in the relative *Esr1* and *Esr2* expression in the ARC and AVPV. Our findings suggest that hypothalamic differences in the relative expression of ERs play a key role in the bimodal regulation of LH release by E2.

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Dissecting the Involvement of Arcuate Nucleus Kisspeptin Neurons in Puberty Onset and LH Secretion

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Puberty is a crucial period of transition to adulthood, marked by an increased activation of gonadotropin-releasing hormone (GnRH) neurons that drives increased pulsatile secretion of pituitary luteinizing hormone (LH). The mechanisms governing GnRH neuron activation at puberty remain unclear but are likely due to enhanced signaling from upstream neuron populations, including kisspeptin neurons. Kisspeptin (encoded by *Kiss1)* directly stimulates GnRH neurons to drive GnRH release and downstream LH secretion. Humans and animals with *Kiss1* mutations fail to reach puberty, demonstrating kisspeptin's importance in puberty onset. Nonetheless, the specific brain area(s) from where kisspeptin signaling arises to trigger puberty remain undetermined. Kisspeptin is primarily expressed in two hypothalamic areas, the arcuate nucleus (ARC) and anteroventral periventricular (AVPV) region. ARC *Kiss1* neurons are known to drive pulsatile GnRH/LH secretion in both sexes whereas AVPV *Kiss1* neurons are sexually dimorphic and mediate the preovulatory GnRH/LH surge in females. We previously showed that *Kiss1* gene expression increases in both the ARC and AVPV across the peri-pubertal period, yet it still remains to be determined whether just one or both of these populations is essential for proper pubertal timing. Indeed, the relative involvement of either ARC or AVPV *Kiss1* neurons in the pubertal process still remains unknown. Here, we hypothesized that ARC *Kiss1* neurons are required for normal puberty timing in both sexes and, conversely, AVPV *Kiss1* neurons are not sufficient on their own to trigger normal puberty. To test this hypothesis, we used transgenic mice expressing diphtheria toxin receptor (DTR) exclusively in *Kiss1* cells (Kiss Cre/iDTR flox) and took advantage of the differential ontogeny of ARC and AVPV *Kiss1* neurons to selectively ablate ARC kisspeptin neurons before puberty, while leaving AVPV neurons intact. We found that targeted deletion of just ARC *Kiss1* neurons during the juvenile period (which does not alter AVPV *Kiss1* cell number) significantly delays puberty onset in both sexes, as measured by vaginal opening, first estrous, and preputial separation. In addition, these mice also exhibit decreased basal and pulsatile LH secretion in adulthood, further supporting a role for ARC kisspeptin neurons in GnRH pulse generation. By contrast, females with ablated ARC *Kiss1* cells still exhibit full estradiol-induced LH surges, ruling out a necessary role of ARC kisspeptin neurons in that process and further supporting AVPV kisspeptin as the primary regulator of the surge. Collectively, our findings demonstrate that ARC *Kiss1* neurons are required for both properly timed activation of the reproductive axis during puberty and proper pulsatile LH secretion in adulthood, while AVPV *Kiss1* neurons are not sufficient to drive normal puberty onset but are sufficient for the preovulatory LH surge.

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Effects of Diet-Induced Obesity on Hypothalamic Kisspeptin-Neurokinin-Dynorphin (KNDy) Neurons and Luteinizing Hormone Secretion in Sex Hormone-Primed Male and Female Rats

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Metabolic stress resulting from a nutrient excess causes infertility in both sexes. Kisspeptin-neurokinin B-dynorphin (KNDy) neurons in the arcuate nucleus (ARC) have been suggested to be key players in reproduction via direct stimulation of gonadotropin-releasing hormone (GnRH) and subsequent gonadotropin release in mammalian species. In this study, we investigated the sex differences in the effects of a high-fat diet (HFD) on KNDy-associated gene expression in the ARC to determine the pathogenic mechanism underlying obesity-induced infertility. Wistar-Imamichi strain male and female rats (7 weeks of age) were fed either a standard diet (10% calories from fat) or high-fat diet (45% calories from fat) for 4 months. In male rats, the HFD caused a significant suppression of *Kiss1*(encoding kisspeptin), *Tac3*(encoding neurokinin B), and *Pdyn*(encoding dynorphin A) gene expression in the ARC, resulting in a decrease in plasma luteinizing hormone (LH) levels. In female rats, 58% of the HFD-fed female rats exhibited irregular estrous cycles, while the other rats showed regular cycles. LH pulses were found, and the numbers of ARC *Kiss1*-,*Tac3*-, and *Pdyn*-expressing cells were high in control animals and almost allHFD-fed female rats, but two out of 10 rats showed profound HFD-induced suppression of LH pulse frequency and reduction in these cells. No statistical differences in LH secretion or ARC KNDy gene expression were observed between HFD-fed and control female rats. Additionally, the number of *Gnrh1*-expressing cells in the preoptic area was comparable between the groups in both sexes. Our findings revealed that HFD-fed male rats showed KNDy-dependent infertility, while irregular menstruation was mainly induced by KNDy-independent pathways during the incipient stage of obese infertility in female rats. Taken together, hypothalamic kisspeptin neurons in male rats may be susceptible to HFD-induced obesity compared with those in female rats.

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Epigenetic Regulation of Gonadotropin Hormone Beta Subunit Gene Expression