tissue.

tions between the ARC nucleus and the PVN (Koutcherov *et al.* 2002). It is therefore possible that in the human, nutritional or hormonal exposure during fetal or early neonatal life may be important in determining the subsequent development of the appetite regulatory system. We have also previously reported that genes for the appetite-regulating neuropeptides – NPY, AgRP, POMC and CART – are each expressed in the ARC nucleus of the fetal sheep hypothalamus by 110 days gestation (term = 147 ± 3 days gestation) (Muhlhausler *et al.* 2004). The long form of the leptin receptor (OB-Rb) is also expressed in both the ARC and ventromedial nucleus (VMN) of the fetal sheep, and to a lesser extent in the dorsomedial nucleus (DMN), consistent with the reported pattern of expression in the adult sheep (Williams *et al.* 1999, Muhlhausler *et al.* 2004). While the sites of OB-Rb expression were similar in the fetal and adult sheep, there were differences in the relative intensity of hybridisation within these hypothalamic nuclei (Fig. 4). Specifically, the intensity of OB-Rb expression was higher in the VMN compared with the ARC nucleus in fetal sheep whereas in the adult hypothalamus, the ARC nucleus is the predominant site of expression of the OB-Rb (Muhlhausler *et al.* 2004). In the adult rodent, the VMN has been implicated as being important for the regulation of thermogenesis in the brown adipose tissue and the higher level of OB-Rb expression in the fetal VMN may indicate that leptin has a greater role in the regulation of the thermogenic activity of brown adipose tissue, rather than 'energy intake', during the perinatal period. In this species it has also recently been demonstrated that intrafetal infusion of glucose in late gestation resulted in a significant increase in POMC mRNA in the ARC nucleus of the fetal sheep hypothalamus (Muhlhausler *et al.* 2005). This occurred in the absence of an increase in circulating leptin, indicating that during fetal life, POMC mRNA expression in the hypothalamus may be responsive to increases in glucose or insulin, acting either alone or in combination. Interestingly, there was no effect of intrafetal glucose infusion on the expression of the orexigenic neuropeptides NPY and AgRP in the fetal sheep hypothalamus (Muhlhausler *et al.* 2005). This is surprising given that circulating glucose and insulin concentrations in the fetus are relatively low compared with those measured in adult life and that fetal hypothalamic NPY content is increased following maternal undernutrition in sheep (Warnes *et al.* 1998). There have been no reports to date of the impact of intrafetal leptin administration on the expression of the appetite regulatory peptides within the fetal hypothalamus. Clearly, further work is required to determine whether there is a critical window during which exposure of the fetal brain to relatively low or high leptin concentrations results in an altered development of the energy balance regulating system within the hypothalamus that persists into postnatal and adult life. In this context it has been demonstrated that low birth weight lambs have a higher relative voluntary food intake during the early postnatal

period and are fatter at body weights up to 20 kg when compared with lambs with normal birth weights (Greenwood *et al.* 1998); however, there is no information on whether this relative hyperphagia is a result of an early programming of the appetite regulatory neuropeptides.

Leptin and the early programming of adult obesity

The role of leptin in the early programming of later obesity has been predominantly investigated in the rodent in a number of models in which the early nutritional experience has been manipulated. The most common manipulations of fetal nutrition in the rodent are through exposure of the pregnant dam to either a global undernutrition through a 30–50% reduction in energy intake or a low-protein diet (generally 9 vs 18% protein content). When rats are undernourished during the first 2 weeks of pregnancy but re-fed during the third week, the male offspring develop significant increases in appetite and obesity when maintained on a high-fat diet (Stephens 1980, Jones & Friedman 1982, Anguita *et al.* 1993, Jones *et al.* 1996). When maternal nutrition is restricted to 30% of control intake throughout the whole of gestation, while the offspring were smaller throughout postnatal life, there was an increase in the relative mass of the retroperitoneal fat pad in these animals at 100 days of age (Vickers *et al.* 2000). Food intake in the offspring of the undernourished rats (cross-fostered on to *ad libitum*-fed mothers) was increased early in postnatal life, increased with increasing age and was amplified by postnatal hypercaloric nutrition (Vickers *et al.* 2000).

When leptin is administered to pregnant dams on days 8, 10 and 12 of pregnancy, the adult offspring have reduced adipose tissue mass and skeletal growth with no change in food intake (Nilsson *et al.* 2003). Maternal protein restriction results in a significant decrease in maternal, but not fetal, leptin concentrations during late gestation (Fernandez-Twinn *et al.* 2003). Administration of leptin to pregnant rats fed a low-protein diet prevented the diet-induced fall in the activity of the placental enzyme, 11 β hydroxysteroid dehydrogenase 2, which normally protects the fetus from excess glucocorticoid exposure (Stocker *et al.* 2004). In these experiments, the male offspring of saline-treated dams gained more weight and had higher plasma leptin levels when transferred to a high-fat diet at 6 weeks, but the offspring of leptin-treated dams did not show this phenotype (Stocker *et al.* 2004). This may be an example of where an experimental induction of an increase in maternal leptin (normally associated with an increase in body fat) programs the ability of the offspring to respond better to a high-fat diet in the postnatal period. This fits with the concept of a maternal 'adaptive' response to overnutrition with the dam programming the subsequent development of her fetus to respond appropriately to an increase in nutrition in postnatal life.

In contrast, exposure of rats to maternal undernutrition (30% energy intake) during gestation leads to an increase in

Figure 4 Autoradiographic images of coronal sections through fetal sheep hypothalamus at 110 days gestation (term $\approx$ 147 days) showing gene expression for NPY, AgRP, POMC, CART and leptin receptor (OB-Rb) (reprinted from Muhlhauser *et al.* (2004)). ARC, arcuate nucleus; ME, median eminence; VMH, ventromedial hypothalamus; DMH, dorsomedial hypothalamus; PVN, paraventricular nucleus; R, reuniens thalamic nucleus; OC, optic chiasm; 3V, 3rd ventricle; LHA, lateral hypothalamic area.

relative adiposity and high circulating leptin concentrations in adult offspring (Vickers *et al.* 2000). The combination of prenatal undernutrition together with postnatal hypercaloric nutrition leads to a major amplification of the hyperleptinaemia. The hyperleptinaemia in the presence of hyperinsulinism in the offspring on the hypercaloric diet suggests that there may be development of leptin resistance both at the hypothalamus and the pancreas (Breier *et al.* 2001).

Thus, in the rat, manipulation of maternal plasma leptin concentrations by altering maternal nutrition, infusion of leptin to the dam or manipulation of transplacental leptin transfer has an impact on the subsequent regulation of leptin synthesis and secretion in the offspring in postnatal life. This impact appears to be dependent to a large extent on the fetal exposure to the maternal leptin signal as there is very limited development of endogenous leptin synthetic capacity before birth.

Leptin and appetite regulation in the rodent in early life

While the appetite regulatory system is relatively immature at birth in the rat, the amount of food consumed during the suckling period plays an important role in determining subsequent food intake in the rat (Oscai & McGarr 1978). When postnatal overnutrition is induced in rats, by rearing in small litters of only three pups, they show an increased early weight gain and fat deposition, followed by an increased appetite (hyperphagia), obesity, hyperleptinaemia, hyperglycaemia, hyperinsulinaemia and insulin resistance (Plagemann *et al.* 1992, 1999). Leptin has a lower inhibitory effect on the appetite stimulatory neurones of the hypothalamus in these animals as young adults (Davidowa & Plagemann 2000, 2001).

Interestingly, a recent study has reported that neural projection pathways from the ARC nucleus are perma-

nently disrupted in leptin-deficient ($\text{Lep}^{\text{ob}}/\text{Lep}^{\text{ob}}$) mice and that treatment of these mice with leptin in neonatal, but not in adult, life rescues the development of projections from the ARC nucleus (Bouret *et al.* 2004). Thus leptin acts to promote the development of hypothalamic pathways that will have a role in later life in regulating food intake and energy consumption. These actions of leptin appear to be specific to the neurones of the ARC nucleus and only occur during a critical window of time that corresponds to a neonatal period when leptin concentrations are usually high. This neonatal 'critical period' also corresponds to the period when the axons from the ARC neurones are guided to their targets (Bouret *et al.* 2004). Thus leptin derived from the maternal circulation, present in breast milk or derived from the neonatal adipocytes may exert an important influence on the development of the appetite regulatory neural network.

It is therefore possible that leptin in the fetal circulation derived either from the maternal circulation (as in the rat in late gestation and potentially the sheep in early gestation) or fetal adipose tissue (as in the sheep and human in late gestation) acts centrally via leptin receptors located on neurones within the fetal hypothalamus to have a 'lipostatic' role. While leptin may play a role in regulating the structural and functional characteristics of the developing adipocyte *in utero*, it is also possible that exposure to high or low leptin concentrations during critical windows of development of the neural network that regulates appetite may have longer term consequences for this network and for the regulation of energy balance after birth.

Summary

There is substantial evidence from epidemiological studies that being of high birth weight, or being of low birth weight and growing rapidly in postnatal life, can each be associated with an increased BMI or an increased visceral adiposity in later life. There is also evidence to indicate that maternal body composition and the level of maternal nutrition may contribute to the programming of obesity in her offspring. While there has been a predominant focus in this field on the impact of poor fetal nutrition on subsequent body composition in adult life, there is a growing awareness of the potential for an intergenerational cycle of obesity as women enter pregnancy with a higher BMI and then have heavier babies who may then be programmed to be heavy in adult life (Kral 2004). There have been many reports, predominantly in the rodent, demonstrating that the nutritional environment in early life can have a lifelong impact on the distribution and amount of body fat mass. It is only relatively recently, however, that the role of specific nutrients and hormones, such as leptin, in the early programming of postnatal obesity has begun to be defined. In the rodent, it is now becoming clear that the maternal nutrient and leptin concentrations may provide the fetal and neonatal rat with key signals of the current, and poten-

tially the future, nutrient environment. In species such as the human and sheep, there is also recent evidence that the synthesis and secretion of adipocyte-derived hormones, such as leptin, are regulated in fetal life; there is also evidence that the hypothalamic neuropeptides which regulate energy intake and expenditure in adult life are also present within the fetal brain and may be regulated by the prevailing level of maternal and hence fetal nutrient and hormonal signals, including leptin. This work is of importance in determining those initiating mechanisms within the 'fat-brain' axis in early life that precede the development of adult obesity and the critical windows during which intervention will have maximum benefit to ensure a lifelong resilience to an increased body fat mass.

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