Chapter 2

Role of Adipose Secreted Factors and Kisspeptin in the Metabolic Control of Gonadotropin Secretion and Puberty[1]

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1. Introduction

1.1. Adipose tissue as an endocrine organ

Recent investigations from many species continue to reinforce and validate adipose tissue as an endocrine organ that impacts physiological mechanisms and whole-body homeostasis. Factors secreted by adipose tissue or "adipokines" continue to be discovered and are linked to important physiological roles (Ahima, 2006) including the innate immune response (Schäffler & Schöolmerich, 2010). In a number of recent experiments transcriptional profiling demonstrated that 5,000 to 8,000 adipose tissue genes were differentially expressed during central stimulation of the melanocortin 4 receptor (Barb et al., 2010a) and several conditions such as fasting (Lkhagvadorj et al., 2009) and feed restriction (Lkhagvadorj et al., 2010). In contrast, 300 to 1,800 genes were differentially expressed in livers in these three studies (Barb et al., 2010a; Lkhagvadorj et al., 2009, 2010). This degree of differential gene expression in adipose depots reflects the potential influence of adipose tissue as a secretory organ on multiple systems in the body. Furthermore, advances in the study of adipose tissue gene expression include high throughput technologies in transcriptome profiling and deep sequencing of the adipose tissue microRNA transcriptome (review, Basu et al., 2012).

Recent proteomic studies of human and rat adipocytes have revealed the true scope of the adipose tissue secretome (Chen et al., 2005; Kheterpal et al., 2011; Lehr et al., 2012; Lim et al., 2008; Zhong et al., 2010). With refined and advanced proteomics techniques, these studies have revealed that many of the adipose tissue secreted factors identified at the gene level do indeed encode secreted proteins (Chen et al., 2005; Kheterpal et al., 2011; Lehr et al., 2012;

[1] Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Lim et al., 2008; Zhong et al., 2010). The presence of an N-terminal secretion signal peptide validates secreted proteins in conditioned media (Renes et al., 2009). In many of these studies, the presence or absence of a signal peptide was used to validate or identify truly secreted adipocyte proteins (Chen et al., 2005; Lehr et al., 2012; Lim et al., 2008; Zhong et al., 2010). In these studies, the percentage of total apparent secreted proteins that were considered secreted (+ signal peptide) ranged from 39 to 75% and the total number of secreted proteins ranged from 164 to 263 (Chen et al., 2005; Lehr et al., 2012; Lim et al., 2008; Zhong et al., 2010). However, the signal peptide approach could underestimate the adipocyte derived proteins present in the extracellular space (review, Renes et al., 2009). For instance, a blocking strategy has been used to distinguish between true secreted proteins and proteins that simply "leak" from the cell (review, Renes et al., 2009). Continued development and refinement of proteomic approaches in the study of the adipose tissue secretome will ultimately confirm the endocrine status of adipose tissue.

1.2. Adipose tissue as a modulator of gonadotropin secretion

Adipose tissue plays a role in whole-body homeostasis by acting as an endocrine organ, which was clearly demonstrated with the discovery of leptin. Evidence indicates a strong link between neural influences and adipocyte expression and secretion of leptin and other adipokines such as other cytokines (interleukins), neurotrophic factors (ciliary neurotrophic factor, CNTF; brain-derived neurotrophic factor, BDNF), insulin-like growth factor (IGF–I, and –II), binding protein (IGFBP-5), and neuropeptides such as neuropeptide Y (NPY) and nesfatin-1 (Table 1). Developmental changes in these relationships are considered important for onset of puberty. Leptin augments secretion of gonadotropins which are essential for initiation and maintenance of normal reproductive function, by acting centrally at the hypothalamus to regulate the gonadotropin-releasing hormone (GnRH) and neuronal activity. The effects of leptin on GnRH are mediated through interneuronal pathways involving NPY, proopiomelanocortin (POMC) and kisspeptin. Increased infertility associated with diet induced obesity or central leptin resistance are likely mediated through the kisspeptin-GnRH pathway. Furthermore, leptin regulates reproductive function by altering the sensitivity of the pituitary gland to GnRH. Other putative metabolic signals are circulating long chain fatty acid which can signal nutrient availability to the central nervous system (CNS) and alter feed intake and glucose availability.

2. Free Fatty Acids (FFA)

2.1. Long-chain fatty acids act in the CNS

The control of appetite and metabolism in response to changes in nutrient availability occurs in part at the level of the hypothalamus (Barb et al., 1999, 2001a; Woods et al., 1998). Thus, macronutrients, such as carbohydrates and lipids, play a role in regulating peripheral concentrations of leptin and insulin (Ahima et al., 1996), which in turn has a direct effect on appetite and energy expenditure primarily through the hypothalamus (Barb et al., 2006; Woods et al., 1998). Levin et al. (1999) reported that hypothalamic neurons may directly detect nutrients. To that extent, treatment with a fatty acid synthase inhibitor *r*educed food

Regulatory – secreted factors			Receptors
adiponectin	IFNG	IL-5	ADIPOR2
adipsin	IGFBP-1	IL-6	BMPR2
agouti	IGFBP-3	IL-8	EDNRB
ANG	IGFBP-4	leptin	ESR1
ANGPTL2, ANGPTL4	IGFBP-5	PAI-1	FGFR1, FGFR4
RBP1, RBP4	IGFBP-7	RANTES	GNRHR2
APO-A1	IL-10	RTN	IFNGR1
APO-CIII	IL-12	TGF- β, TGF-β3	IGF-IR, IGF-IIR
APO-E	IL-15	THBS1	IL-4R, IL-10R
APO-R1	IL-18	TNFα	PGRMC1
BDNF	IL-1A	VEGFC	INSR
bFGF	IL-1B	visfatin	NGFR
CNTF	IL-1RN	CTGF	OB-rb
IGF-I, IGF-II	IL-4	NPY	THRA, THRA2, TSHR
Chemokine ligands 2, 3, 4, 12	Compliment component 1, 2, 4A, 6X, C7	TGF-α	EGFR
BMP-4, BMP-15	CTRP4, CIQTN4	MCP-1	LDLR
RLN	PDGFD	NUCB2, nesfatin-1	LHCGR
LPL			TLR 4
			AGTR1

Abbreviations: ADIPOR2 = adiponectin receptor 2, AGTR1 = angeotensin II receptor, ANG = angiotensin, ANGPTL = angiopoietin-like protein, APO = apolipoprotein, BDNF = brain-derived neurotrophic factor, bFGF = basic fibroblast growth factor, BMP = bone morphogenic protein, BMPR2 = bone morphogenic protein receptor 2, CIQTN4 = complement-c1q tumor necrosis factor-related protein 4, CNTF = ciliary neurotrophic factor, CTGF = connective tissue growth factor, CTRP4 = complement-c1q tumor necrosis factor-related protein 4, EDNRB = endothelin receptor type B, EGFR = epidermal growth factor receptor, ESR1 = estrogen receptor 1, GNRHR2 = gonadotropin-releasing hormone receptor 2, IFNG = interferon gamma, IGF = insulin-like growth factor, IGF-IR = IGF-I receptor, IGFBP = insulin-like growth factor binding protein, IL = interleukin, INSR = insulin receptor, LDLR = low density lipoprotein receptor, LHCGR = luteinizing hormone-choriogonadotropin receptor, LPL = lipoprotein lipase, MCP-1 = monocyte chemoattractant protein-1, NGFR = nerve growth factor receptor, NPY = neuropeptide Y, OB-rb = long form leptin receptor, NUCB2 = nucleobindin 2, PAI-1 = plasminogen activator inhibitor-1, PDGFD = platelet derived growth factor D, PGRMC1 = progesterone receptor membrane component 1, RANTES = chemokine (c-c motif) ligand 5, RBP = retinol binding protein, RLN = relaxin, TGF = transforming growth factor, RTN = reticulon, THR = thyroid hormone receptor, TLR = toll-like receptor, TNF = tumor necrosis factor, TSHR = thyroid-stimulating hormone receptor, VEGFC = vascular endothelial growth factor C.
References: Barb et al., 2010a; Basu et al., 2012; Chen et al., 2005; Hausman & Hausman, 2004; Hausman et al., 2009; Lehr et al., 2012; Lim et al., 2008; Lkhagvadorj et al., 2009, 2010; Renes et al., 2009; Zhong et al., 2010

Table 1. List of representative genes and proteins reported to be expressed by adipose tissue of humans, large animals, and rats.

intake and body weight in mice by reducing expression of NPY in the hypothalamus via a malonyl-Coenzyme A mechanism, which supports the idea that lipid metabolism in the CNS plays a role in the control of appetite (Loftus et al., 2000). Furthermore, long-chain Furthermore, long-chain fatty acyl CoAs (LC-CoAs), such as oleyl-CoA, can activate ATP-sensitive K^+ channels in non-neuronal cells (Larsson et al., 1996). Circulating fatty acids gain rapid access to the brain, where they equilibrate with neuronal LC-CoAs (J.C. Miller et al., 1987; Rapaport, 1996). They are then further metabolized via mitochondria β-oxidation or incorporated into phospholipids (J.C. Miller et al., 1987; Rapaport, 1996). Obici et al. (2002) hypothesized that fatty acids may signal nutritional status to selective neurons in the CNS and activate a feedback loop designed to curtail further influx of nutrients into the circulation. To that extent, Obici and coworkers (2002) reported that intracerebroventricular (i.c.v.) administration of the long-chain fatty acid, oleic acid, suppressed glucose production and feed intake. In addition, this was accompanied by a reduction in hypothalamic expression of NPY. This neuronal circuit plays a role in maintaining energy homeostasis by switching fuel sources from carbohydrates to lipids and by limiting circulating endogenous and exogenous nutrients. Disruption of this circuit may play a role in obesity, type 2 diabetes and other endocrine abnormalities (for a review, see Obici, 2009), which are often accompanied by gonadotropin insufficiency.

2.2. Regulation of gonadotropin secretion by long-chain fatty acids

In the pig, feed deprivation results in a rapid mobilization of FFA from peripheral fat depots, but maintenance of euglycemia suggests increased hydrolysis of triglycerides and FFA oxidation resulting in a glucose sparing effect (Barb et al., 1997). We previously reported that metabolic response to acute feed deprivation occurred more rapidly in prepubertal gilts compared to mature gilts, likely because prepubertal gilts have a higher metabolic rate, smaller energy reserves and thus a greater nutrient intake requirement for growth (Barb et al., 1997). In mature animals, chronic feed restriction resulted in cessation of estrous cycles and lower concentrations of plasma insulin, increased levels of FFA and reduced LH pulse frequency compared to controls (Armstrong & Britt, 1987). This brings into question, therefore, if alterations in serum concentrations of FFA influence hypothalamic-pituitary function. To address this matter, prepubertal gilts received intravenous (i.v.) injection of a lipid emulsion which consisted of the following fatty acids: linoleic (65.87%), oleic (17.7%), palmitic (8.8%), linolenic (4.2%) and stearic (3.43%) acid. The fatty acid content of the lipid emulsion was comparable to that present in the circulation of the pig (Cera et al., 1989). Lipid emulsion injection enhanced the LH response to GnRH (Barb et al., 1991), whereas infusion of lipid emulsion at 1 hour intervals increased serum LH pulse amplitude without effecting LH pulse frequency (Barb et al., 1991). Dispersed cells of the anterior pituitary gland of the pig were cultured to determine whether the effects of FFA *in vivo* occur at the pituitary without the benefit of input from the CNS. The long-chain fatty acids, oleic and linoleic acids increased basal LH release. In contrast oleic acid suppressed the GnRH-induced release of LH (Figure 1). The response for linoleic acid was equivocal (Barb et al., 1995). These events seem to be mediated at the plasma membrane because oleic and linoleic acids did not block the forskolin-induced release of LH (Barb et al., 1995). These results may explain the altered

neuroendocrine activity observed during periods of feed restriction and fast. To that extent, administration of oleic acid into the third ventricle suppressed food intake and hypothalamic expression of NPY in the rat (Obici et al., 2002).

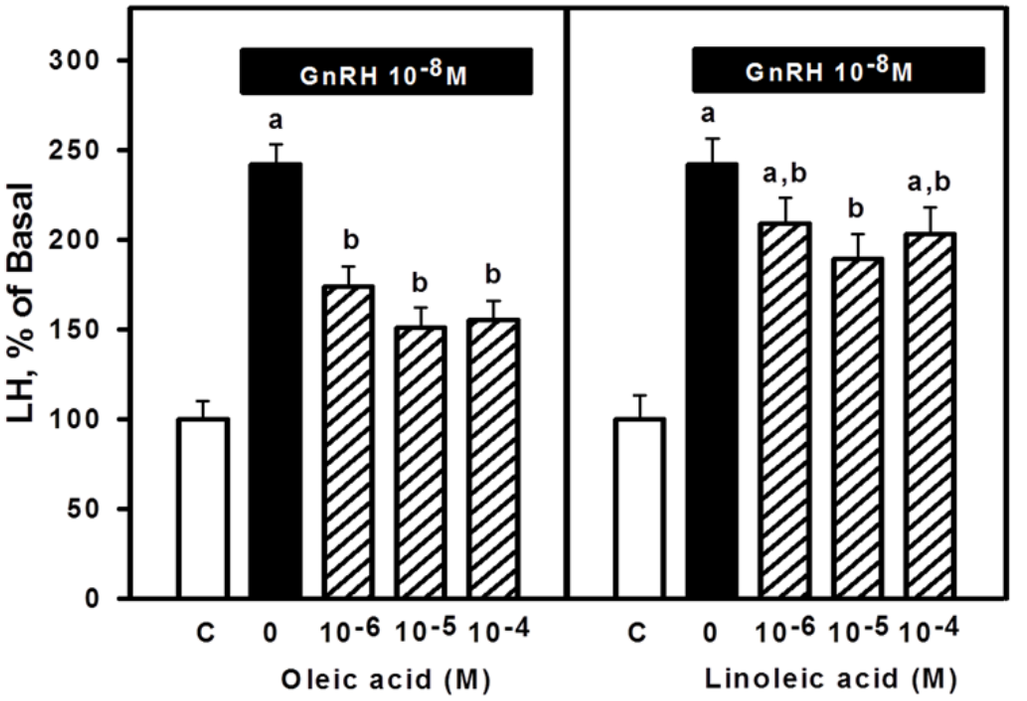

Figure 1. Anterior pituitary cells from prepubertal gilts (n = 11) were cultured in the presence of media alone (C, control wells; basal secretion in absence of any treatment) or gonadotropin releasing hormone (GnRH) at 10^{-8} M. Oleic or linoleic acid were included at 10^{-6} M, 10^{-5} M or 10^{-4} M in wells containing GnRH. Pituitary cells were exposed to oleic or linoleic acid for 30 min before the addition of GnRH. Media was collected 4 h after GnRH treatment. [a]Different from C ($P < 0.03$). [b]Different from GnRH alone ($P < 0.03$). Data from Barb et al. (1995).

An acute 28 h fast increased serum FFA concentrations, and decreased leptin pulse frequency but not mean concentrations of leptin in serum nor LH secretion in the ovariectomized prepubertal gilt (Barb et al., 2001b), while treatment with a competitive inhibitor of glycolysis suppressed LH secretion without affecting serum concentrations of leptin (Barb et al., 2001b). In contrast, short term feed restriction for 8 days decreased leptin secretion and LH pulse frequency in the mature ovariectomized gilt (Whisnant & Harrel, 2002). The ability of the pig to maintain euglycemia during acute fast may account for the failure of acute food deprivation to effect LH secretion (Barb et al., 1997). Although, leptin may serve as a metabolic signal which communicates metabolic status to the brain, the neuroendocrine response to acute energy deprivation may depend on age or mass of adipose tissue.

3. Nesfatin-1

3.1. Nesfatin-1 as an adipokine

While searching for new satiety factors, Oh-I et al. (2006) discovered a troglitazone- (PPARγ ligand) stimulated transcript expressed in SQ-5 (lung squamous carcinoma cell line) cells

that was homologous to the nucleobindin 2 (NUCB2) gene, which codes for a DNA binding/EF hand/acidic protein (NEFA). The NUCB2 gene product is a 396 amino acid protein with several cleavage sites for prohormone convertase. Post-translational processing of the NUCB2 preprotein produces three cleavage products corresponding to amino acid residues 1-82, 85-163, and 166-396. Upon the observation that i.c.v. injection of the first 82 amino acid cleavage product suppressed feed intake resulting in reduced body and fat depot weights in mice, Oh-I et al. (2006) termed the protein nesfatin-1 for NEFA/nucleobindin2-encoded satiety- and fat-influencing protein-1.

Immediately upstream of the nesfatin-1 protein is a 26 amino acid signal sequence indicating that nesfatin-1 is likely a secreted factor that may have endocrine or paracrine action. Expression of NUCB2 mRNA is observed in predifferentiated 3T3-L1 cells (Oh-I et al., 2006; Ramanjaneya et al., 2010) and induction of differentiation resulted in a marked increase in expression of NUCB2 mRNA and secretion of nesfatin-1 into culture media (Ramanjaneya et al., 2010). Nesfatin-1 also is expressed and secreted from human and mouse adipose tissue explants (Ramanjaneya et al., 2010), with subcutaneous adipose tissue having greater expression of NUCB2/nesfatin-1 than omental adipose tissue (Ramanjaneya et al., 2010). Moreover, NUCB2 expression was greater in the adipocyte fraction of adipose tissue than in the stromal vascular fraction (Ramanjaneya et al., 2010) adding further support to the concept of nesfatin-1 as an adipose derived factor. Further studies are needed to define the precise roles of nesfatin-1, or the other NUCB2 gene products, in adipose tissue, but current evidence suggests involvement in chronic inflammatory response of adipose tissue associated with metabolic disease. Treating adipose tissue explants with energy partitioning hormones (insulin, dexamethasone) and cytokines, interleukin-6 (IL-6) and tumor necrosis factor α (TNFα), altered NUCB2 expression and nesfatin-1 secretion (Ramanjaneya et al., 2010). Furthermore, NUCB2 is involved in IL-1β stimulated release of soluble tumor necrosis factor receptor 1 to the extracellular space (Islam et al., 2006).

It is important to note that NUCB2 mRNA and nesfatin-1 protein have been found to be expressed in several endocrine cells and glands throughout the body including gastric glands of digestive tract (Stengel et al., 2009a; Zhang et al., 2010), islet cells of the pancreas (Gonzalez et al., 2009), and Leydig cells of the testes (Garcia-Galiano et al., 2012). This is indicative of the role nesfatin-1 plays in gastric emptying and nutrient absorption (Stengel et al., 2009b), glucose utilization (Gonzalez et al., 2011; Nakata et al., 2011; Su et al., 2010), and testosterone production (Garcia-Galiano et al., 2012). At present, it is unclear how these tissues may contribute to circulating concentrations of nesfatin-1; however, given that adipose tissue is the largest endocrine organ of the body, the contribution that fat depots would have to plasma concentrations of nesfatin-1 seems obvious. Concentrations of nesfatin-1 in the blood are, for the most part, positively correlated with body mass index (BMI) in healthy human subjects (Aydin et al., 2009; Li et al., 2010; Ogiso et al., 2011; Ramanjaneya et al., 2010) as are several single nucleotide polymorphisms within the NUBC2 gene (Zegers et al., 2011). Expression of nesfatin-1 in subcutaneous adipose tissue of mice is suppressed with fasting and increased when mice were fed a high fat diet (Ramanjaneya et al., 2010) indicating that nesfatin-1 concentrations in serum could be regulated by nutritional

status. In point of fact, circulating concentrations of nesfatin-1 were less in patients with anorexia nervosa (Ogiso et al., 2011) and type 2 diabetes (Li et al., 2010). Together with the fact that nesfatin-1 crosses the blood-brain barrier via a nonsaturatable mechanism (Pan et al., 2007; Price et al., 2007), these data collectively indicate that nesfatin-1 is secreted from adipose tissue into the circulation and can enter the brain to regulate appetite.

3.2. Nesfatin-1 as a central regulator of food intake

The anorexogenic effects of nesfatin-1 are observed when nesfatin-1 is given either centrally (Shimizu et al., 2009) or peripherally (Stengel et al., 2009b). It is not clear, however, if suppression of appetite is entirely due to peripherally derived nesfatin-1 or the paracrine action of the protein produced within the hypothalamus. Expression of NUCB2/nesfatin-1 mRNA and protein has been demonstrated in several areas of the CNS. Within the hypothalamus, NUCB2/nesfatin-1 is expressed in nuclei that have important roles for control of appetite including the arcuate (ARC), paraventricular (PVN), lateral hypothalamic area and supraoptic nucleus (Brailoiu et al., 2007; Foo et al., 2008; Kohno et al., 2008; Oh-I et al., 2006). Areas of the brain stem that play pivotal roles in regulating energy homeostasis including the area postrema and the nucleus tractus solitaries (NTS) as well as the nucleus dorsalis of the vagus nerve all express NUCB2/nesfatin-1. Functional evidence that hypothalamic NUCB2/nesfatin-1 is involved in control of energy balance is derived from the observations that NUCB2/nesfatin-1 expression in the PVN is suppressed after fasting in adult and juvenile rats (Garcia-Galiano et al., 2010; Oh-I et al., 2006), and that refeeding activates nesfatin-1 neurons (as assessed by c-Fos) in the PVN (Kohno et al., 2008). Anorexigenic effects of nesfatin-1 require melanocortin receptors (Oh-I et al., 2006) and NPY neurons in hypothalamic slices of the ARC from mice were inhibited by nesfatin-1 *in vitro* (Price et al., 2008); although expression of NPY mRNA in the ARC of the rat *in vivo* was unchanged with nesfatin-1 treatment (Oh-I et al., 2006). Furthermore, alpha melanocyte-stimulating hormone treatment increased NUCB2 expression in the PVN (Oh-I et al., 2006) and nesfatin-1 has potent anorectic action in animals that are resistant to the effects of leptin (Oh-I et al., 2006; Su et al., 2010). This led to the initial thought that nesfatin-1 might be a down-stream effecter of the action of leptin; however, i.c.v. injection of nesfatin-1 antibodies did not block the anorectic effect of leptin in the rat (Oh-I et al., 2006). Instead, the anorexigenic actions of nesfatin-1 appear to be relayed through a mechanism independent from leptin. For instance, nesfatin-1 stimulates oxytocin cells in the PVN which in turn activate POMC neurons in the NTS of the brain stem (Maejima et al., 2009). Moreover, cholecystokinin (CCK) activates NUCB2/nesfatin-1 cell bodies in the PVN and NTS. The inhibition of food intake by CCK is mediated, at least partially, through NUCB2/nesfatin-1 neurons via a corticotrophin-releasing hormone (CRH) 2-receptor. Blocking the action of the CRH2 receptor with an antagonist ameliorated the suppressive effects of nesfatin-1 on food intake (Stengel et al., 2009b).

3.3. Nesfatin-1 as a neuroendocrine regulator of gonadotropin secretion

The neuroanatomical distribution of nesfatin-1 cell bodies in areas of the hypothalamus involved in integration of energy balance and reproduction (i.e., the ARC) and the fact that

peripheral concentrations of nesfatin-1 reflect BMI suggest a role for nesfatin-1 in metabolic regulation of gonadotropin secretion. Hypothalamic expression of NUCB2/nesfatin-1 increases during the pubertal transition in the activity of the gonadotropic axis of rats (Garcia-Galiano et al., 2010). When young pubertal female rats were given i.c.v. injection of nesfatin-1, LH secretion increased two- to threefold; however, the effects of centrally administered nesfatin-1 on LH were much greater (9-fold increase) when rats were fasted for 48 h (Garcia-Galiano et al., 2010). The later observation is likely related to the fact that fasting or less severe but long-term nutrient restriction reduced NUCB2/nesfatin-1 expression in the brain and may explain a possible mechanism whereby fluctuations in energy balance impact gonadotropin secretion in a leptin independent manner. The stimulatory effects of i.c.v. nesfatin-1 on LH were not evident in adult female rats (Garcia-Galiano et al., 2010) suggesting nesfatin-1 plays an important role in regulating gonadotropin secretion during the pubertal transition; a period when increasing adiposity and sensitivity to adipokines is generally thought to be important for activation of the reproductive axis. Consistent with this is the fact that central infusion of nesfatin-1 antisense-morpholino oligonucleotides suppressed LH secretion and delayed puberty (as determined by absence of vaginal opening) in approximately 60% of peripubertal female rats but failed to alter ovulatory surges of LH in adult females (Garcia-Galiano et al., 2010). The effects of nesfatin-1 on LH and follicle-stimulating hormone (FSH) secretion may be sexually dimorphic as i.c.v. treatment with nesfatin-1 stimulated LH and FSH secretion in male rats that were fasted (Tadross et al., 2010). Moreover, nesfatin-1 stimulated release of GnRH from hypothalamic explants taken from male rats (Tadross et al., 2010).

Collectively these data indicated that nesfatin-1 is a protein hormone that participates in metabolic regulation of appetite and energy homeostasis. Reproductive function is sensitive to nutritional status and nesfatin-1 appears to have a role in conferring metabolic state to the gonadotropic axis, particularly during pubertal development. The mechanisms whereby this occurs have not been revealed yet, but likely involve action at the GnRH neuron. Whether this is a direct paracrine action of hypothalamic nesfatin-1 or an alteration in plasma concentrations of nesfatin-1 entering the brain is not known at present. Expression of nesfatin-1 in the testis and its role in regulating testosterone release (Garcia-Galiano et al., 2012) adds further complexity, and raises the possibility that nesfatin-1 can have indirect action on gonadotropin secretion through changes in gonadal steroid feed-back to the hypothalamus or anterior pituitary gland.

4. Leptin

4.1. Effects of leptin on the hypothalamic-pituitary axis

In the pig, presence of biologically-active leptin receptor (OB-rb) in the hypothalamus and pituitary (Lin et al., 2000) and the fact that leptin increased LH secretion from pig pituitary cells (Barb et al., 2004) and GnRH release from hypothalamic tissue (Figure 2; Barb et al., 2004) *in vitro* suggests that leptin acts through the hypothalamic-pituitary axis to modulate

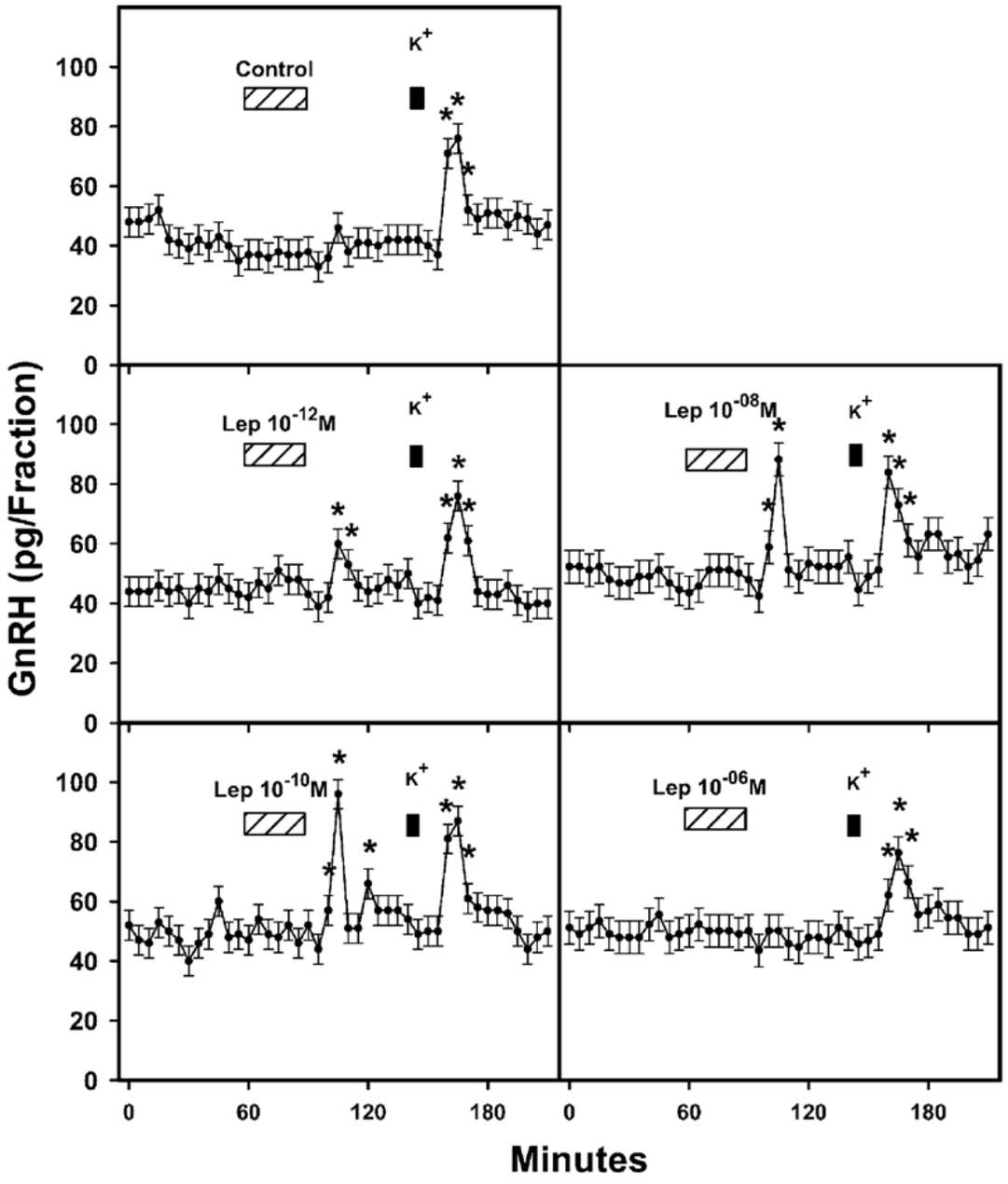

Figure 2. Hypothalamic explants (hypothalamic-preoptic area) were collected from ovariectomized prepubertal gilts and were placed in perfusion culture. Tissue was treated as shown with recombinant human leptin (Lep) at 10^{-12} M (n = 4), 10^{-10} M (n = 4), 10^{-8} M (n = 4), 10^{-6} M (n = 5) or control (n = 5). All fragments were exposed to K^+ (60 mM) to verify tissue viability. Effluent was continuously collected as 5-min fractions (500 μl). *Increased above baseline ($P < 0.05$). Data from Barb et al. (2004).

LH secretion. There is strong evidence from co-localization of leptin receptor mRNA with NPY gene expression that hypothalamic NPY is a potential target for leptin in the pig (Czaja et al., 2002). Moreover, central administration of NPY suppressed LH secretion and stimulated feed intake by reversing the inhibitory action of leptin (Barb et al., 2006). These results support the idea that leptin may serve as a metabolic signal in the activation of the reproductive axis.

Leptin treatment stimulated basal LH secretion directly from pig anterior pituitary cells in culture and GnRH release from hypothalamic-preoptic tissue explants from intact and ovariectomized prepubertal gilts on maintenance rations (Barb et al., 2004). Interestingly, i.c.v. administration of leptin failed to stimulate LH secretion in the well-fed intact prepubertal gilt (Barb et al., 2004). Obviously, hypothalamic explants are deprived of neuro-anatomical connections with other extra-hypothalamic tissues that may convey the heightened negative feedback action of estradiol on the GnRH pulse generator that occurs during pubertal development (Barb et al., 2010a), which may in part explain the failure of a LH response to i.c.v. administration of leptin in the pig.

Intracerebroventricular injection of leptin stimulated LH secretion in steroid-implanted castrated male sheep (D.W. Miller et al., 2002), and chronic i.c.v. administration of leptin stimulated LH secretion in the feed-restricted ovariectomized cow (Amstalden et al., 2002) and ewe (Henry et al., 2001). In contrast, chronic i.c.v. administration of leptin failed to stimulated LH secretion in well nourished ovariectomized ewes with no steroid replacement (Henry et al., 1999), and in intact ewe lambs (Morrison et al., 2001). *In vitro* studies demonstrated that leptin treatment stimulated basal and GnRH-mediated LH secretion from pituitary explants from fasted, but not control-fed cows, while having no effect on GnRH release from hypothalamic explants from either group of cows (Amstalden et al., 2003). Thus, metabolic state appears to be a primary determinant of the hypothalamic-pituitary response to leptin in ruminants.

4.2. The role of leptin in onset of puberty

Onset of puberty may be linked to attainment of a critical body weight or a minimum percentage of body fat (Frisch, 1984). Alternatively, metabolic mass and food intake or its correlated metabolic rate may be the triggering mechanism (Frisch, 1984). Initiation of puberty also may be influenced by metabolic factors of peripheral origin. In this regard, it has been postulated that metabolic signals are important in the initiation of puberty (Barb et al., 1997; Cameron et al., 1985). The discovery of leptin has improved our understanding of the relationship between adipose tissue and energy homeostasis (Campfield et al., 1995). Leptin treatment advanced sexual maturation in restricted and *ad lib* fed animals (Ahima et al., 1997; Barash et al., 1996). In addition, chronic leptin treatment not only reduced food intake and body weight in *ob*/*ob* (leptin deficient) mice, but also restored fertility (Barash et al., 1996). Serum leptin concentrations increased during puberty in the mouse (Chehab et al., 1997), heifer (Garcia et al., 2002) and pig (Qian et al., 1999) and, in the human female, age at first menarche was inversely related to serum leptin concentrations (Matkovic et al., 1997).

There exists, however, controversy as to the precise role of leptin in the onset of puberty. Several reports demonstrated that blood leptin concentrations remain relatively unchanged during pubertal development in the female mouse and rat (Ahima et al., 1998; Bronson, 2001; Cheung et al., 2001), while leptin administration failed to advance puberty onset in well nourished female mice (Cheung et al., 2001). Although, serum leptin concentrations increased during puberty in the gilt, other factors in addition to leptin may regulate onset of

puberty. As indicated above, it is hypothesized that estradiol modulates the hypothalamic-pituitary axis response to leptin (Barb et al., 2004). Moreover, estradiol may regulate the pubertal related changes in Ob-rb gene expression (Figure 3). In the ovariectomized prepubertal gilt, estrogen-induced increase in leptin mRNA expression in adipose tissue occurred at the time of expected puberty but not in younger animals (Qian et al., 1999). This was associated with an increase in LH pulse frequency (Barb et al., 2010b) and an age dependent increase in hypothalamic OB-rb expression (Lin et al., 2001).

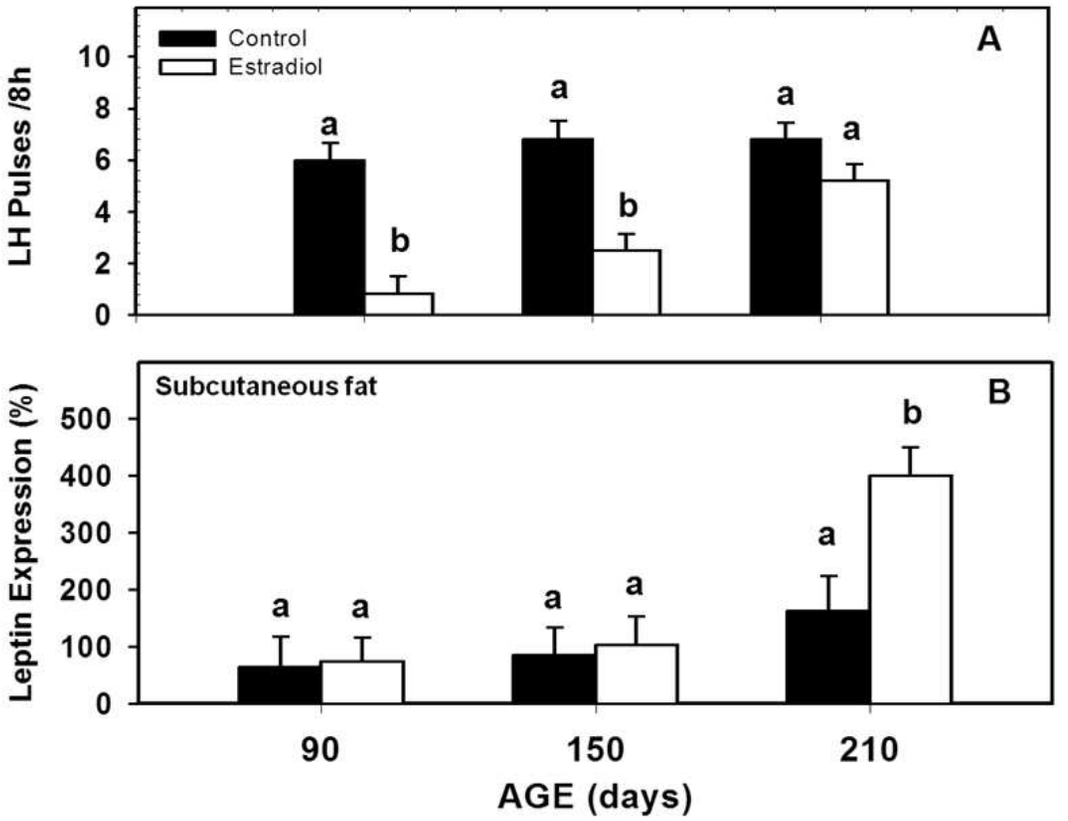

Figure 3. The frequency of luteinizing hormone (LH) pulses (A) and expression of leptin mRNA in subcutaneous (s.q.) adipose tissue (B) of ovariectomized (OVX) gilts. Gilts were OVX at 90, 150, or 210 d of age. Osmotic pumps were implanted s.q. and delivered control (vehicle; polypropylene glycol) or 0.19 mg of estradiol benzoate per kg of body weight daily for 7 d. Messenger RNA for leptin was quantified with RNA protection assays. Means without a common superscript are different; for (A) $^{a,b}P<$ 0.04 and for (B) $^{a,b}P < 0.01$. Data from Qian et al. (1999) and Barb et al. (2010b). Redrawn from Barb et al. (1999).

Several human studies, both cross-sectional and longitudinal, have demonstrated a sharp rise in serum leptin concentrations in young girls starting as early as age 7 and continuing to rise as they progressed through puberty at least age 15 (Ahmed et al., 1999; Blum et al., 1997; Garcia-Mayor et al., 1997). In contrast, in boys, leptin concentrations seem to increase transiently and then decline after Tanner stage 2 to prepubertal concentrations that are approximately one third of those observed in the late-pubertal girl. These changes in concentrations of leptin were paralleled by increasing body fat during female puberty and decreasing body fat during male puberty. Garcia-Mayor et al. (1997) reported in one cross-

sectional study, that the rise in serum concentrations of leptin were well established 2 years prior to marked increases in serum LH and estradiol concentrations were detected. The authors (Garcia-Mayor et al., 1997) suggest this is consistent with the hypothesis that leptin concentrations reach a putative threshold which allows puberty to progress; as opposed to a critical factor that triggers puberty.

Matkovic et al. (1997) examined the idea that if the relationship between body fat and early menarche in humans is mediated by leptin, then leptin concentrations would be related to age at menarche. This study consisted of 343 healthy girls (Tunner stage 2 of puberty) between 8.3 and 13.1 years of age. Menstrual history, height and weight, body composition by dual-energy X-ray absorptiometry, and leptin were measured every 6 to 12 months during a 4-year period. Leptin concentration was highly correlated with body fat mass (r = 0.81). Greater leptin concentrations up to 12 ng/mL were associated with a decline in the age of menarche by approximately 1 month per 1 ng/mL increase in leptin. Furthermore, a group of girls who remained premenarcheal for the entire 4 years of the study had significantly lower leptin concentrations compared to the groups of girls who reached menarche during the study. Matkovic and coworkers (1997) concluded that a threshold blood concentration of leptin may be needed for establishment of normal menses. Furthermore, in a recent review, Kaplowitz (2008) reports that current data supports the idea that leptin plays a permissive role as opposed to a metabolic signal that initiates puberty.

In the prepubertal ruminant, short term feed restriction reduced adipose leptin gene expression and leptin secretion, but increased hypothalamic OB-rb expression (Amstalden et al., 2000; Dyer et al., 1997). This was associated with decreased serum insulin concentration, IGF-I concentration and LH pulse frequency (Amstalden et al., 2000; Morrison et al., 2001). In addition, serum leptin concentrations increased as did leptin gene expression in heifers during pubertal development, which coincided with increases in serum IGF-I concentrations and body weight (Garcia et al., 2002). In contrast to the prepubertal heifer (Amstalden et al., 2000), short-term fasting failed to reduce pulsatile LH secretion in the mature cow (Amstalden et al., 2002). This suggests that there is a heightened sensitivity of the hypothalamic-pituitary axis to variations in energy availability in the heifer. Previous reports demonstrated that inhibition of LH secretion by nutrient restriction in the ovariectomized ewe (Henry et al., 2001) or the ewe lamb (Morrison et al., 2001) was reversed by leptin treatment demonstrating a positive association between LH secretion and leptin. Although leptin treatment reversed the fasting mediated reduction in LH pulse frequency in prepubertal heifers as cited above, chronic administration of ovine leptin by subcutaneous injections twice daily to 12- to 13-month old heifers for 40 days (Maciel et al., 2004) or 3 i.v. leptin injections per hour for 5 hours at 5-week intervals during pubertal development (Zieba et al., 2004) were unable to accelerate LH pulse frequency or onset of puberty. In contrast to data obtained from the cow, it is proposed that the effect of leptin on LH secretion in the pig during pubertal development is associated with stage of sexual maturation and subsequent change in the negative feedback action of estradiol on LH secretion (see Figure 3 and Barb et al., 2004, 2010a).

5. Kisspeptin

5.1. Kisspeptin regulates gonadotropin secretion and pubertal development

Kisspeptin is a hypothalamic neuropeptide and a potent stimulator of gonadotropin secretion (Caraty et al., 2007; Lents et al., 2008; Navarro et al., 2004a, 2005) due to its action directly on GnRH neurons (Constantin et al., 2009; Herbison et al., 2010; Irwig et al., 2004) to stimulate release of GnRH into the hypophysial portal vessels (Messager et al., 2005; Smith et al., 2011). A substantial body of evidence has accumulated that demonstrates kisspeptin plays a pivotal role in the timing of the onset of puberty. Hypothalamic expression of kisspeptin-1 (KiSS-1) and the kisspeptin receptor (GPR54) are developmentally regulated with expression increasing near the expected time of puberty (Castellano et al., 2005; Navarro et al., 2004a; Shahab et al., 2005). Furthermore, expression of KiSS-1 in the ARC and the rostral preoptic area are differentially regulated by gonadal steroids (Estrada et al., 2006; Smith et al., 2005, 2007; Tomikawa et al., 2010). It has recently been shown that increased LH pulsatility during sexual maturation in the ewe is associated with a reduction in the suppressive effects of estradiol on KiSS-1 expression (Redmond et al., 2011). The fundamental importance of kisspeptin in the onset of puberty raises the question as to whether kisspeptin has a central role in the timing of pubertal events associated with metabolic state or body energy reserves.

5.2. Kisspeptin is sensitive to energy balance

Restricted feeding and fasting reduces hypothalamic expression of KiSS-1 in rodents (Castellano et al., 2005; Luque et al., 2007), sheep (Backholer et al., 2010a), and nonhuman primates (Wahab et al., 2011). Expression of KiSS-1 also is suppressed during negative energy balance associated with lactation (True et al., 2011; Yamada et al., 2007). These data demonstrate that kisspeptin neurons in the hypothalamus are an important component to how the reproductive axis senses nutritional state. Castellano et al. (2005) used long-term caloric restriction to inhibit the occurrence of puberty (as defined by absence of vaginal opening and suppressed gonadotropin and estradiol concentrations) in female rats. Treating these rats with kisspeptin rescued gonadotropin secretion and induced puberty (vaginal opening) in approximately 60% of the animals, indicating that kisspeptin may have a role in integrating the effects of energy balance with the pubertal transition in gonadotropin secretion.

Recent data from growth restricted castrate male lambs indicates that the nutritional control of gonadotropin release may also involve alterations in sensitivity to kisspeptin. At 4 weeks after weaning, castrate male lambs were randomly assigned to different diets so that they either continued to grow or maintained body weight. After 12 weeks of treatment, animals in each group were then assigned to receive either i.v. infusion of 0.77 μmoles of kisspeptin or saline control. Area under the LH curve (AUC) for the saline treated animals was similar for growth and restricted lambs; however, the kisspeptin-induced release of LH was greater and lasted longer, as indicated by AUC, in the growth restricted lambs than in the full

growth lambs (Figure 4). Our findings in the growth restricted male lamb corroborate those of Castellano et al. (2005) in rats. These authors used prepubertal male and female rats that were fed either *ad libitum* or were fasted for 72 h. In fed animals, both prepubertal female and male rats demonstrated a 9 to 10 fold increase in LH concentrations in serum 15 minutes after i.c.v. injection of kisspeptin. In contrast, fasted rats demonstrated a much greater 50 to 60 fold increase in LH release. Moreover, the kisspeptin-stimulated release of GnRH from

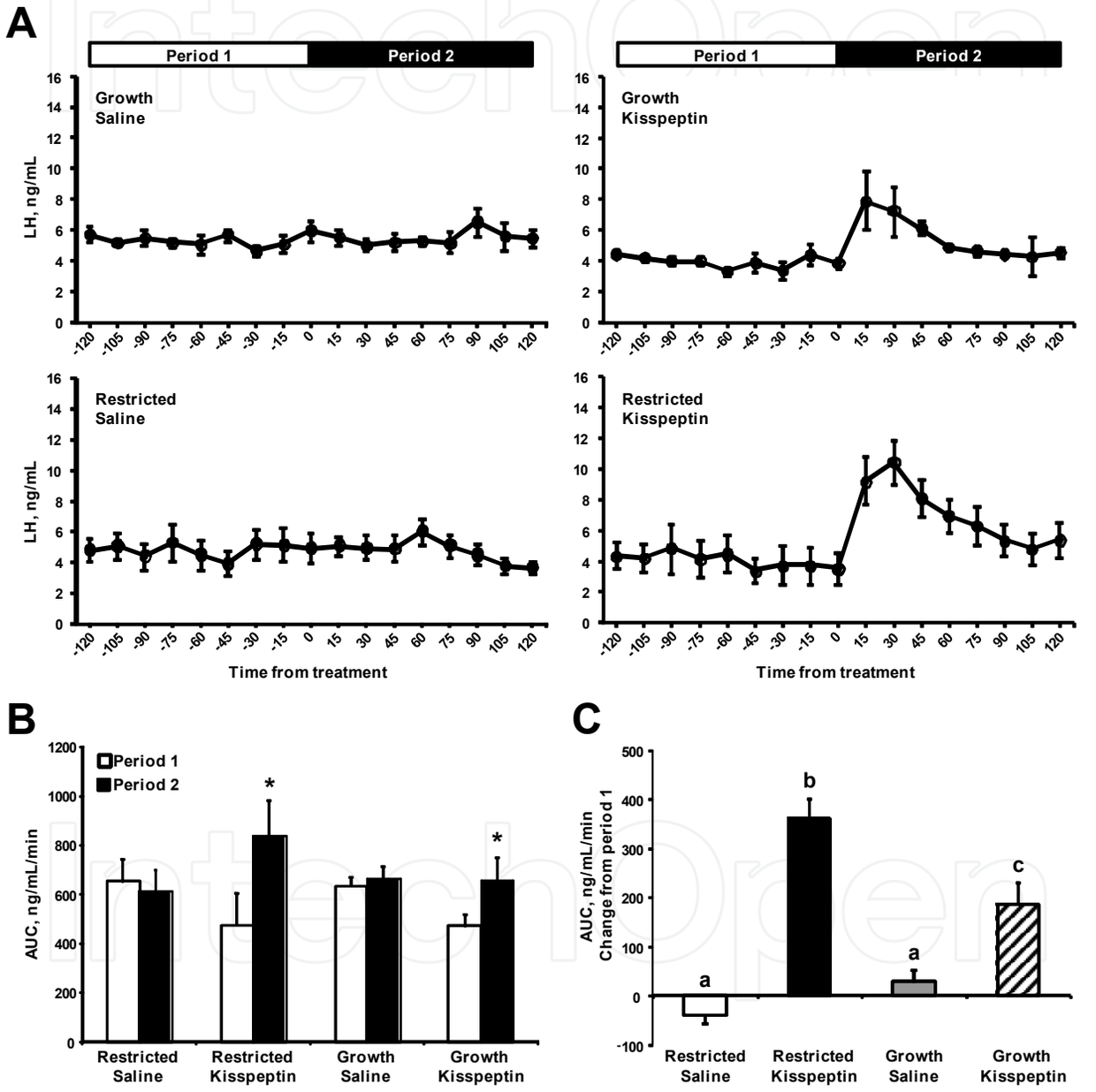

Figure 4. Four weeks after weaning, castrate male lambs (n = 16) were divided and assigned to either continue normal growth (growth) or to maintain body weight (restricted). After 12 weeks, animals in both groups received 0.77 μmoles of kisspeptin or saline as a single intravenous injection (time 0). A) Serum concentrations of luteinizing hormone (LH) during the 120 minutes before (period 1) and after (period 2) injection. B) Area under the curve (AUC) for each period (*$P < 0.05$). C) AUC in period 2 expressed as the change from period 1. [a,b,c]Means without a common superscript are different ($P < 0.05$). Data from C. A. Lents (unpublished).

hypothalamic explants collected from rats that were fasted for 72 h was greater than that from hypothalamic explants collected from *ad libitum* fed rats (Castellano et al., 2005). The increased responsiveness of the hypothalamus to kisspeptin and the subsequently greater release of LH in underfed animals are likely related to changes in expression of kisspeptin receptors. Expression of GPR54 mRNA in the hypothalamus was greater in fasted rats than in *ad libitum* fed controls (Castellano et al., 2005). Thus it appears that the pubertal transition in gonadotropin secretion involves not only increased expression and release of kisspeptin itself (Bentsen et al., 2010), but also a heightened sensitivity of the hypothalamus to the action of kisspeptin (Shahab et al., 2005). Both of these aspects can be modulated by metabolic state and are important for the overall tone of the kisspeptin system (Castellano et al., 2011; Roa et al., 2010).

5.3. Kisspeptin mediates the action of leptin on sexual development and gonadotropin secretion

The effect of energy balance on the kisspeptin system appears to be a consequence of the action of leptin. Expression of KiSS-1 in the ARC of the hypothalamus of *ob*/*ob* mice, which lack functional leptin, is significantly less when compared to expression in wild-type mice (Quennell et al., 2011; Smith et al., 2006); however, KiSS-1 expression in the anteroventral periventricular nucleus (AVPV) of *ob*/*ob* mice was similar to wild-type animals. This indicates that leptin acts on a specific population of kisspeptin cells within the ARC to modulate gonadotropin release. Leptin stimulated firing of kisspeptin neurons in hypothalamic slices of the ARC from guinea pigs (Qiu et al., 2011) and treating either *ob*/*ob* mice or *KiSS1*-Cre mice with leptin stimulated increased hypothalamic expression of kisspeptin mRNA in the ARC (Quennell et al., 2011; Smith et al., 2006) but not the AVPV (Cravo et al., 2011; Quennell et al., 2011; Smith et al., 2006). Leptin probably affects kisspeptin neurons in the ARC directly because kisspeptin cells localized within this hypothalamic area of guinea pigs (Qiu et al., 2011) and mice (Quennell et al., 2011; Smith et al., 2006) express leptin receptor mRNA. Moreover, second messengers that are important in signaling of leptin receptor (i.e., STAT-3) were expressed in kisspeptin cells in the ARC, but not within kisspeptin cells of the AVPV (Quennell et al., 2011). This indicates that increasing concentrations of leptin associated with greater body energy reserves may impact activity of the GnRH pulse generator through increasing the tone of the kisspeptin system via its action on kisspeptin neurons within the ARC.

The consequences of negative energy balance on KiSS-1 expression aren't fully offset by the positive effect of leptin. For example, leptin treatment did not fully reverse the lactation-induced reduction in KiSS-1 expression in rats (Xu et al., 2009). In a similar fashion, continuous i.c.v. infusion of leptin during a 72 h fast of ovariectomized ewes that were thin (made so with chronic nutritional restriction) rescued LH pulses (Backholer et al., 2010b) but KiSS-1 expression was only partially restored when compared with ewes that had greater body fat (Backholer et al., 2010a). Consequently, the suppressive effect of negative energy balance or nutrient deprivation on the gonadotropic axis via the KiSS-1 system

likely involves more than simply altered leptin signaling alone. Other metabolic factors, such as insulin for example, are reflective of metabolic state or availability of food and likely have an important role in regulating the kisspeptin system to augment gonadotropin release.

The possibility that adipocyte derived factors may also inhibit gonadotropin release in undernourished subjects should not be dismissed. Adiponectin is secreted by adipose tissue in response to nutrient restriction and body weight loss. It activates adenosine monophosphate-activated protein kinase (AMPK) to stimulate glucose uptake and β-oxidation of free fatty acids (Gil-Campos et al., 2004). Receptors for adiponectin are expressed not only in the hypothalamus (Kos et al., 2007) but also the anterior pituitary gland (Rodriguez-Pacheco et al., 2007). Furthermore, mice that overexpress adiponectin have an infertile phenotype (Combs et al., 2004). This is indicative of a role for adiponectin in modulating gonadotropin secretion during periods when nutrient intake is insufficient to meet energy demands. Treating anterior pituitary cells *in vitro* (Rodriguez-Pacheco et al., 2007) or LβT2 cells (immortalized embryonic gonadotrope cell line) with adiponectin suppressed both basal and GnRH-stimulated LH release (Lu et al., 2008). When adiponectin was administered i.c.v. to male rats, mean concentrations of LH were decreased owing to a suppression of LH pulse amplitude (Cheng et al., 2011). The later observation would indicate that adiponectin could be functioning to suppress activity of the GnRH neuronal network in subjects experiencing reductions in energy balance. In line with this is the fact that adiponectin inhibits the release of GnRH from GT1-7 cells (immortalized hypothalamic cell line) via an AMPK pathway (Cheng et al., 2011; Wen et al., 2008). It may well be that increased secretion and activity of adiponectin in animals during food deprivation or nutrient restriction off-set, to some degree, the stimulatory action of exogenous leptin on KiSS-1 expression in the hypothalamus. It is yet to be determined, however, if the suppressive effects of adiponectin on GnRH/LH release involve changes in the hypothalamic kisspeptin system.

Expression of KiSS-1 in immortalized hypothalamic N6 cells was increased after treatment with NPY (Luque et al., 2007). This would suggest that neuronal pathways downstream of leptin can impact the kisspeptin system. In the ewe, 13 to 30% of kisspeptin neurons in the ARC are in close apposition to NPY fibers (Backholer et al., 2010a). Moreover, 30 to 40% of kisspeptin cells in the ARC were contacted by POMC fibers (Backholer et al., 2010a). Since both NPY and POMC expressing cells are direct targets for leptin's action, the effects of leptin on gonadotropin secretion may be mediated through kisspeptin neurons indirectly via NPY and POMC pathways. It is also noted that kisspeptin neuronal fibers are located in close apposition to approximately 7% of NPY cell bodies and 20% of POMC cell bodies in the ovine hypothalamus (Backholer et al., 2010a). This anatomical evidence implies that the reproductive axis can influence neuronal pathways to modulate appetite. In fact, i.c.v. injection of kisspeptin increased NPY mRNA and reduced POMC mRNA in the ARC of the hypothalamus of sheep (Backholer et al., 2010a). Thus, other factors that may drive NPY or POMC expression during conditions of underfeeding may further limit the ability of leptin to stimulate increased KiSS-1 expression in the hypothalamus.

5.4. Kisspeptin is involved in the reproductive pathobiology of diabetes and obesity

Metabolic disorders such as diabetes and obesity are accompanied by alterations in adipose tissue biology and impaired fertility. Given the impacts of leptin on the kisspeptin system in the hypothalamus, one could easily speculate that metabolic diseases that impinge upon circulating concentrations of leptin could have negative consequences for reproductive function via alterations in the hypothalamic expression of kisspeptin. Using the streptozotocin-induced diabetic male rat model, Castellano et al. (2006) observed that LH release was rescued when rats were treated with exogenous kisspeptin. Moreover, expression of KiSS-1 was reduced in the hypothalamus of these diabetic male rats. When the authors treated the diabetic rats with leptin, they found that KiSS-1 expression was restored along with increased concentrations of LH and testosterone in serum.

Obesity is an ever growing epidemic and patients that are obese present a number of pathologies. One of these is a reduction in the sensitivity to the action of leptin. Iwasa et al. (2010) observed that female rats which underwent intrauterine growth retardation during their growth as fetuses developed leptin resistance after birth. These leptin resistant female rats demonstrated delayed onset of puberty associated with reduced expression of KiSS-1 in the hypothalamus. Thus, infertility associated with obesity and central leptin resistance may be related to tone of the kisspeptin system within the hypothalamus. Navarro et al. (2004b) found kisspeptin treatment restored LH secretion in *fa/fa* Zucker rats; a genetic model for leptin resistance. Furthermore, diet induced leptin resistance in mice, resulting from prolonged feeding of a high fat diet, was associated with reduced KiSS-1 expression and LH concentrations in serum (Quennell et al., 2011). Therefore, infertility resulting from hypogonadotropism that arises in diabetic or obese patients is likely due to alterations in the expression and secretion of kisspeptin in the hypothalamus.

6. Conclusion

Adipose tissue expresses and secretes a wide array of regulatory factors that have diverse biological roles. These factors contribute to the regulation of energy homeostasis by acting on neural circuits within the hypothalamus. Gonadotropin-releasing hormone is secreted from hypothalamic neurons and acts on gonadotrope cells within the anterior pituitary gland to stimulate the synthesis and release of LH. Activity of this gonadotropic-axis is sensitive to metabolic state. Free fatty acids are released from adipose tissue to have a glucose sparing effect and can be directly sensed by neurons in the hypothalamus. Cyclic changes in availability of FFA associated with meal frequency act to sustain continued release of LH pulses over short periods of time, but chronically elevated FFA likely impairs reproductive function by decreasing the sensitivity of the pituitary gland to GnRH. Conversely, leptin can enhance pituitary GnRH sensitivity and increase LH secretion. Within the hypothalamus, leptin stimulates release of GnRH by acting through interneuronal pathways involving NPY, POMC, and kisspeptin. Other adipose derived factors such as adiponectin and nesfatin-1 can have negative or positive effects on LH

release, respectively (figure 5). Metabolic control of puberty onset likely involves developmental changes in these relationships.

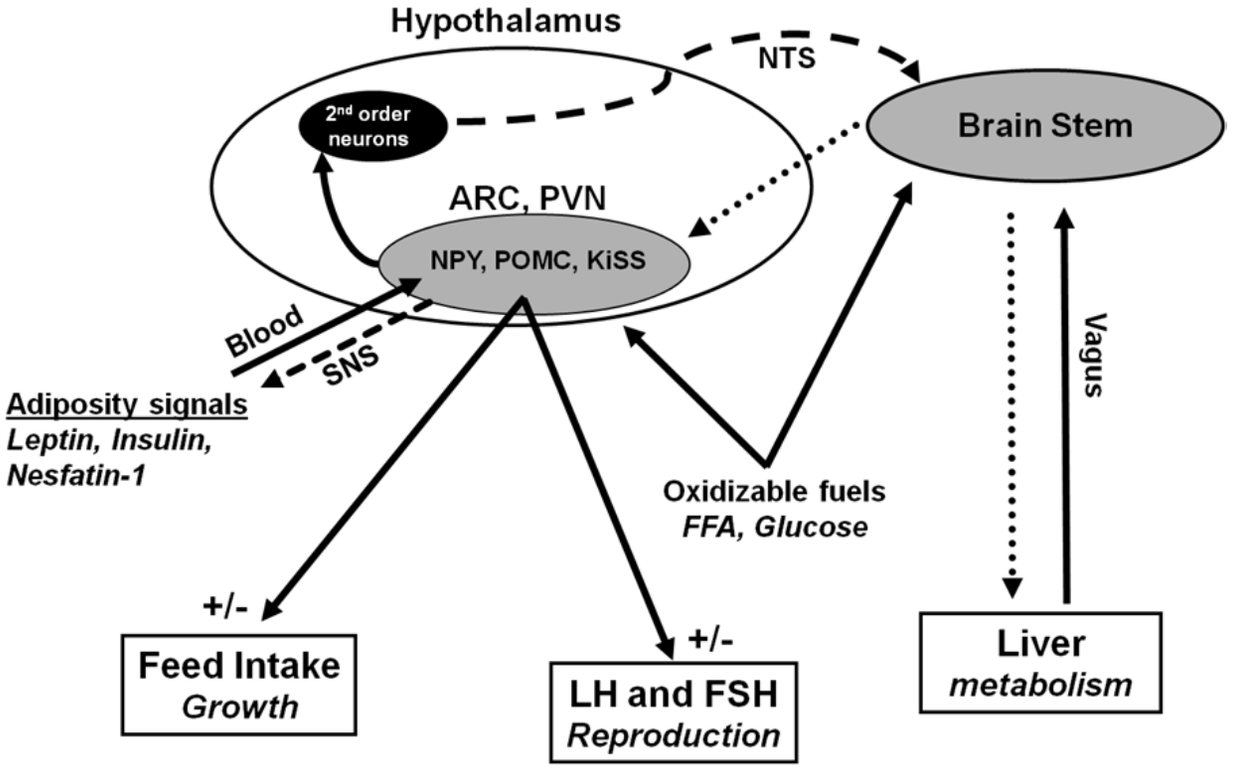

Figure 5. A proposed model for how metabolic signals, including adipokines such as leptin, affect gonadotropin secretion. Insulin fluctuates with consumption of meals at regular intervals to promote adipose accretion. Increased mass of adipose tissue is reflected in concentrations of adipokines such as leptin and nesfatin-1 that circulate in the blood to act as specific neural circuits within the hypothalamus. Leptin suppresses feed intake by modifying activity of POMC and NPY neurons in the arcuate (ARC) and paraventricular (PVN) nuclei, and stimulates release of gonadotropin hormones (LH and FSH). Many neurons in these areas of the hypothalamus express leptin receptor and directly innervate adipose tissue, thus constituting a hypothalamic-adipose neuroendocrine axis involving the sympathetic nervous system (SNS). Leptin directly activates kisspeptin (KiSS) cell bodies to stimulate GnRH release and to cause an upregulation of LH pulses. Nesfatin-1, which also stimulates LH release, suppresses food intake by acting through second order neurons to modulate activity of POMC systems in the nucleus tractus solitaries (NTS) of the hind brain as well as the nucleus dorsalis of the vagus nerve; thus altering liver function, which results in shifting availability of oxidizable fuels. Elevated free fatty acids (FFA) have a glucose sparing affect and can be directly sensed by neurons in the hypothalamus to sustain continued release of LH pulses over a short period.

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Abbreviations

ADIPOR2	adiponectin receptor 2	KiSS-1	kispeptin-1
AGTR1	angeotensin II receptor	LC-CoAs	long-chain fatty acyl CoAs
AMPK	adenosine monophosphate-activated protein kinase	LDLR	low density lipoprotein receptor
		LH	luteinizing hormone
ANG	angiotensin	LHA	lateral hypothalamic area
ANGPTL	angiopoietin-like protein	LHCGR	luteinizing hormone-choriogonadotropin receptor
APO	apolipoprotein		
ARC	arcuate	LPL	lipoprotein lipase
ARS	Agricultural Research Service	LβT2	immortalized embryonic gonadotrope cell line
AUC	Area under curve		
AVPV	anteroventral periventricular nucleus	MCP-1	monocyte chemoattractant protein-1
BDNF	brain-derived neurotrophic factor	N6	neuronal 6 cells
		NEFA	DNA binding/EF hand/acidic amino acid rich protein
bFGF	basic fibroblast growth factor		
BMI	body mass index	NGFR	nerve growth factor receptor
BMP	bone morphogenic protein	NPY	neuropeptide Y
BMPR2	bone morphogenic protein receptor 2	NTS	nucleus tractus solitaries
		NUCB2	nucleobindin 2
CCK	cholecystokinin	OB-rb	biologically-active long form leptin receptor
CIQTN4	complement-c1q tumor necrosis factor-related protein 4		
		PAI-1	plasminogen activator inhibitor-1
CNS	central nervous system	PDGFD	platelet derived growth factor D
CNTF	ciliary neurotrophic factor	PGRMC1	progesterone receptor membrane component 1

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CRH	corticotrophin-releasing hormone	POMC	proopiomelanocortin
CTGF	connective tissue growth factor	PPARγ	proxisome proliferator activated receptor γ
CTRP4	complement-c1q tumor necrosis factor-related protein 4	PVN	paraventricular
EDNRB	endothelin receptor type B	RANTES	chemokine (c-c motif) ligand 5
EGFR	epidermal growth factor receptor	RBP	retinol binding protein
ESR1	estrogen receptor 1	RLN	relaxin
FFA	free fatty acids	RRC	Richard B. Russell Research Center
FSH	follicle-stimulating hormone	RTN	reticulon
GnRH	gonadotropin-releasing hormone	SQ-5	lung squamous carcinoma cell line 5
GNRHR2	gonadotropin-releasing hormone receptor 2	STAT-3	signal transducer and activator of transcription 3
GPR54	g protein coupled receptor 54	TGF	transforming growth factor
GT1-7	GT1-7 cells (immortalized hypothalamic cell line)	THR	thyroid hormone receptor
i.c.v.	intracerebroventricular	TLR	toll-like receptor
i.v.	intravenous	TNF	tumor necrosis factor
IFNG	interferon gamma	TNFα	tumor necrosis factor α
IGF	insulin-like growth factor	TSHR	thyroid-stimulating hormone receptor
IGF-IR	IGF-I receptor	USDA	United States Department of Agriculture
IGFBP	insulin-like growth factor binding protein	USMARC	U.S. Meat Animal Research Center
IL	interleukin	VEGFC	vascular endothelial growth factor C
INSR	insulin receptor		

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