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

Dori R. Pitinsky, Francis W. Flynn, and Donal C. Skinner
Department of Zoology and Physiology, University of Wyoming

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 and acts on the suprachiasmatic nucleus to suppress the vasopressin-kisspeptin signaling cascade. Puberty is delayed in animals with genetically elevated FGF-21. Additionally, stress is a known suppressor of the reproductive axis and salt is purported to act as the stress axis. 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/HT; 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 antisera (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-VIAR (Santa Cruz, Dallas, Texas), followed by goat anti-rabbit conjugated to Alexa Fluor 488 (Jackson ImmunoResearch; West Grove, PA). Sections were coveredipped 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:

EXP 1:
- FGF-21 levels
- Corticosterone levels

EXP 2:
- Salt intake

EXP 3:
- Total Salt Consumed

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

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, VIAR, 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: