

Energy Metabolism and Leptin: Effects on Neuroendocrine Regulation of Reproduction in the Gilt and Sow

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Contents

It is well established that reproductive function is metabolically gated. However, the mechanisms whereby energy stores and metabolic cues influence appetite, energy homeostasis and fertility are yet to be completely understood. Adipose tissue is no longer considered as only a depot to store excess energy. Recent findings have identified numerous genes, several neurotrophic factors, interleukins, insulin-like growth factor binding protein-5, ciliary neurotrophic factor and neuropeptide Y (NPY) as being expressed by adipose tissue during pubertal development. These studies demonstrated for the first time the expression of several major adipokines or cytokines in pig adipose tissue which may influence local and central metabolism and growth. Leptin appears to be the primary metabolic signal and is part of the adipose tissue-hypothalamic regulatory loop in the control of appetite, energy homeostasis and luteinizing hormone (LH) secretion. Leptin's actions on appetite regulation are mediated by inhibition of hypothalamic NPY and stimulation of proopiomelanocortin. Its effects on gonadotropin-releasing hormone (GnRH)/LH secretion are mediated by NPY and kisspeptin. Thus, leptin appears to be an important link between metabolic status, the neuroendocrine axis and subsequent fertility in the gilt and sow.

Introduction

It is generally accepted that there are two modes of luteinizing hormone (LH) secretion in the pig (Kraeling and Barb 1990), pulsatile secretion and surge secretion. These patterns of LH secretion reflect the pattern of gonadotropin-releasing hormone (GnRH) released from neurosecretory neurones within the hypothalamus into the hypothalamic-hypophysial portal system (Williams 1989). The importance of pulsatile GnRH/LH secretion was demonstrated in studies that induced precocious oestrus and ovulation in intact pre-pubertal gilts with hourly intravenous (i.v.) injections of GnRH (Lutz et al. 1984; Pressing et al. 1992). In addition, hourly administration of GnRH to anestrus post-partum sows induced oestrus and ovulation (Cox and Britt 1988). Thus, GnRH neurones secrete their product in an episodic manner, but interoceptive and exteroceptive factors detected by the central nervous system are translated by neuroendocrine mechanisms into signals which alter the pattern of GnRH and subsequent LH secretion. For example, interoceptive signals, such as gonadal and adrenal steroids, metabolites, and other neuronal signals act to modulate frequency and amplitude of GnRH pulses.

The importance of nutrition and metabolic state in initiating and maintaining reproductive function and growth is well established (reviewed by Prunier et al. 1993; Prunier and Quesnel 2000; Barb et al. 2002). Dietary nutrients influence expression of metabolic

pathways that allow animals to achieve their full genetic potential for reproduction and growth. These pathways are complex and involve appetite regulation along with regulation of the reproductive and growth axis, as well as gonadal function (Wade et al. 1996; Barb et al. 1999; Muller et al. 1999). Identification of blood-borne metabolic signals that activate the GnRH/LH pulse generator, alter the pattern of growth hormone (GH) secretion, and regulate appetite remains elusive. Recent reports have identified adipose tissue as a source of putative metabolic signals that regulate the neuroendocrine axis (Schwartz et al. 1996; Barb and Kraeling 2004; Barb et al. 2006a).

Nutrition and Reproduction

Research has examined backfat levels in gilts relative to future reproductive performances. Several reports demonstrated that gilts with higher backfat subsequently had a shorter weaning to first oestrus interval, larger litter size, and higher farrowing rate as second parity sows compared to gilts with lower amounts of backfat (Tummaruk et al. 2001). Whittemore (1996) suggested that primiparous sows should not be allowed to have backfat depth fall below 14 mm or rise above 25 mm (lipid/protein > 1:1 and < 2:1) for optimum reproductive performance. It is widely accepted that severe underfeeding or overfeeding of the breeding female will adversely affect reproductive performance, though the endocrine control of this relationship is not well defined (Aherne and Kirkwood 1985).

During lactation, feed intake of sows is often inadequate to meet nutrient requirements for maintenance and lactation. There is increasing evidence that nutrition, reduction in backfat, changes in metabolic state, and associated changes in metabolites and metabolic hormones such as insulin, insulin-like growth factor (IGF)-I, GH and leptin, influence the reproductive axis in the sow (Prunier and Quesnel 2000). Through mobilization of fat and protein reserves, sows overcome the nutritional demands of lactation (Shields et al. 1985). However, if the sow mobilizes too much body protein and fat, a decrease in litter growth and ovarian function will occur (Clowes et al. 2003; Quesnel et al. 2005). Loss of body protein during lactation may be of greater importance than loss of body fat (King and Martin 1989; Brendemuhl et al. 1989); particularly it has a greater impact on first-litter sows, because these animals are still growing during their first lactation (King and Williams 1984). High feed intake, weight gain and increased backfat during gestation will lead to a significant decrease of feed intake and weight loss during

lactation (Weldon et al. 1994; Whittemore 1996). Almeida et al. (2001) showed that moderate feed restriction during the luteal phase of the oestrous cycle in the gilt affects ovulation rate and the progesterone rise after the LH surge.

The role of insulin on LH secretion in the post-partum sow is somewhat controversial. Tokach et al. (1992) reported that insulin levels during early lactation were correlated with LH peak amplitude and thus appear to be associated with reproductive function. Koketsu et al. (1998) reported greater lactation feed intake was associated with greater concentrations of insulin and glucose, greater LH pulse frequency prior to weaning and shorter farrowing-to-oestrus interval. In addition, exogenous insulin administered on the day of weaning for 4 consecutive days in primiparous sows decreased the average interval from weaning to oestrus and increased the percentage of sows in oestrus (Whitley et al. 1998). One model used to study the effect of insulin on the hypothalamic-pituitary axis is the diabetes-induced animal. In the diabetic ovariectomized gilt, withdrawal of insulin therapy for 4 days prevented the oestradiol-induced preovulatory-like LH surge, but did not affect pulsatile LH secretion (Angell et al. 1996). This finding indicates that diabetes mellitus alters the sensitivity of the hypothalamic-pituitary axis to oestradiol and pituitary responsiveness to GnRH. Pituitary cell culture experiments confirmed that the sensitivity of the pituitary gland to GnRH decreased after removal of insulin therapy for 7 days in diabetic pigs (Angell et al. 1996), suggesting that insulin may play a role in maintaining pituitary responsiveness to GnRH. In contrast, insulin administration on 5 consecutive days prior to weaning in feed-restricted sows did not affect weaning to oestrus interval or ovulation rate (Quesnel and Prunier 1998). In primiparous sows, neither glucose nor insulin concentrations were correlated with LH secretion during lactation, after weaning or with the weaning to oestrus interval (van den Brand et al. 2000). Moreover, van den Brand et al. (2001) found in primiparous sows that plasma IGF-I concentrations on day 22 (weaning) were positively correlated with LH pulse frequency while Quesnel et al. (1998) reported no relationship between IGF-I concentrations and LH pulse frequency before or after weaning in primiparous sows. Thus, the definitive role of IGF-I and insulin on LH secretion and subsequent reproductive performance in the post-partum sow has yet to be determined.

Serum and milk leptin concentrations in the primiparous and multiparous lactating sow were positively correlated with backfat thickness and level of dietary energy fed during gestation, as well as feed consumption (Estienne et al. 2000, 2003). A positive correlation was observed among plasma insulin, leptin and LH concentrations in lactating sows fed *ad libitum* but not in feed-restricted sows. Moreover, the weaning to oestrus interval was greater in the feed-restricted sows compared to controls (Mao et al. 1999). De Rensis et al. (2005) reported that in sows classified as fat, medium or thin based on backfat thickness at farrowing, serum leptin concentrations were greater in fat sows compared to medium and thin animals at weaning. In addition, there was no relationship between leptin concentrations and

reproductive performance after weaning. However, plasma leptin concentrations were associated with backfat depth, and loss of backfat was associated with reproductive performance. These findings provide evidence that circulating leptin, LH concentrations and feed consumption during lactation are influenced by dietary energy intake during pregnancy or lactation in the sow, suggesting that leptin may serve as a permissive metabolic signal that may be necessary for activation of the reproductive axis in the post-partum sow.

Growth and Metabolic Signals

In addition to developmentally related maturation of the neuroendocrine axis, permissive peripheral signals are associated with attainment of a minimum percentage of body fat (Frisch 1984); one example is leptin which may play a role in the timing of puberty (Barb et al. 2001a; Barb and Kraeling 2004). During the pre-pubertal period, expression of a number of hypothalamic genes associated with appetite and growth regulation appear to be developmentally regulated. For example, hypothalamic expression of the biological form of the leptin receptor (OB-rb), adipose tissue leptin expression and serum leptin concentrations increased by 3.5 months of age (Qian et al. 1999; Lin et al. 2001). In addition, Kojima et al. (2007) demonstrated a positive relationship between pre-weaning weight and post-weaning hypothalamic agouti-related protein, orexin, type-2 orexin receptor and NPY gene expression. These genes may play an important role in post-weaning growth and development in the gilt. Furthermore, their encoded proteins are well positioned anatomically to interact with GnRH (Kraeling and Barb 1990) and GHRH (Leshin et al. 1994) neurones and appetite regulating neurones (Lawrence et al. 1999; Matteri 2001). Consistent with this idea, central administration of leptin increased GH secretion and suppressed feed intake in the pre-pubertal gilt (Barb et al. 1998) and *in vitro* leptin stimulated GnRH release from porcine hypothalamic explants (Barb et al. 2004). These changes in the growth/reproductive axis appear to be in concert with the timing of puberty.

We previously reported that metabolic response to acute feed deprivation occurred more rapidly in pre-pubertal gilts compared to mature gilts, likely because pre-pubertal gilts have a higher metabolic rate, smaller energy reserves and thus a greater nutrient intake requirement for growth (Barb et al. 1997). An acute 24 h fast increased serum free fatty acid concentrations, and decreased leptin pulse frequency but not mean serum leptin concentrations in the ovariectomized pre-pubertal gilt (Barb et al. 2001a). Furthermore, short term feed restriction decreased leptin secretion and LH pulse frequency in the mature ovariectomized gilt (Whisnant and Harrell 2002) and decreased LH secretion in the intact pre-pubertal gilt (Booth et al. 1996), while a 3-day fast reduced adipose tissue leptin mRNA in castrate male pigs (Spurlock et al. 1998). These results support the idea that leptin may serve as a metabolic signal in the activation of the reproductive axis.

In pre-pubertal gilts short term feed restriction to 33% of control diet for 8 days failed to affect LH or

Table 1. Microarray analysis of several fat depots demonstrated that thyroid hormone receptors were collectively upregulated while several other receptors (MCIR, FGF4) and lipogenic (LPL, SCD) enzymes were downregulated with fasting^a

Fat depot	Gene	Estimate	SE	T-value	p-value
Perirenal	Thyroid hormone receptor alpha 2 (<i>Sus scrofa</i>)	-0.20083	0.051199	-3.92248	0.000254
Mesenteric	Thyroid hormone receptor, alpha	-0.16131	0.047101	-3.42473	0.001195
Leaf	Thyroid hormone receptor, alpha	-0.15323	0.030834	-4.96954	0.000007
Leaf	Thyroid hormone receptor alpha 2 (<i>Sus scrofa</i>)	-0.31586	0.089853	-3.51524	0.000909
Leaf	Melanocortin 1 receptor	0.357846	0.175065	2.044075	0.045929
Perirenal	Melanocortin 1 receptor	0.396369	0.117805	3.364614	0.001431
Leaf	Fibroblast growth factor receptor 4	0.40214	0.121846	3.300404	0.001731
Perirenal	Fibroblast growth factor receptor 4	0.44749	0.139023	3.218823	0.002199
Perirenal	Stearoyl-CoA desaturase	0.914822	0.323623	2.826815	0.006618
	Lipoprotein lipase	0.43321	0.125486	3.452266	0.0011
Mesenteric	Stearoyl-CoA desaturase	1.40823	0.304109	4.630675	0.000024
	Lipoprotein lipase	0.14577	0.069703	2.091302	0.04131
Leaf	Stearoyl-CoA desaturase	0.170878	0.071794	2.380111	0.020937
	Lipoprotein lipase	0.380183	0.079236	4.798133	0.000013

^aAnalysis of the microarray data was conducted with either LOWESS normalization based or an ANOVA normalization based method to determine the influence of fasting on gene expression.

leptin secretion or to affect backfat leptin, Ob-rb, adipocyte fatty acid binding protein, or the transcription factors peroxisome proliferator-activated receptor- γ 2 and CCAAT-enhancer-binding protein- α expression (Hart et al. 2007). However, based on maintenance requirements for the pre-pubertal gilt (NRC 1998) used in that study, the feed restricted gilts were actually fed 124% of the maintenance requirement. This may explain the failure of feed restriction to affect LH or leptin concentrations. Although feed restriction did prevent body weight and backfat gain adipocyte function was altered as evidenced by upregulation of thyroid hormone receptor- α in perirenal, leaf and mesenteric fat depots in feed restricted animals; this coincided with elevated thyroxin concentration (GJ Hausman, CR Barb, HA Hart, unpublished data; Table 1). These changes may in part represent an attempt to maintain thermogenesis, as well as immune and neuroendocrine homeostasis.

Adipose Tissue as an Endocrine Organ

Adipose tissue plays a more dynamic role than previously thought in physiological mechanisms and whole-body homeostasis. Studies now show that the role of adipose tissue includes responding to nutrient, neural and hormonal signals, and secreting factors or 'adipokines' that control feeding, thermogenesis, immunity, and neuroendocrine function (review by Ahima et al. 2006). The current evidence indicates that of all the adipose tissue secreted factors, or 'adipokines', interleukins (IL)-6, IL-8, plasminogen activator inhibitor-1, leptin and adiponectin can be considered true endocrine factors (review by Hauner 2005). Furthermore, the secretory function of adipose tissue is adversely influenced by both obesity (Hauner 2005) and impaired adipose tissue accretion (review by Guerre-Millo 2004). Therefore, body condition or adiposity by virtue of secreted adipokines could impact or regulate, in part, physiological states such as reproductive condition or status. Adipose tissue constitutes the largest amount of stored energy in the body (Loftus 1999), and regulation of energy expenditure

involves a balance of several factors, such as feeding behaviour, adipose tissue mass and activation of catabolic processes (e.g. lactation). Changes in body weight or nutritional status are characterized by alterations in serum concentrations of many hormones and growth factors that regulate adipocyte function and leptin secretion (Barb et al. 2001b). Recent microarray analyses by our laboratory revealed that 21 genes encoding secreted proteins were expressed 40-fold over background in neonatal porcine adipose tissue and porcine pre-adipocyte cultures (Hausman et al. 2006). Additionally, agouti gene expression was detected by RT-PCR in pig adipose tissue. Proteomic analysis of adipocyte culture conditioned media identified several secreted proteins, including several neurotrophic factors, IL-1A, IL-1B, IL-8, IL-6, IL-15, and IGF binding protein-5. Through microarray and RT-PCR analyses, we recently demonstrated that in addition to these, several other cytokines, e.g., ciliary neurotrophic factor and NPY, are expressed by adipose tissue during pubertal development (Hausman et al. 2007). These studies demonstrate for the first time the expression of several major adipokines or cytokines in pig adipose tissue which may influence local and central metabolism and growth. Thus, these reports support the idea that adipose tissue functions as an endocrine organ.

Morphological studies have revealed that adipose tissue is innervated by adrenergic nerve fibres (Hausman and Richardson 1987). Further, immunocytochemical data revealed that most of the subpopulations of the adrenergic leptin receptor immuno-reactive (OBR-IR) neurones supplying fat tissue in the pig were positive for NPY and tyrosine hydroxylase immunoactivity (Czaja et al. 2002). Moreover, immuno-positive neurones for OBR were located in the paraventricular nucleus, ventromedial nucleus, anterior hypothalamic area, pre-optic area, arcuate nucleus and supraoptic nucleus (Czaja et al. 2003). Collectively, these studies provide morphological data demonstrating that hypothalamic OBR containing neurones are transsynaptically connected to the perirenal fat depot. Therefore, the above evidence supports a direct link between hypothalamic

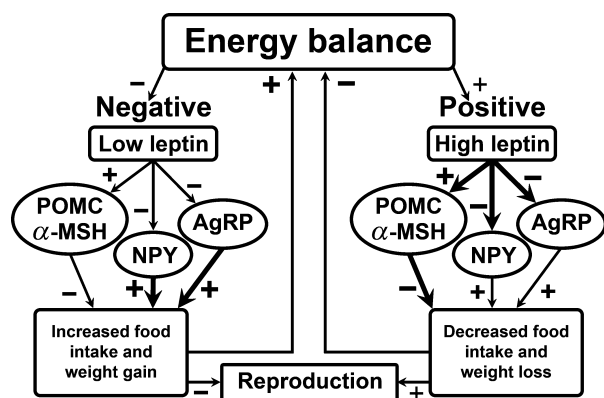


Fig. 1. Schematic illustration of leptin coordination of energy homeostasis and neuroendocrine function: Leptin is secreted in response to changes in energy balance and acts on the hypothalamus to control food intake, reproduction and adipocyte function. Positive energy balance increases blood leptin concentrations, suppresses expression of neuropeptide Y (NPY) and agouti related peptide (AgRP), and upregulates proopiomelanocortin (POMC) and α -melanocyte stimulating hormone (α -MSH). Negative energy balance decreases blood leptin concentrations, stimulates expression of NPY and AgRP and downregulates POMC and α -MSH. Thick black lines represent strong regulator tone and thin black lines weak regulator tone. Modified from Loftus (1999)

neurons in the regulation of fat metabolism and reproduction.

Under changing metabolic states, which may increase adipose tissue mass and circulating leptin concentrations and stimulate catabolic pathways within the hypothalamus, conversely decreased adipose mass and reduced blood leptin concentrations stimulate anabolic pathways and inhibit catabolic pathways (Fig. 1). These two pathways comprise a number of genes that not only regulate appetite and energy balance but also impact the reproductive axis either directly in the case of NPY, or indirectly by altering adipocyte function and hence the expression of genes and their protein products. For example, hypothalamic NPY and proopiomelanocortin (POMC) associated products α -melanocyte stimulating hormone (α -MSH) and beta endorphin can affect hypothalamic GnRH release and subsequent feeding behaviour (Barb et al. 1994, 1998, 2006b; Crown et al. 2007). Peripherally, adipocyte leptin expression and secretion can suppress appetite (Barb et al. 1998), stimulate hypothalamic GnRH release (Barb et al. 2004) and reverse the inhibitory effect of energy deprivation on LH secretion (Whisnant and Harrell 2002). Thus, as previously stated, reproductive function is metabolically gated, but mechanisms interfacing energy stores or metabolic cues and fertility are not completely understood.

Identification of targets of leptin in the hypothalamus

It is well established that reproductive function is metabolically gated. However, the mechanisms whereby energy stores and metabolic cues influence fertility are yet to be completely understood. The effects of leptin appear to be mediated through modulation of hypothalamic NPY expression (Campfield et al. 1996). In the pig, the presence of biologically-active OBR in the

hypothalamus and pituitary (Lin et al. 2000) and the fact that leptin increased LH secretion from pig pituitary cells and GnRH release from hypothalamic tissue *in vitro* (Barb et al. 2004) suggest that leptin acts through the hypothalamus. There is strong evidence from colocalization of leptin receptor mRNA with NPY gene expression that hypothalamic NPY is the primary potential target for leptin in the pig (Czaja et al. 2002). Moreover, central administration of NPY suppressed LH secretion and stimulated feed intake and reversed the inhibitory action of leptin on feed intake (Barb et al. 2006b). However, NPY alone may not mediate the action of leptin, since leptin failed to effect NPY release from pig hypothalamic-preoptic area tissue fragments (Barb et al. 2004). Furthermore, metabolic signals may in part be communicated to GnRH neurones via other neuropeptides such as galanin-like peptide (Rich et al. 2007), α -MSH (Crown et al. 2007), β -endorphin (Barb et al. 1994), or kisspeptin (Arreguin-Arevalo et al. 2007; Luque et al. 2007).

The kisspeptins are potent stimulators of the GnRH/LH axis (Castellano et al. 2006; Arreguin-Arevalo et al. 2007; Luque et al. 2007). The kisspeptins are a group of structurally related peptides that are products of the kisspeptin-1 (KiSS-1) gene (Ohtaki et al. 2001; Kotani et al. 2001). Synthesized as a pre-prohormone, it is cleaved to liberate a 54 amino acid peptide which can be proteolytically processed (Takino et al. 2003) to shorter variants, all of which share the same amidated C-terminus and retain full biological activity. Kisspeptins acting through their cognate receptor, GPR54, are thought to be an important determinant in the onset of puberty (Shahab et al. 2005; Smith and Clarke 2007). Thus, hypothalamic kisspeptin and its receptor, GPR54, may serve as an essential gatekeeper of GnRH neurones and hence of reproductive function. Kisspeptins play a role in the timing of puberty onset (Tena-Sempere 2006c) and act on the gonadotropic axis *via* the release of hypothalamic GnRH (Tena-Sempere 2006a; Dungan et al. 2006). The hypothalamic expression of KiSS-1 gene is under the control of sex steroids, and KiSS-1 neurones are involved in mediating the negative and positive feedback effects of oestradiol on gonadotropin secretion (Smith et al. 2005, 2006). Moreover, the hypothalamic KiSS-1 system may also convey the modulatory action of metabolic signals to the GnRH neurones (Tena-Sempere 2006b; Luque et al. 2007). A recent report demonstrated that short-term fasting resulted in a decline in hypothalamic KiSS-1 and GPR54 mRNA levels at 12 and 24 h which preceded the reduction in GnRH gene expression at 48 h (Luque et al. 2007). Moreover, leptin and not IGF-I or insulin stimulated KiSS-1 expression in mouse hypothalamic cell line N6. Furthermore, hypothalamic KiSS-1 expression was decreased in NPY null mice and this was reversed by NPY administration (Luque et al. 2007). These reports support the idea that leptin and NPY are key mediators of metabolic regulation of the hypothalamic KiSS-1 system and subsequent GnRH release.

Lents et al. (2008) recently demonstrated that administration of kisspeptin into the lateral ventricle of the brain stimulated LH and follicle stimulating hormone (FSH) secretion but not GH in the pre-pubertal gilt,

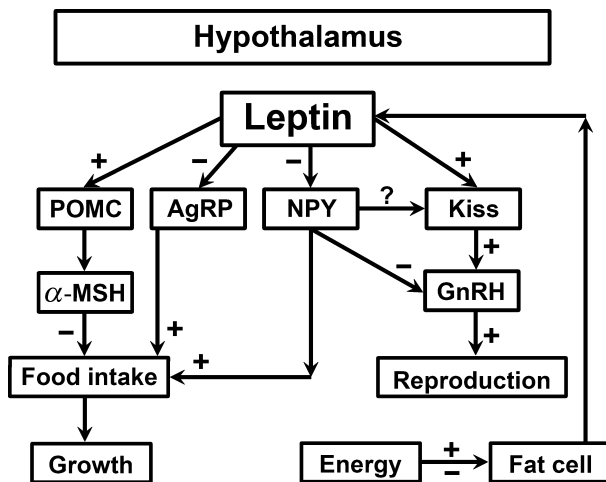


Fig. 2. Schematic illustration of leptin coordination of energy homeostasis and neuroendocrine function: Leptin is secreted in response to changes in energy balance. Leptin acts on the hypothalamus to control food intake, reproduction and, by suppressing expression of neuropeptide Y (NPY) and agouti related peptide (AGRP) and upregulation of proopiomelanocortin (POMC), α -melanocyte stimulating hormone (α -MSH) and kisspeptin (KiSS)

while peripheral administration of kisspeptin increased serum concentrations of LH but not FSH or GH. These data illustrate that kisspeptin can activate the brain-pituitary axis and may be part of an important mechanism regulating activation of the GnRH/LH release and initiating onset of puberty in swine. Moreover it is tempting to speculate that the kisspeptin neuronal pathway may play a role in interfacing metabolic status of the post-partum sow with the GnRH/gonadotropic axis and hence may affect the post-weaning fertility of the sow.

In conclusion evidence presented supports the concept that metabolic signals, which reflect changes in energy balance, affect both the hypothalamic GnRH/LH pulse generator and appetite. Changes in fat metabolism in response to changes in feed intake and energy balance alter adipocyte function and circulating concentrations of IGF-I and leptin. A preponderance of evidence cited above suggests that leptin may serve as the primary metabolic signal interacting with neuropeptides such as kisspeptin and NPY that link energy status with the neuroendocrine axis and subsequent reproduction (Fig. 2).

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