to HFD, *Ghsr* mRNA increased by 64% (P=0.0154) when compared to control fed males. Among CR20 females, *Gh* expression was unchanged with HFD but *Ghrhr* expression was blunted by 54.7% (P=0.0363). However, *Gh* mRNA was reduced by 34% (P= 0.0265) when compared to control females on the HFD. Collectively, these data show that mild undernutrition causes a prematurely high leptin surge and sex-specific differences in growth and responses to a HFD, including a potential resistance to a HFD in underfed males.

References: (1) TK Miles et al., J Endocrinol. 2020 Sep 1; (2) F Delahaye et al., Endocrinology. 2008 Feb; 149(2):470. (3) S Yura et al., Cell metabolism. 2005 1(6):371.

Neuroendocrinology and Pituitary
NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Musashi as a Regulator of the Follicle-Stimulating Hormone in the Gonadotropes

Ana Rita Silva Moreira, MS[1], Alexandra Lagasse, BS[1], Anessa Haney, BS[1], Ulrich Boehm, PhD[2], Michael G. Kharas, PhD[3], Christopher J. Lengner, PhD[4], Melanie C. MacNicol, PhD[1], Angus M. MacNicol, PhD[1], Gwen V. Childs, PhD[1], Angela Katherine Odle, PhD[1].

[1]University of Arkansas for Medical Sciences, Little Rock, AR, USA, [2]University of Saarland School of Medicine, Homburg, Germany, [3]Memorial Sloan Kettering Cancer Center, New York, NY, USA, [4]University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA, USA.

The cyclic expression of gonadotropin releasing-hormone receptors (GnRHR), luteinizing hormone (LH), and follicle-stimulating hormone (FSH) by pituitary gonadotropes is critical in the female reproductive process. We have shown that the translational regulator Musashi (MSI) binds to *Gnrhr* mRNA and inhibits its translation, and the gonadotrope-specific deletion of *Msi1* and *Msi2* (Gon-*Msi*-null) leads to increased pituitary GnRHR protein levels. An *in silico* analysis of gonadotropin mRNAs revealed 5 different MSI binding elements in the 3'UTR of *Fshb* mRNA. We hypothesize that, in addition to *Gnrhr*, MSI may also bind and repress *Fshb* mRNA translation in the gonadotropes. To test if MSI does target the *Fshb* transcript in the pituitary, we performed RNA immunoprecipitation (IP) on pooled control female mouse pituitaries using a MSI1 antibody and measured *Fshb* mRNA by qRT-PCR. To study the *in vivo* effects of MSI on *Fshb*, we harvested the pituitaries of the Gon-*Msi*-null (MUT) female mice and their littermate controls (CTL) during the estrous cycle. We collected serum and protein for EIAs to measure the levels of FSH and LH, and RNA for *Fshb* qRT-PCR. We harvested proestrous ovaries and fixed them for embedding, sectioning, and H&E staining. Our RNA IP experiments show a 7-fold enrichment for *Fshb* with the MSI1 antibody. The Gon-*Msi*-null females have significantly higher pituitary FSH protein content than controls on estrous morning (MUT: 4.8±1.3 vs. CTL: 1.8±2.6 ng/ml/μg protein, p<0.0001, n=9-10/group). These mice also have increased serum FSH levels (MUT: 56.9±6.4 vs. CTL: 44.5±9.6 ng/ml, p=0.0147, n=9-10/group). No changes at the *Fshb* mRNA level were detected. Analysis of Gon-*Msi*-null ovaries revealed a

50% decrease in the number of follicles, with significant decreases in the average numbers of maturing follicles (p<0.0175) and corpora lutea (p<0.0215). Interestingly, the LH levels in these mice were also altered. The Gon-*Msi*-null females show a decrease in the pituitary LH protein content in the evening of proestrus (MUT: 11.8±1.4 vs. CTL: 15.1±2.0 ng/ml/μg protein, p=0.0333, n=7/group), in addition to a delayed and blunted LH surge (MUT: 2.6±1.9 vs. CTL: 7.3±3.5 ng/ml, p=0.0089, n=7-11/group). Taken together, our data indicate that *Fshb* is a Musashi target in the gonadotropes. By deleting MSI from the pituitary gonadotropes, we observe an increase in FSH protein content and serum levels. These Gon-*Msi*-null female mice have significantly fewer maturing follicles and corpora lutea, which might suggest lower levels of estrogens and progesterone. This, together with the increased GnRHR pituitary protein content, affects LH secretion, leading to a blunted LH surge in the Gon-*Msi*-null females. Our studies thus reveal a novel translational regulatory mechanism to govern levels of critical reproductive hormones in the pituitary.

Neuroendocrinology and Pituitary
NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Mutation of the *GnRHR* Proximal Promoter AP-1 Element in Mice Results in Suboptimal GnRH Induction of LH and an Abnormal Reproductive Phenotype

Krist N. Hausken, Ph.D.[1], Sekoni D. Noel, Ph.D.[1], Han Kyeol Kim, BS[1], Rona Stephanie Carroll, PhD[1], Ursula B. Kaiser, MD[2].
[1]Brigham and Women's Hospital, Boston, MA, USA, [2]Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA.

Reproduction is regulated by the gonadotropins, LH and FSH, which are synthesized and secreted by pituitary gonadotrophs in response to hypothalamic GnRH in a pulse frequency dependent manner. The gonadotroph decodes GnRH pulsatility via the GnRH receptor (GnRHR), which increases in expression and cell surface density before estrus and is responsible for downstream signaling cascades that differentially favor gonadotropin expression. The gonadotroph *Gnrh* promoter contains a tripartite enhancer, including an AP-1 element that is necessary for full GnRH induction of *Gnrhr* expression *in vitro*. We previously generated an AP-1 knock-in (KI) mouse model with a single point mutation (C-269T) in the *Gnrhr* promoter AP-1 binding motif that resulted in an abnormal reproductive phenotype in female mice. Compared to wildtype (WT) littermates, female KI mice had a significant delay in first estrus, disrupted estrous cyclicity, fewer corpora lutea, and smaller litters. Males had no apparent reproductive phenotype. Basal serum gonadotropin levels were similar between WT and KI mice, but gonadectomy induced a significantly lower rise in serum LH levels of KI mice relative to WT mice, concomitant with significantly lower pituitary *Gnrhr*, *Lhb*, and *Fshb* mRNA levels in both sexes. We have now extended the characterization of these mice by measuring LH pulsatility and assessing GnRH induction of LH *in vivo* and *in vitro*. The frequency and amplitude of LH

pulses over three hours were similar in ovariectomized WT and KI mice; however, KI mice had significantly reduced LH secretion, as measured by area under the curve. Similarly, GnRH treatment induced a diminished LH response in intact KI compared to WT males. *In vitro* cultures of hemi-pituitaries from gonadectomized WT and KI males were exposed to 0.01 nM GnRH and LH secretion into culture media was measured by ELISA at 0, 0.5, 1, 2, and 4 hours. There was no difference in basal LH secretion between WT and KI pituitaries but GnRH induction of LH was significantly lower in cultures from AP-1 mutant mice, indicating a direct impairment of GnRH action at the level of the pituitary. Taken together, these data indicate that the gonadotroph *Gnrhr* AP-1 promoter motif is critical for normal reproductive function. Prevention of AP-1 binding to the *Gnrhr* proximal promoter element decreases GnRH-induced *Gnrhr, Lhb, and Fshb* levels, impairs GnRH-stimulated LH secretion, and disrupts pubertal development and reproductive cyclicity in female mice.

Neuroendocrinology and Pituitary
NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Neuroendocrine Basis for Disrupted Ovarian Cyclicity in Females During Chronic Undernutrition: A Mouse Model

Kirollos S. Tadrousse, UC San Diego Undergraduate,
Richard B. McCosh, PhD, Michael J. Kreisman, MS,
Kellie M. Breen, PhD.
University of California, San Diego, La Jolla, CA, USA.

Chronic undernutrition is a type of metabolic stress that impairs reproduction across species and, in women, is implicated in the development of functional hypothalamic amenorrhea. Although the tight coupling of energy balance to reproductive capacity is recognized *in principle*, the neuroendocrine loci and molecular mechanisms that mediate ovarian cycle dysfunction during undernutrition remain poorly understood. Ovarian cyclicity is dependent on a population of kisspeptin (Kiss1) neurons in arcuate nucleus (ARC^{Kiss1}) for luteinizing hormone (LH) pulses and in the anteroventral periventricular nucleus (AVPV^{Kiss1}) for LH surge secretion. Here, we present a series of studies in which we tested the hypothesis that inhibition of both Kiss1 cell populations underlies the impairment of the cycle by undernutrition. During a baseline period, body weight, feed intake, and ovarian cycle stage (via vaginal cytology) were evaluated in female c57bl6 mice. Then, animals were randomly assigned into one of two groups (n=6-8/grp): 1) ad libitum fed controls or 2) feed restricted (70% of feed consumed during the baseline period). Control animals displayed clear and regular cycles throughout the 4-week treatment period. In contrast, feed restriction caused a significant and rapid cessation of ovarian cyclicity (4.8±0.3 vs. 1.5±0.5 estrus cycles/4 weeks; control vs. restricted, p<0.05), causing all females to enter and remain mostly in diestrus. Based on these results, we conducted two experiments to directly test the hypothesis that undernutrition inhibits both modes of LH secretion (and both Kiss1 cell populations) using two well-defined estradiol (E) replacement paradigms. We first evaluated LH pulses in mice that were ovariectomized and implanted subcutaneously with a pellet containing a diestrus level of E (100 ng, OVX+LowE). Following 3 days of feed restriction or control diet (n=3/grp), serial blood samples were collected every 8 min for 88 min. Undernutrition prevented LH pulses and significantly reduced mean LH (5.2±0.6 vs. 0.6±0.2 ng/mL; control vs. restricted, p<0.05). Fixed neural tissue was evaluated by immunohistochemistry to determine whether undernutrition impairs ARC^{Kiss1} neuronal activation, using c-Fos as a marker. The percent of ARC^{Kiss1} neurons expressing cFos was reduced by 90% (p<0.05). We next evaluated the LH surge. After 3 days, control or feed restricted mice were OVX and implanted subcutaneously with a surge-inducing estradiol implant (OVX+HighE, 1 µg, n=3-4/grp). Undernutrition completely blocked the E-induced LH surge (1.9±0.3 vs. 0.2±0.02 ng/mL; control vs. restricted, p<0.05) and diminished *Kiss1* mRNA abundance in micropunches of the AVPV (42%, p<0.05). Collectively, these studies clearly show that undernutrition impairs both ARC^{Kiss1} control of LH pulses and AVPV^{Kiss1} induction of the LH surge, via mechanisms that remain to be identified.

Neuroendocrinology and Pituitary
NEUROENDOCRINOLOGY AND PITUITARY BASIC RESEARCH ADVANCES

Neuromedin B Receptor Antagonist Suppresses Pomc Expression in AtT-20 Cells and Human Corticotroph Adenoma Cells

Tomonori Sekizaki, MD, Hiraku Kameda, MD, PhD,
Akinobu Nakamura, PhD, Hiroshi Nomoto, PhD, Kyu Yong Cho,
PhD, Yukitomo Ishi, MD, Hiroaki Motegi, PhD, Hideaki Miyoshi,
PhD, Tatsuya Atsumi, PhD.
HOKKAIDO University GRAD SCHL OF Medical, Sapporo, Japan.

Objective: We previously reported that Neuromedin B (NMB) is expressed in murine pituitary corticotrophs under adrenal insufficiency (1). Because NMB is also expressed in several cancer cells and stimulates ACTH secretion, we hypothesized that NMB is related to corticotroph adenoma cell proliferation and hormone secretion. To examine this hypothesis, we investigated the expression of NMB and its receptor NMBR in human corticotroph adenoma and the effects of a NMBR antagonist on AtT-20 cells, a mouse corticotroph adenoma cell line, and patient-derived corticotroph adenoma cells. **Methods:** 1. NMB and NMBR expression in human pituitary adenoma: We performed real-time qPCR and immunostaining on human pathological specimens of corticotrophs, somatotrophs, and non-functioning pituitary adenoma to investigate NMB and NMBR expression. 2. Experiments in AtT-20 cells: We extracted mRNAs and proteins from AtT-20 cells after incubation with 100nM NMBR antagonist PD168368, and performed real-time qPCR and western blotting analyses to investigate Pomc expression. 3. Experiments in patient-derived corticotroph adenoma cells: We isolated surgically resected human corticotroph adenoma cells from patients who underwent trans-sphenoidal surgery and investigated POMC mRNA expression by real-time qPCR after incubation with PD168368. Statistical analysis: One-way