Salt and Puberty: Self-Regulated Salt Intake and the Effect of Varying Levels of Salt on Puberty

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Objectives:

Puberty is the culmination of a complex series of events and is essential for reproductive success. High salt (8%) significantly delays the timing of puberty, even when paired with a high fat diet. Fibroblast growth factor 21 (FGF-21) is increased during starvation[1] and acts on the suprachiasmatic nucleus to suppress the vasopressin-kisspeptin signaling cascade[2]. Puberty is delayed in animals with genetically elevated FGF-21[2]. Additionally, stress is a known suppressor of the reproductive axis[3] and salt is purported to activate the stress axis[4]. We hypothesized that elevated salt would concomitantly elevate FGF-21 and corticosterone levels. Our original experiment used 8% salt in the diet. Here, we varied salt levels to further elucidate how salt affects puberty. To determine how much salt a rat would self-ingest and thereby establish the appropriate salt composition of diets, we gave rats the option of saline or water in addition to their salt-containing diets.

Methods:

EXP 1: From weaning (P21) to P45, female Sprague Dawley rats were fed Control (10% kcal fat/70% carb/20% protein/0.3% salt), High Salt (HS; 10% kcal fat/70% carb/20% protein/8% salt), High Fat (HF; 60% kcal fat/20% carb/20% protein/0.3% salt), or High Salt & High Fat (HS/HF; 60% kcal fat/20% carb/20% protein/8% salt) diets. **Tissue Processing:** Sections were incubated in either sheep anti-Kiss (Alain Caraty) or rabbit anti-NKB antiserum (Pierce Antibodies; Rockford, IL) and then incubated in donkey anti-sheep Alexa 594 and FAB conjugated with Alexa 594 respectively (Jackson ImmunoResearch; West Grove, PA). Sections were then incubated in rabbit anti-V1AR (Santa Cruz, Dallas, Texas), followed by goat anti-rabbit conjugated to Alexa Fluor 488 (Jackson ImmunoResearch; West Grove, PA). Sections were coverslipped using Vectashield with DAPI (Vector Laboratories; Burlingame, CA). **Hormone assays:** ELISAs were used to estimate plasma FGF-21 (BioVendor; Asheville, NC) and Corticosterone (Enzo, Farmingdale, NY).
EXP 2: Rats were fed no salt (NS; 0.01% salt), Control (0.3% salt), 2% salt, and 4% salt, and monitored for vaginal opening (VO).
EXP 3: Rats had the option of drinking saline (0.5% salt) and water. Rats were fed NS, Control, and 2% salt, and monitored for VO. Water and food intake were measured daily.

Results:

EXP1:

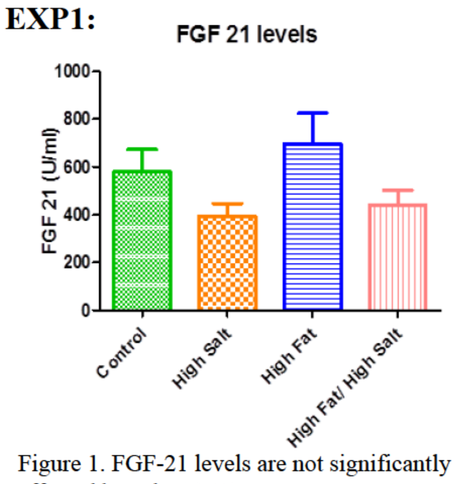

Figure 1. FGF-21 levels are not significantly affected by salt. ANOVA

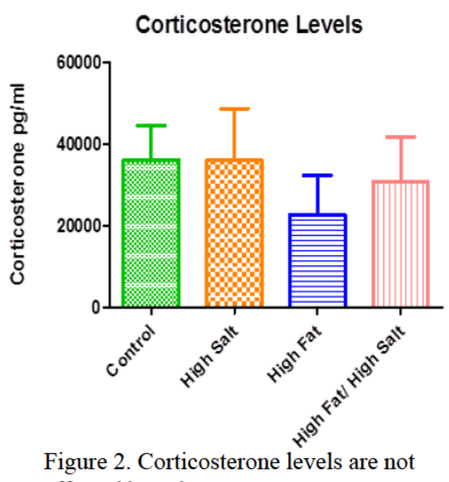

Figure 2. Corticosterone levels are not affected by salt. ANOVA

EXP2:

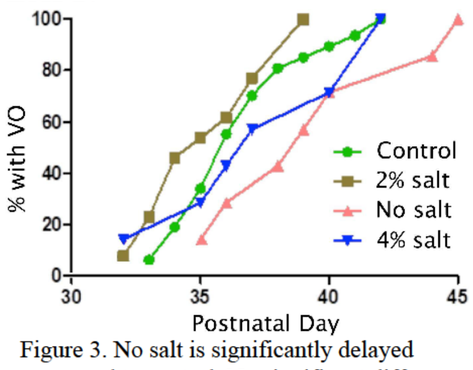

Figure 3. No salt is significantly delayed compared to control. No significant difference between other groups.

EXP3: Total Salt Consumed

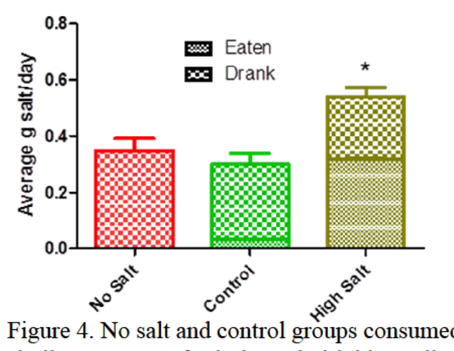

Figure 4. No salt and control groups consumed similar amounts of salt through drinking saline. The high salt group consumed significantly more sodium via drinking saline and ingesting sodium through the diet.

Preliminary Data

Kisspeptin and Neurokinin B Cells in the Arcuate Nucleus Contain Vasopressin 1A Receptors

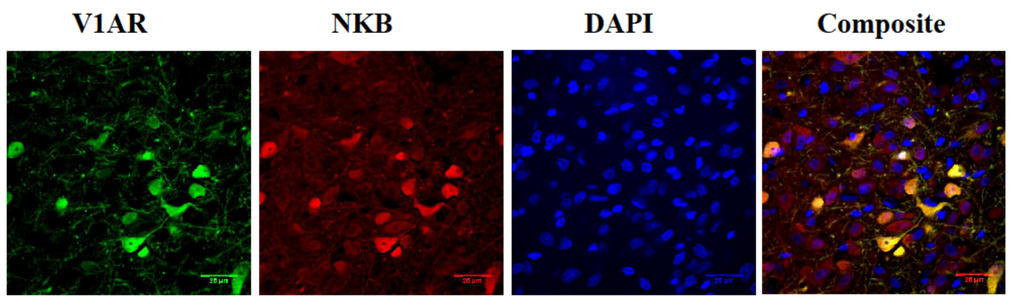

Figure 5. V1AR (Green), NKB (Red) and colocalization of V1AR and NKB (Yellow) in arcuate nucleus neurons.

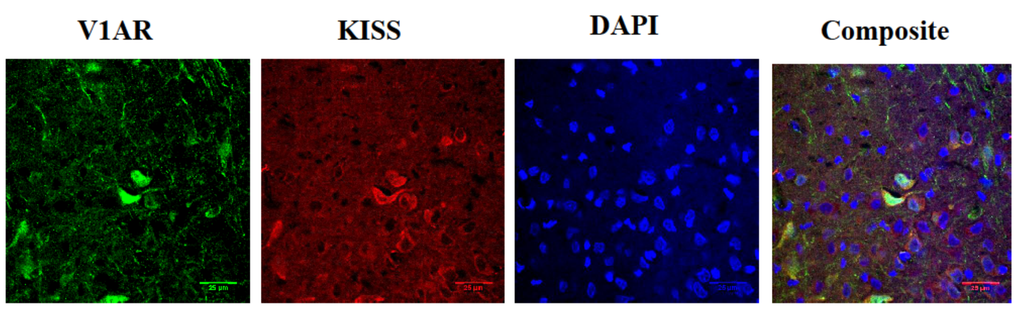

Figure 6. V1AR (Green), KISS (Red) and colocalization of V1AR and KISS (Yellow) in arcuate nucleus neurons.

Conclusions:

- High salt significantly delays puberty
- The high salt-mediated delay in puberty is not caused by a change in FGF-21
- Corticosterone levels are not altered by high salt in juvenile rats, indicating that the stress axis is not activated in response to high salt.
- The vasopressin receptor, V1AR, is expressed by kisspeptin and neurokinin B neurons in the arcuate nucleus.
- 2% and 4% high salt diets are within the physiological ranges of salt consumption in rats

Future Directions

- Infusing a vasopressin antagonist to determine if the high salt-induced pubertal delay is transduced through vasopressin

References:

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3. Li, X. F., Knox, A. M. I. & O'Byrne, K. T. *Brain Res.* **1364,** 153–163 (2010).
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