Chapter

Leptin: A Metabolic Signal for the Differentiation of Pituitary Cells

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Abstract

Pituitary cell function is impacted by metabolic states and therefore must receive signals that inform them about nutritional status or adiposity. A primary signal from adipocytes is leptin, which recent studies have shown regulates most pituitary cell types. Subsets of all pituitary cell types express leptin receptors and leptin has been shown to exert transcriptional control through classical JAK/STAT pathways. Recent studies show that leptin also signals through post-transcriptional pathways that involve the translational regulatory protein Musashi. Mechanistically, post-transcriptional control would permit rapid cellular regulation of critical pre-existing pituitary transcripts as energy states change. The chapter will review evidence for transcriptional and/or post-transcriptional regulation of leptin targets (including *Gnrh*r, activin, *Fshb, Gh, Ghrhr,* and *Pou11f1*) and the consequences of the loss of leptin signaling to gonadotrope and somatotrope functions.

Keywords: Leptin, somatotropes, gonadotropes, Musashi, post-transcriptional, *Pou1f1*, *Ghrhr*, *Gnrhr*, *Fshb*

1. Introduction

To perform their vital functions, anterior pituitary cells must respond appropriately to their unique hypothalamic releasing hormones, while also responding to extrinsic signals informing them of the body's nutritional and metabolic state. Leptin is one of the most important of these extrinsic signals. However, recent studies show that leptin does more than simply signal levels of fat stores [1–11]. Leptin plays a trophic role that optimizes and maintains differentiation of at least two of these cell types, somatotropes and gonadotropes.

Anterior pituitary somatotropes produce growth hormone (GH) to support growth in muscles and bones before puberty and build muscle, bone, and reduce fat to optimize body composition in the adult [12, 13]. Gonadotropes produce the gonadotropins, luteinizing hormone (LH) and follicle stimulating hormone (FSH), which differentially regulate gonadal functions, ovulation and reproductive cyclicity [14]. Both somatotrope and gonadotrope functions are impacted by the nutritional state and therefore it is not surprising that they exhibit a dependency on leptin. Early studies showed significant reductions in numbers of gonadotropes in leptin-deficient animals [6, 15–19]. Similarly, rodents that lack leptin or leptin

receptors (LEPR) had reduced numbers of somatotropes [20, 21]. Our studies on the distribution of pituitary LEPR showed expression in nearly all cells [1, 22].

A dependency on normal levels of serum leptin was seen in our studies of 24 h fasted rats, when we correlated the reduction in serum leptin with reduced numbers of immunolabeled somatotropes and gonadotropes, along with reduced receptivity for gonadotropin releasing hormone (GnRH) and growth hormone releasing hormone (GHRH) [23]. As these findings pointed to potential trophic actions by leptin, we continued *in vitro* studies to determine if leptin would rescue either cell population, restoring hormone stores lost during the acute fast. We cultured pituitary cells from fasted rats overnight and then incubated them with 10-100 pg./ml leptin for 1 h. This brief treatment rapidly restored stores as detected by increases in numbers of immunolabeled somatotropes and gonadotropes [23], confirming direct effects of leptin on these cell populations.

These findings agree with recent *in vivo* studies of rodents by Luque et al. [24], which showed that both GH secretion and *Ghrhr* mRNA levels were restored by leptin in leptin-deficient *ob/ob* mice. Furthermore, studies of non-human primates by this same group confirmed both somatotropes and gonadotropes as leptin targets in primates [25, 26], reporting that leptin stimulated release of GH and follicle stimulating hormone (FSH) *in vitro* [25].

Leptin's restorative or stimulatory effects directly on somatotropes and gonadotropes have since led to studies that explored the significance of this regulatory influence as well as basic mechanisms of action, including the identification of signaling pathways and transcription factors. This chapter will review the studies which have identified critical leptin target molecules that are vital to the differentiated function of gonadotropes and somatotropes. We will also review signaling pathways used by leptin to stimulate production of these targets. Finally, we will show how leptin may contribute to plasticity of the pituitary by supporting multihormonal cell populations.

2. Leptin regulation of reproduction

The overall importance of leptin to reproduction was established soon after its discovery [5]. Leptin alone will restore fertility in leptin-deficient animals and humans [4, 15, 16, 27–36]. There are distinct sex differences in serum leptin levels in the adult. After puberty, adult males have relatively low leptin levels, when compared with females [37–41]. This sex difference may reflect the differential regulation of leptin by gonadal steroids. Androgens inhibit leptin secretion to prevent leptin inhibition of testicular function (reviewed in [5]). In females by contrast, estrogens stimulate leptin secretion. The rise in estrogen early in the cycle may contribute to the 2-3-fold increase in leptin levels known as the midcycle leptin surge [37, 39].

With respect to gonadotrope function, studies have also reported a synchrony between nocturnal leptin and LH pulses in normal cycling women [36, 37]. Indeed, a comprehensive study of 259 cycling women reported that the highest levels of leptin were correlated with the timing of the LH surge [37]. In contrast, anovulatory cycles were associated with overall low leptin levels.

3. Leptin regulation of gonadotropes

Shortly after leptin was discovered, pioneering studies by Yu et al. [10] demonstrated that leptin stimulated LH and FSH release, *in vitro*, from hemi-anterior

pituitaries. They reported a dose dependent increase in LH after 3 h in 10^{-11}-10^{-9} M leptin. Higher concentrations, however, were not stimulatory suggesting the development of leptin resistance. It is interesting to note that the relatively narrow concentration range that stimulates gonadotropin release matches that of the normal cyclic rise in leptin [36, 39]. Yu et al. reported that leptin alone stimulated LH release, *in vitro* and it did not add to the stimulatory effect of GnRH [11]. Their studies also identified nitric oxide as a signaling pathway for leptin regulation of gonadotropes. They showed that a competitive inhibitor of nitric oxide synthase (NOS), N6-monomethyl-L-arginine (NMMA), inhibited the leptin stimulation of LH release *in vitro* [11] suggesting that leptin may use the NOS pathway to stimulate gonadotropes directly.

Our studies on the importance of leptin to gonadotropes began with the detection of leptin receptors (LEPR) in dispersed pituitary cells from male and cycling female rats and mice [1]. The expression of LEPR varied with the stage of the cycle and was seen in 40-50% of anterior pituitary cells from males and females in metestrus or diestrus. LEPR expression increased to 60-80% of AP cells in proestrous and estrous females, which coincided with the midcycle rise in serum leptin [1].

To determine if the increase reflected changes in gonadotrope receptivity, dual labeling was performed for LEPR and gonadotropins. The results showed that 90% of gonadotropes identified by the stores of luteinizing hormone (LH) or follicle stimulating hormone (FSH) expressed LEPR in males and throughout all stages of the cycle in females [1]. Some of the increase in LEPR in proestrous females was due to an increase in cells expressing LH and LEPR, which occurs just before the LH surge.

The findings showing an overall increase in LEPR early in the estrous cycle stimulated studies to determine potential regulators for this expression. We treated one day cultures of anterior pituitary cells from diestrous female mice with estradiol overnight and then treated a subset of these cultures with 10 or 100 pg./ml neuropeptide Y (NPY) for 3 h. **Figure 1** shows that estradiol or NPY alone (100 pg./ml) stimulated a significant 2--fold increase in LEPR-bearing cells and that the effects of the two were not additive. In contrast, NPY did not stimulate LEPR expression in anterior pituitary cells from male mice (data not shown). Collectively, these data support the hypothesis that rising estradiol early in the cycle may stimulate an increase in pituitary receptivity to leptin which may serve as a gateway for leptin's permissive actions [7].

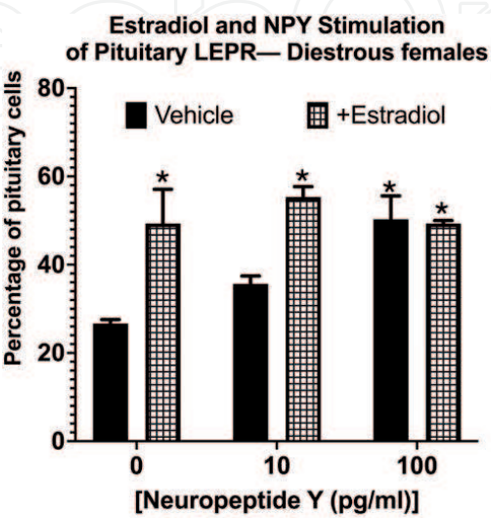

Figure 1.
*Estradiol and NPY stimulate LEPR expression in 1-day cultures of anterior pituitary cells. * = significantly higher values than all other values; ANOVA + Bonferroni's post hoc test. Note: These are original data, not published elsewhere.*

Having established the presence of the receptor population in gonadotropes, we determined if leptin acted on gonadotropes through the Janus Kinase-Signal Transducer and Activator of Transcription (JAK–STAT) pathway. Following leptin stimulation for 10-60 min *in vitro*, pituitary cells from diestrous females were dual immunolabeled for phosphorylated STAT3 and LH or FSH [1]. In 30-60 min, leptin stimulation increased the overall percentage of LH or FSH-bearing gonadotropes and the percentages of gonadotropes bearing pSTAT3 [1]. Thus, leptin acts through both NOS [11] and the JAK–STAT [1] pathways to increase LH or FSH stores in gonadotropes.

4. The importance of leptin to gonadotrope function

The next series of studies investigated leptin's importance to gonadotrope function by selectively ablating LEPR in gonadotropes with Cre-LoxP technology. This work fills a critical knowledge gap, because, as summarized in our recent review [5], much of the research surrounding leptin's role in reproduction has been focused in the hypothalamus. There was a growing body of evidence showing that leptin's regulatory actions were mediated through its stimulation of Kisspeptin neuronal pathways that regulate GnRH neurons (reviewed in [5]).

Our first set of studies used Cre-recombinase driven by the *rLhb* promoter to delete either the JAK binding site (floxed LEPR exon 17) [1] or the signaling peptide of the LEPR (floxed LEPR exon 1). Deletion of LEPR exon 17 resulted in a non-signaling receptor. Deletion of exon 1 resulted in ablation of all isoforms of LEPR. because the deletion removed the signal peptide thereby preventing the protein from entering the rough endoplasmic reticulum. Ongoing studies are using Cre-recombinase driven by the *Gnrhr* promoter to delete LEPR exon 1.

The first question to be addressed related to the impact of loss of LEPR in gonadotropes on pubertal development, growth, and fertility of the mice [1]. When LEPR exon 17 was deleted in gonadotropes with the Cre-*Lhb* driver, mice showed no evidence of delayed puberty or growth. Mutant males showed no evidence of impaired fertility. However, mutant females exhibited a 36% increase in time to first pregnancy and the litters contained significantly fewer pups. Pup survival was 98% from either parent and there was no evidence of growth defects in weanlings from mutant females. Therefore, mutant females appeared to lactate normally.

We analyzed hormone levels in mice lacking LEPR exon 17 and reported that loss of LEPR resulted in several deficits [1]. In mutant diestrous females, serum levels of LH, FSH, and GH were reduced. In contrast, mutant males showed reductions in GH, prolactin (Prl), and thyroid stimulating hormone (TSH), but no reductions in gonadotropins. The loss of LEPR resulted in reduced *Fshb* mRNA levels in both males and females but no reductions in *Lhb, Cga* (in females) or *Gnrhr* mRNA levels. In addition, there was a reduction in inhibin/activin beta subunit mRNA (*Actβa* and *Actβb*) in females. The most striking reduction, however, was in GnRHR proteins, as detected by immunolabeling or binding to a biotinylated analog of GnRH [1]. The reduced binding was most severe in diestrous females, a stage where GnRHR expression is normally at the highest levels.

However, during this phase of the study, we detected Cre-recombinase in the testes and therefore continued these studies focusing only on mutant females [7] bearing Cre-*Lhb* and floxed LEPR exon 1. The deletion of this exon was impactful because it results in loss of all isoforms of the receptor. Tests of fertility showed normal litter numbers from three breeding cages of F2 generation heterozygous females (bearing only one deleted allele of LEPR exon 1). However, the study showed severe subfertility/infertility in F3 generation mutant homozygous and

heterozygous females [7]. Out of the five F3 generation homozygous females, only two were fertile, producing litters more slowly than control females (one every 30-45 days). One of the litters did not survive. In addition, three F3 homozygous females and two F3 heterozygous females were completely infertile in breeding tests that lasted from 65 to 281 days with a fertile male [7].

We were able to study cyclicity in the progeny from the two F3 fertile females. These mutant F4 female progeny cycled irregularly. Two of them remained in diestrus and the remaining females spent more time in diestrus than normal females. Collectively, these breeding studies showed that ablation of all isoforms of LEPR in gonadotropes had a profound impact on a subset of females; less than half could cycle and were fertile [7]. This highlighted the importance of leptin to gonadotrope functions. However, because of the infertility issues in the line expressing Cre-LH X LEPR exon 1, our ongoing studies have now switched to mice bearing Cre-recombinase driven by the *Gnrhr* promoter. Whereas the mutant females in this line are still subfertile, they produce sufficient progeny for our ongoing and continuing studies of this line.

5. Leptin regulates target genes through different pathways

After we characterized the deletion mutants lacking LEPR in gonadotropes, we hypothesized that rising leptin early in the cycle may have a permissive effect on the rise in pituitary GnRHR levels [7], which could serve as a gateway that permitted full receptivity to GnRH and facilitates the LH surge. We treated pituitary cells from normal diestrous female mice with 10 nM leptin and showed a significant increase in GnRHR proteins [7]. We also detected leptin-stimulated increases in pituitary activin (but not inhibin) mRNA (*Actβa* and *Actβb)* in the same sets of experiments. However, leptin did not stimulate increases in *Gnrhr* mRNA levels [7], which correlated well with the lack of change in mRNA levels evident in the LEPR-null gonadotropes. Thus, we identified three targets of leptin in our animal model, and proposed that leptin may activate these by different pathways.

5.1 Transcriptional regulation of FSH and activin by leptin

We have demonstrated that expression of *Fshb* and activin transcripts are dependent on a normal leptin signal [1, 7]. Other workers have shown that activin and FSH may be dependent on the timing of this leptin signal during postnatal development, which is characterized by a rapid rise in serum leptin. Wen et al. [42] studied the link between the postnatal rise in leptin and FSH and reported that full co-expression of GnRHR and FSH is seen by postnatal day 7, which coincides with the peak leptin surge. A parallel rise in *Fshb* and *Actβa* and *Actβb* mRNA levels during the postnatal leptin rise has also been reported [43–46]. Researchers investigating the impact of altering the neonatal leptin surge on the reproductive system reported that blockade or alteration of the leptin surge decreased testicular or ovarian growth, delayed puberty, and reduced FSH in rat pups [47]. In addition, females showed reduced numbers of ovarian primordial follicles [48].

Another link between leptin and FSH was reported by studies that restored LEPR in gonadotropes from mice that were genetically engineered to be globally deficient in LEPR [49]. As expected, fertility was not restored, because the mice were morbidly obese, and kisspeptin and GnRH neuronal function was still deficient. However, they did report elevated FSH levels in these mice. It was not determined whether restoration of the leptin signal influenced GnRHR expression.

The reduced *Fshb* mRNA detected in our gonadotrope LEPR-null mutants correlates well with the reduced activin (*Actβa* and *Actβb*) mRNA [1], which is a critical regulator of *Fshb* transcription [50, 51]. Our studies show that leptin stimulates activin mRNA [7], which could thus serve as a pathway for FSH stimulation. Leptin regulation of FSH also agrees with studies of rats [10, 11] and non-human primates [25] in which leptin directly stimulated FSH secretion, *in vitro*. Collectively, these findings suggest that leptin may be an important transcriptional regulator of FSH production both postnatally and early in the cycle, either directly or indirectly. Additional studies are needed to determine if this pathway is mediated through JAK–STAT activation or NOS [10, 11].

Figure 2 illustrates how the ovary and adipocytes may partner in the remodeling of gonadotropes to support the development of the follicles with key cellular regulatory pathways and outputs indicated. This cartoon focuses mainly on leptin, FSH and estrogen. We propose that normal levels of leptin permit a rise in FSH early in the cycle regulating FSH directly or through activin. This could be an important checkpoint if leptin levels drop due to fasting, for example [23] as this may signal poor nutrition and reduce FSH production. The cartoon then shows that FSH stimulates ovarian follicles to produce and secrete estrogen, which stimulates a rise in serum leptin. The growth in ovarian follicles and subsequent rise in estrogen also has positive feedback actions on GnRH neurons (shown in ref. [5]) and the gonadotropes. Estrogen may also stimulate a rise in pituitary LEPR (**Figure 1**), which renders the gonadotropes more responsive to leptin.

Not shown in this cartoon is GnRH, which is secreted in response to estradiol positive feedback to stimulate gonadotrope production of gonadotropins and GnRHR (pathway shown in ref. [5]). GnRH and estradiol both stimulate *Gnrhr* mRNA during this time (reviewed in [9]). Leptin's role is to de-repress Musashi's actions on *Gnrhr*

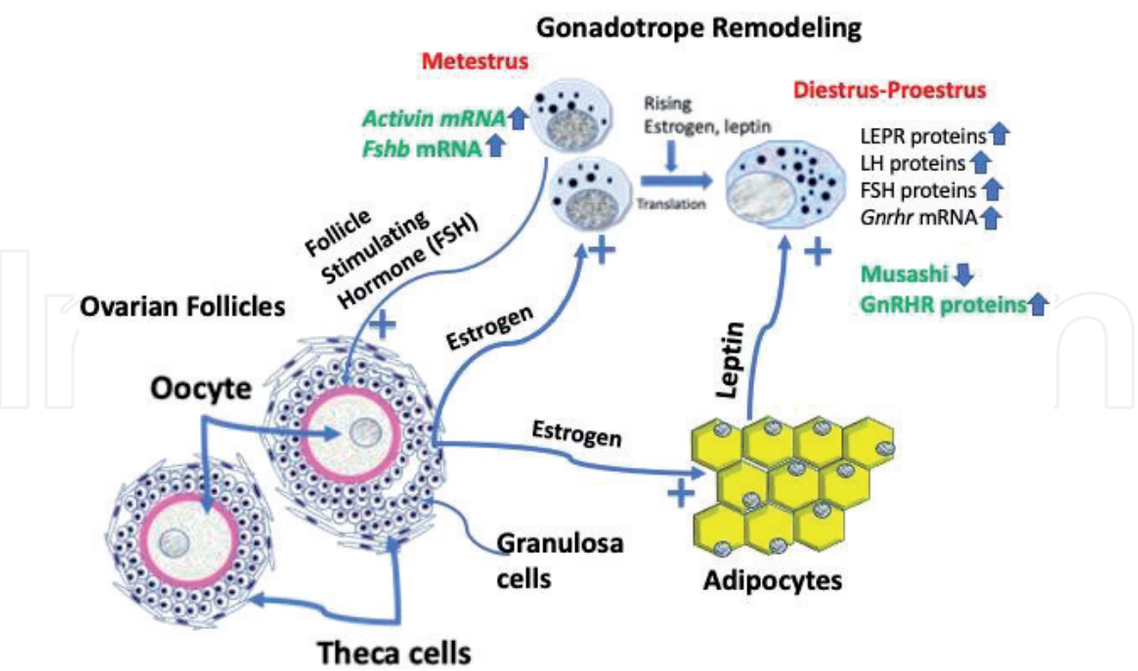

Figure 2.
Gonadotropes are remodeled early in the cycle by estrogens, GnRH, and leptin to support the ovary. We postulate that normal levels of leptin permit FSH release directly or through activin. FSH stimulates the growing population of follicles, which produce more estrogen. This rise in estrogen may stimulate leptin release from adipocytes and the expression of LEPR in gonadotropes. Estrogen also exerts positive feedback on the neuronal circuit that regulates GnRH, which produce more rapid GnRH pulses, which also stimulate Gnrhr *mRNA. As* Gnrhr *mRNA rises, leptin works post-transcriptionally to permit translation of GnRHR proteins by de-repressing the actions of the translational regulator Musashi (MSI). Leptin also causes a reduction in expression of MSI. This is an original figure drawn by the corresponding author and not published elsewhere.*

mRNA and permit translation. Leptin also reduces Musashi expression [7, 8]. Thus, our studies show that, whereas leptin does not regulate *Gnrhr* mRNA directly, it works in partnership with estradiol and GnRH to permit its translation by regulating MSI. This is another checkpoint in reproductive cycles [7, 9]. Reduced leptin, due to fasting for example, may signal poor nutrition and thus reduce translation of GnRHR [7–9] and GnRH binding sites [23]. Ultimately, leptin reduction of ablation slows or prevents reproduction. Our animal models lacking LEPR in gonadotropes support this hypothesis [1, 5, 7].

5.2 Post-transcriptional regulation of GnRHR by leptin

Figure 2 also shows the pathway that regulates the third target for leptin, GnRHR. This receptor appears to be regulated post-transcriptionally by leptin, because *Gnrhr* mRNA is unchanged when diestrous female or male gonadotrope LEPR-null mutants were compared with control males or diestrous females. Additionally, stimulation of control diestrous female pituitary cultures by leptin increases GnRHR, but not *Gnrhr* mRNA levels [7, 8]. We investigated post-transcriptional mediators of leptin action and determined that a putative miRNA repressor of *Gnrhr* mRNA translation, *miR-581/669d,* was increased in LEPR-null gonadotropes [7]. The most promising regulation, however, came from the translational regulatory protein, Musashi (MSI), as we identified 3 consensus binding elements for Musashi (MBEs) in the 3' UTR of murine *Gnrhr* mRNA [8]. The evolutionarily conserved Musashi family of sequence-specific RNA binding proteins (Musashi1 and Musashi2) have long been known to be expressed in stem and progenitor cell populations, where they act to oppose differentiation and promote stem cell self-renewal [52]. Although originally identified as a repressor of target mRNA translation, Musashi was subsequently shown capable of directing translational activation of target mRNAs in a context-dependent manner [53].

Our studies of leptin stimulation of GnRHR proteins showed a dose response relationship between leptin and expression of GnRHR (detected by enzyme assays) or Biotinylated GnRH binding to living pituitary cells (detected cytochemically) [8] After we confirmed that leptin stimulated GnRHR proteins, but not mRNA, we determined by electrophoretic mobility shift assays that Musashi1 interacted directly with the *Gnrhr* 3' UTR [8] . This pituitary association was confirmed by immunoprecipitation with anti-Musashi antibody and the detection of an enrichment of the endogenous *Gnrhr* mRNA (17-fold over control immunoprecipitates). Moreover, the use of luciferase mRNA reporter assays showed that Musashi1 repressed translation of the *Gnrhr* 3' UTR. Tests of leptin actions on Musashi showed that leptin stimulation caused a reduction in Musashi protein levels in gonadotropes, suggesting that leptin may inhibit Musashi expression [8].

To summarize, our studies of leptin actions on gonadotropes have shown severe functional deficiencies in gonadotropes lacking exon 1 of LEPR. The total absence of the LEPR caused infertility in a subset of females [7]. Collectively, studies of these animal models point to key gene products that are affected by loss of leptin signals. Leptin may be important in the transcription of *Fshb* mRNA either directly and/or through the transcription of activin. In addition, leptin's actions may serve to regulate the translation of GnRHR protein [7–9]. Our studies suggest that leptin opposes Musashi-dependent repression of target mRNAs and/or reduces expression of Musashi directly in gonadotropes, leading to enhanced translation of the *Gnrhr* mRNA. This may provide a pathway which permits full expression of GnRHR early in the cycle to reach peak levels in diestrus and proestrus. Estradiol may also stimulate the expression of LEPR, which peaks on proestrus (**Figure 1**). Rising leptin may then partner with estradiol to promote the production of GnRHR (**Figure 2**). We hypothesize that leptin's

permissive actions on GnRHR may be to de-repress actions of the translational regulatory protein Musashi and promote full receptivity of the gonadotrope to GnRH [7–9].

6. Impact of ablation of LEPR in gonadotropes on other pituitary cell types

The loss of leptin receptors in gonadotropes also had a broader impact on pituitary function. We reported a profound reduction in serum GH, in both mutant males and females (**Figure 3**) [1]. This reduction would expect to result in growth hormone deficiency, which, in our other models has resulted in significant changes in body weight (adult-onset obesity) [22]. However, when mice were weighed regularly for nearly a year, these deletion mutant animals grew normally and did not gain more weight than normal mice during their first year of life [1]. In addition, male mutants show reduced levels of serum TSH and prolactin [1]. There were also sex-specific differences in mRNA levels. As stated earlier, in deletion mutant females, *Fshb* and activin were reduced. In contrast, male deletion mutants showed reduced mRNA levels of *Fshb, Cga, Gh*, and *Ghrhr*. This phenotype may be the result of deficits in the production of paracrine factors from gonadotropes, which are needed to regulate the function of these cell types. Alternatively, we hypothesized that this phenotype may simply result from of the loss of multihormonal function in subsets of gonadotropes themselves. Evidence for the presence of multihormonal gonadotropes is reviewed in the next few paragraphs.

The presence of multihormonal gonadotropes in the rodent pituitary cell population is not unexpected since our group previously reported cells that stored gonadotropins and either ACTH [54–56] or GH [57, 58]. Early studies of gonadotropes purified by centrifugal elutriation reported a fraction that contained 91-93% immunolabeled LH-FSH cells (a 9-fold enrichment). This group of cells were enriched based on their response to GnRH. The stimulated secretion caused them to enlarge. Which allowed them to be separated and enriched in a fraction containing larger cells. The fraction also contained gonadotropes that immunolabeled for other hormones. In the female gonadotrope fraction, we detected: 29.2% GH cells, 4% prolactin cells, 6.8% adrenocorticotropin (ACTH) cells, and 2.8% thyroid stimulating hormone cells (TSH [59]).

More recently we bred a Cre-reporter gene into our Cre-LH line to purify gonadotropes by fluorescence activated cell sorting mice [60]. Floxed tdTomato (red fluorescence) was expressed in all pituitary cells. However, in cells bearing Cre-recombinase (Cre-Lhb), the tdTomato was ablated promoting the expression of

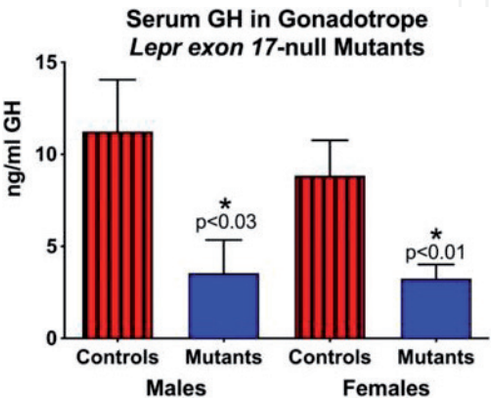

Figure 3.
Mutants lacking Lepr exon 17 in gonadotropes show significantly reduced levels of serum GH. This colored figure has not been published elsewhere. However, the data were published as separate figures (separating sexes) in ref. [1] in a completely different, black and white graph.

eGFP (green fluorescence). Thus, all non-gonadotropes (not producing Cre-*Lhb*) fluoresced red and all gonadotropes bearing LH expressed the green eGFP fluorescence. The red and green fractions were then separated by Fluorescence Activated Cell Sorting (FACS). The FACS and assay methods are identical to those used for the Cre-GH line, as described by Odle et al. [60].

Figure 4 shows the FACS separation profiles for non-fluorescent pituitary cells (**Figure 4A**); fluorescent Cre-negative populations bearing only tdTomato (**Figure 4B**) and Cre-positive populations, which contain the eGFP cells (**Figure 4C**). Assays for content of gonadotropins and GnRH receptors show that over 95% of the total content is in the eGFP fraction (**Figure 4D–F**). Over 90% of eGFP cells were immunolabeled for LH (**Figure 4G**). However, multihormonal expression is evident as shown in **Figure 4H–K**. The eGFP fraction contains 30% of the GH and TSH content and 10% of the ACTH and prolactin content. In contrast, the non-gonadotrope, tdTomato fraction (red bars) contain over 70% of the content of GH and TSH and 90% of the content of ACTH and prolactin.

When mRNA levels were assayed by qPCR, similar results were seen. **Figure 5A** shows the 72-88% enrichment in gonadotropin and *Gnrhr* mRNAs in the

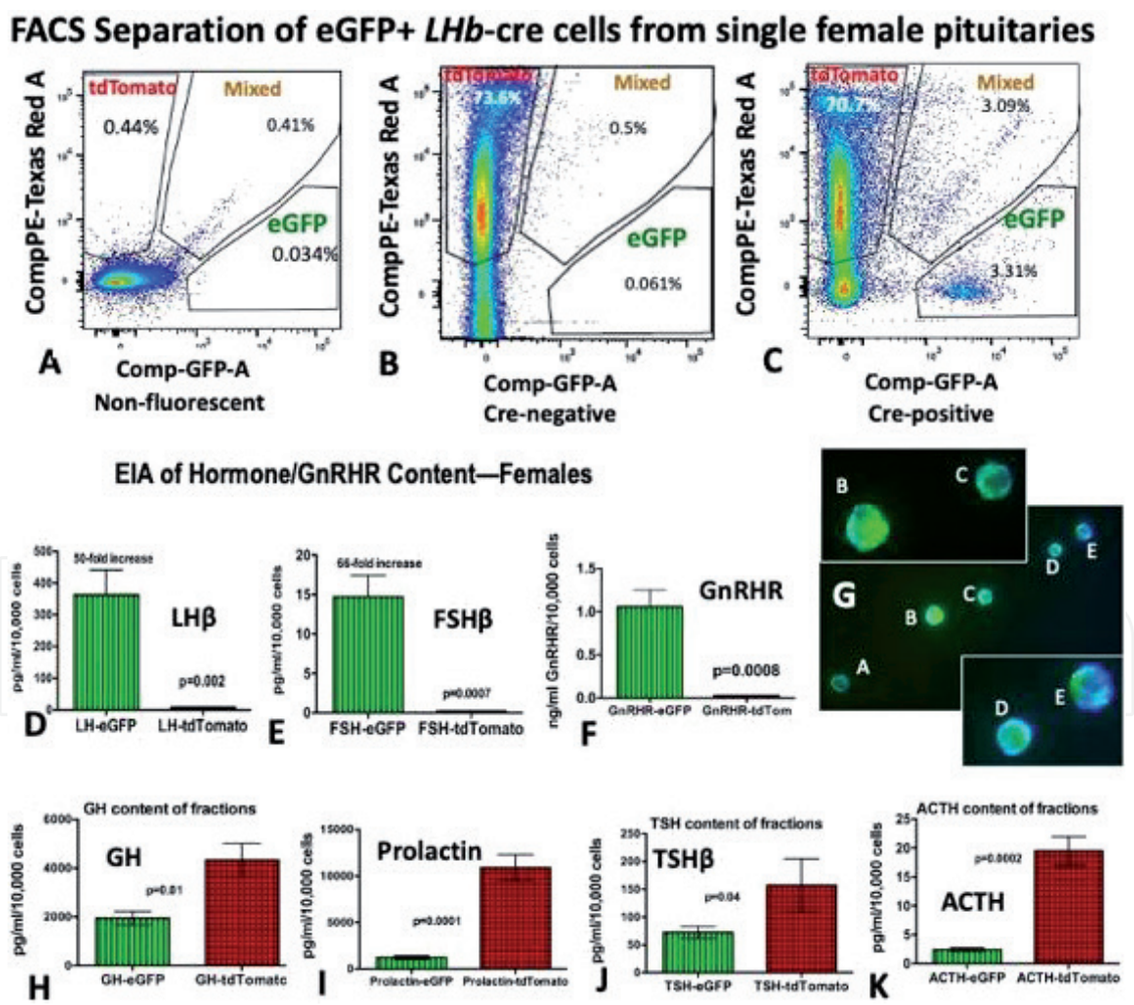

Figure 4.
FACS experiment comparing non-fluorescent cells (4A) with those bearing tdTomato-eGFP, but no Cre-recombinase (4B) and those bearing Cre-recombinase, which ablates the tdTomato, allowing expression of eGFP (4C). Only the profile in 4C shows the presence of eGFP cells. (D–F) show that >90% of LH, FSH, and GnRHR is assayed in the eGFP fraction and barely detectable in the tdTomato fraction. Immunolabeling in (G) shows that >90% of the eGFP cells are labeled for LH (cyan shows blue label over eGFP green). (H–K) detect 70-90% of other pituitary hormones in the tdTomato fraction, but 10-30% of these hormones are found in the pure gonadotrope fraction. These data have never been published elsewhere and are original to this chapter.

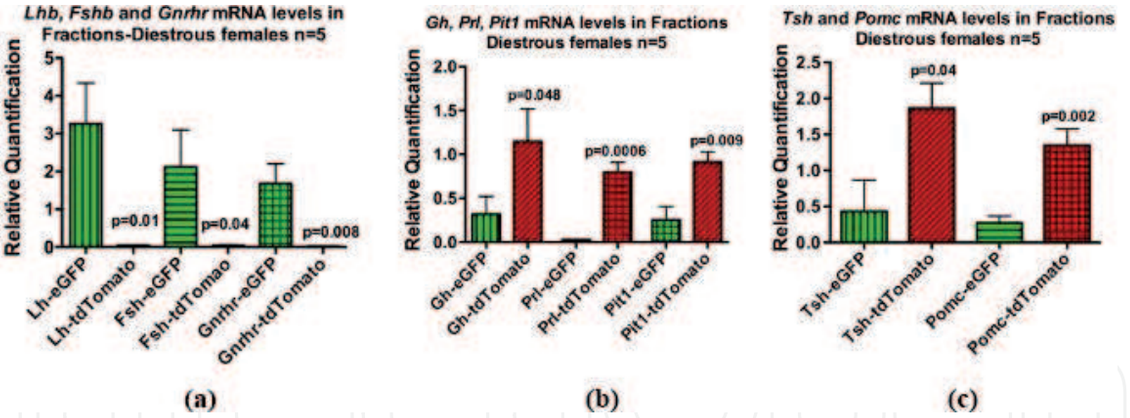

Figure 5.
(A) qPCR assays show enrichment in Lhb, Fshb, and Gnrhr mRNA in eGFP fraction. These data have not been published elsewhere and are original. (B) Shows enrichment of Gh, Prl, and Pit1 mRNA levels in tdTomato fractions, but expression of about 20% of the total in the gonadotrope fraction. (C) Shows Tshb and Pomc mRNA levels and about 20% are in the gonadotrope fraction and 80% in the tdTomato fraction. These data have not been published elsewhere and are original to this chapter.

eGFP fractions, with little evidence for expression in the tdTomato fraction. **Figure 5B** shows that 10-20% of the levels of *Gh, Tsh*, and *Pomc* mRNA were also found in the gonadotrope fraction with the remaining tdTomato fraction containing the bulk of these RNAs (80%). It is also interesting to note that *Pou1f1* (also known as *Pit1*) mRNA, a transcription factor important in the production of *Gh, Tshb* and *Prl* is also found in the eGFP-gonadotrope fraction at about the same levels as that of *Gh* and *Tshb*. This expression would be important as Pou1f1 would be available to support the transcription of *Gh, Tsh,* and *Prl.*

FACS fractions from male mice from this line were also analyzed for mRNA content and similar enrichment of gonadotropins and *Gnrhr* mRNA levels was evident as well as similar levels of *Gh*, *Prl* and *Tshb* mRNAs in the eGFP-gonadotrope fractions (data not shown). Also, it is interesting to compare the expression profile of these purified gonadotrope fractions with that of the purified fractions produced in mice bearing Cre-GH. As previously reported, the Cre-GH/eGFP fraction contains most of the GH but very little LH, FSH or ACTH (see Figure 5 in [60]). The pure somatotropes contain significant amounts of Pou1f1, prolactin and TSH proteins and mRNA. Thus, somatotropes also include a multihormonal subset, but the expression profile is different.

More recently, multihormonal pituitary cell populations have been detected by single cell RNA-sequencing, especially in the study by Ho et al. [61], which investigated changes in multihormonal cell transcriptome patterns in mice subjected to different physiological stresses. As we have outlined in a recent review [62], these pools of multihormonal cells may serve to support pituitary plasticity and add to the functions of pituitary populations as physiological needs arise. We hypothesize that these cells may include progenitor cells. Our data showing that gonadotrope LEPR-null mice have deficiencies in other hormones suggest that leptin may regulate multihormonal expression from this group of cells. The fact that secretion of a particular hormone is reduced in animals with LEPR-null gonadotropes highlights the importance of leptin to multihormonal function and suggests a role for leptin in the regulation of pituitary plasticity.

7. Leptin regulation of somatotropes

Somatotropes are vital metabolic sensors because they directly regulate stores of fat as they build muscle, bone, and regulate optimal body composition [63].

Most somatotropes bear leptin receptors [64, 65] and leptin deficiency results in reduced somatotrope functions [4, 20, 66–68]. As stated in the introduction, leptin treatment of leptin deficient *ob/ob* mice restores pituitary GH secretion and *Ghrhr* mRNA levels, but not hypothalamic production or secretion of GHRH [24].

Our studies of leptin's regulation of somatotropes began with the ablation of LEPR exon 17 or exon 1 with Cre-recombinase driven by the rat GH promoter [22, 69]. Both models showed GH deficiency, adult-onset obesity and metabolic dysfunction. At the level of the pituitary, this deficiency was seen as a reduction in GH and GHRHR.

We also reported sex-specific deficiencies during postnatal development with the discovery that leptin may target two transcription factors important in the production of GH, GHRHR, PRL, and TSH. These included Prophet of Pit1 (Prop1) and Pou1f1 [70]. Ablation of LEPR exon 1 in somatotropes reduced Pou1f1 in neonatal females along with serum prolactin. GH stores detected by immunolabeling were also reduced in both neonatal males and females. Interestingly, the lack of LEPR promoted an increase in Prop1 in neonatal males.

The studies of the impact of loss of LEPR were continued on FACS purified somatotropes [60]. Purified somatotropes showed reductions in GH, as expected, however they also contained a subset of multihormonal cells storing TSH and/or prolactin. In somatotrope LEPR-null females, TSH and prolactin stores were reduced in the pure somatotrope fraction [60]. Taken together, our analysis of somatotropes that lack LEPR shows that this multihormonal subset is significantly reduced, suggesting once more that leptin may play a role in maintaining multihormonal expression and promoting pituitary cell plasticity. Finally, these studies also demonstrated that Pou1f1 was reduced in pure somatotropes, which may explain the reduction in any or all hormones dependent on this transcription factor (GH, GHRHR, TSH and prolactin) [60].

We continued the investigation of leptin signaling pathways in somatotropes and reported that they included both transcriptional and posttranscriptional regulators [3]. Our tests of pathway inhibitors showed that full GH expression may be maintained by leptin through the JAK/STAT3 pathway but not nitric oxide. This contrasts with leptin pathways that regulate gonadotropins, which include NOS. Leptin regulation is likely to be transcriptional as loss of LEPR in somatotropes reduced *Gh* and *Ghrhr* mRNA and proteins [3]. In addition, leptin regulation of the *Pou1f1* transcription factor may also serve as a pathway for the transcriptional regulation of *Gh* and *Ghrhr* [2, 3, 60].

However, regulation of POU1F1 by leptin appears to be via post-transcriptional mechanisms as loss of LEPR in somatotropes causes reduction in mRNA levels of the Pou1f1 protein, but not the *Pou1f1* mRNA [2, 60]. Conversely, leptin stimulation results in increased expression of Pou1f1 proteins, but not mRNA [60].

An *in silico* analysis detected eight Musashi binding elements in the 3'UTR of the *Pou1f1* mRNA and tests of Musashi binding showed direct interaction of Musashi with this region and repression of translation, which was reversed by leptin [2]. Furthermore, Musashi immunoprecipitation of whole pituitary extract showed co-association of Musashi and the endogenous *Pou1f1* mRNA. Our analyses of transcripts by scRNA-sequencing studies of normal pituitary cells showed that *Msi1* mRNA was expressed in somatotropes. This was confirmed in pure somatotrope populations [2].

8. Leptin regulation of pituitary musashi

Our studies of animal models in which LEPR was ablated in gonadotropes or somatotropes opened the door to the discovery that leptin may regulate some of its

target gene products by post-transcriptional pathways. The post-transcriptional targets included *Gnrhr* mRNA in gonadotropes [1, 7, 8] and the mRNA encoding the POU1F1 transcription factor in somatotropes [2, 60, 70].

The concept that Musashi would be involved in the translational regulation of either *Gnrhr* or *Pou1f*1 mRNA was novel, as both transcripts are important in the function and differentiation of somatotropes and gonadotropes. The expression and involvement of Musashi in differentiated hormone-producing cell lineages was surprising as Musahsi is typically implicated in stem and progenitor cell self-renewal. Nonetheless, while *Msi1* and *Msi2* are expressed in pituitary stem cells as expected, our scRNA sequencing clearly demonstrated that *Msi1* and *Msi2* mRNAs were also expressed in all hormone-producing cell lineages of the anterior pituitary [2].

Since our findings indicated that Musashi was involved in the repression of translation of *Gnrhr* or *Pou1f*1 mRNAs, we hypothesized that normal signals from leptin were needed to reverse this repression [2, 7]. This was based on the fact that the loss of leptin signals resulted in a reduction in the proteins (but not the mRNA). We were able to demonstrate a role for leptin in regulating the actions of Musashi s in reporter assays, where leptin mediated the reversal of Musashi-dependent repression [2].

Our studies also showed that leptin may directly reduce expression of Musashi in its target cells. In pituitaries from proestrous female mice lacking LEPR in gonadotropes, *Msi* mRNA expression is higher. Furthermore, leptin treatment of normal pituitaries from proestrous females resulted in reduced levels of *Msi1* mRNA [8]. More specifically, leptin treatment of normal pituitaries reduced Musashi1 immunolabeling in gonadotropes, identified by their expression of binding to biotinylated GnRH [8].

Similarly, Musashi1 protein and *Msi1* mRNA levels were increased in pure somatotropes lacking LEPR [2]. Furthermore, leptin treatment of pure somatotropes significantly reduced their expression of Musashi1 proteins. Collectively these findings point to a post-transcriptional pathway for leptin, which would reverse repression of translation of key target molecules in somatotropes or gonadotropes by regulating the function and expression of the Musashi family of translational regulatory proteins.

Our *in silico* analyses have identified other potential Musashi targets in the anterior pituitary, which may be regulated by leptin as well. Notably, there are MBEs in the *Fshb, Tshb*, *Prl* and *Pomc* mRNAs. However, no MBEs are found in *Lhb, Gh, or Ghrhr* mRNAs. It is interesting to speculate that this differential targeting may reflect specific roles for Musashi in regulating differentiated pituitary cells. Musashi could act in multihormonal cells by repressing translation of one set of hormones but not another. Furthermore, our studies of leptin regulation of Musashi, GnRHR, and Pou1f1 protein levels suggest that leptin may use this Musashi pathway to promote selective differentiation of a given cell type depending on the body's needs.

9. Conclusions

Pituitary gonadotropes and somatotropes were initially shown to be most vulnerable to the global loss in leptin signals as demonstrated by their reduction in numbers in the population, even following acute fasting. As little as 10-100 pg./ml leptin directly restored hormone levels in these populations, so they could once more be detected by immunolabeling. We now have much more information about the impact of loss of leptin signaling to model animals including infertility when they carried LEPR-null gonadotropes and adult-onset obesity and GH deficiency when they carried LEPR-null somatotropes. We have identified specific leptin targets

in each of these cell types and determined that leptin regulation may involve both transcriptional and post-transcriptional pathways. The target molecules are vital to the differentiated function of these cells, which highlights a role for leptin in maintaining their differentiated state. Regarding post-transcriptional pathways, we have shown that leptin also regulates expression of the translational regulatory protein, Musashi. Our studies have led to the discovery of novel roles for Musashi, implicating this regulator in the repression of targets in specific pituitary cell types. This broadens the scope of Musashi's regulatory role beyond that of regulation of stem cells. Finally, our studies of purified somatotropes and gonadotropes have confirmed the presence of multihormonal expression in a subpopulation of cells and have led to the discovery that leptin signaling is needed to maintain this subset. The presence of these multihormonal pituitary cells is also evident in single cell RNA-sequencing studies. Future studies are needed that focus on the role leptin plays in maintaining this cell population, which supports pituitary plasticity. Future investigation will elucidate the role Musashi may play in the selective regulation of specific hormones or their transcription factors.

Acknowledgements

The authors are grateful for the outstanding technical assistance of Anessa Haney, Linda Hardy, and Alex Lagasse. The authors acknowledge funding from the following sources; National Institute of Health (NIH) R01 HD059056 (GVC); NIH R01 HD-087057 (GVC, AMM); NIH R01 HD-093461 (AMM, GVC, and MCM); NIH R01 DK113776 (GVC, AMM, and MCM); and NIH R01 DK 127723 (GVC, AMM, and MCM). We also acknowledge pilot studies grants from the Sturgis Foundation (AMM, GVC) and Development Enhancement Awards (GVC, AO) from UAMS. We also acknowledge support for core facilities from Center grants-NIGMS P20 GM103425 and P30 GM11070 (Dr. Edgar Garcia-Rill PI).

Conflict of interest

The authors declare no conflict of interest.

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References

[1] Akhter N, CarlLee T, Syed MM, Odle AK, Cozart MA, Haney AC, et al. Selective deletion of leptin receptors in gonadotropes reveals activin and GnRH-binding sites as leptin targets in support of fertility. Endocrinology. 2014;155(10):4027-4042. DOI: 10.1210/en.2014-1132:

[2] Allensworth-James M, Banik J, Odle A, Hardy L, Lagasse A, Moreira ARS, et al. Control of the Anterior Pituitary Cell Lineage Regulator POU1F1 by the Stem Cell Determinant Musashi. Endocrinology. 2021;162(3).DOI: 10.1210/endocr/bqaa245:

[3] Allensworth-James ML, Odle AK, Lim J, LaGasse AN, Miles TK, Hardy LL, et al. Metabolic signalling to somatotrophs: Transcriptional and post-transcriptional mediators. J Neuroendocrinol. 2020;32(11):e12883. DOI: 10.1111/jne.12883:

[4] Carro E, Pinilla L, Seoane LM, Considine RV, Aguilar E, Casanueva FF, et al. Influence of endogenous leptin tone on the estrous cycle and luteinizing hormone pulsatility in female rats. Neuroendocrinology. 1997;66(6):375-377.DOI: 10.1159/000127262:

[5] Childs GV, Odle AK, MacNicol MC, MacNicol AM. The Importance of Leptin to Reproduction. Endocrinology. 2021;162(2).DOI: 10.1210/endocr/bqaa204:

[6] Lloyd RV, Jin L, Tsumanuma I, Vidal S, Kovacs K, Horvath E, et al. Leptin and leptin receptor in anterior pituitary function. Pituitary. 2001;4(1-2):33-47.DOI: 10.1023/a:1012982626401:

[7] Odle AK, Akhter N, Syed MM, Allensworth-James ML, Benes H, Melgar Castillo AI, et al. Leptin Regulation of Gonadotrope Gonadotropin-Releasing Hormone Receptors As a Metabolic Checkpoint and Gateway to Reproductive Competence. Front Endocrinol (Lausanne). 2017;8:367.DOI: 10.3389/fendo.2017.00367:

[8] Odle AK, Benes H, Melgar Castillo A, Akhter N, Syed M, Haney A, et al. Association of Gnrhr mRNA With the Stem Cell Determinant Musashi: A Mechanism for Leptin-Mediated Modulation of GnRHR Expression. Endocrinology. 2018;159(2):883-894. DOI: 10.1210/en.2017-00586:

[9] Odle AK, MacNicol MC, Childs GV, MacNicol AM. Post-Transcriptional Regulation of Gnrhr: A Checkpoint for Metabolic Control of Female Reproduction. International Journal of Molecular Sciences. 2021;22(7):3312. DOI: 10.3390/ijms22073312:

[10] Yu WH, Kimura M, Walczewska A, Karanth S, McCann SM. Role of leptin in hypothalamic-pituitary function. Proc Natl Acad Sci U S A. 1997;94(3):1023-1028.DOI: 10.1073/pnas.94.3.1023:

[11] 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):5055-5058.DOI: 10.1210/endo.138.11.5649:

[12] Veldhuis JD, Roemmich JN, Richmond EJ, Bowers CY. Somatotropic and gonadotropic axes linkages in infancy, childhood, and the puberty-adult transition. Endocr Rev. 2006;27(2):101-140.DOI: 10.1210/er.2005-0006:

[13] Veldhuis JD, Roemmich JN, Richmond EJ, Rogol AD, Lovejoy JC, Sheffield-Moore M, et al. Endocrine control of body composition in infancy, childhood, and puberty. Endocr Rev.

2005;26(1):114-146.DOI: 10.1210/er.2003-0038:

[14] Childs GV. Gonadotropes and Lactotropes. In Physiology of Reproduction; J Neill and E Knobil, Eds, Elsevier Press, NY. 2006:1483-579

[15] Casanueva FF, Dieguez C. Neuroendocrine regulation and actions of leptin. Frontiers in Neuro endocrinology. 1999;20(4):317-363. DOI: 10.1006/frne.1999.0187:

[16] Chan JL, Mantzoros CS. Leptin and the hypothalamic-pituitary regulation of the gonadotropin-gonadal axis. Pituitary. 2001;4(1-2):87-92.DOI: 10.1023/a:1012947113197:

[17] Montague CT, Farooqi IS, Whitehead JP, Soos MA, Rau H, Wareham NJ, et al. Congenital leptin deficiency is associated with severe early-onset obesity in humans. Nature. 1997;387(6636):903-908.DOI: 10.1038/43185:

[18] Welt CK. Will leptin become the treatment of choice for functional hypothalamic amenorrhea? Nat Clin Pract Endocrinol Metab. 2007;3(8): 556-557.DOI: 10.1038/ncpendmet0561:

[19] Welt CK, Chan JL, Bullen J, Murphy R, Smith P, DePaoli AM, et al. Recombinant human leptin in women with hypothalamic amenorrhea. N Engl J Med. 2004;351(10):987-997.DOI: 10.1056/NEJMoa040388:

[20] 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-S24.DOI: 10.1507/endocrj.46.suppl_s17:

[21] Popovic V, Damjanovic S, Dieguez C, Casanueva FF. Leptin and the pituitary. Pituitary. 2001;4(1-2):7-14.DOI: 10.1023/a:1012938308654:

[22] Childs GV, Akhter N, Haney A, Syed M, Odle A, Cozart M, et al. The somatotrope as a metabolic sensor: deletion of leptin receptors causes obesity. Endocrinology. 2011;152(1): 69-81.DOI: 10.1210/en.2010-0498:

[23] Crane C, Akhter N, Johnson BW, Iruthayanathan M, Syed F, Kudo A, et al. Fasting and glucose effects on pituitary leptin expression: is leptin a local signal for nutrient status? J Histochem Cytochem. 2007;55(10): 1059-1074.DOI:10.1369/jhc.7A7214. 2007:

[24] 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-E899.DOI: 10.1152/ajpendo.00258.2006:

[25] Sarmento-Cabral A, Peinado JR, Halliday LC, Malagon MM, Castano JP, Kineman RD, et al. Adipokines (Leptin, Adiponectin, Resistin) Differentially Regulate All Hormonal Cell Types in Primary Anterior Pituitary Cell Cultures from Two Primate Species. Sci Rep. 2017;7:43537.DOI: 10.1038/srep43537:

[26] Vazquez-Borrego MC, Gahete MD, Martinez-Fuentes AJ, Fuentes-Fayos AC, Castano JP, Kineman RD, et al. Multiple signaling pathways convey central and peripheral signals to regulate pituitary function: Lessons from human and non-human primate models. Mol Cell Endocrinol. 2018;463:4-22.DOI: 10.1016/j.mce.2017.12.007:

[27] Caprio M, Fabbrini E, Isidori AM, Aversa A, Fabbri A. Leptin in reproduction. Trends Endocrinol Metab. 2001;12(2):65-72.DOI: 10.1016/s1043-2760(00)00352-0:

[28] 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): 659-665.DOI: 10.1530/eje.0. 1450659:

[29] Jin L, Burguera BG, Couce ME, Scheithauer BW, Lamsan J, Eberhardt NL, 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):2903-2911.DOI: 10.1210/jcem.84.8.5908:

[30] Jin L, Zhang S, Burguera BG, Couce ME, Osamura RY, Kulig E, et al. Leptin and leptin receptor expression in rat and mouse pituitary cells. Endocrinology. 2000;141(1):333-339. DOI: 10.1210/endo.141.1.7260:

[31] Ogura K, Irahara M, Kiyokawa M, Tezuka M, Matsuzaki T, Yasui T, et al. Effects of leptin on secretion of LH and FSH from primary cultured female rat pituitary cells. Eur J Endocrinol. 2001;144(6):653-658.DOI: 10.1530/ eje.0.1440653:

[32] 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):R476-R485.DOI: 10.1152/ ajpregu.2000.278.2.R476:

[33] Schneider JE, Buckley CA, Blum RM, Zhou D, Szymanski L, Day DE, et al. Metabolic signals, hormones and neuropeptides involved in control of energy balance and reproductive success in hamsters. Eur J Neurosci. 2002;16(3):377-379.DOI: 10.1046/j.1460-9568.2002.02118.x:

[34] Schneider JE, Wade GN. Availability of metabolic fuels controls estrous cyclicity of Syrian hamsters. Science. 1989;244(4910):1326-1328.DOI: 10.1126/science.2734610:

[35] Schneider JE, Zhou D. Interactive effects of central leptin and peripheral fuel oxidation on estrous cyclicity. Am J Physiol. 1999;277(4 Pt 2):R1020-R1024. DOI: 10.1152/ajpregu.1999.277.4.R1020:

[36] Schoeller DA, Cella LK, Sinha MK, Caro JF. Entrainment of the diurnal rhythm of plasma leptin to meal timing. J Clin Invest. 1997;100(7):1882-7.DOI: 10.1172/JCI119717 [doi]:

[37] Licinio J, Negrao AB, Mantzoros C, Kaklamani V, Wong ML, Bongiorno PB, et al. Synchronicity of frequently sampled, 24-h concentrations of circulating leptin, luteinizing hormone, and estradiol in healthy women. Proc Natl Acad Sci U S A. 1998;95(5):2541-2546.DOI: 10.1073/pnas.95.5.2541:

[38] Luukkaa V, Pesonen U, Huhtaniemi I, Lehtonen A, Tilvis R, Tuomilehto J, et al. Inverse correlation between serum testosterone and leptin in men. J Clin Endocrinol Metab. 1998;83(9):3243-3246.DOI: 10.1210/ jcem.83.9.5134:

[39] Riad-Gabriel MG, Jinagouda SD, Sharma A, Boyadjian R, Saad MF. Changes in plasma leptin during the menstrual cycle. Eur J Endocrinol. 1998;139(5):528-531.DOI: 10.1038/ srep43537:

[40] Wabitsch M, Blum WF, Muche R, Braun M, Hube F, Rascher W, et al. Contribution of androgens to the gender difference in leptin production in obese children and adolescents. J Clin Invest. 1997;100(4):808-813.DOI: 10.1172/ JCI119595:

[41] Wauters M, Considine RV, Van Gaal LF. Human leptin: from an adipocyte hormone to an endocrine mediator. Eur J Endocrinol. 2000;143(3):293-311.DOI: 10.1530/ eje.0.1430293:

[42] Wen S, Ai W, Alim Z, Boehm U. Embryonic gonadotropin-releasing

hormone signaling is necessary for maturation of the male reproductive axis. Proc Natl Acad Sci U S A. 2010;107(37):16372-16377.DOI: 10.1073/pnas.1000423107:

[43] Bjelobaba I, Janjic MM, Kucka M, Stojilkovic SS. Cell Type-Specific Sexual Dimorphism in Rat Pituitary Gene Expression During Maturation. Biol Reprod. 2015;93(1):21.DOI: 10.1095/biolreprod.115.129320:

[44] Dohler KD, Wuttke W. Changes with age in levels of serum gonadotropins, prolactin and gonadal steroids in prepubertal male and female rats. Endocrinology. 1975;97(4):898-907. DOI: 10.1210/endo-97-4-898:

[45] Wilson ME, Handa RJ. Ontogeny of gene expression in the gonadotroph of the developing female rat. Biol Reprod. 1997;56(2):563-568.DOI: 10.1095/biolreprod56.2.563:

[46] Wilson ME, Handa RJ. Activin subunit, follistatin, and activin receptor gene expression in the prepubertal female rat pituitary. Biol Reprod. 1998;59(2):278-283.DOI: 10.1095/biolreprod59.2.278:

[47] Leonhardt M, Lesage J, Croix D, Dutriez-Casteloot I, Beauvillain JC, Dupouy JP. Effects of perinatal maternal food restriction on pituitary-gonadal axis and plasma leptin level in rat pup at birth and weaning and on timing of puberty. Biol Reprod. 2003;68(2):390-400.DOI: 10.1095/biolreprod.102.003269:

[48] Attig L, Larcher T, Gertler A, Abdennebi-Najar L, Djiane J. Postnatal leptin is necessary for maturation of numerous organs in newborn rats. Organogenesis. 2011;7(2):88-94.DOI: 10.4161/org.7.2.14871:

[49] Allen SJ, Garcia-Galiano D, Borges BC, Burger LL, Boehm U, Elias CF. Leptin receptor null mice with reexpression of LepR in GnRHR expressing cells display elevated FSH levels but remain in a prepubertal state. American journal of physiology Regulatory, integrative and comparative physiology. 2016;310(11):R1258-R1266. DOI: 10.1152/ajpregu.00529.2015:

[50] Gregory SJ, Kaiser UB. Regulation of gonadotropins by inhibin and activin. Seminars in reproductive medicine. 2004;22(3):253-267.DOI: 10.1055/s-2004-831901:

[51] Pernasetti F, Vasilyev VV, Rosenberg SB, Bailey JS, Huang HJ, Miller WL, et al. Cell-specific transcriptional regulation of follicle-stimulating hormone-beta by activin and gonadotropin-releasing hormone in the LbetaT2 pituitary gonadotrope cell model. Endocrinology. 2001;142(6):2284-2295.DOI: 10.1210/endo.142.6.8185:

[52] Fox RG, Park FD, Koechlein CS, Kritzik M, Reya T. Musashi signaling in stem cells and cancer. Annu Rev Cell Dev Biol. 2015;31:249-267.DOI: 10.1146/annurev-cellbio-100814-125446:

[53] MacNicol MC, Cragle CE, MacNicol AM. Context-dependent regulation of Musashi-mediated mRNA translation and cell cycle regulation. Cell Cycle. 2011;10(1):39-44.DOI: 10.4161/cc.10.1.14388:

[54] Childs GV, Ellison DG, Ramaley JA. Storage of Anterior Lobe Adrenocorticotropin in Corticotropes and a Sub-population of Gonadotropes During the Stress-Nonresponsive Period in the Neonatal Male Rat. Endocrinology. 1982;110(5):1676-1692

[55] Moriarty GC, Garner LL. Immunocytochemical studies of cells in the rat adenohypophysis containing both ACTH and FSH. Nature. 1977;265(5592):356-358.DOI: 10.1038/265356a0:

[56] Childs GV. Multipotential Pituitary Cells that Contain Adrenocorticotropin (ACTH) and Other Pituitary Hormones. Trends in Endocrinology and Metabolism. 1991;2(3):112-117.DOI: 10.1016/s1043-2760(05)80007-4:

[57] Childs GV. Growth hormone cells as co-gonadotropes: partners in the regulation of the reproductive system. Trends Endocrinol Metab. 2000;11(5):168-175.DOI: 10.1016/s1043-2760(00)00252-6:

[58] 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):990-997.DOI: 10.1210/endo.134.2.8299592:

[59] Childs GV, Unabia G. Epidermal growth factor and gonadotropin-releasing hormone stimulate proliferation of enriched population of gonadotropes. Endocrinology. 2001;142(2):847-853.DOI: 10.1210/endo.142.2.7953:

[60] Odle A, Allensworth-James M, Akhter N, Syed M, Haney A, MacNicol M, et al. A Sex-Dependent Tropic Role for Leptin In The Somatotrope As A Regulator of POU1F1 and POU1F1-dependent Hormones. Endocrinology,. 2016;157(10):3958-3971.DOI: 10.1210/en.2016-1472:

[61] Ho Y, Hu P, Peel MT, Chen S, Camara PG, Epstein DJ, et al. Single-cell transcriptomic analysis of adult mouse pituitary reveals sexual dimorphism and physiologic demand-induced cellular plasticity. Protein Cell. 2020.DOI: 10.1007/s13238-020-00705-x:

[62] Childs GV, MacNicol AM, MacNicol MC. Molecular Mechanisms of Pituitary Cell Plasticity. Front Endocrinol (Lausanne). 2020;11:656. DOI: 10.3389/fendo.2020.00656:

[63] Buzi F, Bontempelli AM, Alberti D, Jones J, Pilotta A, Lombardi A, et al. Growth, insulin-like growth factor I (IGF-I), and IGF-binding proteins 1 and 3 in children with severe liver disease before and after liver transplantation: a longitudinal and cross-sectional study. Pediatr Res. 1998;43(4 Pt 1):478-483. DOI: 10.1203/00006450-199804000-00007:

[64] Sone M, Osamura RY. Leptin and the pituitary. Pituitary. 2001;4(1-2):15-23.DOI: 10.1023/a:1012978525492:

[65] 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):351-356.DOI: 10.1007/s004410100407:

[66] 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):6231-6235. DOI: 10.1073/pnas.93.13.6231:

[67] Carro E, Seoane LM, Senaris R, Casanueva FF, Dieguez C. Leptin increases in vivo GH responses to GHRH and GH-releasing peptide-6 in food-deprived rats. Eur J Endocrinol. 2000;142(1):66-70.DOI: 10.1530/eje.0.1420066:

[68] Asada N, Takahashi Y, Honjo M. Effects of 22K or 20K human growth hormone on lipolysis, leptin production in adipocytes in the presence and absence of human growth hormone binding protein. Horm Res. 2000;54(4):203-207.DOI: 10.1159/000053260:

[69] Allensworth-James M, Odle AK, Haney A, Childs GV. Sex Differences in Somatotrope Dependency on Leptin Receptors in Young Mice: Ablation of

LEPR Causes Severe Growth Hormone Deficiency and Abdominal Obesity in Males. Endocrinology. 2015;156(9):3253-3264.DOI: 10.1210/EN.2015-1198:

[70] Allensworth-James ML, Odle A, Haney A, MacNicol M, MacNicol A, Childs G. Sex-specific changes in postnatal GH and PRL secretion in somatotrope LEPR-null mice. J Endocrinol. 2018;238(3):221-230.DOI: 10.1530/JOE-18-0238.