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

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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.

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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

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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 *Gnrhr* 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, a*nd *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