

Gene expression in the brain-pituitary adipose tissue axis and luteinising hormone secretion during pubertal development in the gilt

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The occurrence of puberty in the female is due to the interplay of central and peripheral mechanisms in which the hypothalamic-pituitary-ovarian axis regulates growth and gonadal function, as well as adipocyte hormone secretion. Hypothalamic GnRH mRNA expression increased at 3.5 months of age and declined by 6 months of age. Concomitant with the age related reduction in the oestrogen negative feedback on LH secretion was a decline in hypothalamic oestrogen receptor- α (ER α) expression and increased expression of repressor of ER activity gene (REA) at 210 days of age. Hypothalamic proopiomelanocortin expression increased at 6 months of age followed by increased expression of progesterone receptor (PR) membrane compliment-1 and steroid membrane binding protein gene at 210 days of age. This represents development of the endogenous opioid peptide-progesterone dependent LH inhibitory pathway. Adipose tissue leptin and insulin like growth factor -I (IGF-I) gene expression increased with age and adiposity. Pituitary transcription factors, steroidogenic factor 1 (SF1) and Lhx3, and LH β and FSH β gene expression increased with age. These results identify key hypothalamic and pituitary genes associated with changes in LH secretion and growth during pubertal development and adipose tissue genes and secreted proteins related to maturation of the neuroendocrine axis and puberty.

Introduction

The occurrence of puberty in the female is the manifestation of the interplay between central and peripheral mechanisms in which the hypothalamic-pituitary-ovarian axis regulates growth and gonadal function, as well as adipocyte hormone secretion. The integral components of this axis are functional before the normal onset of puberty (Kraeling and Barb, 1990). Little is known, however, about the mechanisms within the brain which bring these various components into the proper temporal relationship to initiate puberty. Induction of oestrus and ovulation in prepubertal gilts with hourly i.v. injections of GnRH demonstrated the importance of pulsatile GnRH secretion (Lutz *et al.*, 1985). The gonadostat hypothesis is one of the most widely accepted theories regarding neuroendocrine mechanisms controlling onset of puberty

and contends that as an animal ages, sensitivity to oestrogen negative feedback on pulsatile LH secretion declines (Ramirez and McCann, 1963). The pig appears to conform to the gonadostat hypothesis because onset of puberty is immediately preceded by a significant increase in pulsatile LH secretion in the face of increasing oestrogen secretion (Lutz et al., 1984; Barb et al., 2000). In addition, the neuroendocrine axis became less sensitive to the suppressive effects of exogenous oestrogen on LH secretion as gilts progressed from the prepubertal to peripubertal state (Barb et al., 2000). This change in sensitivity could be the result of brain maturational processes which reflect decreased inhibitory inputs and (or) increased stimulatory inputs to GnRH neurons. Lastly, there is a positive genetic correlation between backfat thickness and average daily gain with first detectable oestrus and first breeding in the gilt (Mabry et al., 1985; Tummaruk et al., 2000). Thus, adipose derived factors may play a role in the timing of puberty. The purpose of this review is: first, to identify hypothalamic and pituitary genes associated with changes in LH secretion and growth during pubertal development and second, to examine adipose tissue gene expression related to maturation of the neuroendocrine axis and puberty.

GnRH/ LH pulse generator

In general, in the gilt, mean serum LH concentration and serum LH pulse frequency increased from 15 days of age to maximum levels between 110 and 125 days of age, then decreased until 150 days of age and remained suppressed (juvenile nadir) until the peripubertal period (Pelletier et al., 1981; Diekman et al., 1983; Lutz et al., 1984; Camous et al., 1985; Kraeling and Barb, 1990). Further, a reduction in the negative feedback effect of oestrogen on LH secretion occurred during the peripubertal period in the gilt. There is little information regarding mechanisms within the brain that brings the various components of the reproductive axis together in a proper temporal relationship to initiate puberty (Esbenshade et al., 1982; Prunier et al., 1993; Clapper et al., 1993a; Clapper et al., 1993b). Hypothalamic GnRH mRNA expression increased markedly by 3.5 months of age compared to 7 day-old pigs and declined by 6 months of age (Lin et al., 2001; Table 1). This expression pattern is consistent with the LH secretory pattern during development; particularly around the time of the juvenile nadir. However, in a subsequent study hypothalamic GnRH mRNA expression remained unchanged between 150 and 210 days of age in the prepubertal gilt (C. R. Barb, unpublished). Similarly, in the intact female primate, hypothalamic GnRH content did not vary during the juvenile to pubertal transition (Ma et al., 1994). Furthermore, in the intact male monkey, GnRH gene expression was similar among juvenile and adult animals (Vician et al., 1991). In the female rat, hypothalamic GnRH mRNA expression increased gradually during development between day 10 and 41 (Gore et al., 1996) and increased significantly on the day of vaginal opening. This occurred after establishment of a diurnal pattern of LH secretion (Ojeda and Urbanski, 1994b), suggesting that level of hypothalamic GnRH gene expression may not be a necessary antecedent for puberty.

Excitatory and inhibitory inputs. Glutamatergic N-methyl-D-aspartate (NMDA) receptor activation accelerated puberty (Brann and Mahesh, 1997) and NMDA receptor blockade delayed puberty in the rat (Ojeda and Terasawa, 2002). In juvenile male monkeys, NMDA receptor activation initiated a pubertal pattern of LH secretion (Gay and Plant, 1988). In agreement with the studies above, stimulation of NMDA receptor enhanced circulating concentrations of LH in the prepubertal gilt (Estienne et al., 1995). Furthermore, administration of GnRH antiserum abolished the ability of NMDA to increase LH secretion (Sesti and Britt, 1992). This is consistent with the idea that activation of the glutamatergic system stimulates GnRH release in the prepubertal gilt. Several studies have proposed that the pubertal increase in GnRH secretion may result from a reduction in inhibitory input as well as an increased flow of excitatory input (Ojeda and Urbanski, 1994; Terasawa and Fernandez, 2001; Plant and Barker-Gibb, 2004).

Table 1. Hypothalamic expression of OB-rb, preproorexin, corticotropin releasing factor (CRF), somatostatin (SS), gonadotropin releasing hormone (GnRH) and proopiomelanocortin (POMC) mRNA, and adipose tissue expression of leptin (LS mean \pm SE) for 106 day-old foetuses (n=3) and 3.5 month-old (n=3) and 6 month-old (n=2) gilts.^a

Item	106 day-old foetus	7 day-old pig	3.5 month-old pig	6 month-old pig	P-Value
OB-rb	0.15 \pm 0.05 ^d	0.19 \pm 0.05 ^d	0.44 \pm 0.06 ^e	0.38 \pm 0.06 ^e	<0.05
Preproorexin	0.70 \pm 0.06 ^d	0.59 \pm 0.06 ^d	0.96 \pm 0.06 ^e	0.75 \pm 0.07 ^d	<0.05
CRF	0.81 \pm 0.17 ^d	0.44 \pm 0.21 ^d	0.10 \pm 0.17 ^d	0.78 \pm 0.21 ^d	NS ^b
SS	0.52 \pm 0.11 ^d	0.62 \pm 0.11 ^d	1.32 \pm 0.13 ^e	0.36 \pm 0.13 ^d	<0.02
GnRH	0.21 \pm 0.03 ^d	0.19 \pm 0.03 ^d	0.74 \pm 0.04 ^e	0.38 \pm 0.04 ^d	<0.02
POMC	0.31 \pm 0.05 ^d	0.30 \pm 0.05 ^d	0.26 \pm 0.05 ^d	0.73 \pm 0.06 ^e	<0.01

^a Expressed as a ratio of OB-rb, neuropeptide of leptin mRNA relative to 18s rRNA.

^b NS: Non significant.

^c ND: Not determined.

^{d,e,f} Means in a row with different superscripts differ; P, level of significance.

From Lin *et al.*, 2001.

The gamma-aminobutyric acid (GABA) neuronal pathway represents a major inhibitory neurotransmitter system that contributes to the inhibitory input to GnRH neurons (Ojeda and Urbanski, 1994; Terasawa and Fernandez, 2001). The pubertal increase in GnRH secretion may result from a reduction in GABAergic inhibition and a concomitant increase in glutamatergic excitatory input (Terasawa and Fernandez, 2001). Other neurosecretory pathways may provide inhibitory and excitatory input to the GnRH system during pubertal development such as NPY and EOP and norepinephrine (for a review, see Kraeling and Barb, 1990; Terasawa and Fernandez, 2001; Ojeda and Terasawa, 2002). Therefore, regulation of hypothalamic GnRH expression may not play a critical role in onset of puberty, but the summation of increased excitatory and decreased inhibitory input on GnRH neurons may be the determining factor.

Steroid and age dependent changes

Oestrogen

To determine if there is an age and steroid dependent change in adipose leptin expression associated with LH secretion, ovariectomised (OVX) prepubertal gilts at 90, 150 and 210 days of age were implanted with oestrogen and blood and adipose tissue samples were collected 7 days post-implant. Oestrogen-induced leptin mRNA expression in adipose tissue occurred in the 210 day-old animals (time of expected puberty) but not in younger animals (Qian *et al.*, 1999). This was associated with a reduction in the negative feedback regulation of pulsatile LH secretion (Barb *et al.*, 2000; Fig. 1) and an age dependent increase in hypothalamic long form leptin receptor (OB-rb) expression (Lin *et al.*, 2001; Table 1) and serum leptin concentrations (Qian *et al.*, 1999). These events coincided with increased hypothalamic expression of repressor of oestrogen receptor activity (REA) between 150 to 210 days of age in the pig (C. R. Barb, unpublished; Table 2). This gene was reported to suppress oestrogen receptor (ER) transcriptional activity (Montano *et al.*, 1999). Thus, REA may play an important role in determining sensitivity of oestrogen target neurons during pubertal development.

Maturational change in sensitivity to oestrogen may reflect decreased GnRH neuronal ER concentration, a reduction in inhibitory inputs and (or) an increase in stimulatory inputs to the GnRH neuron (Ojeda and Terasawa, 2002; Sisk and Foster, 2004) and (or) a decline in ER concentrations in an intermediary neuronal pathway that regulates GnRH secretion (Dierschke *et al.*, 1974; Day *et al.*, 1984). To that extent, Day *et al.* (1984) observed in the heifer that the number of ER in the anterior hypothalamus and medial basal hypothalamus declined in associa-

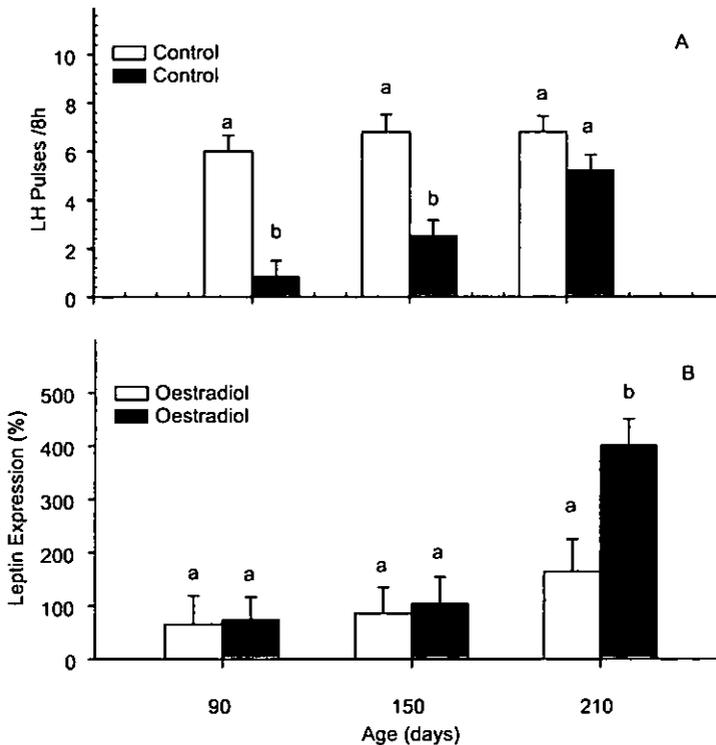


Fig. 1 (A) Luteinising hormone pulse frequency (mean \pm SE) for 90, 150 and 210 day-old gilts implanted with osmotic pumps containing oestradiol or vehicle control ($n = 6$ per treatment/age, except 150 day-old control, $n = 5$). Bars with different superscripts differ ($P < 0.05$). (B) Leptin mRNA expression for 90, 150 and 210 day-old pigs implanted with osmotic pumps containing oestradiol or vehicle control. Quantification of leptin mRNA expression by laser densitometry was expressed as a percentage of leptin mRNA relative to 28S ribosomal RNA and normalized for an mRNA pool run on each gel. Means with different superscripts differ ($P < 0.01$). Modified from Qian *et al.* 1999 and Barb *et al.*, 2000).

Table 2. Microarray analysis of age related changes in hypothalamic gene expression from 90 to 150 ($n=5$), 150 to 210 ($n=5$) and 90 to 210 ($n=5$) days of age in the prepubertal gilt.

Gene Description	90 to 150 days old	150 to 210 days old	90 to 210 days old	P value
Steroid Related				
Progesterone receptor	Nc	Up	Up	2.8×10^{-5}
Membrane comp. 1	Nc	Up*	Up**	$*2.8 \times 10^{-5}$; ** 0.006
Steroid membrane BP	Nc	Up*	Up**	$*0.0002$; **0.002
Repressor estrogen receptor activity	Nc	Up*	Up**	$*0.0002$; **0.002
Oestrogen receptor α	Nc	Down	Nc	0.002
Oestrogen receptor β	Nc	Nc	Nc	
Appetite Related				
MC3-R	Nc	Up*	Up**	$*0.0003$; **0.002
MCH precursor	Down	Up	Nc	2.8×10^{-5}
GH Related				
Somatostatin	Nc	Up	Nc	0.0002

MC3R: Melanocortin-3 receptor; MCH precursor: Melanin-concentrating hormone precursor; MC4R: Melanocortin-4 receptor; Nc: No change. * P value for 150 to 210 day-old gilts; ** P value for 90 to 210 day-old gilts.

tion with increased LH secretion and onset of puberty. In the prepubertal pig, concentrations of hypothalamic nuclear ER β were not different at 4 and 5.5 months of age (Diekman and Anderson, 1983). We observed no change in hypothalamic ER β expression between 150 and 210 days of age, but ER α declined during this time (C. R. Barb, unpublished; Table 2). Thus, it is reasonable to question whether or not a developmental change in hypothalamic ER α mRNA expression represents a change in sensitivity to steroid negative feedback. Alternatively, the type of ER expressed may be of greater importance. Work in the rat demonstrated that 85% of the GnRH neurons contained ER β (Hrabovszky *et al.*, 2001). In the female mouse, both ER α and ER β are involved in oestrogen negative feedback of LH secretion *in vivo*. However, only ER α appears to be critical for oestrogen-negative feedback suppression of GnRH mRNA expression (Dorling *et al.*, 2003). Whether or not the pubertal decrease in sensitivity to oestrogen negative feedback is a result of related changes in GnRH neuronal steroid hormone receptor expression remains unclear. It is possible that changes in the gonadostat may be mediated by developmental upstream changes in neuronal regulatory control of GnRH secretion. GnRH neuronal activity is regulated by multiple synaptic inputs (Ojeda and Urbanski, 1994) that are subject to regulation by ovarian steroids (Herbison, 1998). Moreover, Herbison (1998) suggested that two potentially independent oestrogen-sensitive neuronal systems exist, an inhibitory and stimulatory pathway for GnRH secretion.

Progesterone

We have previously reported that EOP modulation of LH secretion is progesterone dependent in the mature gilt (Barb *et al.*, 1985; Barb *et al.*, 1986; Barb *et al.*, 1988). Furthermore, EOP act at the hypothalamus to modulate GnRH release (Barb *et al.*, 1994). Evidence in the gilt suggests that puberty is initiated in part by a decrease in intrinsic central nervous system (CNS) inhibitory input and/or a reduction in the sensitivity to the negative feedback action of ovarian steroids (Kraeling and Barb, 1990; Kraeling *et al.*, 1992). We suggest that the EOP-progesterone dependent pathway that inhibits LH secretion is the result of a maturational process (Barb *et al.*, 1988). In this regard direct synaptic contacts between proopiomelanocortin (POMC) and GnRH containing neurons were found in the arcuate nucleus of the hypothalamus in the rat (Thind and Goldsmith, 1988; Chen *et al.*, 1989). In the pig, POMC containing neurons are located in the area within the hypothalamus that is involved in GnRH secretion (Kineman *et al.*, 1988; Kineman *et al.*, 1989). Moreover, hypothalamic POMC expression increased by 6 months of age, a time when GnRH gene expression was suppressed in the prepubertal gilt (Lin *et al.*, 2001; Table 1). These events preceded increased hypothalamic expression of progesterone receptor (PR) membrane complement-1 and steroid membrane binding protein gene at 210 days of age (C. R. Barb, unpublished; Table 2). Taken together the above data represent maturational changes in key components of the EOP-progesterone dependent pathway that inhibits LH secretion in the mature gilt.

Pituitary

The anterior pituitary gland integrates signals of hypothalamic and peripheral origin. Hormones secreted from the anterior pituitary gland regulate growth, reproduction and energy homeostasis among other physiological functions. There are a number of regulatory proteins, steroid hormone receptors and transcription factors that play a role in anterior pituitary development (Savage *et al.*, 2003). Regulation of LH β and FSH β subunits is a critical rate limiting step in the production of biologically active LH and FSH. Thus, factors that control synthesis of these glycoprotein hormone subunits are necessary for reproductive function (Savage *et al.*, 2003).

Two candidate genes known to play a critical role in pituitary development and production of hormones, which are necessary for growth and reproduction in rodents and humans, have been identified in the pig. Steroidogenic factor 1 (SF1) and Lhx3 LIM homeodomain transcription factor genes were recently mapped to an area on chromosome 1, which is associated with a QTL for growth and puberty (Smith *et al.*, 2001). The Lhx3 is required for anterior/intermediate pituitary development and differentiation of GH, PRL, TSH, LH, FSH cells while SF1, a nuclear receptor, is required for gonadotrope development (Savage *et al.*, 2003). The absence of these genes resulted in loss of GH, LH, PRL, FSH and TSH secretion, gonadal agenesis, reduced growth and failure to reach puberty (Netchine *et al.*, 2000). In the pig, a transient increase in Lhx3 gene expression occurred during the time of pituitary organogenesis, and pituitary cell differentiation with a high level of expression in the mature pituitary gland (Smith *et al.*, 2001). Consistent with these developmental changes in Lhx3 and SF1 expression, we recently observed an increase in pituitary LH β and FSH β gene expression by 150 days of age in the gilt. Thus, identification of other pituitary regulatory genes that are developmentally regulated may provide potential candidate genes for genetic analysis of growth and reproductive traits.

Oestrogen receptor

The biological action of oestrogen on the anterior pituitary gland is manifested through the ER. Like many other reproductive tissues, the pituitary gland expresses two isoforms, ER- α and - β . We observed no change in pituitary ER β and PR expression during development in gilts, but ER α expression increased by 210 days of age compared to 150 days of age (C. R. Barb, unpublished; Table 2). Similarly, pituitary concentrations of cytoplasmic or nuclear ER β and progesterone receptor (PR) were similar among 1, 2.5, 4 or 5.5 month-old gilts (Diekman and Anderson, 1982). In general, the biological role of ER α is to regulate PRL and gonadotrope gene expression and lactotrope cell growth, where as ER β stimulates PR expression and is involved in GnRH self-priming in the gonadotrope (Savage *et al.*, 2003). A recent report by Sanchez-Criado *et al.* (2004) utilizing selective receptor agonists for ER α and - β demonstrated that ER α mediated oestrogen stimulation of PRL secretion and basal and GnRH-induced LH and FSH secretion, whereas oestrogen increased the number of pituitary cells expressing PR via the ER β in the rat. Thus, the ER appears to play a critical role in development of the pituitary's capability to secrete LH and FSH.

Growth and metabolic signals

In addition to developmentally related maturation of the neuroendocrine axis, permissive peripheral signals associated with attainment of a minimum percentage of body fat (Frisch 1984), which are of adipocyte origin, such as leptin or IGF-I (Barb *et al.*, 1998a; Barb *et al.*, 2002) may play a role in the timing of puberty. During the prepubertal period, expression of a number of hypothalamic genes associated with appetite and growth regulation appear to be developmentally regulated (Table 1 and 2). For example hypothalamic OB-rb mRNA expression, adipose tissue leptin expression and serum leptin concentrations increased by 3.5 months of age (Qian *et al.*, 1998; Lin *et al.*, 2001). In addition, hypothalamic melanocortin 3 receptor (MC3-R), and melanin-concentrating hormone (MCH) precursor expression were up-regulated between 150 and 210 days of age (C. R. Barb, unpublished; Table 2). These genes and their encoded proteins are well positioned anatomically to interact with GnRH (Kraeling and Barb, 1990) and GHRH (Leshin *et al.*, 1994) neurons and appetite regulating neurons (Lawrence *et al.*, 1999; Matteri 2001). Consistent with this idea, central administration of leptin increased GH secretion and suppressed feed intake in the gilt (Barb *et al.*, 1998b). Furthermore, 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.

The early growth pattern of the gilt is almost linear. Growth sharply increased from the postnatal period (Robison 1976). However, serum GH concentrations and pituitary response to GHRH declined with age in the pig (Dubreuil *et al.*, 1987). Greater hypothalamic somatostatin mRNA expression at 3.5 months (Lin *et al.*, 2001) and 210 days of age (C. R. Barb, unpublished) compared to 6 month and 150 days of age, respectively, support the idea of an age related decline in GH secretion due to increased somatostatin secretion. Furthermore, Drisko *et al.* (1998) reported a temporal relationship between hypophysial-portal blood concentration of somatostatin and generation of serum GH pulses in the pig.

In the pig, the pubertal rise in circulating IGF-I concentration (Lee *et al.*, 1991) occurred concomitantly with increased IGF-I expression in adipose tissue (G. J. Hausman, unpublished; Table 3), decreased pituitary response to GHRH (Dubreuil *et al.*, 1987) and increased LH secretion (Lutz *et al.*, 1984; Fig. 2). These observations are consistent with the idea that IGF-I may modulate hypothalamic release of GnRH and factors regulating GH secretion. Spencer *et al.* (1991) reported that ICV administration of IGF-I suppressed GH secretion in the foetal pig. Although the central effect of IGF-I on GH secretion was lost during maturation in the pig (Barb *et al.*, 2002), pituitary sensitivity to IGF-I modulation of the GH response to GHRH from porcine pituitary cells in culture was dependent on age of the pituitary donor (Barb *et al.*, 2002). Furthermore, IGF-I-induced LH secretion was greater in porcine pituitary cells from follicular phase gilts compared to luteal phase and OVX gilts (Whitley *et al.*, 1995). Thus, during pubertal development, endogenous IGF-I may contribute to the regulation of LH and GH secretion from the anterior pituitary, and therefore, the timing of puberty in the pig.

Table 3. Microarray analysis of age related changes from 90 to 150 (n = 5), 150 to 210 (n = 5) and 90 to 210 (n = 5) days of age in adipose tissue gene expression in the inner subcutaneous layer of the prepubertal gilt.

Gene description	90 to 150 days old	150 to 210 days old	90 to 210 days old	P value
Leptin	Up	Nc	Up	2.8 x 10 ⁻⁵
IGF-2	Nc	Nc	Up	0.001
IGF-1	Nc	Up	Nc	0.005
INSL3	Nc	Up	Up	0.005
IFN γ , IL-1 α	Nc	Nc	Up	0.003

IGF: Insulin-like growth factor; INSL-3: Insulin-like growth factor-3; IL-1 α : Interleukin-1 α ; INF- γ : Interferon- γ ; Nc: No change.

Adipose tissue, an endocrine organ

In addition to maturational changes at the CNS, the onset of puberty may be linked to attainment of a critical body weight or a minimum percentage of body fat (Frisch, 1984). Metabolic mass and food intake and subsequent metabolic rate may play a permissive role in the timing of puberty (Frisch, 1984; Barb and Kraeling, 2004). Moreover, initiation of puberty may also be influenced by metabolic factors of peripheral origin. It was postulated that metabolic signals are important in initiation of puberty in the pig and primate by Cameron *et al.* (1985) and Barb *et al.* (1997), respectively. Identification of such signals has remained elusive, primarily because there is a large number of peripherally originating substances that can act centrally to modify neuronal activity. Recent studies demonstrated that adipose tissue plays a dynamic role in modulating physiological mechanisms and whole-body homeostasis (Ahima and Flier, 2000). Adipose tissue constitutes the largest amount of stored energy in the body (Loftus, 1999). Energy homeostasis depends on a balance among several factors, such as feeding behaviour, adi-

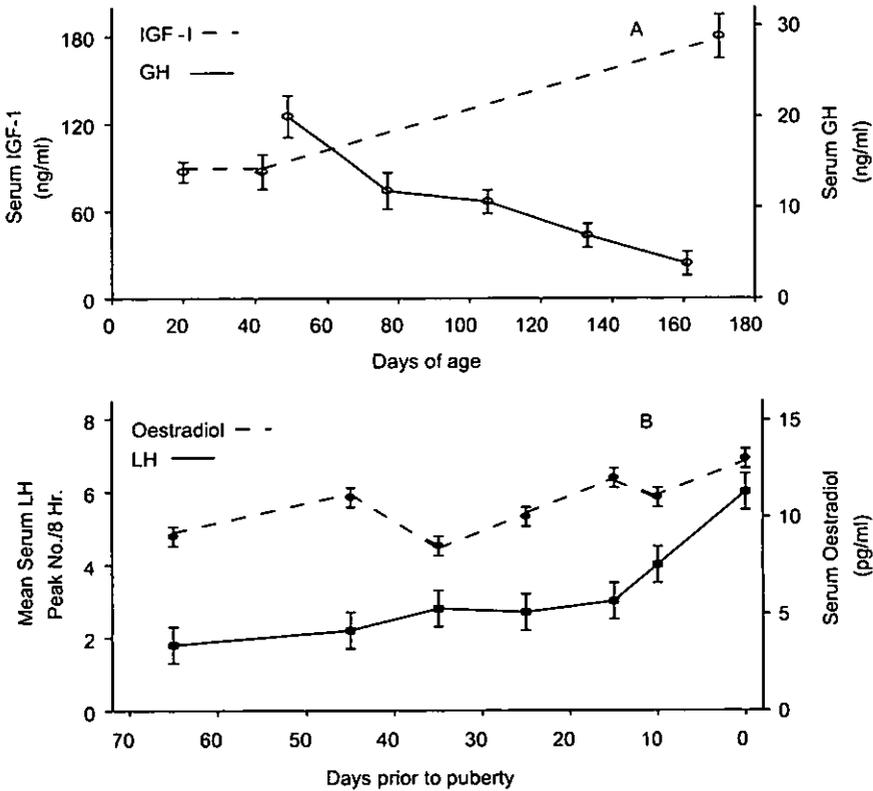


Fig. 2 (A) Serum IGF-I and GH concentrations at 20, 40, 60, 80, 100, 120, 140 and 160 days of age. (B) Serum oestradiol and LH concentrations at day 65, 45, 35, 25, 15 and 10 before oestrus and the day of oestrus. Modified from Lee *et al.*, 1991; Dubreuil *et al.*, 1987; Lutz *et al.*, 1984.

pose tissue mass and activation of catabolic processes, such as reproduction. Alterations in serum concentrations of many hormones and growth factors that regulate adipocyte function and leptin secretion are associated with developmental changes in body weight, nutritional status or adipose tissue mass (Barb *et al.*, 2001). Microarray analysis revealed that 21 secreted protein genes were expressed 40 fold in neonatal porcine adipose tissue and porcine preadipocyte cultures, such as agouti-related protein (AGRP), growth differentiation factor-9 β (GDF-9 β), and tumour growth factor- α (TGF α ; G.J. Hausman, unpublished). Moreover, RT-PCR analysis identified the agouti gene in pig adipose tissue. Proteomic analysis of conditioned media from adipose tissue and preadipocyte cultures identified several secreted proteins, such as relaxin, interleukin-8 (IL-8) and insulin like growth factor binding protein (IGFBP-5; G. J. Hausman, unpublished). Furthermore, there was an age related change in gene expression in the inner subcutaneous adipose tissue layer (G. J. Hausman, unpublished). Leptin expression increased between 150 and 210 days of age when compared to 90 day-old gilts. In addition, IGF-I and insulin-like factor-3 (INSL-3) gene expression increased between 150 and 210 days of age (G. J. Hausman unpublished; Table 3). These studies demonstrate expression of several major secreted proteins in pig adipose tissue, which may act as autocrine /paracrine factors to regulate adipocyte activity and centrally to influence the neuroendocrine axis. As cited above, hypothalamic OB-rb mRNA expression increased by 3.5 months of age and remained elevated at 6 months of age (Lin *et al.*, 2000) and the fact that leptin stimulated LH secretion from pig pituitary cells and GnRH release from hypothalamic tissue *in vitro* (Barb *et al.*, 2004) suggests that leptin acts through the hypothalamic-pituitary axis. Thus,

these reports support the idea that adipose tissue functions as an endocrine organ and may play a role in establishing onset of puberty.

Conclusion

Evidence has been presented that suggests puberty, and its associated increase in LH pulse frequency in the pig, is brought about by an integration of adipose tissue derived metabolic signals along with maturational changes in the hypothalamic-pituitary axis. The proposed sequence of developmental events involves increased excitatory and reduced inhibitory input on GnRH neurons. A concomitant age related reduction in oestrogen negative feedback on LH secretion and an associated increase in hypothalamic ER α and REA expression, an age related increase in anterior pituitary LH β and FSH β gene expression, and increased expression of adipose derived metabolic signals, such as leptin and IGF-I play a role in establishing onset of puberty (Fig. 3).

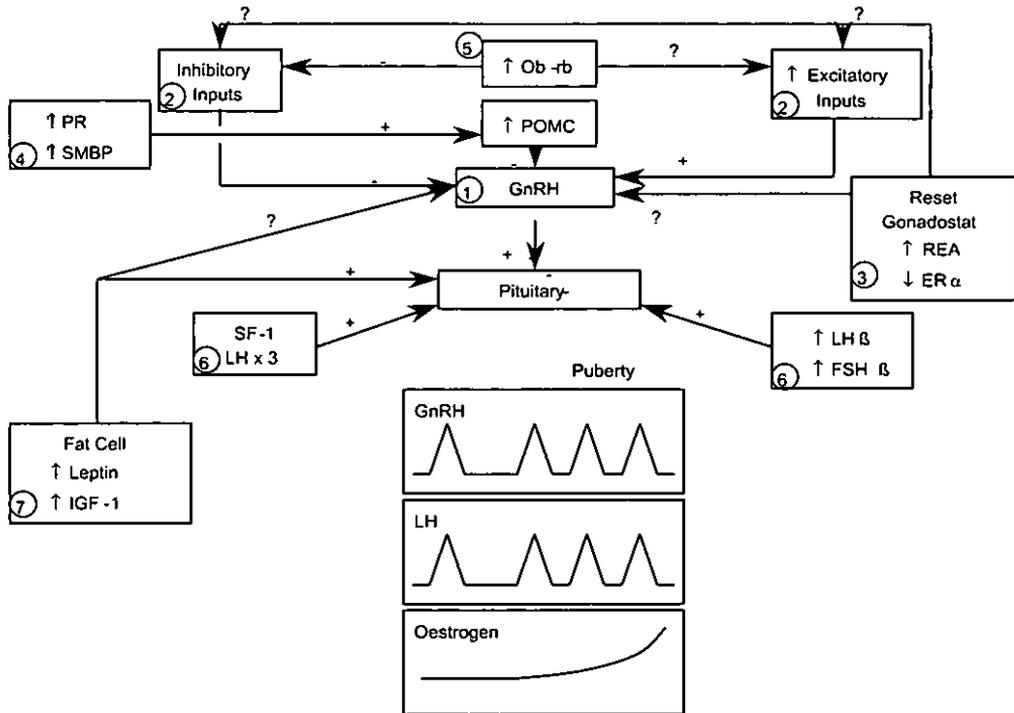


Fig. 3 Proposed hypothalamic and pituitary genes associated with changes in LH secretion and growth during pubertal development and adipose tissue genes and secreted proteins related to maturation of the neuroendocrine axis and puberty in the gilt. 1) Hypothalamic GnRH mRNA expression increased at 3.5 months of age and declined by 6 months of age. 2) Increased excitatory and reduced inhibitory input on GnRH neurons. 3) Concomitant age related reduction in oestrogen negative feedback on LH secretion and decreased oestrogen receptor- α (ER α) and increased repressor of oestrogen receptor activity gene (REA) expression at 210 days of age. 4) Development of the endogenous opioid peptide (EOP)-progesterone dependent LH inhibitory pathway. Pathway components: increased proopiomelanocortin (POMC); progesterone receptor (PR) membrane complement-1; steroid membrane binding protein (SMBP) gene expression at 210 days of age. 5) Expression of the long form leptin receptor (OB-rb) in the leptin responsive sites increased at 3.5 months of age. 6) Pituitary transcription factors, steroidogenic factor 1 (SF1) and Lhx3, and LH β and FSH β gene expression increased with age. 7) Age and adiposity related increase in leptin and insulin like growth factor -I (IGF-I) gene expression. (\uparrow , increases; \downarrow , decreases; ?, unknown/unclear).

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