Pituitary Leptin-A Paracrine Regulator of Gonadotropes: A Review

Noor Akhter, Christopher Crane and Gwen V. Childs*

Department of Neurobiology and Developmental Sciences, College of Medicine, University of Arkansas for Medical Sciences, Little Rock, AR, USA

Abstract: Leptin, a potent anorexigenic hormone secreted primarily by adipocytes, is known to be expressed in the anterior pituitary. Studies in our laboratory found leptin proteins and mRNA predominantly in somatotropes in normal male and cycling female rats. In contrast, leptin expression predominated in gonadotropes during pregnancy and lactation. Leptin expression varied with the cycle and was enhanced, *in vitro* by gonadotropin releasing hormone and Neuropeptide Y. In contrast, ghrelin inhibited pituitary leptin expression. Pituitary leptin in somatotropes or gonadotropes was reduced by nutritional deprivation for 24 h. However, growth hormone (GH), luteinizing hormone (LH) and pituitary leptin recovered if fasted animals were given glucose water. The glucose-mediated recovery suggests that the system is sensitive to changes in serum glucose. Somatotropes and gonadotropes also recovered if pituitary cells from fasted rats were stimulated *in vitro* with 1-100 pg/ml leptin. This *in vitro* leptin-mediated recovery suggests that leptin is important in the maintenance of somaotropes and gonadotropes. Collectively, the data suggest that pituitary leptin might serve as a "glucostat", sensing levels of serum glucose and reporting this nutritional state to somaotropes and gonadotropes in a paracrine manner. A decrease in pituitary leptin might signal nutritional deprivation to somatotropes and gonadotropes, lowering LH and GH production and allowing for conservation of resources. Lower GH would help conserve fat stores and lower LH would promote survival over reproduction.

Keywords: Leptin, somatotropes, gonadotropes, growth hormone, gonadotropins, pituitary, paracrine.

DISCOVERY OF LEPTIN AND ITS ROLE IN REPRODUCTION

Leptin is a recently discovered cytokine produced by white fat cells encoded by the *Lep* gene [1]. It regulates satiety as well as energy expenditure [2, 3]. This cyokine suppresses appetite by stimulating specific anorexigenic neurons in the arcuate nucleus [2, 4-6] that produce CART/POMC (cocaine and amphetamine RNA transcript / pro-opiomelanocortin) and á-MSH (alpha-melanocyte-stimulating hormone)**.** High leptin levels also inhibit functions of orexigenic neurons producing neuropeptide Y/agouti related peptide (NPY/AgRP) [7, 8], which stimulate appetite [7-12].

Leptin is also an "adipostat," because it informs the body of the levels of fat stores. However, the widespread distribution of leptin receptors throughout the body has suggested that this cytokine performs additional functions [13]. Schneider *et al.* [14-18] have reviewed leptin's effects on fuel utilization, showing that leptin works with insulin to facilitate the use of glucose and free fatty acids.

Leptin may also regulate neuroendocrine systems, particularly in the reproductive system [19-21]. Neuroendocrinologists have suggested that circulating leptin plays a critical role in reproduction because a threshold level of fat is vital for normal puberty and fertility [22-28]. Rats without functional leptin receptors (Zucker *fa/fa)* and mice without functional leptin proteins (*ob/ob*) both exhibit characteristics of morbid obesity, insulin resistance, delayed or impaired pubertal development, and pituitaries with low numbers of somatotropes or gonadotropes [29, 30]. Puberty is delayed or absent, and fertility is severely impaired in the absence of leptin receptors or in animals with low leptin levels [28, 31-33].

Infertility is also seen in obese humans bearing a mutation in the leptin gene (*LEP*) that causes leptin deficiency [34-36]. Humans lacking the full-signaling leptin receptor (*LEPR exon 16*) are both infertile and have impaired growth [37]. In adult humans, low gonadotropin levels and functional hypothalamic amenorrhea occur when a relative energy deficit (from weight loss, excessive exercise, or eating disorders) disrupts the pituitary–gonadal axis. Women with hypothalamic amenorrhea have low leptin levels and do not express the normal diurnal leptin rhythm [38-45].

Leptin therapy has normalized reproductive hormone levels in a leptin-deficient prepubertal child [34], as well as in adult men [46] and in women with functional amenorrhea [44, 45]. Giving exogenous leptin to leptin-deficient obese animals will cure their infertility [24, 47]. Leptin has also normalized gonadotropins (in women with functional hypothalamic amenorrhea), even if the behavior that led to it has not changed [43-45].

Although leptin may be important for fertility, it may not be directly responsible for the onset of puberty. Early studies showed that leptin accelerated puberty [22, 48, 49], suggesting that leptin might be a metabolic trigger for its

*Address correspondence to this author at the Department of Neurobiology and Developmental Sciences, College of Medicine, 4301 W. Markham, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA; Tel: 501 686-7020; Fax: 501 686-6382; E-mail: Childsgwenv@uams.edu

1876-5289/11

time of onset. However, later studies did not report a good correlation between normal prepubertal serum leptin levels and the timing of puberty in normal rodents [50-52] or primates [53-56]. Therefore, Cheung *et al*. [52], Urbanski [33], and Mann and Plant [32] suggested that leptin was not the trigger for puberty. It is considered a permissive factor because infertility develops when leptin is deficient [57]. Mechanisms behind its actions in reproduction are only beginning to be understood.

Leptin may regulate reproduction by direct or indirect effects on the hypothalamic-pituitary axis. Although the exact target cells in the hypothalamic part of the circuit are uncertain [25, 58-60], leptin's importance in regulating secretion from Gonadotropin releasing hormone (GnRH) neurons in the hypothalamus is well established [25, 50, 58, 61-66]. The uncertainty relates to the fact that GnRH neurons in rats and monkeys may not have leptin receptors (LEPR) [25, 58, 59], although LEPR are found on immortalized GnRH neurons [60]. Thus it is possible that LEPR are expressed early in developing GnRH neurons. Collectively, the data indicate that leptin regulates GnRH, directly or indirectly.

Thus, the literature suggests that leptin stimulates the secretion of both Luteinizing hormone (LH) and Gonadotropin releasing hormone (GnRH) either directly or indirectly [58, 64, 67-69]. Leptin also restores LH secretion in fasted mice, rats, hamsters and monkeys [14-17, 20, 58, 64, 67, 68]. Leptin also directly stimulates LH secretion from pituitary cells *in vitro* [70-74]. In addition, leptin regulates Growth hormone (GH) [19], although there is lack of agreement about the exact direction of its regulation [19, 75-78]. Leptin stimulates GH secretion mediated by GH releasing peptide (GHRP [77, 79] and restores GH pulses in fasted rats [76, 78]. Luque *et al.* [80] reported that leptin restored GH secretion in *ob/ob* mice that were infused with exogenous leptin for 7 days. In addition, leptin caused an increase in Growth hormone releasing hormone (GHRH) receptors. No changes were noted in GHRH levels themselves, which suggested that GHRH did not mediate the restoration.

Collectively, the evidence supports the hypothesis that leptin may serve as a mediator or modulator in the neuroendocrine circuit regulating gonadotropes and somatotropes. The hypothesis is further supported by evidence showing a reduction in both gonadotropes and somatotropes in rats or mice that are leptin deficient [29, 30]. Because somatotropes and gonadotropes are present in leptin-deficient rodents, **t**hese data suggest that leptin may not be needed for the embryonic birth of these cell types. Rather, the reduced numbers suggest that leptin may promote later developmental events necessary for the full functioning of these cells.

Support for a role later in development in rodents comes from studies by Ahima *et al.* [81], who mapped postnatal changes in serum leptin. They reported a discrete surge in leptin levels during days 7–10 of postnatal development in mice coinciding with a 6–10-fold increase in leptin mRNA in the adipose tissue, suggesting that the source was indeed adipocytes. Their studies showed that this surge did not depend on changes in fat mass or food intake, which suggests that it may be linked to an independent source of regulation not identified in their study. Similar findings in mice and rats were reported by Devaskar *et al.* [82], who showed a rise in leptin levels as early as 2 postnatal days followed by a decline in leptin after postnatal day 14. Ahima *et al*. [81] correlated this rise in leptin with a potential role for leptin as a neuroendocrine hormone and linked it to significant changes along the neuroendocrine axes.

How would these changes relate to development in the human? In fact the timing of events in rodants and humans correlate well, because rodents are relatively immature at birth. The postnatal ages that correspond to the rise in leptin are equivalent to the midgestational stage of human fetal development [83, 84]. Reitman *et al*. [85] described a comparable surge of leptin in the human fetus, showing a 100–150-fold rise from the first to the second trimesters. The source of leptin is mostly the placenta, which is so important that Ong *et al*. [86], correlating leptin levels in cord blood, found them to be inversely proportional to weight gain after birth.

Collectively, this suggests important homeostatic roles for leptin in early human development. This second trimester rise in leptin correlates with a similar rise in GH secretion [87]. Shimon *et al.* [88] reported both the presence of leptin receptors, and leptin stimulation of GH secretion in cultured fetal human pituitary cells, suggesting that pituitary cells from this stage are responsive to leptin. The expansion of gonadotropes also coincides with the rise in leptin during the second trimester [87]. There an increase in GnRH and gonadotropin levels, especially in females [84, 88-92]. Castillo *et al.* [89] reported that GnRH stimulated LH release from cultured fetal human pituitary cells, with higher levels coming from female fetuses.

THE CASE FOR LEPTIN AS A PARACRINE REGULATOR IN THE ANTERIOR PITUITARY

Most of the early studies assumed that leptin's functions are mediated by circulating leptin from adipocytes. However, a growing body of evidence suggests that the adipocyte source of leptin may not be able to regulate rapid neuroendocrine responses. The rise in serum leptin that is seen at midcycle is too slow for it to be a regulator of the LH surge [93]. Furthermore, Schneider *et al* report that nutrition alone can restore LH pulses lost by fasting [14, 15]. The timing of this response did not match that for the restoration of adipocyte leptin [50, 94, 95]. This has led workers to consider additional sources of leptin for the regulation (permissive or facilitatory) of neuroendocrine pathways. The most obvious sources might be from the neuroendocrine cells themselves.

Evidence is growing that leptin is produced by the anterior pituitary and the hypothalamus [31, 93, 96-100]. A pituitary source would point to a paracrine role for this cytokine. Studies have identified leptin expression in the pituitary and the hypothalamus of humans and rodents although during the early part of the decade, studies did not agree as to the cell type producing leptin. One report of rats and mice found leptin only in TSH cells [97]. Another group suggested that leptin was in gonadotropes or thyrotropes, but not in somatotropes [100]. In humans, leptin proteins were found in 70% of corticotropes, 21% of somatotropes and 29-33% of gonadotropes, prolactin cells or thyrotropes [30].

Vidal and Cohen reported labeling for leptin in secretory granules of all pituitary cell types but prolactin cells [101]. In human pituitary adenomas, leptin mRNA and leptin secretion was detected in subsets of all tumorous pituitaries [102, 103]. Thus, a pituitary source of leptin could mediate its local, paracrine effects. Later sections of this review will present data from this laboratory that show both the cell types that produce leptin and also potential regulators for this source of leptin.

The hypothesis that pituitary leptin might be a paracrine factor is further supported by the fact that subsets of all pituitary cells have leptin receptors (LEPR) [30, 96, 97, 99-101]. LEPR are related to the class 1 cytokine receptor superfamily [104, 105]. The long form of the receptor, LEPRb, has a single-pass transmembrane domain and a 302-amino-acid cytosolic domain that binds and activates the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway [106]. In addition, there are multiple splice-variant short isoforms of this receptor, which may be expressed in a tissue-specific manner [107]. They include LEPRa, LEPRc, and LEPRd, each of which contains a single transmembrane domain and 34-, 32-, and 40-amino-acid cytosolic domains, respectively. Another isoform, LEPRe, is soluble and lacks the transmembrane domain. Of all of the isoforms, only LEPRb contains the complete cytosolic domain that activates JAK/STAT pathways. The exact physiologic significance of the other isoforms is uncertain; however, there is speculation that they are involved in transport in serum and through the blood–brain barrier [107]. In *db/db* mutant mice, deletion of exon 16 of the *Lepr* interferes with the JAK/STAT pathway binding and renders the mice hyperphagic and obese [108-111]. A similar, rare deletion of *LEPR* exon 16 has been reported in a human family by Clement *et al.* [37].

LEPR mRNA was first detected in rat pituitary cells over a decade ago by Zamerano *et al.* [57]. Cai *et al*. [112] reported increased expression of *Leprb* in the anterior pituitaries and somatotropes of mice expressing a human GHRH transgene [113]. Shimon *et al*. [88] reported *LEPR* mRNA expression in adult and fetal human pituitaries, as well as in GH- and prolactin-secreting adenomas. Giusti *et al.* [114] also reported high *LEPR* expression levels in human GH-secreting adenomas. Jin *et al.* [96, 97] used RT-PCR to show that *LEPR*a and *LEPR*b mRNAs were expressed in most, if not all, of the 12 types of human pituitary tumors, including those secreting GH. Later studies of porcine pituitaries detected *LEPR* mRNA [115] and also quantified the expression of LEPR in each of the cell types [116]. LEPR immunoreactivity was shown in 69% of somatotropes, which fits well with what we have reported in rats and mice [93, 98, 117]. Sone *et al.* [99, 100] detected LEPR in 97% of rat pituitary somatotropes.

LEPR expression by gonadotropes was first reported by Jin *et al*. [96, 97], who detected LEPRa and LEPRb isoforms in human LH adenomas and two mouse cell lines: LβT2 cells, which produce LH and FSH; and αT3-1 cells, which produce αGSU. In porcine pituitaries [116], LEPR were expressed in 90% of gonadotropes in the pars tuberalis and in 29% of remaining gonadotropes. Later studies by Kaminiski *et al*. [118] showed regulation of LEPRb with the estrous cycle, reporting that expression of LEPRb peaked during the luteal phase of the cycle and early in pregnancy in porcine pituitaries. Igbal *et al* [116] reported LEPR in 70% of somatotropes in ovine pituitaries. In contrast to the foregoing, Sone *et al*, [99, 100] showed the leptin receptors in 97% of somatotropes (but not the other cell types). They suggested from this evidence that leptin might be produced by gonadotropes for the paracrine regulation of somatotropes. Sone *et al*. [99, 100] found LEPR in less than 1% of gonadotropes. However, our recent studies have confirmed Kaminiski *et al*. [118], showing significant expression of LEPR by most gonadotropes [93].

Our studies of pituitary leptin began early this decade with tests of the hypothesis proposed by Sone *et al*. [98]. We reasoned that if leptin was produced primarily by gonadotropes, we would see changes in leptin expression with the reproductive state. In addition, we recognized that we needed to prove that leptin was actually produced by pituitary cells by detecting leptin mRNA. Our concern, as we began these studies, was that some of the leptin proteins identified by immunolabeling might reflect circulating leptin bound to leptin receptors. Thus the first set of tests identified cells that expressed leptin mRNA, which provided needed proof for leptin production by pituitary cells [93, 98]. The remaining sections of this report discuss our findings, which prove leptin production by specific cell types and our follow up studies, which show evidence that pituitary leptin may act as a paracrine or autocrine regulator in the pituitary [93, 117]. These latest findings are discussed in light of our current understanding of a role for leptin in the regulation of the pituitary.

CHANGES IN PITUITARY LEPTIN EXPRESSION WITH THE REPRODUCTIVE STATE

To detect changes in leptin-bearing cells with the physiological state, dual immunolabeling for leptin and GH, FSHβ, or LHβ was done, as previously described [98]. In situ hybridization was carried out as described previously [98, 119] with the use of a 48 bp biotinylated oligonucleotide probe complementary to nucleotides 342-389 located within the coding sequence for rat leptin (accession number NM_013076). qRT-PCR assays for leptin mRNA are also described in previous studies [98, 106]. The statistics and power analyses are also described in these studies.

The percentages of cells with leptin proteins rose from a low of 21.0 ± 4.0% on the AM of estrus to a peak of 55.0 ± 3.0% of AP cells on the afternoon of proestrus [93]. This peak during proestrus is higher than that in all other groups ($p<0.001$), including male rats, which had 39.6 ± 1.0% of AP cells with leptin proteins. All data reported in this and later sections represent the average ± SEM.

The counts of pituitary cells with leptin mRNA show leptin production by 32-37% of pituitary cells in male or in estrous, metestrous, or diestrous female rats [93]. Early in the cycle (estrus and metestrus), there are significantly more cells with leptin mRNA than leptin proteins. In situ hybridization and QRT-PCR assays both detected an interesting significant decline in leptin mRNA on the morning of proestrus [93]. The QRT-PCR assays show that leptin mRNA is maintained at relatively low levels through estrus. Then, levels rise 2—fold and by diestrus, they are higher than all other groups. The increase in mRNA from

metestrus to diestrus was also detected by densitometric analysis of cytochemical label, which showed that the total area of label for leptin mRNA increased and thus signified a higher level of production of leptin mRNA by individual cells. Finally, the most unexpected and interesting finding in this survey was in pregnant animals, which exhibited the highest percentages of AP cells with leptin proteins (60.0 ± 2.0%) or mRNA (44.0 ± 2.0%) [93]. Intermediate levels of leptin-bearing cells, in the range of those seen early in the cycle, were seen in females taken on the 3rd day of lactation.

These studies brought out several significant points about the expression of leptin in pituitary cells [93]. First, leptin expression varies with the stage of the cycle or during pregnancy. Leptin proteins reach a peak just before the LH surge and then are reduced by the morning of estrus. Perhaps leptin stores may have been secreted, although we cannot rule out degradation as a cause of the reduction. The leptin-bearing cells could still be detected, however, by their content of mRNA.

Second, the differential expression of leptin mRNA is important evidence that confirms the production of leptin by pituitary cells. This addresses the concern that pituitary might be there only as a result of uptake of circulating leptin. Pituitary leptin mRNA is detected by both in situ hybridization and QRT-PCR.

Third, more specific paracrine role for pituitary leptin is suggested by the collective evidence showing that leptin is known to be a secretagogue for LH, both *in vivo* and *in vitro* [67, 70, 73, 74, 120]. There is no rapid periovulatory rise in serum leptin coming from adipocytes to permit or facilitate an LH surge. However, the rapid rise and fall in pituitary leptin from proestrus to estrus may be timed to adequately facilitate LH secretion. The need for adequate leptin to permit gonadotrope function will be brought out and discussed further in a later section focused on the effects of fasting on LH cells.

The dramatic rise in leptin transcripts on diestrus AM and PM coincides with a time when LH protein synthesis is enhanced. If leptin is important in the regulation of the LH surge, the timing of leptin expression would suggest that it could permit or facilitate LH synthesis or secretion during diestrus and proestrus. The rapid decline in leptin mRNA during estrus is intriguing and consistent with the hypothesis that leptin transcripts are tightly regulated during the cycle, perhaps by rapid degradation.

Our data on pregnant animals suggest that pituitary leptin might be regulated to support gonadotropic or other reproductive functions during pregnancy. This could relate to its role in facilitating the utilization of nutrients, like glucose and free fatty acids [14-18, 121]. It may also play a role in maintaining gonadotropes, or it may regulate placental or gonadal functions.

PITUITARY CELLS PRODUCING LEPTIN

Effects of the Reproductive State

Tests of regulators for pituitary leptin depended upon our results from studies that determined which cell types produced leptin, especially during the reproductive cycle. Our original hypothesis was based on data from Sone *et al* [99, 100], which indicated that leptin might be produced by gonadotropes to regulate somatotropes. The changes overall in pituitary leptin certainly supported production by gonadotropes. Dual immunolabeling was therefore done on some of these experimental groups to test this hypothesis. The main question was whether or not somatotropes or gonadotropes, or both, contributed to the rise in the percentage of leptin-bearing cells during proestrus and pregnancy.

Cells co-labeled for leptin proteins and GH proteins were significantly increased on the morning of diestrus to 37.0 ± 4.0% of the population on the morning of proestrus. Further more, the 12 percentage point increment matched the overall increment in pituitary cells bearing leptin [93]. Because the overall percentage of pituitary cells with GH proteins did not change significantly, this may reflect the mobilization of somatotropes that are stimulated to express more leptin during diestrus. Thus, these cells appear to be significant contributors to the rising overall leptin during this period.

The comparative percentages of gonadotropes in the population suggest that these cells probably do not contribute to the rise in pituitary leptin. Gonadotropes with leptin proteins represented 6-8% of pituitary cells in cycling females. Similarly, in male rats, cells co-expressing leptin and GH were most abundant (26% of pituitary cells) and only 4.0% of pituitary cells co-expressed leptin and gonadotropins.

Significant plasticity in the cell types expressing leptin was seen in populations from pregnant rats, as leptin-bearing cells shifted from being predominantly somatotropes to a population that is predominantly gonadotropes [93]. Whereas there were no significant changes in the overall percentages of cells with GH proteins in pregnant or lactating rats, fewer GH cells co-expressed leptin proteins. At the same time, there was a significant rise in percentage of pituitary cells that co-expressed LH or FSH and leptin to 42.0 ± 1.0% or 36.0 ± 4.0% of pituitary cells, respectively. Cells from lactating females also had significantly more cells with LH and leptin (18.7 ± 0.4%) or FSH and leptin (17.0 ± 1.0%) than normal cycling rats [93].

Thus the proportion of leptin-bearing cells with GH declined from 60-70% in normal males or cycling females to less than 20% in pregnant or lactating females [93].

In contrast, the proportion of leptin-bearing cells with gonadotropin rose from 17% in cycling females to 60-70% in pregnant rats [93]. The analysis of dual labeling shows overlap in the percentages of leptin bearing cells with LH or FSH, which supports the hypothesis that leptin is expressed in part by bihormonal gonadotropes.

When, in situ hybridization was done to detect leptin mRNA in cells with GH or LH proteins [93], the data confirmed that from the immunolabeling. Most leptin-mRNA bearing cells in diestrous animals expressed GH and less than 10% expressed LH. In males, 73% of leptin-mRNA bearing cells express GH and 10% express LH. [93]

Thus, our data detecting leptin proteins or mRNA did not support the original hypothesis [99, 100], that leptin production was predominantly in gonadotropes in normal animals. On the other hand, the hypothesis was supported in pregnant and lactating females. There was a significant

decline in cells co-expressing GH and leptin mRNA. Thus, there is plasticity in the expression of leptin across cell types. This association between gonadotropins and leptin in pregnancy and lactation may correlate with the enhanced needs of the animal during these reproductive states. One might speculate that, if it can be secreted, gonadotrope leptin might provide an important continuing source of this hormone, as stores of fat are used to support the pregnancy or lactation. Selective knockout animals in which leptin is ablated from gonadotropes, would be needed to test this hypothesis.

Effects of the Nutritional State

The predominance of leptin production by normal somatotropes alerted us to the possibility that leptin may be regulated by different nutritional states, which would be consistent with the one of the somatotropes' functions, to break down fat and thus optimize body composition. Therefore, the studies of leptin production by the pituitary were continued in male rats following a 24 h fast [117]. It was already known that significant changes in the adipocyte leptin mRNA expression can be seen as early as 8 hours [1, 122, 123], with losses in leptin mRNA of 50% [124] or 85-90% [12, 125] in 24 h. These studies led to tests of potential paracrine actions by pituitary leptin, so they will be described in more detail.

Food deprivation brought about changes in the expression of pituitary leptin. As illustrated by (Fig. **3**) in reference [117], there was a significant 64% reduction in the overall percentages of pituitary cells with leptin mRNA from 33 ± 2 to 12 ± 1% of anterior pituitary cells ($p<0.001$) along with a 40% reduction in the integrated optical density of label ($p=0.03$). There was also a 22% reduction in the percentages of cells with leptin proteins, from 36 ± 2% to 29 ± 2 % of AP cells (±SEM; $p<0.029$)[117].

The next objective of these studies was to learn which pituitary cell types were most affected by the food deprivation, specifically in their production of leptin. Fig. (**4**) in ref. [117] illustrates dual labeling for all pituitary hormones, which accounted for over 90% of cells with leptin proteins, with 61% of leptin-bearing cells co-expressing GH, 8-10% co-expressing LH, TSH, or ACTH and only 3% co-expressing prolactin. The remaining 10% could be folliculostellate cells, or monohormonal FSHβ cells, which were not detected. Food deprivation caused a significant loss in leptin protein expression by somatotropes or gonadotropes. In contrast, corticotropes maintained their expression of leptin at 30% of ACTH cells.

When we focused on the pituitary hormone bearing cells themselves to learn about the overall impact on their expression, we found that fasting caused declines in percentages of pituitary cells with GH, prolactin and LH [117]. The percentages of thyrotropes appeared unaffected by fasting. In contrast however, fasting increased percentages of corticotropes over 2X from 10 to 21% of AP cells. The overall increase in corticotropes resulted in a 1.94-fold increase in pituitary cells with leptin and ACTH, from 3.6% to 7% of AP cells ($p=0.03$). The analysis of leptin in fasted rat pituitary populations accounted for 86% of the leptin cell population, with 45% of leptin cells storing GH (reduced from 61%) and 24% storing ACTH (increased from 8%). Illustrations of these changes are found in photographs in Fig. (**4**), [117].

We focused then on gonadotropes and somatotropes for continued studies of leptin mRNA expression, because they were most severely affected by the food deprivation. Our analysis showed an 80% decline in cells that co-expressed leptin mRNA and GH proteins from 25 ± 3% to 5 ± 2% of AP cells ($p<0.014$) [117]. Similarly, fasting caused a significant decline in the co-expression of leptin mRNA in gonadotropes from 3 ± 0.5% of AP cells to 0.7 ± 0.02% of AP cells ($p<0.029$). The sum of the losses in gonadotropes and somatotropes accounted for the overall loss in leptin mRNA. Fig. (**5**) in reference [117] illustrates the dual labeling for leptin mRNA and GH in fed and fasted rat populations.

These studies have once more shown leptin plasticity in the anterior pituitary, only this time it was in response to metabolic changes as a result of fasting. The finding highlights the importance of leptin expression by somatotropes and brings out another set of leptin producers, the corticotropes. They will ultimately provide the basis for follow-up studies of the significance of each site. However, this report will focus on our ongoing studies of somatotropes and gonadotropes.

REGULATION OF PITUITARY LEPTIN

Growth Hormone Releasing Hormone (GHRH)

The first set of studies focused on GHRH as a regulator for pituitary leptin, primarily because most leptin producing cells are somatotropes [98]. Biotinylated GHRH was used to detect target cells with cytochemical (avidin peroxidase) techniques [126]. In diestrous animals, 24 ± 3% (values ± sd) of pituitary cells bound Bio-GHRH and also stored leptin. This co-expression involved 84 ± 10% (values ± sd) of Biotinylated GHRH target cells. Analysis of populations from estrous rats (in which leptin was found in only 20% of pituitary cells), showed that only 11 ± 1.7% of pituitary cells co-expressed Bio-GHRH binding sites and leptin. The patches of peripheral labeling could be on the surface, or in vesicles and early endosomes.

The next set of studies tested responses to GHRH. Estrous rats were used for these experiments to determine if GHRH would stimulate more leptin-bearing cells to levels like those seen in proestrus. Neither estradiol (100 pM) overnight nor 2 nM GHRH alone for 3 hours stimulated expression of more leptin-bearing cells. However, stimulation with both estrogen and GHRH increased expression of leptin to levels similar to those found in diestrous or proestrous animals (41 ± 9%; $p=0.02$). The changes in the leptin-bearing cells are illustrated in ref [98]. No GHRH stimulated secretion of leptin was noted in these experiments, however.

These studies thus showed an increase in leptin expression by somatotropes to levels like those from diestrous animals. This suggests that rising estrogen may increase GHRH receptivity and hence increase leptin expression. The lack of evidence for secretion of leptin could reflect an autocrine role for this cytokine.

Ghrelin

Ghrelin is a relatively new secretagogue for GH. It was discovered as a product of the stomach in 1999 [127], found in the secretory granules of X/A-enteroendocrine cells [128]. It was named ghrelin on the basis of the Proto-Indo-European word root ***ghre***, which means "to grow." Ghrelin was reported to be a potent secretagogue for growth hormone (GH) in humans [129] and rats [130]. In both species, ghrelin also stimulated the release of adrenocorticotropin (ACTH) and thyroid-stimulating hormone [129, 130]. Studies and reviews of ghrelin's actions have proliferated, including those showing its orexigenic functions [128, 131-135]. Ghrelin is released 30 min after the stomach is empty, and signals hunger, stimulating orexigenic neuropeptides [131, 133, 136-140].

Because ghrelin is stimulatory for somatotropes, recent studies were initiated to determine if it also affected pituitary leptin. Fig. (**1A**) shows that 3 h in 1 or 10 nM ghrelin had no significant effects on the expression of pituitary leptin proteins, as measured by integrated optical density (IOD) of immunolabeling in dispersed pituitary cells. In contrast, ghrelin caused a significant reduction in the expression of pituitary leptin mRNA, as quantified by IOD of fields labeled by *in situ* hybridization. Counts of leptin mRNA-bearing cells (Fig. **1B**) also showed a significant decline with 1 nM (p=0.018) or 10 nM (p=0.013) ghrelin (from 32.3 ± 0.3% to 18.3 ± 0.6% or 17 ± 16%, respectively). The response to ghrelin is illustrated by photographs in Fig. (**1C**) and (**1D**).

Dual labeling was then performed to determine if the loss in leptin mRNA was in GH cells. Fig. (**2**) is a graph showing the significant decline in overall percentages of anterior pituitary (AP) cells dual labeled for leptin and GH proteins ($p<0.001$). When the counts are expressed as a percentage of leptin bearing cells, 1 nM ghrelin resulted in a decline in the percentage of leptin mRNA-bearing cells that contained GH from 59 ± 6% to less than 4.7 ± 3% of leptin cells (p=0.001). Similarly, 1 nM ghrelin produced a decline in percentages of GH cells with leptin mRNA from 70 ± 3.5% to 18.6 ± 6% of GH cells ($p<0.03$). The apparent increase with 10 nM was not significantly different from values seen with 1 nM. The decline is illustrated in Fig. (**3A**) and (**3B**). In the vehicle control (Fig **3A**), there are at least 4-5 dual labeled cells, however the field treated with ghrelin has only one (Fig. **3B**). Also, note that the labeling for GH proteins has diminished with the ghrelin to pale amber (note circled cells).

These studies suggest that ghrelin may be a potent regulator of pituitary leptin mRNA, specifically in somatotropes. Ghrelin is an appetite stimulator and it is

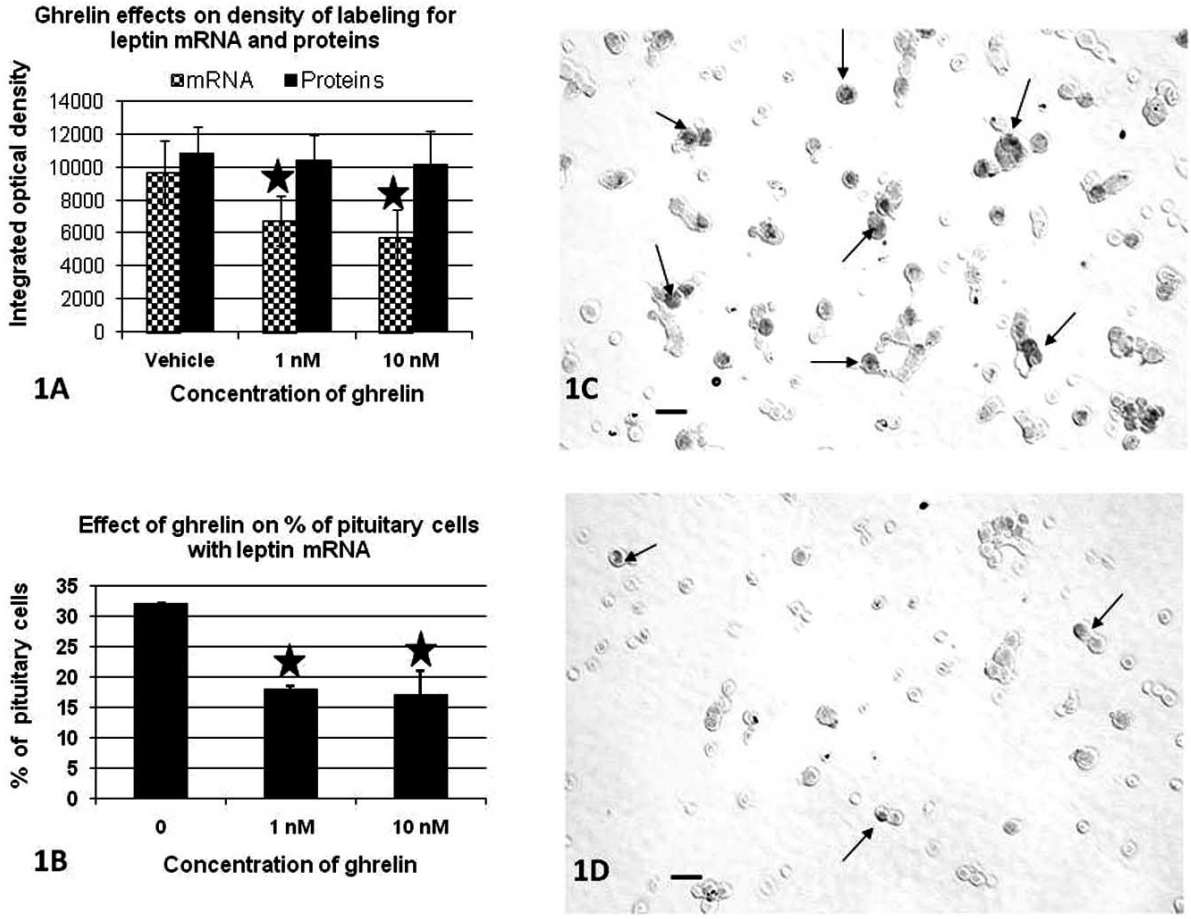

Fig. (1). Effect of 3 h in ghrelin *in vitro* on expression of pituitary (AP) leptin mRNA or proteins. Figure 1A shows a reduction in density of label for leptin mRNA (IOD) and Figure 1B shows a decrease in percentages of cells with leptin mRNA. This decrease in mRNA-bearing cells is illustrated in Figures 1C (vehicle control) compared with Figure 1D (ghrelin treated). Bar=15 μm.

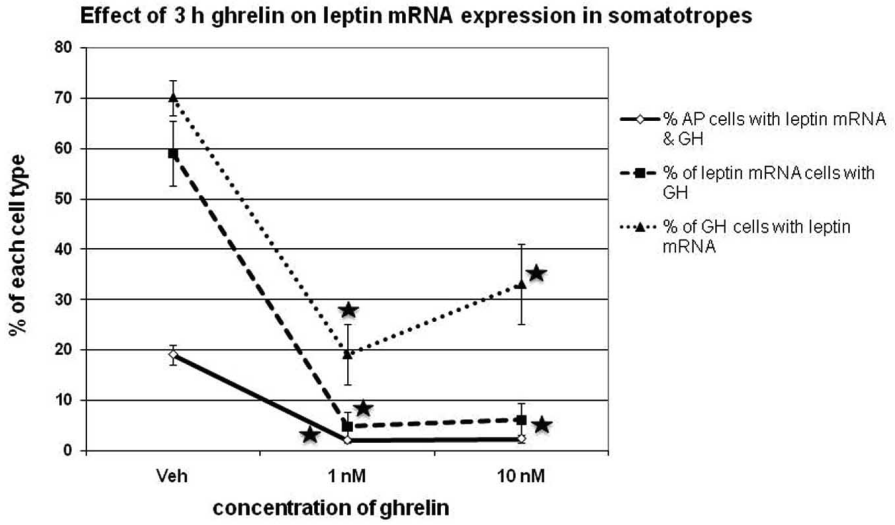

Fig. (2). Cells from the experiment in Figure 1 were dual-labeled for leptin mRNA and GH proteins to detect changes, if any in expression in somatotropes. The overall percentage of pituitary (AP) cells declines significantly ($p<0.001$) after 1 nm along with the percentage of leptin-bearing cells that store GH ($p=0.001$). Both of these data points suggest that the effects of ghrelin on leptin mRNA are predominantly in somatotropes. There is also a significant decline in the percentages of somatotropes that express leptin mRNA ($p<0.03$). There are no significant differences between values detected after exposure to 1 or 10 nM.

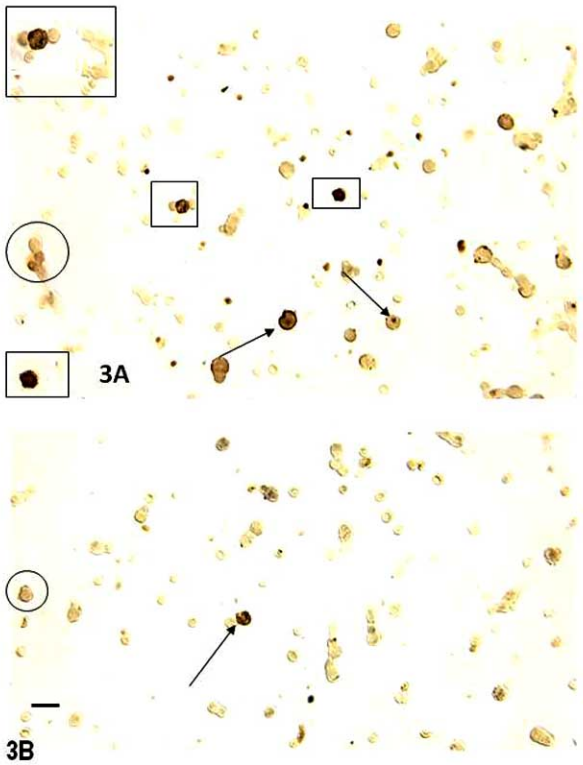

Fig. (3). Photographs illustrate the dramatic reduction in leptin mRNA (dark labeling) in somatotropes in the ghrelin-treated field (Figure 3B) compared with the numerous dual labeled cells in the vehicle control (Figure 3A). Bar=15 μm. Circles show GH cells without leptin mRNA. Squares and arrows show dual labeled cells (GH cells with leptin mRNA). The inset in Figure 3A shows a higher magnification of one of the dual labeled cells.

possible that the inhibition of leptin mRNA in somatotropes may reflect a feedback circuit that would prevent somatotrope leptin from contributing to the circulating pool and thus inhibiting appetite. This feedback loop could also stimulate GH, which is lipolytic and reduces leptin expression by adipocytes, thereby lowering leptin.

The role of ghrelin as a mediator of paracrine actions by pituitary leptin is less certain, however. One clue might

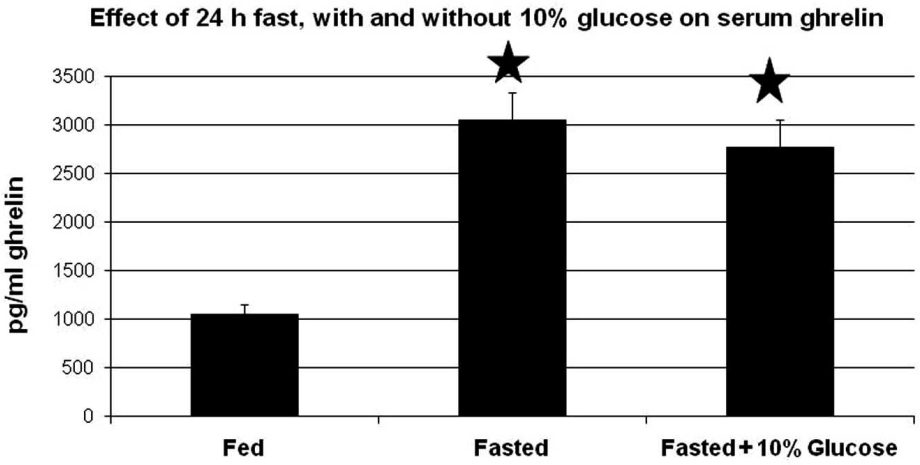

Fig. (4). Serum from the fasted groups of animals studied as reported in reference [117] was collected and assayed for ghrelin by EIA. A third group of animals were fasted and given water containing 10% glucose. In both groups of fasted animals, serum ghrelin was elevated significantly (star; $p<0.001$).

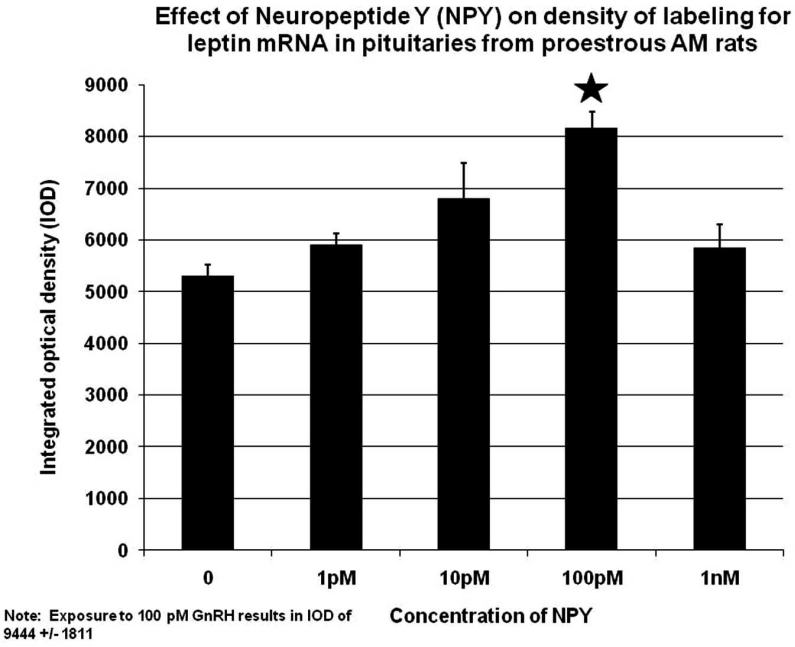

Fig. (5). Freshly dispersed pituitary cells from rats taken on the AM of proestrus were treated for 1 h with neuropeptide Y (NPY) and significant increases in labeling were detected following exposure to 100 pM NPY ($p<0.001$). The comparable values for 100 pM GnRH are shown in numbers in the lower left of the Figure.

come from reported actions of ghrelin on gonadotropes and luteinizing hormone secretion. Investigators have shown that ghrelin had inhibitory effects on LH and mixed effects on reproductive functions, depending on sex and physiological state [141-143]. Other workers showed reductions in LH pulse frequency in rats [144] or humans [145]. Recently, Iqbal *et al.* [146] reported that an i.c.v. infusion of ghrelin lowered plasma LH in sheep. Fig. (**4**) shows serum ghrelin results from the fasting experiments reported in the first section of this review. As expected, serum ghrelin is elevated during the fast, even if glucose water is provided. This ghrelin response to fasting would explain the lower LH and leptin reported by our study [117] and insure that the animal's survival needs are met before reproductive needs. The higher ghrelin might thus be responsible for the mediation of the lower pituitary leptin. Furthermore, if pituitary (somatotrope) leptin is important for gonadotrope function through paracrine mechanisms, lowering somatotrope leptin might contribute to the lower LH or reduced pulses of LH [145].

Gonadotropin Releasing Hormone (GnRH)

Gonadotropes are regulated by gonadotropin releasing hormone (GnRH) neurons, which are scattered in the preoptic and anterior regions of the hypothalamus and extending to the arcuate nucleus [147-149]. GnRH is secreted in pulses and stimulates both LH and FSH synthesis and release [150-156]. Estrogen from the ovary exerts positive feedback to gonadotropes from diestrus to proestrus and increases the production of GnRH receptors [157] on

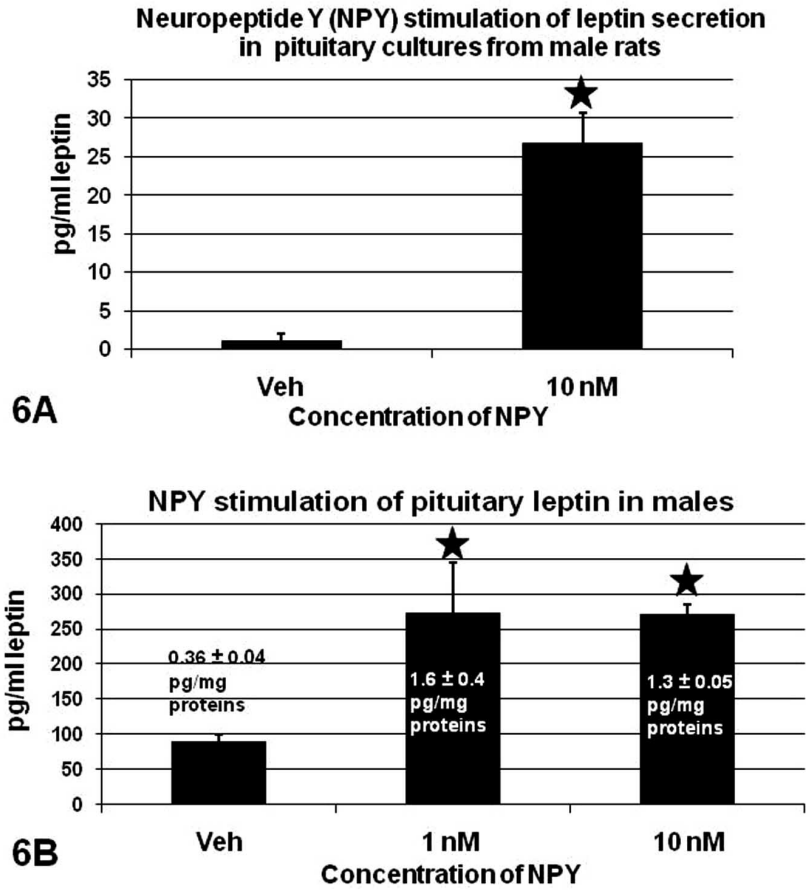

Fig. (6). A. NPY is effective in the stimulation of leptin secretion from freshly dispersed male rat pituitary cells ($p<0.001$; Star=significantly different from vehicle-veh control). Figure 6B shows that NPY also stimulated more leptin protein expression in pituitary extracts from male rats. Star=significantly different from Vehicle (veh) control. The numerical values above or on the bars show the data expressed per mg proteins.

gonadotropes. GnRH pulses are slower during diestrus, thereby favoring FSH synthesis, but faster during proestrus, favoring LH secretion [157-159].

Strong evidence for a stimulatory effect of leptin directly on gonadotropes comes from studies in which the fertility of leptin-deficient *ob/ob* mice was completely restored by exogenous leptin [24]. Leptin also restored LH secretion in fasted mice, rats, and monkeys [14-17, 20, 58, 64, 67, 68]. Leptin increased LH levels in ovariectomized, estrogen-primed rats [72, 73]. Antiserum to leptin suppressed LH secretion and disrupted the estrous cycles [160]. All of this information assumes that the source of leptin comes from adipocytes.

We reasoned that, if pituitary leptin plays a role in regulating gonadotropes, it might be regulated by GnRH. We realized that pituitary leptin expression by gonadotropes is relatively low except in lactating or pregnant animals. However, the increased expression of leptin seen during proestrus along with that in lactating and pregnant animals suggested that reproductive regulatory factors like GnRH and estrogen may be important in regulating leptin expression.

The first set of studies [93] detected binding sites for GnRH on pituitary cells that contain leptin with 1 nM biotinylated GnRH [161-164] and dual labeling. In diestrous rats, cells with GnRH receptors and leptin proteins are 11.5 ± 2.0% of all pituitary cells. This represented 30.0 ± 3.0% of leptin-bearing cells and 73.0 ± 3.0% of GnRH target cells. The GnRH labeling actually identifies more leptin in cells that are gonadotropes than does labeling for gonadotropins. This is explained further at the end of this section.

Estrogen is a well established modulator of GnRH receptors and our previous studies have shown that 100 pM increases the percentage of GnRH-target cells, when given overnight to diestrous rats [157]. Our studies showed that, whereas estrogen does increase the overall percentage of GnRH-target cells as in our previous studies [157], it does not significantly increase the number of leptin-bearing cells that bind GnRH, which remain at 13.25 ± 2.0% of AP cells [93].

The next phase of our study was designed to learn if estrogen and GnRH could increase leptin mRNA expression by gonadotropes labeled for LH or FSH proteins [93]. Three groups of cells from diestrous rats were treated with and without 100 pM estradiol overnight and then given vehicle or 1 nM GnRH for 1 h the next morning. They were then fixed and labeled for leptin mRNA followed by immunolabeling for LHβ or FSHβ. Neither estrogen nor GnRH alone stimulated more gonadotropes to express leptin mRNA. However, estrogen and GnRH together stimulated a

significant increase in percentage of pituitary cells that co-express LHβ and leptin mRNA from 7.0 ± 2.0% to 11.0 ± 3.0% ($p<0.03$) of AP cells and an even greater increase in the percentages of cells with leptin mRNA and FSHβ from 8.0 ± 3.0% to 15.0 ± 6.0% ($p<0.02$)[93]. These findings support the hypothesis that the increase in leptin expression by gonadotropes in pregnant and lactating animals may be brought about by higher estrogen and GnRH stimulation.

Because GnRH receptors are at a peak late in diestrus, extending to the morning of proestrus [157, 164] cells from proestrous AM female rats were studied to learn more about the specific effects of GnRH on leptin mRNA and protein expression [93]. There is a significant increase in the average density of labeling for leptin mRNA or proteins (IOD) after 1 h in 100 pM GnRH ($p<0.001$), which plateaus at 500 pM. There is a significant decrease in IOD of labeling for leptin proteins after 1 nM GnRH ($p<0.03$), when compared with that following 500 pM. The value for 1 nM GnRH is still higher than the IOD for the vehicle treated group ($p=0.009$)[93].

The average IOD for leptin mRNA in the vehicle control is about 5—fold lower than that for the proteins, which reflects the lower expression of mRNA on the AM of proestrus [93]. GnRH increased the density of label for leptin mRNA (the increase with 100 pM is significant by Student's T test ($p<0.001$) and there is a further increase to reach a peak with 500 pM ($p<0.008$). The IOD following 1 nM is not different from that with 500 pM).

GnRH also stimulated secretion of leptin from cultures of pituitary cells taken from diestrus, proestrus or pregnant females. In Fig. (**8**) in reference [93], we compared basal and GnRH stimulated leptin secretion in these groups. Basal secretion is significantly higher when one compares media from pregnant rat AP cells with that from diestrous rats ($p=0.012$). GnRH- stimulated secretion is increased over basal in each of the groups (diestrous $p=0.016$; proestrus $p<0.015$ and pregnant $p<0.01$). The cells from pregnant rats show the highest responses to GnRH, when compared with all others. All of these data correlated well with the counts of leptin bearing cells and the findings that leptin is most abundant in gonadotropes in pregnant rats.

This series of studies brings forth more clues about the significance of pituitary leptin. Our studies of GHRH and estrogen indicate that together they increase leptin expression by somatotropes, however they do not stimulate leptin secretion. In contrast, GnRH is a potent secretagogue for leptin as it stimulates an increase in leptin mRNA and proteins. This suggests that GnRH may be one driving force in the plasticity of leptin expression by the pituitary cells.

As stated earlier in this section, it is noteworthy that counts of cells with leptin and GnRH-receptors detected twice as many cells with the potential for gonadotropic activity (defined by their GnRH binding) than are detected by labeling for leptin and gonadotropins. This apparent discrepancy is explained by the appearance, in diestrus and proestrus, of somatotropes, which express LH and FSH mRNAs and GnRH receptors [164, 165]. These "somatogonadotropes" represent 11-16% of the AP population during proestrus and may play a co-gonadotropic role in the support of the reproductive system [166]. The predominance of somatotropes in the leptin-bearing cell population suggests that the GnRH might be affecting leptin from the somatogonadotrope subtype.

Thus, our studies also show that estrogen can work with GnRH to increase the population of gonadotropes that express leptin [93]. In addition GnRH has stimulatory effects on expression of cellular leptin mRNA and proteins in proestrous rats, which express maximal numbers of GnRH receptors [157]. Furthermore, as we will see in a later section in this review, Neuropeptide Y also stimulates increased expression of leptin by gonadotropes.

Whereas there is a significant reduction in expression of leptin mRNA seen on the AM of proestrus, it is worthwhile to note that cells from proestrous females were still able to respond to GnRH by the production of more leptin transcripts and secretion [93]. Thus, the emerging view is of a subset of somatogonadotropes that increase leptin production during the periovulatory period, under the influence of GnRH. Estrogen's role may be to enhance expression of the GnRH (and the GHRH) receptors. It also stimulates expression of NPY receptors in the proestrous animal. Estrogen may be acting on this converging population of somatogonadotropes. This hypothesis will be tested in future studies that knock out leptin selectively in somatotropes or gonadotropes with Cre-LoxP technology.

Neuropeptide Y

NPY was also chosen as a candidate regulator of pituitary leptin because of the high expression of leptin proteins in proestrus [93]. Neuropeptide Y (NPY) regulates gonadotropes just before the LH surge [167-172]. It is produced by neurons in the arcuate nucleus from which it stimulates appetite and feeding, in a complex circuitry that is regulated by circulating leptin [173-175]. Circulating leptin rises after feeding to inhibit NPY production and thus reduce appetite.

However, NPY secretion is also required for a full GnRH-mediated LH surge in rodents [167-172]. NPY knockout mice have blunted LH surges, which can be enhanced by exogenous NPY [171]. NPY Y1 receptors, stimulated by estrogens, are believed to mediate its actions in the pituitary [176]. To regulate gonadotropes, NPY requires high amplitude GnRH pulses [66, 167, 169, 170], estrogen [176] or progesterone [177]. All of these conditions are seen on the afternoon of proestrus, just before the LH surge. Therefore, our studies of NPY stimulation focused on proestrous rats. Cells from these rats were stimulated for 1 h with 0–1000 pM NPY. Some of the pituitary cells were used for immunocytochemistry and *in situ* hybridization for leptin (followed by dual labeling for leptin and gonadotropins). Analysis showed that 0.1 nM NPY stimulated a 1.9-fold increase in the density of label for leptin mRNA/cell (Fig. **5**). The effects of GnRH and NPY were not additive in any of these experiments (data not shown).

When we expanded the studies to include male pituitary cells, Fig. (**6A**) shows that 3 h in 1 nM NPY also stimulated secretion of leptin. Fig. (**6B**) also shows that NPY stimulated a significant increase in pituitary leptin proteins detected by EIA. The data are expressed as pg leptin/ml in the graph,

however we also include values expressed as per milligram protein near the graph bars.

Gonadotropes are believed to be the major expression site for NPY receptors. Therefore, single and dual labeling for leptin mRNA and LH proteins was done in cells from male rat pituitaries after NPY stimulation to learn more about the effects of NPY on expression of leptin. Counts of cells bearing leptin mRNA or proteins and LH proteins showed NPY stimulation of all three products (Fig. **7**). There was a 25% increase in the percentages of leptin-bearing cells along with impressive increases in percentages of cells with LH proteins or in percentages of pituitary cells dual labeled for leptin mRNA and LH after stimulation by NPY. Collectively these data suggest that NPY may be a potent driver in the regulation of pituitary leptin, particularly that in gonadotropes.

Thus, to summarize, this section of the review identified three hormones that increased expression of pituitary leptin: GHRH, GnRH and NPY. The actions of GHRH had to be potentiated by estrogen, however. Ghrelin, which normally stimulates somatotropes, decreased expression of leptin from this group of cells. Two of the hormones, GnRH and NPY, stimulated secretion of pituitary leptin and increased expression by gonadot5ropes. Collectively, these data show that pituitary leptin can be regulated, mainly by hormones that regulate somatotropes and the reproductive system. Furthermore, in the studies of leptin secretion, there was an excellent correlation between the abundance of leptin protein-bearing cells in a pituitary cell population and their secretory activity [93].

With respect to secretory pathways inside the cells, it is worthwhile noting that Vidal *et al.* [101] detected leptin co-expression with pituitary hormones at the electron microscopic level and their photographs depict labeling for leptin in the same granules that store LH or GH. This suggests that the leptin secretory cycle could be similar to that of gonadotropins or GH. Collectively, our experiments with GnRH and the morphological data from Vidal *et al* [101] support regulatory pathways for leptin that are similar to those that regulate pituitary hormones.

PITUITARY LEPTIN AND PARACRINE INTERACTIONS

Having established regulators for leptin secretion, the final section of this review will describe studies that tested a role for pituitary leptin as a paracrine signaling hormone. However, first, we will present studies that provide evidence for direct interactions of leptin on pituitary gonadotropes and somatotropes.

In earlier sections of this review, the importance of leptin to pituitary gonadotropes and somatotropes was brought out by the reduced numbers of these cell types in animals or humans that are leptin deficient. However, the exact cause of this reduction is controversial. Are the low numbers of somatotropes or gonadotropes caused by the lack of direct leptin stimulation? Alternatively, one may also argue indirect causes from the metabolic disease and/or the hypogonadal condition of these mice. Steroid stimulation of GH cells was needed for their maintenance [106, 119, 178] and GnRH stimulation was needed to promote gonadotrope development. Thus, there is no question that leptin works indirectly through the hypothalamic circuits to affect gonadotropes and somatotropes [25, 50, 58, 61-66]. Indeed, as stated earlier in this review, strong evidence for a stimulatory effect of leptin on gonadotropes comes from studies in which the fertility of leptin-deficient *ob/ob* mice was completely restored by exogenous leptin [24]. Leptin also restored LH secretion in fasted mice, rats, and monkeys [14-17, 20, 58, 64, 67, 68], GH pulses in fasted rats [68, 76,

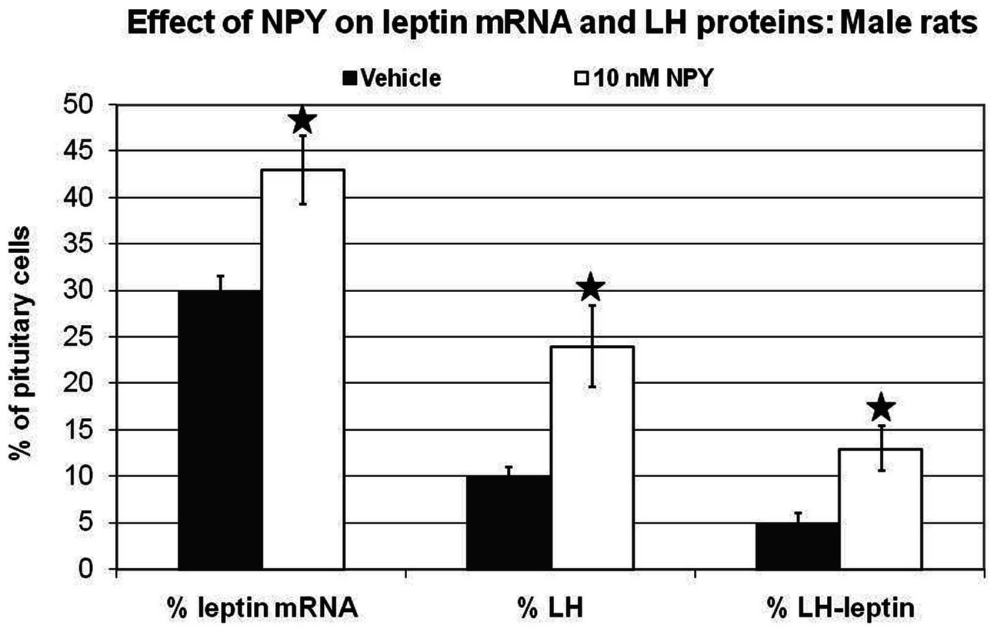

Fig. (7). Data from cells from male rats, which were dual labeled for leptin mRNA and LH proteins to detect any changes in co-expression after 1 h in 10 nM NPY. There was an overall increase in the percentages of cells with leptin mRNA ($p<0.001$) and an increase in percentages of cells with LH proteins ($P=0.019$). Dual labeling showed a significant increase in the percentages of anterior pituitary cells dual-labeled for leptin mRNA and LH ($P=0.006$). Star=significant difference.

78], and GH and GHRH receptor expression in leptin-deficient *ob/ob* mice [80]. Leptin increased LH levels in ovariectomized, estrogen-primed rats [72, 73]. Antiserum to leptin suppressed LH secretion and disrupted the estrous cycles [160].

One of the above *in vivo* studies highlights the pituitary as an important site of LEPR for the regulation of somatotropes. Luque *et al*. [80] reported that leptin restored GH secretion in *ob/ob* mice that were infused with exogenous leptin for 7 days. In addition, leptin caused an increase in GHRH receptors. Interestingly, no changes were noted in hypothalamic GHRH levels themselves, which suggested that GHRH did not mediate the restoration. In addition, several *in vitro* studies showed that leptin directly stimulated LH secretion [70-74] and may stimulate or inhibit GH [23, 68, 76, 77, 79, 80, 88, 114, 179-184], depending on experimental conditions.

In light of these findings, we hypothesized that, if it was a paracrine regulator, pituitary leptin might itself be regulated by the nutrient status. We thus developed an animal model of food deprivation to test this hypothesis. The model was developed based on information about changes in adipocyte leptin mRNA expression [1, 122, 123] after food deprivation in which losses in leptin mRNA of 50% [124] or 85-90% [12, 125] were seen in 24 h. As shown by (Fig. **4**) in reference [117], food deprivation reduced leptin expression by somatotropes and gonadotropes significantly. However, that by corticotropes was increased, largely due to the overall increase in number of corticotropes caused by the stress of fasting [117].

We then modified this model, to determine if pituitary leptin was responsive to an elevation of serum glucose [117]. We added a third group of animals, which were fasted, but had been given 10% glucose water during the period of the fast. This concentration promoted recovery in serum glucose to levels seen in the control animals [117]. We tested serum leptin as well as leptin expression by pituitary cells. Fig. (**6**) in reference [117] shows the loss in serum leptin in the fasted animals along with a partial recovery in serum leptin from animals that were fasted, but given glucose water during the food deprivation period. The QRT-PCR assays of pituitary leptin mRNA also showed a similar pattern[117], only the animals given glucose water completely recovered leptin mRNA expression. This recovery in expression of pituitary leptin mRNA was confirmed by in situ hybridization; the percentages of leptin-bearing cells were reduced in the fasted animals, but recovered in animals given glucose water[117]. Similarly, parallel changes in expression of LH mRNA-bearing cells were seen, with a loss in fasted animals and a recovery in fasted animals given glucose water.

Recovery was seen in LH protein expression as well. Dual immunolabeling detected GH, LH or Leptin in fed and the two groups of fasted animals and the graph is shown in Fig. (**7**) in reference [117]. There was a complete recovery in the percentages of GH and LH cells and a partial recovery in the percentages of leptin protein bearing cells in the fasted animals given glucose water.

At this point, the evidence for leptin as a driving force behind the recovery in somatotropes and gonadotropes was circumstantial. All we had shown was a parallel recovery in pituitary leptin (partial) and LH and GH. This might have occurred simply because of the partial recovery in serum leptin seen in the glucose treated animals [117].

Therefore, the next set of experiments tested exogenous leptin, to learn if it, alone could effect a recovery in gonadotropes and somatotropes in freshly dispersed cells from fasted animals, which had reduced expression of LH, GH and leptin. After treatment for 1 hour with vehicle, the cell cultures showed low percentages of LH, GH and leptin-bearing cells. However, as little as 10 pg/ml leptin for 1 h stimulated a recovery in numbers of somatotropes and gonadotropes. There was also a partial recovery in numbers of leptin-bearing cells. Photographs of the cell populations are illustrated in Fig. (**7**), [117].

The overall objective of this last set of studies was to determine the physiological significance of pituitary leptin. This has been challenging to prove *in vivo* because of the abundance of circulating leptin from adipocytes, and the presence of both leptin and leptin receptors in all pituitary cell types. Animal models with leptin or leptin receptor deficiencies have other confounding variables, like hyperglycemia and diabetes, with inherent independent effects on somaotropes and gonadotropes. These models are deficient in gonadotropes and somatotropes and do not respond normally to tests like fasting [185]. We recognize that, ultimately, selective knockouts in the pituitary would be needed to fully test pituitary leptin's significance.

The parallel restoration of leptin and LH mRNA, or leptin, LH and GH proteins in the glucose treated rats provided circumstantial evidence that further supports an association between these gene products in the pituitary. Food deprivation (signaled by a drop in nutrients, like glucose) is known to result in the attenuation of LH or GH pulses and it is thought that a drop in serum leptin signals some of this. However, *in vivo* studies have shown that the LH pulses can be restored within hours of giving nutrition, before adipocytes have recovered. Thus, the adipocyte-derived leptin can be too sluggish as a neuroendocrine modulator. LH pulses can also be restored, *in vivo*, in fasted animals by exogenous leptin as long as glucose can be utilized [14, 15, 17]. Thus, workers have theorized that leptin reports nutritional information, like glucose levels, to the hypothalamic and pituitary cells, permitting reproduction and normal GH or LH cell pulses if nutrition is adequate. Because of the slow nature of that from adipocytes, we suggest that pituitary leptin might serve as a rapid relay for nutritional information.

Our dual labeling evidence showed that the reduction in pituitary leptin following food deprivation is mostly in somatotropes. It is important to recall that ghrelin was stimulated by fasting (Fig. **4**) in these studies and ghrelin inihibits pituitary leptin specifically in somatotropes (Fig. **2** and **3**). Thus, a rise in serum ghrelin could be an important regulatory mechanism behind the lowering of somatotrope leptin.

It is interesting to note that leptin was not reduced in the expanding population of corticotropes. This suggests that the

regulation of the leptin gene is not uniform across all pituitary cell types. Furthermore, assuming the new corticotropes could secrete their leptin in this environment, it was clearly not sufficient to correct for the losses in somatotropes and gonadotropes. Perhaps the actions of ghrelin on somatotropes were critical in reducing this source of leptin as they were selective for somatotropes.

One of the clues to paracrine regulation is the sensitivity of the response to a candidate factor. In other words, target cells may respond to very low concentrations of a candidate factor in physiologic tests. This sensitivity develops because the candidate factor is not diluted by secretion and transport through the entire blood stream. A local vascular, or extracellular route from cell to cell might allow for effective regulation by very small amounts of a paracrine factor.

Thus, it is significant that, in our study of fasted rats [117], the losses in somatotropes or gonadotropes could be corrected rapidly by a 1 h incubation in as little as 1-10 pg/ml exogenous leptin. Also, these pg/ml levels match those secreted by normal rat pituitary cultures, so the responses match the availability of secreted pituitary leptin.

The recovery in gonadotropes and somatotropes confirms *in vivo* studies in which exogenous leptin restored LH or GH pulses [20, 58, 67, 68, 76, 78]. The assumption has been made, however that leptin acts mainly on the hypothalamus. In agreement with Luque *et al*, [80], our current studies suggest that leptin may also act directly on pituitary target cells and that restoration is not limited to actions on the hypothalamus.

Exogenous leptin was not able to fully restore the expression of pituitary leptin. This points to independent regulators for pituitary leptin and our studies show that they include estrogen and GHRH [98] and GnRH [93], all of which would be reduced in a fasted state [20, 58, 67, 68, 76, 78, 186, 187]. NPY would not be reduced, however receptors for this neuropeptide would likely be reduced because of the requirement for estrogen, at least in female rats.

To summarize, we suggest from this evidence that pituitary leptin is needed to maintain LH and GH cell functions. Most of the leptin comes from somatotropes, especially in the male. The findings support the hypothesis that a decline in somatotrope leptin below certain threshold levels might signal nutritional distress and cause parallel reductions in LH and GH cell functions. Endogenous pituitary leptin could thus serve as either a paracrine, or an autocrine regulator.

In our fasted rat model, once serum glucose became normal, there were parallel increases in pituitary leptin, GH and LH. This suggests that pituitary leptin may be sensitive to serum glucose. Thus, pituitary leptin might serve as a glucostat, which would provide one mechanism by which it senses the nutritional state. Recent landmark studies [188, 189] reported glucokinase expression in a subset of pituitary cells including thyrotropes and gonadotropes, which would allow them to monitor the changes in serum glucose. Our studies suggest that pituitary leptin may also be affected by this sensor. A drop in glucose might signal nutritional distress and cause a drop in pituitary leptin. This reduction would, in turn, report nutritional distress to somatotropes and gonadotropes, slowing down their functions and promoting survival activities (hunting for food, and fat conservation) rather than lipolysis (induced by GH) and reproduction (mediated by gonadotropins).

ACKNOWLEDGEMENTS

This study was supported by bridging funds from the University of Arkansas for Medical Sciences Research Council and R03 HD059066 and 1R01HD059056 to GVC. The studies were also supported by core facilities funded by NCRR P20 RR020146 and P30 NS047546. The authors thank Dr. A Parlowe and the Hormone Distribution Program for the antisera to rat GH.

REFERENCES

[1] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.

[2] Rowland NE, Morien A, Li BH. The physiology and brain mechanisms of feeding. Nutrition 1996; 12: 626-39.

[3] Mizuno T, Bergen H, Kleopoulos S, Bauman WA, Mobbs CV. Effects of nutritional status and aging on leptin gene expression in mice: importance of glucose. Horm Metab Res 1996; 28: 679-84.

[4] Baranowska B, Chmielowska M, Wolinska-Witort E, Roguski K, Wasilewska-Dziubinska E. The relationship between neuropeptides and hormones in starvation. Neuro Endocrinol Lett 2001; 22: 349-55.

[5] Neary NM, Goldstone AP, Bloom SR. Appetite regulation: from the gut to the hypothalamus. Clin Endocrinol (Oxf) 2004; 60: 153-60.

[6] Vasselli JR. Behavioral and biological determinants of leptin resistance. Appetite 2001; 37(2): 115-7.

[7] Mizuno TM, Makimura H, Silverstein J, Roberts JL, Lopingco T, Mobbs CV. Fasting regulates hypothalamic neuropeptide Y, agouti-related peptide, and proopiomelanocortin in diabetic mice independent of changes in leptin or insulin. Endocrinology 1999; 140: 4551-7.

[8] Mizuno TM, Mobbs CV. Hypothalamic agouti-related protein messenger ribonucleic acid is inhibited by leptin and stimulated by fasting. Endocrinology 1999; 140(2): 814-7.

[9] Chua SC, Jr., Leibel RL, Hirsch J. Food deprivation and age modulate neuropeptide gene expression in the murine hypothalamus and adrenal gland. Brain Res Mol Brain Res 1991 Jan; 9(1-2): 95-101.

[10] Ebihara K, Ogawa Y, Katsuura G, *et al.* Involvement of agouti-related protein, an endogenous antagonist of hypothalamic melanocortin receptor, in leptin action. Diabetes 1999; 48: 2028-33.

[11] Korner J, Savontaus E, Chua SC, Jr., Leibel RL, Wardlaw SL. Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. J Neuroendocrinol 2001; 13: 959-66.

[12] Mizuno TM, Bergen H, Funabashi T, *et al.* Obese gene expression: reduction by fasting and stimulation by insulin and glucose in lean mice, and persistent elevation in acquired (diet-induced) and genetic (yellow agouti) obesity. Proc Natl Acad Sci U S A 1996 16; 93: 3434-8.

[13] Fruhbeck G. Intracellular signalling pathways activated by leptin. Biochem J 2006; 393: 7-20.

[14] Schneider JE, Blum RM, Wade GN. Metabolic control of food intake and estrous cycles in syrian hamsters. I. Plasma insulin and leptin. Am J Physiol Regul Integr Comp Physiol 2000; 278(2): 476-85.

[15] Schneider JE, Buckley CA, Blum RM, *et al.* Metabolic signals, hormones and neuropeptides involved in control of energy balance and reproductive success in hamsters. Eur J Neurosci 2002; 16(3): 377-9.

[16] Schneider JE, Goldman MD, Tang S, Bean B, Ji H, Friedman MI. Leptin indirectly affects estrous cycles by increasing metabolic fuel oxidation. Horm Behav 1998; 33(3): 217-28.

[17] Schneider JE, Zhou D. Interactive effects of central leptin and peripheral fuel oxidation on estrous cyclicity. Am J Physiol 1999; 277: 1020-4.
[18] Schneider JE, Wade GN. Availability of metabolic fuels controls estrous cyclicity of Syrian hamsters. Science 1989; 244(4910): 1326-8.
[19] Casanueva FF, Dieguez C. Neuroendocrine regulation and actions of leptin. Front Neuroendocrinol 1999; 20(4): 317-63.
[20] Ahima RS, Prabakaran D, Mantzoros C, *et al.* Role of leptin in the neuroendocrine response to fasting. Nature 1996; 382(6588): 250-2.
[21] Moschos S, Chan JL, Mantzoros CS. Leptin and reproduction: a review. Fertil Steril 2002; 77(3): 433-44.
[22] Ahima RS, Dushay J, Flier SN, Prabakaran D, Flier JS. Leptin accelerates the onset of puberty in normal female mice. J Clin Invest 1997; 99(3): 391-5.
[23] Casanueva FF, Dieguez C. Neuroendocrine Regulation and Actions of Leptin. Front Neuroendocrinol 1999; 20(4): 317-63.
[24] Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. Nat Genet 1996; 12(3): 318-20.
[25] Cunningham MJ, Clifton DK, Steiner RA. Leptin's actions on the reproductive axis: perspectives and mechanisms. Biol Reprod 1999 ; 60(2): 216-22.
[26] Mannucci E, Ognibene A, Becorpi A, *et al.* Relationship between leptin and oestrogens in healthy women. Eur J Endocrinol 1998; 139(2): 198-201.
[27] McMinn JE, Liu SM, Liu H, *et al.* Neuronal deletion of Lepr elicits diabesity in mice without affecting cold tolerance or fertility. Am J Physiol Endocrinol Metab 2005; 289(3): E403-11.
[28] Yura S, Ogawa Y, Sagawa N, *et al.* Accelerated puberty and late-onset hypothalamic hypogonadism in female transgenic skinny mice overexpressing leptin. J Clin Invest 2000; 105(6): 749-55.
[29] Isozaki O, Tsushima T, Miyakawa M, Nozoe Y, Demura H, Seki H. Growth hormone directly inhibits leptin gene expression in visceral fat tissue in fatty Zucker rats. J Endocrinol 1999; 161(3): 511-6.
[30] Popovic V, Damjanovic S, Dieguez C, Casanueva FF. Leptin and the pituitary. Pituitary 2001; 4(1-2): 7-14.
[31] Lloyd RV, Jin L, Tsumanuma I, *et al.* Leptin and leptin receptor in anterior pituitary function. Pituitary. 2001; 4(1-2): 33-47.
[32] Mann DR, Plant TM. Leptin and pubertal development. Semin Reprod Med 2002; 20(2): 93-102.
[33] Urbanski HF. Leptin and puberty. Trends Endocrinol Metab 2001; 12(10): 428-9.
[34] Farooqi IS, Jebb SA, Langmack G, *et al.* Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med 1999; 341(12): 879-84.
[35] Montague CT, Farooqi IS, Whitehead JP, *et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature 1997; 387(6636): 903-8.
[36] Strobel A, Issad T, Camoin L, Ozata M, Strosberg AD. A leptin missense mutation associated with hypogonadism and morbid obesity. Nat Genet 1998; 18(3): 213-5.
[37] Clement K, Vaisse C, Lahlou N, *et al.* A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. Nature 1998; 392(6674): 398-401.
[38] Kaufman BA, Warren MP, Dominguez JE, Wang J, Heymsfield SB, Pierson RN. Bone density and amenorrhea in ballet dancers are related to a decreased resting metabolic rate and lower leptin levels. J Clin Endocrinol Metab 2002; 87(6): 2777-83.
[39] Laughlin GA, Yen SS. Hypoleptinemia in women athletes: absence of a diurnal rhythm with amenorrhea. J Clin Endocrinol Metab 1997; 82(1): 318-21.
[40] Miller KK, Parulekar MS, Schoenfeld E, *et al.* Decreased leptin levels in normal weight women with hypothalamic amenorrhea: the effects of body composition and nutritional intake. J Clin Endocrinol Metab 1998; 83(7): 2309-12.
[41] Thong FS, Graham TE. Leptin and reproduction: is it a critical link between adipose tissue, nutrition, and reproduction? Can J Appl Physiol 1999; 24(4): 317-36.
[42] Thong FS, McLean C, Graham TE. Plasma leptin in female athletes: relationship with body fat, reproductive, nutritional, and endocrine factors. J Appl Physiol 2000; 88(6): 2037-44.
[43] Warren MP, Voussoughian F, Geer EB, Hyle EP, Adberg CL, Ramos RH. Functional hypothalamic amenorrhea: hypoleptinemia and disordered eating. J Clin Endocrinol Metab 1999; 84(3): 873-7.
[44] Welt CK. Will leptin become the treatment of choice for functional hypothalamic amenorrhea? Nat Clin Pract Endocrinol Metab 2007; 3(8): 556-7.
[45] Welt CK, Chan JL, Bullen J, *et al.* Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med 2004; 351(10): 987-97.
[46] Chan JL, Heist K, DePaoli AM, Veldhuis JD, Mantzoros CS. The role of falling leptin levels in the neuroendocrine and metabolic adaptation to short-term starvation in healthy men. J Clin Invest 2003; 111(9): 1409-21.
[47] Barash IA, Cheung CC, Weigle DS, *et al.* Leptin is a metabolic signal to the reproductive system. Endocrinology 1996; 137(7): 3144-7.
[48] Chehab FF, Mounzih K, Lu R, Lim ME. Early onset of reproductive function in normal female mice treated with leptin. Science 1997; 275(5296): 88-90.
[49] Cheung CC, Thornton JE, Kuijper JL, Weigle DS, Clifton DK, Steiner RA. Leptin is a metabolic gate for the onset of puberty in the female rat. Endocrinology 1997; 138(2): 855-8.
[50] Bronson FH. Food-restricted, prepubertal, female rats: rapid recovery of luteinizing hormone pulsing with excess food, and full recovery of pubertal development with gonadotropin-releasing hormone. Endocrinology 1986; 118(6): 2483-7.
[51] Bronson FH. Puberty in female mice is not associated with increases in either body fat or leptin. Endocrinology 2001; 142(11): 4758-61.
[52] Cheung CC, Thornton JE, Nurani SD, Clifton DK, Steiner RA. A reassessment of leptin's role in triggering the onset of puberty in the rat and mouse. Neuroendocrinology 2001; 74(1): 12-21.
[53] Mann DR, Akinbami MA, Gould KG, Castracane VD. Leptin and thyroxine during sexual development in male monkeys: effect of neonatal gonadotropin-releasing hormone antagonist treatment and delayed puberty on the developmental pattern of leptin and thyroxine secretion. Eur J Endocrinol 2002; 146(6): 891-8.
[54] Mann DR, Bhat GK, Ramaswamy S, Stah CD, Plant TM. Regulation of circulating leptin and its soluble receptor during pubertal development in the male rhesus monkey (Macaca mulatta). Endocrine 2007; 31(2): 125-9.
[55] Plant TM, Durrant AR. Circulating leptin does not appear to provide a signal for triggering the initiation of puberty in the male rhesus monkey (Macaca mulatta). Endocrinology 1997; 138(10): 4505-8.
[56] Urbanski HF, Pau KY. A biphasic developmental pattern of circulating leptin in the male rhesus macaque (Macaca mulatta). Endocrinology 1998; 139(5): 2284-6.
[57] Zamorano PL, Mahesh VB, De Sevilla LM, Chorich LP, Bhat GK, Brann DW. Expression and localization of the leptin receptor in endocrine and neuroendocrine tissues of the rat. Neuroendocrinology1997; 65(3): 223-8.
[58] Finn PD, Cunningham MJ, Pau KY, Spies HG, Clifton DK, Steiner RA. The stimulatory effect of leptin on the neuroendocrine reproductive axis of the monkey. Endocrinology 1998; 139(11): 4652-62.
[59] Hakansson ML, Brown H, Ghilardi N, Skoda RC, Meister B. Leptin receptor immunoreactivity in chemically defined target neurons of the hypothalamus. J Neurosci 1998; 18(1): 559-72.
[60] Magni P, Vettor R, Pagano C, *et al.* Expression of a leptin receptor in immortalized gonadotropin-releasing hormone-secreting neurons. Endocrinology 1999; 140(4): 1581-5.
[61] Chan JL, Mantzoros CS. Leptin and the hypothalamic-pituitary regulation of the gonadotropin-gonadal axis. Pituitary 2001; 4(1-2): 87-92.
[62] Lebrethon MC, Vandersmissen E, Gerard A, Parent AS, Bourguignon JP. Cocaine and amphetamine-regulated-transcript peptide mediation of leptin stimulatory effect on the rat gonadotropin-releasing hormone pulse generator *in vitro*. J Neuroendocrinol 2000; 12(5): 383-5.
[63] Lebrethon MC, Vandersmissen E, Gerard A, Parent AS, Junien JL, Bourguignon JP. *In vitro* stimulation of the prepubertal rat gonadotropin-releasing hormone pulse generator by leptin and neuropeptide Y through distinct mechanisms. Endocrinology 2000 ; 141(4): 1464-9.

[64] Nagatani S, Guthikonda P, Thompson RC, Tsukamura H, Maeda KI, Foster DL. Evidence for GnRH regulation by leptin: leptin administration prevents reduced pulsatile LH secretion during fasting. Neuroendocrinology 1998; 67(6): 370-6.

[65] Spicer LJ. Leptin: a possible metabolic signal affecting reproduction. Domest Anim Endocrinol 2001; 21(4): 251-70.

[66] Watanobe H. Leptin directly acts within the hypothalamus to stimulate gonadotropin-releasing hormone secretion *in vivo* in rats. J Physiol 2002; 545(Pt 1): 255-68.

[67] Gonzalez LC, Pinilla L, Tena-Sempere M, Aguilar E. Leptin(116-130) stimulates prolactin and luteinizing hormone secretion in fasted adult male rats. Neuroendocrinology 1999; 70(3): 213-20.

[68] Nagatani S, Zeng Y, Keisler DH, Foster DL, Jaffe CA. Leptin regulates pulsatile luteinizing hormone and growth hormone secretion in the sheep. Endocrinology 2000; 141(11): 3965-75.

[69] Tezuka M, Irahara M, Ogura K, *et al.* Effects of leptin on gonadotropin secretion in juvenile female rat pituitary cells. Eur J Endocrinol 2002; 146(2): 261-6.

[70] De Biasi SN, Apfelbaum LI, Apfelbaum ME. *In vitro* effect of leptin on LH release by anterior pituitary glands from female rats at the time of spontaneous and steroid-induced LH surge. Eur J Endocrinol 2001; 145(5): 659-65.

[71] Ogura K, Irahara M, Kiyokawa M, *et al.* Effects of leptin on secretion of LH and FSH from primary cultured female rat pituitary cells. Eur J Endocrinol 2001; 144(6): 653-8.

[72] Walczewska A, Yu WH, Karanth S, McCann SM. Estrogen and leptin have differential effects on FSH and LH release in female rats. Proc Soc Exp Biol Med 1999; 222(2): 170-7.

[73] Yu WH, Kimura M, Walczewska A, Karanth S, McCann SM. Role of leptin in hypothalamic-pituitary function. Proc Natl Acad Sci USA 1997; 94(3): 1023-8.

[74] Yu WH, Walczewska A, Karanth S, McCann SM. Nitric oxide mediates leptin-induced luteinizing hormone-releasing hormone (LHRH) and LHRH and leptin-induced LH release from the pituitary gland. Endocrinology 1997; 138(11): 5055-8.

[75] Aubert ML, Pierroz DD, Gruaz NM, *et al.* Metabolic control of sexual function and growth: role of neuropeptide Y and leptin. Mol Cell Endocrinol 1998; 140(1-2): 107-13.

[76] Pombo M, Pombo CM, Astorga R, *et al.* Regulation of growth hormone secretion by signals produced by the adipose tissue. J Endocrinol Invest 1999; 22(5 Suppl): 22-6.

[77] Roh SG, Nie GY, Loneragan K, Gertler A, Chen C. Direct modification of somatotrope function by long-term leptin treatment of primary cultured ovine pituitary cells. Endocrinology 2001; 142(12): 5167-71.

[78] Vuagnat BA, Pierroz DD, Lalaoui M, *et al.* Evidence for a leptin-neuropeptide Y axis for the regulation of growth hormone secretion in the rat. Neuroendocrinology 1998; 67(5): 291-300.

[79] Chen C, Roh SG, Nie GY, *et al.* The *in vitro* effect of leptin on growth hormone secretion from primary cultured ovine somatotrophs. Endocrine 2001; 14(1): 73-8.

[80] Luque RM, Huang ZH, Shah B, Mazzone T, Kineman RD. Effects of leptin replacement on hypothalamic-pituitary growth hormone axis function and circulating ghrelin levels in ob/ob mice. Am J Physiol Endocrinol Metab 2007; 292(3): E891-9.

[81] Ahima RS, Prabakaran D, Flier JS. Postnatal leptin surge and regulation of circadian rhythm of leptin by feeding. Implications for energy homeostasis and neuroendocrine function. J Clin Invest 1998; 101(5): 1020-7.

[82] Devaskar SU, Ollesch C, Rajakumar RA, Rajakumar PA. Developmental changes in ob gene expression and circulating leptin peptide concentrations. Biochem Biophys Res Commun 1997; 238(1): 44-7.

[83] Clancy B, Kersh B, Hyde J, Darlington RB, Anand KJ, Finlay BL. Web-based method for translating neurodevelopment from laboratory species to humans. Neuroinformatics 2007 Spring; 5(1): 79-94.

[84] Terasawa E, Fernandez DL. Neurobiological mechanisms of the onset of puberty in primates. Endocr Rev 2001; 22(1): 111-51.

[85] Reitman ML, Bi S, Marcus-Samuels B, Gavrilova O. Leptin and its role in pregnancy and fetal development--an overview. Biochem Soc Trans 2001; 29: 68-72.

[86] Ong KK, Ahmed ML, Dunger DB. The role of leptin in human growth and puberty. Acta Paediatr Suppl 1999; 88(433): 95-8.

[87] Kaplan SL, Grumbach MM, Shepard TH. The ontogenesis of human fetal hormones. I. Growth hormone and insulin. J Clin Invest 1972; 51(12): 3080-93.

[88] Shimon I, Yan X, Magoffin DA, Friedman TC, Melmed S. Intact leptin receptor is selectively expressed in human fetal pituitary and pituitary adenomas and signals human fetal pituitary growth hormone secretion. J Clin Endocrinol Metab 1998; 83(11): 4059-64.

[89] Castillo RH, Matteri RL, Dumesic DA. Luteinizing hormone synthesis in cultured fetal human pituitary cells exposed to gonadotropin-releasing hormone. J Clin Endocrinol Metab 1992; 75(1): 318-22.

[90] Kaplan SL, Grumbach MM. The ontogenesis of human foetal hormones. II. Luteinizing hormone (LH) and follicle stimulating hormone (FSH). Acta Endocrinol (Copenh) 1976; 81(4): 808-29.

[91] Li JY, Claustrat B, Begeot M, Dubois PM. Human fetal antehypophysis *in vitro*. II. Immunocytological study and radioimmunoassay of LH and FSH (author's transl). Ann Endocrinol (Paris). 1977; 38(6): 389-90.

[92] Siler-Khodr TM, Khodr GS. Studies in human fetal endocrinology: II. LH and FSH content and concentration in the pituitary. Obstet Gynecol 1980; 56(2): 176-81.

[93] Akhter N, Johnson BW, Crane C, *et al.* Anterior pituitary leptin expression changes in different reproductive states: stimulation, *In vitro*, by gonadotropin releasing hormone (GnRH). J Histochem Cytochem 2007; 55: 151-66.

[94] Bronson FH, Heideman PD. Short-term hormonal responses to food intake in peripubertal female rats. Am J Physiol 1990; 259: R25-31.

[95] Cameron JL. Regulation of reproductive hormone secretion in primates by short-term changes in nutrition. Rev Reprod 1996; 1(2): 117-26.

[96] Jin L, Burguera BG, Couce ME, *et al.* Leptin and leptin receptor expression in normal and neoplastic human pituitary: evidence of a regulatory role for leptin on pituitary cell proliferation. J Clin Endocrinol Metab 1999; 84(8): 2903-11.

[97] Jin L, Zhang S, Burguera BG, *et al*. Leptin and leptin receptor expression in rat and mouse pituitary cells. Endocrinology 2000; 141(1): 333-9.

[98] McDuffie IA, Akhter N, Childs GV. Regulation of leptin mRNA and protein expression in pituitary somatotropes. J Histochem Cytochem 2004; 52(2): 263-73.

[99] Sone M, Nagata H, Takekoshi S, Osamura RY. Expression and localization of leptin receptor in the normal rat pituitary gland. Cell Tissue Res 2001; 305(3): 351-6.

[100] Sone M, Osamura RY. Leptin and the pituitary. Pituitary 2001; 4(1-2): 15-23.

[101] Vidal S, Cohen SM, Horvath E, *et al.* Subcellular localization of leptin in non-tumorous and adenomatous human pituitaries: an immuno-ultrastructural study. J Histochem Cytochem 2000 Aug; 48(8): 1147-52.

[102] Korbonits M, Chitnis MM, Gueorguiev M, *et al.* Leptin in pituitary adenomas--a novel paracrine regulatory system. Pituitary 2001; 4(1-2): 49-55.

[103] Korbonits M, Chitnis MM, Gueorguiev M, *et al.* The release of leptin and its effect on hormone release from human pituitary adenomas. Clin Endocrinol (Oxf) 2001; 54(6): 781-9.

[104] Scarpace PJ, Matheny M, Zhang Y, Cheng KY, Tumer N. Leptin antagonist reveals an uncoupling between leptin receptor STAT3 signaling and metabolic responses with central leptin resistance. J Pharmacol Exp Ther 2007; 320(2): 706-12.

[105] Zhang J, Matheny MK, Tumer N, Mitchell MK, Scarpace PJ. Leptin antagonist reveals that the normalization of caloric intake and the thermic effect of food following high-fat feeding are leptin dependent. Am J Physiol Regul Integr Comp Physiol 2007; 292(2): R868-74.

[106] Iruthayanathan M, Zhou YH, Childs GV. Dehydroepiandrosterone restoration of growth hormone gene expression in aging female rats, *in vivo* and *in vitro*: evidence for actions via estrogen receptors. Endocrinology 2005; 146(12): 5176-87.

[107] Schulz LC, Widmaier EP. Leptin receptors. In: Castracane VD, Henson MC, Eds. Leptin. New York, NY: Springer Science; 2006; Chp. 2: 11-33.

[108] Baumann H, Morella KK, White DW, *et al*. The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. Proc Natl Acad Sci U S A. 1996; 93(16): 8374-8.

[109] Chen H, Charlat O, Tartaglia LA, *et al.* Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. Cell 1996; 84(3): 491-5.

[110] Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC. Defective STAT signaling by the leptin receptor in diabetic mice. Proc Natl Acad Sci U S A 1996; 93(13): 6231-5.

[111] Vaisse C, Halaas JL, Horvath CM, Darnell JE, Jr., Stoffel M, Friedman JM. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. Nat Genet 1996; 14(1): 95-7.

[112] Cai A, Hyde JF. Upregulation of leptin receptor gene expression in the anterior pituitary of human growth hormone-releasing hormone transgenic mice. Endocrinology 1998; 139(1): 420-3.

[113] Cai A, Hyde JF. The human growth hormone-releasing hormone transgenic mouse as a model of modest obesity: differential changes in leptin receptor (OBR) gene expression in the anterior pituitary and hypothalamus after fasting and OBR localization in somatotrophs. Endocrinology 1999; 140(8): 3609-14.

[114] Giusti M, Bocca L, Florio T, *et al*. *In vitro* effect of human recombinant leptin and expression of leptin receptors on growth hormone-secreting human pituitary adenomas. Clin Endocrinol (Oxf) 2002; 57(4): 449-55.

[115] Lin J, Barb CR, Matteri RL, *et al.* Long form leptin receptor mRNA expression in the brain, pituitary, and other tissues in the pig. Domest Anim Endocrinol 2000; 19(1): 53-61.

[116] Iqbal J, Pompolo S, Considine RV, Clarke IJ. Localization of leptin receptor-like immunoreactivity in the corticotropes, somatotropes, and gonadotropes in the ovine anterior pituitary. Endocrinology 2000; 141(4): 1515-20.

[117] Crane C, Akhter N, Johnson BW, *et al.* Fasting and Glucose Effects on Pituitary Leptin Expression: Is Leptin a Local Signal for Nutrient Status? J Histochem Cytochem 2007; 55(10): 1059-74.

[118] Kaminski T, Smolinska N, Gajewska A, *et al*. Leptin and long form of leptin receptor genes expression in the hypothalamus and pituitary during the luteal phase and early pregnancy in pigs. J Physiol Pharmacol 2006; 57(1): 95-108.

[119] Childs GV, Iruthayanathan M, Akhter N, Unabia G, Whitehead-Johnson B. Bipotential effects of estrogen on growth hormone synthesis and storage *in vitro*. Endocrinology 2005; 146(4): 1780-8.

[120] Gonzalez LC, Pinilla L, Tena-Sempere M, Dieguez C, Casanueva FF, Aguilar E. Effect of acute immunoneutralization of endogenous leptin on prolactin and LH secretion during the afternoon of pro-oestrus or in steroid-treated ovariectomized female rats. J Reprod Fertil 2000; 118(1): 39-45.

[121] Schoeller DA, Cella LK, Sinha MK, Caro JF. Entrainment of the diurnal rhythm of plasma leptin to meal timing. J Clin Invest 1997 Oct 1; 100(7): 1882-7.

[122] Gui Y, Silha JV, Mishra S, Murphy LJ. Changes in adipokine expression during food deprivation in the mouse and the relationship to fasting-induced insulin resistance. Can J Physiol Pharmacol 2003; 81(10): 979-85.

[123] Kowalska I, Straczkowski M, Gorski J, Kinalska I. The effect of fasting and physical exercise on plasma leptin concentrations in high-fat fed rats. J Physiol Pharmacol 1999; 50(2): 309-20.

[124] Igel M, Kainulainen H, Brauers A, Becker W, Herberg L, Joost HG. Long-term and rapid regulation of ob mRNA levels in adipose tissue from normal (Sprague Dawley rats) and obese (db/db mice, fa/fa rats) rodents. Diabetologia 1996; 39(7): 758-65.

[125] MacDougald OA, Hwang CS, Fan H, Lane MD. Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. Proc Natl Acad Sci USA 1995; 92(20): 9034-7.

[126] Childs GV, Unabia G, Miller BT, Collins TJ. Differential expression of gonadotropin and prolactin antigens by GHRH target cells from male and female rats. J Endocrinol 1999; 162(2): 177-87.

[127] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 1999; 402(6762): 656-60.

[128] Kojima M, Hosoda H, Matsuo H, Kangawa K. Ghrelin: discovery of the natural endogenous ligand for the growth hormone secretagogue receptor. Trends Endocrinol Metab 2001; 12(3): 118-22.

[129] Takaya K, Ariyasu H, Kanamoto N, *et al*. Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab 2000; 85(12): 4908-11.

[130] Wren AM, Small CJ, Ward HL, *et al.* The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion. Endocrinology 2000; 141(11): 4325-8.

[131] Malagon MM, Luque RM, Ruiz-Guerrero E, *et al*. Intracellular signaling mechanisms mediating ghrelin-stimulated growth hormone release in somatotropes. Endocrinology 2003; 144(12): 5372-80.

[132] Pinilla L, Barreiro ML, Tena-Sempere M, Aguilar E. Role of ghrelin in the control of growth hormone secretion in prepubertal rats: interactions with excitatory amino acids. Neuroendocrinology 2003; 77(2): 83-90.

[133] Kappeler L, Zizzari P, Grouselle D, Epelbaum J, Bluet-Pajot MT. Plasma and hypothalamic peptide-hormone levels regulating somatotroph function and energy balance in fed and fasted states: a comparative study in four strains of rats. J Neuroendocrinol 2004; 16(12): 980-8.

[134] Korbonits M, Goldstone AP, Gueorguiev M, Grossman AB. Ghrelin--a hormone with multiple functions. Front Neuroendocrinol 2004; 25(1): 27-68.

[135] van der Lely AJ, Tschop M, Heiman ML, Ghigo E. Biological, physiological, pathophysiological, and pharmacological aspects of ghrelin. Endocr Rev 2004; 25(3): 426-57.

[136] Chen C. Growth hormone secretagogue actions on the pituitary gland: multiple receptors for multiple ligands? Clin Exp Pharmacol Physiol 2000; 27(5-6): 323-9.

[137] Frohman LA, Kineman RD, Kamegai J, *et al.* Secretagogues and the somatotrope: signaling and proliferation. Recent Prog Horm Res 2000; 55: 269-90; discussion 90-1.

[138] Gracia-Navarro F, Castano JP, Malagon MM, *et al.* Research progress in the stimulatory inputs regulating growth hormone (GH) secretion. Comp Biochem Physiol B Biochem Mol Biol 2002; 132(1): 141-50.

[139] McKee KK, Palyha OC, Feighner SD, *et al.* Molecular analysis of rat pituitary and hypothalamic growth hormone secretagogue receptors. Mol Endocrinol 1997; 11(4): 415-23.

[140] Park S, Peng XD, Frohman LA, Kineman RD. Expression analysis of hypothalamic and pituitary components of the growth hormone axis in fasted and streptozotocin-treated neuropeptide Y (NPY)-intact (NPY+/+) and NPY-knockout (NPY-/-) mice. Neuroendocrinology 2005; 81(6): 360-71.

[141] Fernandez-Fernandez R, Navarro VM, Barreiro ML, *et al.* Effects of chronic hyperghrelinemia on puberty onset and pregnancy outcome in the rat. Endocrinology 2005; 146(7): 3018-25.

[142] Fernandez-Fernandez R, Tena-Sempere M, Navarro VM, *et al.* Effects of ghrelin upon gonadotropin-releasing hormone and gonadotropin secretion in adult female rats: *in vivo* and *in vitro* studies. Neuroendocrinology 2005; 82(5-6): 245-55.

[143] Fernandez-Fernandez R, Tena-Sempere M, Navarro VM, *et al.* Effects of Ghrelin upon Gonadotropin-Releasing Hormone and Gonadotropin Secretion in Adult Female Rats: *In vivo* and *in vitro* Studies. Neuroendocrinology 2006; 82(5-6): 245-55.

[144] Furuta M, Funabashi T, Kimura F. Intracerebroventricular administration of ghrelin rapidly suppresses pulsatile luteinizing hormone secretion in ovariectomized rats. Biochem Biophys Res Commun 2001; 288(4): 780-5.

[145] Vulliemoz NR, Xiao E, Xia-Zhang L, Germond M, Rivier J, Ferin M. Decrease in luteinizing hormone pulse frequency during a five-hour peripheral ghrelin infusion in the ovariectomized rhesus monkey. J Clin Endocrinol Metab 2004; 89(11): 5718-23.

[146] Iqbal J, Kurose Y, Canny B, Clarke IJ. Effects of central infusion of ghrelin on food intake and plasma levels of growth hormone, luteinizing hormone, prolactin, and cortisol secretion in sheep. Endocrinology 2006; 147(1): 510-9.

[147] Clayton RN. Gonadotrophin-releasing hormone: its actions and receptors. J Endocrinol 1989; 120(1): 11-9.

[148] Conn PM, editor. The molecular mechanism of gonadotropin-releasing hormone action in the pituitary. 2nd ed. New York: New York: Raven Press 1994.

[149] Conn PM, Huckle WR, Andrews WV, McArdle CA. The molecular mechanism of action of gonadotropin releasing hormone (GnRH) in the pituitary. Recent Prog Horm Res 1987; 43: 29-68.

[150] Belchetz PE, Plant TM, Nakai Y, Keogh EJ, Knobil E. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. Science 1978; 202(4368): 631-3.

[151] Burger LL, Dalkin AC, Aylor KW, Haisenleder DJ, Marshall JC. GnRH pulse frequency modulation of gonadotropin subunit gene transcription in normal gonadotropes-assessment by primary transcript assay provides evidence for roles of GnRH and follistatin. Endocrinology 2002; 143(9): 3243-9.

[152] Crowley WF, Jr., Filicori M, Spratt DI, Santoro NF. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. Recent Prog Horm Res 1985; 41: 473-531.

[153] Haisenleder DJ, Dalkin AC, Ortolano GA, Marshall JC, Shupnik MA. A pulsatile gonadotropin-releasing hormone stimulus is required to increase transcription of the gonadotropin subunit genes: evidence for differential regulation of transcription by pulse frequency *in vivo*. Endocrinology 1991; 128(1): 509-17.

[154] Kaiser UB, Jakubowiak A, Steinberger A, Chin WW. Differential effects of gonadotropin-releasing hormone (GnRH) pulse frequency on gonadotropin subunit and GnRH receptor messenger ribonucleic acid levels *in vitro*. Endocrinology 1997; 138(3): 1224-31.

[155] Kaiser UB, Sabbagh E, Katzenellenbogen RA, Conn PM, Chin WW. A mechanism for the differential regulation of gonadotropin subunit gene expression by gonadotropin-releasing hormone. Proc Natl Acad Sci USA 1995; 92(26): 12280-4.

[156] Levine JE, Ramirez VD. Luteinizing hormone-releasing hormone release during the rat estrous cycle and after ovariectomy, as estimated with push-pull cannulae. Endocrinology 1982; 111(5): 1439-48.

[157] Lloyd JM, Childs GV. Changes in the number of GnRH-receptive cells during the rat estrous cycle: biphasic effects of estradiol. Neuroendocrinology 1988; 48(2): 138-46.

[158] Loumaye E, Catt KJ. Homologous regulation of gonadotropin-releasing hormone receptors in cultured pituitary cells. Science 1982; 215(4535): 983-5.

[159] Savoy-Moore RT, Schwartz NB, Duncan JA, Marshall JC. Pituitary gonadotropin-releasing hormone receptors during the rat estrous cycle. Science 1980; 209(4459): 942-4.

[160] Carro E, Pinilla L, Seoane LM, *et al.* Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. Neuroendocrinology 1997; 66(6): 375-7.

[161] Childs GV, Naor Z, Hazum E, Tibolt R, Westlund KN, Hancock MB. Localization of biotinylated gonadotropin releasing hormone on pituitary monolayer cells with avidin-biotin-peroxidase complexes. J Histochem Cytochem 1983; 31(12): 1422-5.

[162] Childs GV, Naor Z, Hazum E, Tibolt R, Westlund KN, Hancock MB. Cytochemical characterization of pituitary target cells for biotinylated gonadotropin releasing hormone. Peptides 1983; 4(4): 549-55.

[163] Childs GV, Unabia G. Cytochemical studies of the effects of activin on gonadotropin-releasing hormone (GnRH) binding by pituitary gonadotropes and growth hormone cells. J Histochem Cytochem 1997; 45(12): 1603-10.

[164] Childs GV, Unabia G, Miller BT. Cytochemical detection of gonadotropin-releasing hormone-binding sites on rat pituitary cells with luteinizing hormone, follicle-stimulating hormone, and growth hormone antigens during diestrous up-regulation. Endocrinology 1994; 134(4): 1943-51.

[165] Childs GV, Unabia G, Rougeau D. Cells that express luteinizing hormone (LH) and follicle-stimulating hormone (FSH) beta-subunit messenger ribonucleic acids during the estrous cycle: the major contributors contain LH beta, FSH beta, and/or growth hormone. Endocrinology 1994; 134(2): 990-7.

[166] Childs GV. Growth hormone cells as co-gonadotropes: partners in the regulation of the reproductive system. Trends Endocrinol Metab 2000; 11(5): 168-75.

[167] Sutton SW, Toyama TT, Otto S, Plotsky PM. Evidence that neuropeptide Y (NPY) released into the hypophysial-portal circulation participates in priming gonadotropes to the effects of gonadotropin releasing hormone (GnRH). Endocrinology 1988; 123(2): 1208-10.

[168] Bauer-Dantoin AC, McDonald JK, Levine JE. Neuropeptide Y potentiates luteinizing hormone (LH)-releasing hormone-stimulated LH surges in pentobarbital-blocked proestrous rats. Endocrinology 1991; 129(1): 402-8.

[169] Watanobe H, Takebe K. Evidence that neuropeptide Y secretion in the median eminence increases prior to the luteinizing hormone surge in ovariectomized steroid-primed rats: estimation by push-pull perfusion. Neurosci Lett 1992; 146(1): 57-9.

[170] Woller MJ, McDonald JK, Reboussin DM, Terasawa E. Neuropeptide Y is a neuromodulator of pulsatile luteinizing hormone-releasing hormone release in the gonadectomized rhesus monkey. Endocrinology 1992; 130(4): 2333-42.

[171] Xu M, Hill JW, Levine JE. Attenuation of luteinizing hormone surges in neuropeptide Y knockout mice. Neuroendocrinology 2000; 72(5): 263-71.

[172] Xu M, Urban JH, Hill JW, Levine JE. Regulation of hypothalamic neuropeptide Y Y1 receptor gene expression during the estrous cycle: role of progesterone receptors. Endocrinology 2000; 141(9): 3319-27.

[173] Morrison CD, Morton GJ, Niswender KD, Gelling RW, Schwartz MW. Leptin inhibits hypothalamic Npy and Agrp gene expression via a mechanism that requires phosphatidylinositol 3-OH-kinase signaling. Am J Physiol Endocrinol Metab 2005; 289(6): E1051-7.

[174] Schwartz MW, Baskin DG, Bukowski TR, *et al.* Specificity of leptin action on elevated blood glucose levels and hypothalamic neuropeptide Y gene expression in ob/ob mice. Diabetes 1996; 45(4): 531-5.

[175] Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG. Identification of targets of leptin action in rat hypothalamus. J Clin Invest. 1996; 98(5): 1101-6.

[176] Hill JW, Urban JH, Xu M, Levine JE. Estrogen Induces Neuropeptide Y (NPY) Y1 receptor gene expression and responsiveness to NPY in gonadotrope-enriched pituitary cell cultures. Endocrinology 2004; 145(5): 2283-90.

[177] Bauer-Dantoin AC, Tabesh B, Norgle JR, Levine JE. RU486 administration blocks neuropeptide Y potentiation of luteinizing hormone (LH)-releasing hormone-induced LH surges in proestrous rats. Endocrinology 1993; 133(6): 2418-23.

[178] Childs GV, Iruthayanathan M, Akhter N, Johnson BW. Estrogen mediated cross talk between the ovary and pituitary somatotrope. Pre-ovulatory support for reproductive activity. Mol Cell Endocrinol 2006; 247(1-2): 60-3.

[179] Isozaki O, Tsushima T, Miyakawa M, Demura H, Seki H. Interaction between leptin and growth hormone (GH)/IGF-I axis. Endocr J 1999; 46(Suppl): S17-24.

[180] Baratta M, Saleri R, Mainardi GL, Valle D, Giustina A, Tamanini C. Leptin regulates GH gene expression and secretion and nitric oxide production in pig pituitary cells. Endocrinology 2002; 143(2): 551-7.

[181] Saleri R, Giustina A, Tamanini C, *et al.* Leptin stimulates growth hormone secretion via a direct pituitary effect combined with a decreased somatostatin tone in a median eminence-pituitary perifusion study. Neuroendocrinology 2004; 79(4): 221-8.

[182] Saleri R, Grasselli F, Tamanini C. Effects of different culture conditions and leptin on GH mRNA expression and GH secretion by pig pituitary cells. Horm Metab Res 2005; 37(4): 214-9.

[183] Mizuno I, Okimura Y, Takahashi Y, Kaji H, Abe H, Chihara K. Leptin stimulates basal and GHRH-induced GH release from cultured rat anterior pituitary cells *in vitro*. Kobe J Med Sci 1999; 45(5): 221-7.

[184] Zieba DA, Amstalden M, Morton S, *et al.* Effects of leptin on basal and GHRH-stimulated GH secretion from the bovine adenohypophysis are dependent upon nutritional status. J Endocrinol 2003; 178(1): 83-9.

[185] Hardie LJ, Rayner DV, Holmes S, Trayhurn P. Circulating leptin levels are modulated by fasting, cold exposure and insulin administration in lean but not Zucker (fa/fa) rats as measured by ELISA. Biochem Biophys Res Commun 1996; 223(3): 660-5.

[186] Luque RM, Park S, Kineman RD. Severity of the catabolic condition differentially modulates hypothalamic expression of growth hormone-releasing hormone in the fasted mouse: potential role of neuropeptide Y and corticotropin-releasing hormone. Endocrinology 2007; 148(1): 300-9.

[187] Maciel MN, Zieba DA, Amstalden M, Keisler DH, Neves JP, Williams GL. Leptin prevents fasting-mediated reductions in pulsatile secretion of luteinizing hormone and enhances its gonadotropin-releasing hormone-mediated release in heifers. Biol Reprod 2004; 70(1): 229-35.

[188] Sorenson RL, Stout LE, Brelje TC, Jetton TL, Matschinsky FM. Immunohistochemical evidence for the presence of glucokinase in the gonadotropes and thyrotropes of the anterior pituitary gland of rat and monkey. J Histochem Cytochem 2007; 55(6): 555-66.

[189] Zelent D, Golson ML, Koeberlein B, *et al.* A glucose sensor role for glucokinase in anterior pituitary cells. Diabetes 2006; 55(7): 1923-9.

Received: January 08, 2010 Revised: March 31, 2010 Accepted: October 16, 2010