SYMPOSIUM REPORT

From feeding one to feeding many: hormone-induced changes in bodyweight homeostasis during pregnancy

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Pregnancy is associated with hyperphagia, increased fat mass, hyperleptinaemia and hyperprolactinaemia. The neuroendocrine control of bodyweight involves appetite-regulating centres in the hypothalamus, containing both orexigenic and anorexigenic neurons that express leptin receptors (LepR). In the rat, central leptin resistance develops during mid pregnancy, well after hyperphagia becomes apparent, to negate the appetite suppressing effects of leptin. We have investigated the hypothalamic response to leptin during pregnancy and examined the role of pregnancy hormones in inducing these changes. We have shown that there are multiple levels of leptin resistance during pregnancy. Despite elevated serum leptin, neuropeptide Y and agouti related peptide mRNA in the arcuate nucleus are not suppressed and may even be increased during pregnancy. LepR mRNA and leptin-induced pSTAT3 expression, however, are relatively normal in the arcuate nucleus. In contrast, both LepR and leptin-induced pSTAT3 are reduced in the ventromedial hypothalamic nucleus. Injecting α-melanocyte-stimulating hormone (α-MSH) into the brain, to bypass the first-order leptin-responsive neurons in the arcuate nucleus, also fails to suppress food intake during pregnancy, suggesting that pregnancy is also a melanocortin-resistant state. Using a pseudopregnant rat model, we have demonstrated that in addition to the changes in maternal ovarian steroid secretion, placental lactogen production is essential for the induction of leptin resistance in pregnancy. Thus, hormonal changes associated with pregnancy induce adaptive changes in the maternal hypothalamus, stimulating food intake and then allowing elevated food intake to be maintained in the face of elevated leptin levels, resulting in fat deposition to provide energy stores in preparation for the high metabolic demands of late pregnancy and lactation.

Introduction: metabolic adaptation to pregnancy

Pregnancy places significant physiological demands on a female mammal, with a conflict arising between the needs of the developing fetuses and the requirements to maintain the health of the mother. To cope with these novel demands, the hormones of pregnancy induce a coordinated range of adaptations to physiological functions in the mother. These adaptations include changes in the cardiovascular and respiratory systems,

changes in immune function, behavioural changes, and extensive changes in neuroendocrine mechanisms governing homeostatic processes (Russell *et al.* 2001). One of the most profound adaptations is in energy homeostasis, where the mother faces competing needs of meeting her own energy demands, supplying nutrients to the growing fetus, and also establishing a positive energy balance to promote storage of energy resources in preparation for the huge metabolic demands of lactation. There are differences in nutrient partitioning to ensure adequate nutrition to the offspring, and changes in metabolic processing within different tissues. Perhaps the most important adaptation, however, is an increase in appetite and food intake. The process of 'eating for two', or 'eating for many', in the case of rodents, starts

long before the actual metabolic demand affects the mother. Hence, the hormone-induced increase in food intake represents a true adaptive response to prepare the female for motherhood. In this review, we summarize the pregnancy-induced changes in the homeostatic systems that normally regulate food intake, and discuss the neuroendocrine mechanisms underlying these changes.

Neuroendocrine regulation of body weight

Body weight in most individuals is held relatively stable, even in the face of daily fluctuations in energy intake and energy expenditure, by complex interactions between short- and long-term regulators of energy balance. Hormones produced by the stomach (ghrelin) and gut (cholecystokinin and peptide YY) stimulate and suppress food intake, respectively, on a daily basis (Saper *et al.* 2002). Long-term regulators of bodyweight include the adipocyte-derived hormone, leptin, and to a lesser extent, insulin. Both are produced in the periphery and act centrally to maintain bodyweight homeostasis. Before the discovery of leptin (Zhang *et al.* 1994), it had been postulated that a secreted molecule circulates in the blood, increases in proportion to total body fat and modulates energy intake and energy expenditure over long periods of time (Weigle, 1994). Leptin fulfilled the criteria of an adiposity signal; circulating levels of leptin in humans and rodents correlate with body adiposity and levels change in accordance with nutritional status (Maffei *et al.* 1995; Considine *et al.* 1996). The essential role of leptin in the control of food intake has been confirmed, and is extensively reviewed elsewhere (Elmquist *et al.* 1998*b*; Friedman & Halaas, 1998; Schwartz *et al.* 2000; Gao & Horvath, 2007). Leptin enters the central nervous system (CNS), binds to receptors in hypothalamic nuclei involved in energy intake, and decreases food intake. Unfortunately for dieters, leptin works best as a starvation signal; leptin levels fall during fasting, triggering counter measures to conserve energy and increase appetite (Ahima *et al.* 1996). The evolutionary role of leptin suggests that rather than acting as an obesity signal, low levels of leptin stimulate appetite and food intake and promote fat storage to return bodyweight to a previous 'set-point' (Schwartz *et al.* 2000).

Leptin action in the CNS

Much work on leptin action in the hypothalamus has focused on two populations of neurons in the arcuate nucleus that have opposing effects on food intake. LepR are found in the mediobasal hypothalamus, in nuclei that have been implicated in feeding, thermogenesis and hormone regulation, and also in a variety of non-hypothalamic sites and the brainstem (Mercer *et al.* 1996*b*; Schwartz *et al.* 1996*b*; Cheung *et al.* 1997; Fei *et al.* 1997; Elmquist *et al.* 1998*a*). Within the arcuate nucleus, LepR have been localized on a population of

orexigenic neurons (Mercer *et al.* 1996*a*) that coexpress neuropeptide Y (NPY) and agouti-related peptide (AGRP) (Broberger *et al.* 1998). Leptin acts to inhibit these neurons and regulates the level of NPY mRNA (Stephens *et al.* 1995; Ahima *et al.* 1996; Schwartz *et al.* 1996*b*). In fasted rodents, and *ob/ob* and *db/db* mice, NPY gene expression is increased, and this increase is blunted by leptin treatment (Stephens *et al.* 1995; Ahima *et al.* 1996; Schwartz *et al.* 1996*b*). Anorexigenic proopiomelanocortin (POMC) neurons in the arcuate nucleus also contain LepR mRNA (Cheung *et al.* 1997) and leptin increases the level of POMC mRNA (Schwartz *et al.* 1997). In fasted rodents or *ob/ob* mice, POMC mRNA is reduced, and this decrease is prevented by leptin administration (Schwartz *et al.* 1997). POMC neurons produce the anorectic peptide α-melanocyte-stimulating hormone (α-MSH), and mutations in the POMC gene which prevent α-MSH production result in obesity in mice and humans (Spiegelman & Flier, 2001). Electrophysiological studies on transgenic mice have shown that leptin activates POMC neurons directly and decreases the firing rate of NPY/AGRP neurons that normally exhibit an inhibitory GABAergic tone onto POMC neurons (Cowley *et al.* 2001).

Mechanisms of leptin resistance

Despite the clear role of leptin to suppress appetite, most obese individuals have high plasma leptin concentrations, proportional to their increased body fat (Maffei *et al.* 1995; Considine *et al.* 1996) and leptin administration has weight-reducing effects in only a subset of obese people (Heymsfield *et al.* 1999). More commonly, obese humans are resistant to elevated endogenous leptin and to exogenously administered leptin. Thus, mechanisms of leptin resistance are of key interest in the efforts to understand rising levels of obesity. Resistance to leptin is evident in mice (*db/db*) and rats (*fa/fa*) with mutant leptin receptors, and in mice that develop obesity due to other reasons such as diet-induced obesity, age-related obesity, New Zealand Obese (NZO) mice and mice that lack melanocortin-4 receptors (MC4-R) (Halaas *et al.* 1997; Zhang & Scarpace, 2006). Various mechanisms have been identified in these animal models of obesity and leptin resistance. Diet-induced obese and NZO mice have peripheral leptin resistance (Halaas *et al.* 1997) due to impaired leptin transport into the brain, and this can be bypassed by central leptin injections (El-Haschimi *et al.* 2000; Hileman *et al.* 2002). Peripheral leptin resistance is also seen in obese Otsuka Long–Evans fatty rats but again, they still respond to central leptin (Niimi *et al.* 1999). In humans there is a correlation between plasma and CSF leptin levels, and the ratio of these two factors is lower in obese individuals (Schwartz *et al.* 1996*a*), implicating reduced leptin transport into CSF as a possible

cause of leptin resistance. Leptin transport into the brain is thought to be via a saturable transport mechanism, possibly involving short-form LepR at the blood–brain barrier (Hileman *et al.* 2002). Therefore, human leptin resistance may be caused by a lack of leptin transport into the brain (Caro *et al.* 1996; Banks, 2004). More recently, however, it has been shown that functional uptake of leptin can occur directly in regions of the brain outside the blood brain barrier, via arcuate nucleus neurons extending processes down into the median eminence and having direct contact with the peripheral circulation (Faouzi *et al.* 2007). Thus, the arcuate nucleus displays a more rapid response and increased sensitivity to circulating leptin than other deeper hypothalamic sites. In DIO mice, the arcuate nucleus remains unresponsive to central leptin injection (Munzberg *et al.* 2004) (whereas other hypothalamic nuclei were responsive), which suggests that in some leptin resistant models, resistance also occurs centrally. In fact, age-related obesity is associated with central leptin resistance involving decreased LepR mRNA and protein (Fernandez-Galaz *et al.* 2001) and impaired leptin signal transduction (Scarpace *et al.* 2000; Zhang & Scarpace, 2006). Rats on a high-fat diet become obese and unresponsive to central leptin gene therapy due to decreased LepR expression (Wilsey *et al.* 2003), which is reversed if animals are calorie-restricted (Wilsey & Scarpace, 2004). Agouti (A^y) mice are hyperleptinaemic due to antagonism of the melanocortin system responsible for down-stream regulation of food intake (Fan *et al.* 1997), and are hence, obese. While they do not respond to leptin with a decrease in food intake, they are not technically leptin resistant as they show normal intracellular responses to leptin. This demonstrates that leptin resistance may involve defects in multiple mechanisms at the hypothalamic level.

Physiological regulation of leptin responses

Pregnancy is an example of transient leptin resistance. This is beneficial to allow build up of long-term energy stores required for successful reproduction. Pregnancy and lactation are both states of physiological hyperphagia, a desirable adaptation that supports the growing conceptus and provides adequate energy in preparation for lactation. Similar changes can occur in response to other physiological demands, such as seasonal breeding (Tups *et al.* 2004). Our studies have focused on pregnancy in the rat as a model to better understand homeostatic regulation of body weight and to contribute to understanding leptin resistance, which has implications for obesity. We have investigated the mechanisms triggering leptin resistance (Augustine & Grattan, 2007), and the mechanisms that limit the actions of leptin during the pregnancy-induced state of hyperleptinaemia (Ladyman & Grattan, 2004, 2005). During pregnancy there are peripheral actions of

leptin involving placental/fetal interactions that have been reviewed in great depth (Henson & Castracane, 2000, 2006). Therefore, this review will focus on neuroendocrine adaptations that occur in the maternal brain during this physiologically demanding time.

Food intake during pregnancy

During pregnancy and lactation, adequate nutrition of the mother is crucial for survival of the young. Hence, leptin resistance, which can be considered as a state in which a starvation-like signal is maintained despite sufficient food intake, is a physiological adaptation that is necessary to maintain increased food intake and fat deposition in the face of rising levels of leptin. In rats, food intake increases by up to 50% during pregnancy, decreases the day prior to parturition, then increases by up to 300% during lactation compared to non-pregnant rats (Cripps & Williams, 1975; Shirley, 1984; Ladyman & Grattan, 2004) (see Fig. 1). Importantly, increased food intake during pregnancy precedes the metabolic demand, establishing a positive energy balance, resulting in an increase in maternal body fat (Shirley, 1984). These stores are later depleted due to suckling-induced mobilization of fat stores (Naismith *et al.* 1982). The initial increase in appetite is likely to be due to the absence of the anorectic effect of oestradiol, plus high circulating concentrations of progesterone. Oestradiol specifically reduces meal size (Eckel & Geary, 2001; Asarian & Geary, 2002) and increases energy utilization (Wade & Gray, 1979). Ovariectomised (OVX) rats and mice have increased food intake and weight gain (Tarttelin & Gorski, 1971; Clegg *et al.* 2007), which is reversed if treated with oestrogen. Progesterone is orexigenic and administration to either ovariectomised or intact rats and mice increases food intake (Hervey & Hervey, 1967). Plasma leptin concentrations increase as pregnancy advances, reaching peak levels on day 19 followed by a rapid decline prior to parturition (Kawai *et al.* 1997; Amico *et al.* 1998; Ladyman & Grattan, 2004). The source of increased leptin secretion during pregnancy is species dependent. In humans, placental production of leptin significantly contributes to leptin levels in the maternal circulation (Masuzaki *et al.* 1997). In the rat, the placenta is not a major source of hyperleptinaemia during pregnancy, and high serum leptin is more likely to be due to increased leptin mRNA expression in maternal fat (Kawai *et al.* 1997). Also, in rats and mice, increased levels of circulating binding proteins of placental origin are thought to contribute to hyperleptinaemia during pregnancy (Gavrilova *et al.* 1997; Seeber *et al.* 2002). Despite elevated plasma leptin concentrations during pregnancy, increased food consumption is maintained, a paradox considering leptin's pivotal role in suppressing food intake. The presence of leptin-binding proteins in the plasma during pregnancy (Gavrilova *et al.* 1997; Seeber

et al. 2002) may impair leptin action by restricting leptin access to the brain. Our group and others, however, have shown that leptin resistance occurs at the hypothalamic level in pregnant rats, with a region-specific loss of hypothalamic LepR mRNA (Garcia *et al.* 2000; Ladyman & Grattan, 2005) and suppression of leptin signal transduction (Garcia *et al.* 2000; Ladyman & Grattan, 2004, 2005). Pregnant females do not show suppression of food intake in response to exogenous leptin (Mounzih *et al.*

1998; Johnstone & Higuchi, 2001; Ladyman & Grattan, 2004; Lecklin *et al.* 2005).

Changes in leptin action during pregnancy

Despite high leptin, NPY mRNA is either unchanged (Rocha *et al.* 2003) or even increased during pregnancy (Garcia *et al.* 2003), while levels of POMC remain unchanged (Garcia *et al.* 2003; Rocha *et al.* 2003).
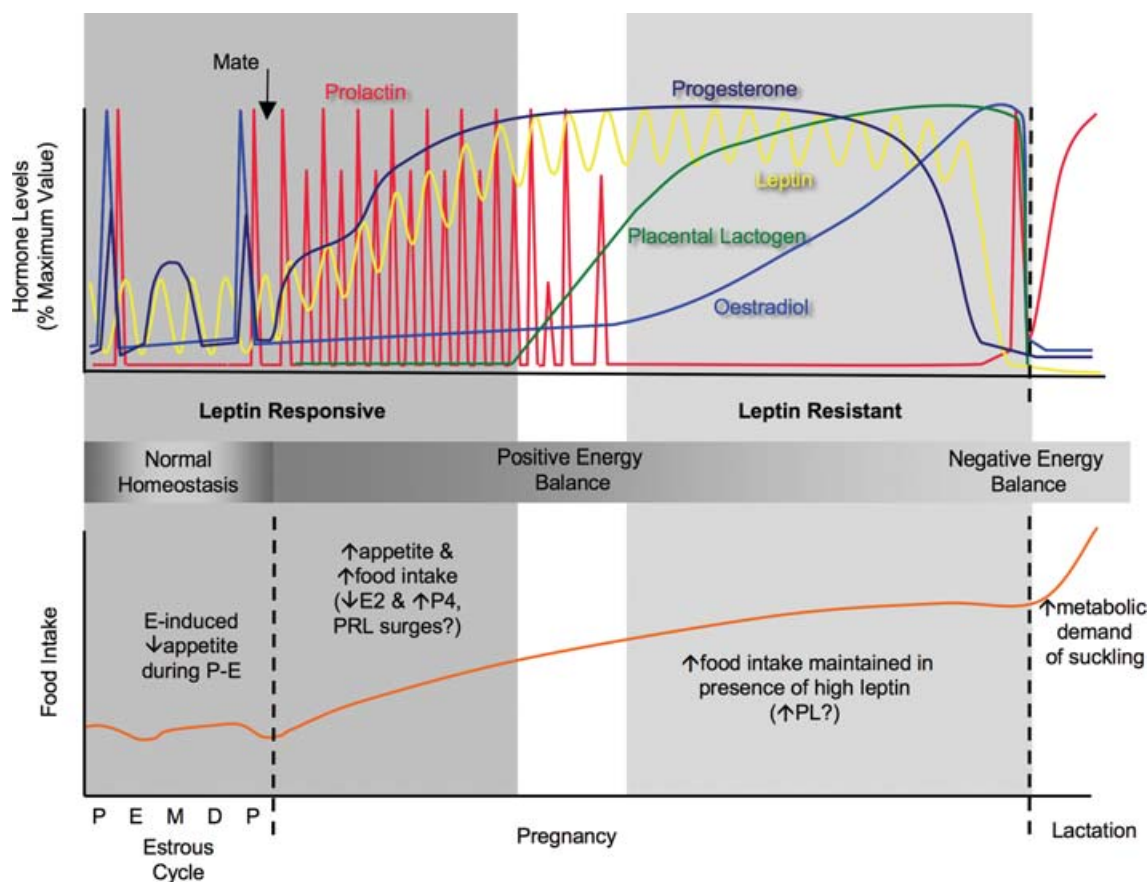

Figure 1. Schematic representation of the changing hormonal milieu and food intake during the rat four-day oestrous cycle, followed by pregnancy and lactation (see text for sources of data)
Levels are expressed as a percentage of maximum levels. During the oestrous cycle surges of oestradiol, progesterone and prolactin occur on pro-oestrus (P). Food intake on oestrous (E) displays the oestrogen-induced decrease in appetite, while food intake increases on metoestrus (M) and dioestrus (D), coincident with increased progesterone levels at this time. After mating, prolactin surges are initiated, which provide luteotrophic support for corpus luteum production of progesterone to be maintained throughout pregnancy. The placenta forms around day 7–8 of pregnancy and secretes placental lactogen at relatively constant levels, resulting in inhibition of endogenous prolactin secretion via negative feedback. Oestradiol levels gradually rise during pregnancy, and just prior to parturition the changing ratio between increasing oestradiol and decreasing progesterone causes the late-parturition surge of prolactin. Prolactin levels are then constantly elevated during lactation due to the suckling stimulus. Leptin levels show a circadian fluctuation that persists into pregnancy, when overall levels rise to be significantly elevated by day 7 of pregnancy and remain elevated until the day prior to parturition. Even in the face of elevated leptin, appetite and food intake are increased during pregnancy (significantly by day 4), creating a state of positive energy balance. Non-pregnant and early pregnant rats are responsive to the satiety actions of leptin. However by day 14 of pregnancy, rats are leptin resistant. Coincident with the timing of pregnancy-induced leptin resistance is the changing pattern of lactogen hormone secretion, from phasic prolactin surges to chronic placental lactogen secretion. During lactation the rat is in a state of negative energy balance, as food intake is increased by up to 300% to meet the high metabolic demands of the suckling young.

An increase in NPY mRNA would be expected to occur during a fast when leptin levels are low, or in the absence of leptin (Schwartz *et al.* 1996*b*). Thus, these results suggest that the maternal brain is receiving a starvation-like signal from the periphery and therefore promotes hyperphagia, in the face of high leptin concentrations, by increasing orexigenic signalling in the arcuate nucleus. The mechanism of this leptin resistance is not known. Leptin signalling in the CNS has been extensively reviewed, and multiple pathways are involved (Bjorbaek & Kahn, 2004; Fruhbeck, 2006). The most important pathway activated is thought to be the janus kinase-signal transducer and activator of transcription (JAK/STAT3) pathway. Neuron specific deletion of STAT3 leads to hyperphagia and obesity (Gao *et al.* 2004). We have shown that LepR mRNA in the arcuate is normal during pregnancy, and hence used the phosphorylation of STAT3 as a functional marker to assess leptin-induced signalling in arcuate neurons. We observed reduced overall levels of pSTAT3 activation in the arcuate nucleus after leptin administration in pregnant rats (Ladyman & Grattan, 2004), although the number of cells showing leptin-induced pSTAT3 was not different from non-pregnant animals (Ladyman & Grattan, 2005). Despite the fact that the number of cells showing leptin-induced pSTAT3 in the arcuate nucleus during pregnancy is normal, the NPY and POMC neurons are not responding appropriately to leptin, suggesting that some other signalling pathway may be altered.

Recent data show that leptin action is also dependent on a range of additional signal transduction pathways, including the phosphoinositol-3 kinase/protein kinase B (PI3K/PKB) and mitogen-activated protein kinase/ extracellular regulated protein kinase (MAPK/ERK) pathways. Leptin action on NPY and AgRP may be independent of STAT3 (Bates *et al.* 2003), and appears to be mediated by PI3K/PKB (Spanswick *et al.* 1997).

The tyrosine residue Tyr^{1138}, located on the intra-cellular tail of the leptin receptor, is critical for leptin receptor activation via the JAK/STAT3 pathway. Mice that have had Tyr^{1138} replaced with a serine residue (s/s knock-in mouse), are unable to activate STAT3 and are obese and hyperphagic and have decreased energy expenditure, but have normal reproductive function and increased linear growth (Bates *et al.* 2003). NPY mRNA levels are near normal in the s/s mice, while POMC mRNA in the hypothalamus is decreased, suggesting that the STAT3-signalling pathway is important for POMC/α-MSH signalling, whereas STAT3-independent signalling may be important for leptin action on NPY gene expression. The acute regulation of orexigenic arcuate neurons by leptin, as measured by c-fos immunoreactivity and electrophysiological changes, does not require STAT3-mediated transcription (Munzberg *et al.* 2007). Other LepR-mediated signals may therefore contribute to leptin's inhibition on orexigenic neurons. Thus, it is possible that leptin signalling through the PI3K/PKB or MAPK pathway may be altered during pregnancy. One potential mechanism whereby this pathway might be compromised would be the activation of the forkhead transcription factor (FOXO1), an important mediator in insulin signalling in the hypothalamus. Activated FOXO1 blocks the action of both leptin- and insulin-induced decreases in food intake (Kim *et al.* 2006; Kitamura *et al.* 2006), directly stimulating NPY/AgRP expression and suppressing POMC by antagonizing transcriptional actions of activated STAT3 (Kim *et al.* 2006; Kitamura *et al.* 2006). Leptin and insulin suppress appetite, at least in part, by inactivating FOXO1 through a PKB-mediated phosphorylation of FOXO1. It is possible that increased FOXO1 in the hypothalamus during pregnancy might cause loss of leptin responses, even in the presence of normal pSTAT3. These signalling pathways are illustrated in Fig. 2.

Figure 2. Diagrammatic representation of interactions between leptin (Lep) and insulin (Ins) signal transduction pathways

P = phosphorylation events. The three pathways discussed (MAPK/ERK, STAT3, PI3K/PKB) are highlighted in blue. The Jak/STAT3 pathway is the best characterized; LepR mediates signalling via the receptor-associated Jak2 tyrosine kinase, which phosphorylates the tyrosine residue Tyr_{1138} on the intracellular tail of the LepR. Phosphorylation of Tyr_{1138} recruits the cytoplasmic transcription factor STAT3, which is phosphorylated by Jak2. Phosphorylated STAT3 molecules form dimers, and translocate into the nucleus to activate gene transcription. Note, leptin can activate all three pathways, and recent work suggests that the PKB pathway is required for STAT-mediated signalling, possibly by phosphorylating and thereby inactivating FOXO1, which can otherwise act to inhibit STAT-mediated gene transcription.
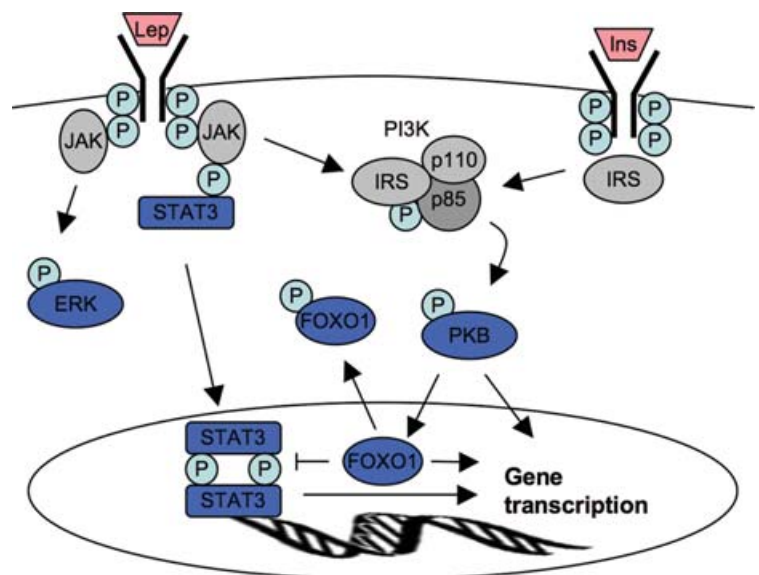
The melanocortin system

The melanocortin system is one downstream pathway activated in response to leptin, triggered through leptin action on POMC neurons. α-MSH is a derivative of the POMC precursor and acts on MC3 and MC4 receptors which have been localized using *in situ* hybridization in the paraventricular, dorsomedial hypothalamic, ventromedial hypothalamic and lateral hypothalamic areas (Mountjoy *et al.* 1994; Harrold *et al.* 1999). MC3-R and MC4-R knockout mice are obese and a small percentage of severely obese humans appear to be linked to MC4R mutations (reviewed by Spiegelman & Flier, 2001). Intra-cerebroventricular (I.C.V.) injection of melanotan II, a melanocortin agonist, inhibits feeding in hyperphagic animal models, while administration of SHU9119, a specific melanocortin antagonist, completely blocks this inhibition in feeding (Fan *et al.* 1997). To bypass any leptin resistance in first-order arcuate neurons in pregnant rats, and determine whether other parts of the leptin response pathway were altered, we injected α-MSH I.C.V. Such a treatment effectively reduces food intake in mice made leptin resistant with DIO (Hansen *et al.* 2001). Pregnant rats did not respond to central α-MSH administration with a decrease in food intake, as was seen in non-pregnant rats, suggesting that pregnancy is also an α-MSH-resistant state (S. R. Ladyman, unpublished data). Other animal models of hyperphagia and obesity respond to α-MSH or the melanocortin agonist, melanotan-II (Scarpace *et al.* 2003; Zhang *et al.* 2004), suggesting that this phenomenon might be unique to pregnancy. The mechanism for this is unknown. However, AGRP is an endogenous antagonist of melanocortin receptors, and therefore we hypothesized that there might be an increase in AGRP during pregnancy (as reported previously by Rocha *et al.* (2003). AGRP and NPY are coexpressed in a high percentage of arcuate neurons (Broberger *et al.* 1998), and over-expression of AGRP results in obesity similar to that seen in the Agouti and MC4-R knockout mice. Using *in situ* hybridization, we found AGRP mRNA did not change during pregnancy (S. R. Ladyman, unpublished data), although other groups have shown an increase during pregnancy (Rocha *et al.* 2003). Reasons for this discrepancy are unclear at this time but may be due to different time points or methodologies used. Despite this discrepancy, either no change or elevated AGRP in the face of elevated leptin during pregnancy is further evidence for leptin resistance at the level of the arcuate nucleus.

The arcuate nucleus is thought to be central in the control of appetite and body weight regulation because it contains anorexigenic and orexigenic neurons that release neuropeptides that control appetite. LepR are colocalized on these neurons and animals lacking leptin or its receptor have altered gene expression of these neuropeptides. However, unlike the obesity seen in *db/db* mice lacking functional LepR in all tissue, conditional gene-knockout of LepR just from POMC neurons (Balthasar *et al.* 2004) only results in a mildly obese phenotype. Similarly, specific deletion of the NPY gene in *ob/ob* mice only partially restores body weight (Erickson *et al.* 1996). This suggests that additional neuronal populations, elsewhere in the hypothalamus, must also be involved in mediating the anorectic actions of leptin. The ventromedial hypothalamic nucleus (VMH) has been known for some time to be involved in suppressing food intake. Early studies showed that lesions of the VMH resulted in hyperphagia, morbid obesity and impaired thermo-regulation, while lesions in the lateral hypothalamus resulted in death from starvation, thereby declaring these areas as key sites in the control of energy balance (reviewed by Elmquist *et al.* 1999). Recently, interest in the VMH has regained strength, after retrograde tracing studies show inputs from arcuate nucleus neurons into the VMH and LHA (Sternson *et al.* 2005). Leptin receptors are expressed in the VMH (Elmquist *et al.* 1998*a*) and conditional knockout of LepR on specific neurons in the VMH leads to obesity of a similar magnitude to that observed in POMC-specific LepR knockouts (Dhillon *et al.* 2006). We have shown that in leptin-resistant pregnant rats, there is a decrease in the long form of LepR in the VMH and decreased leptin-induced pSTAT3 levels, indicative of reduced leptin signalling in this area (Ladyman & Grattan, 2005). This evidence, and the role of leptin-responsive neurons in the VMH, suggests that the VMH is also a first-order leptin responsive area and is an important site for the control of food intake, especially during pregnancy (see Fig. 3).

Hormonal mechanisms inducing leptin resistance during pregnancy

Leptin resistance during pregnancy is likely to be driven by the hormonal changes characteristic of this state, such as elevated progesterone, leptin, prolactin and placental lactogen and loss of the cyclical elevations in serum oestradiol (see Fig. 1). Leptin levels in blood are elevated during pregnancy (Kawai *et al.* 1997; Amico *et al.* 1998; Ladyman & Grattan, 2004), and chronic high leptin has been shown to induce leptin resistance (Sahu, 2002). However, it is unlikely that leptin resistance during pregnancy is caused by elevated leptin, as levels of leptin rise slowly during pregnancy, and are only elevated two-to threefold for a few days prior to development of leptin resistance (Ladyman & Grattan, 2004). To induce leptin resistance with leptin, prolonged infusions (> 14 days) of high levels of leptin are required (Sahu, 2002; Zhang & Scarpace, 2006). Pregnant *ob/ob* mice treated with leptin throughout pregnancy become leptin resistant at mid-pregnancy, but it is unlikely to be a leptin-induced

effect, as non-pregnant *ob/ob* mice can be identically treated with leptin for up to 6 months with no observed resistance to leptin action (Mounzih *et al.* 1998). Gonadal steroid hormone concentrations are altered dramatically during pregnancy, and are known to influence feeding behaviour. Oestradiol has an inhibitory effect on food intake, specifically reducing meal size (Eckel & Geary, 2001; Asarian & Geary, 2002) and increasing energy utilization (Wade & Gray, 1979). Oestradiol acts within the brain to increase leptin sensitivity (Clegg *et al.* 2006), and thus, low oestradiol during pregnancy might reduce leptin responsiveness. In contrast, administration of progesterone or progesterone metabolites to either OVX or intact rats increases food intake (Hervey & Hervey, 1967; Wade, 1975; Chen *et al.* 1996). Progesterone-treated rats have an increased body weight and food intake but maintain normal plasma and CSF leptin levels (Grueso *et al.* 2001), suggesting progesterone may inhibit CNS action of leptin. Elevated levels of prolactin and the closely related hormone, placental lactogen, are also characteristic of pregnancy and lactation (Grattan, 2001) and may contribute to the hyperphagia at these times. In virgin female rats, prolactin infusion induces hyperphagia in a dose-dependent manner (Gerardo-Gettens *et al.* 1989; Sauve & Woodside, 1996), and this is a central effect, involving direct actions of prolactin in the hypothalamus (Sauve & Woodside, 2000).

To investigate the hormonal mechanisms involved in inducing leptin resistance during pregnancy, we have examined leptin responses in pseudopregnant rats, which have identical hormone profiles to early pregnancy.

Like pregnancy, pseudopregnancy is associated with high progesterone, hyperprolactinaemia and hyperphagia (Tarttelin & Gorski, 1971; Smith *et al.* 1975; Augustine & Grattan, 2007). Thus, pseudopregnancy in the rat provided an ideal model to investigate the effect of maternal hormones on appetite regulation, without the confounding influence of placental hormones. Central leptin resistance does not develop until midpregnancy in the rat, whereas hyperphagia develops almost immediately (Ladyman & Grattan, 2004). Thus, pregnancy-induced hyperphagia is not primarily caused by leptin resistance, but would be facilitated by the subsequent development of leptin resistance, which probably plays a role in the rapid increase in food intake later in pregnancy (Johnstone & Higuchi, 2001; Rocha *et al.* 2003). Leptin resistance develops between day 7 and 14 of pregnancy (Ladyman & Grattan, 2004), coincident with the development of the placenta and onset of secretion of placental lactogen (see Fig. 1). Thus, we hypothesized that placental lactogen may be a critical factor influencing appetite regulatory centres in the brain. Early pregnant, and pseudopregnant rats are characterized by pulsatile prolactin secretion and hyperphagia, but continue to have a relatively normal response to leptin (Ladyman & Grattan, 2004; Augustine & Grattan, 2007). In contrast, during the second half of pregnancy, when placental lactogen is chronically elevated, animals show a loss of response to leptin (Ladyman & Grattan, 2004). Therefore, it is possible that the pattern of prolactin secretion, whether phasic or continually high, might exert different physiological responses in regards to appetite regulation. Twice-daily injections of prolactin

Figure 3. The leptin responsive network in the hypothalamus

Inset (top, left) shows a representative *in situ* hybridization for the long form of the leptin receptor (LepR). Note strong signal in the arcuate (Arc), ventromedial (VMH) and dorsomedial (DMH) hypothalamic nuclei. Insets (top, middle and top, right) show leptin-induced activation of pSTAT3, as a functional marker for leptin responsive neurons. Note distribution in the same hypothalamic nuclei. The main panel shows a diagram of a sagittal section through the hypothalamus, showing the known and postulated (?) interactions between key hypothalamic nuclei. Leptin stimulates anorexigenic POMC neurons and inhibits orexigenic NPY/AGRP neurons in the arcuate nucleus. These neurons project to other hypothalamic nuclei, notably the paraventricular nucleus (PVN), stimulating and antagonizing melanocortin receptors (MC4R) in this nucleus, and thus regulating further downstream pathways. Leptin also acts directly in the VMH and DMH, to influence the appetite regulatory pathways in as yet poorly defined ways. Leptin actions in the VMH appear to be particularly important in the adaptive response to pregnancy.
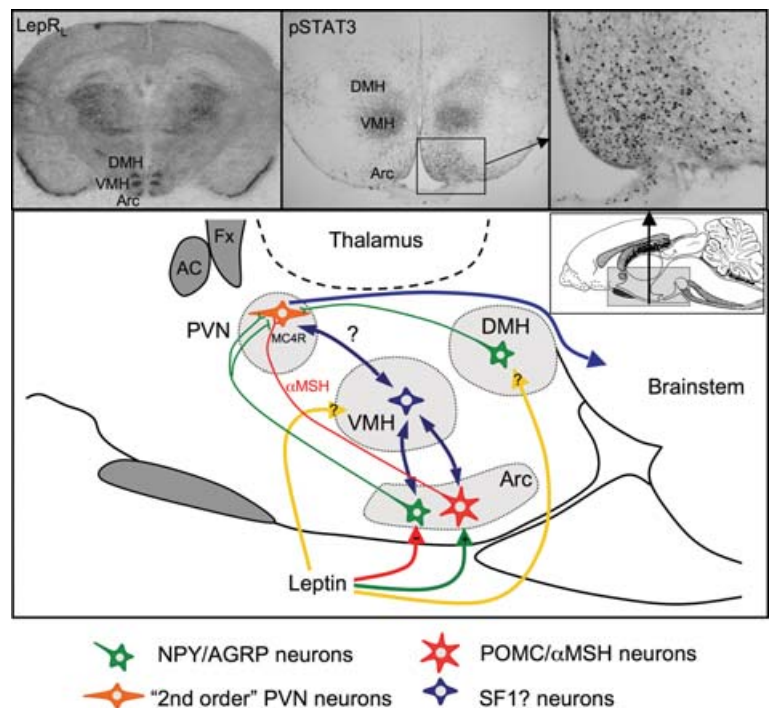
into the paraventricular nucleus of virgin rats induces a feeding response of similar magnitude to that observed during the first week of pregnancy (Sauve & Woodside, 2000), but chronically elevated prolactin, such as seen in lactating rats, increases food intake to a greater extent (Woodside, 2007). It seems likely that leptin resistance might contribute to this effect of chronic prolactin, as leptin infusion results in only a transient decrease in food intake during lactation (Woodside *et al.* 2000). Hence, we investigated if the pattern of prolactin secretion caused changes in feeding and hypothalamic responses to leptin, by giving pseudopregnant rats continuous exposure to high concentrations of prolactin. Under these conditions, the leptin-induced decrease in food intake was completely suppressed (Augustine & Grattan, 2007). Thus, by giving chronic i.c.v. prolactin infusions to mimic the pattern of placental lactogen secretion during the second half of pregnancy, we were able to induce leptin resistance in pseudopregnant female rats in the absence of any metabolic load induced by pregnancy or fetal development (Augustine & Grattan, 2007). The exact neuroendocrine changes that are occurring in the hypothalamus in response to chronic lactogenic infusion are currently unknown but may involve a loss in functional leptin receptors in key hypothalamic nuclei, or a loss in leptin signalling via intracellular signalling cascades.

Conclusion

Pregnancy is characterized by hyperphagia and fat deposition, with the subsequent development of leptin resistance allowing the hyperphagia to be maintained even in the presence of elevated leptin secretion from the growing fat deposits. Both the initial increase in food intake and the subsequent development of leptin resistance are driven by the unique pattern of hormonal changes associated with pregnancy. Our work suggests that the establishment of the placenta and the associated pattern of chronically high levels of placental lactogen secretion are essential causes of the leptin resistance seen during the latter parts of pregnancy. Indeed, leptin resistance can be induced in the absence of pregnancy, merely by mimicking the pattern of placental lactogen secretion. Thus, the developing conceptus provides a key neuroendocrine signal to the mother, inducing a resetting of maternal homeostatic mechanisms to facilitate a change in behaviour that will eventually help both mother and offspring cope with the metabolic demands of their new life together.

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