NPY expression of low-birth-weight offspring in nutrient restricted utero

Janine E. Levandowski, Holly Young, Erin M. Rhinehart
Department of Neuroscience, Susquehanna University, Selinsgrove, PA

ABSTRACT

In a clinical setting, sexually dimorphic factors of obesity have become well characterized; however, understanding the underlying mechanisms of the physiology that leads to obesity between both male and female lacks comprehension. Along with the many effects of obesity, expression of neuropeptide Y (NPY) and other peripheral factors like leptin and ghrelin are major contributors to the onset of obesity. Previous research investigates how maternal undernutrition in utero produces low-birth weight offspring with obese-like tendencies. Nutrition restricted gestational programming leads to over activation of NPY systems to increase food drive and decrease energy balance. Because NPY is prevalent in consumption mechanisms, we sought to determine if there are sexually dimorphic and quantitative differences in the expression of NPY in low-birth weight offspring in both the hypothalamus and hindbrain. To test this hypothesis, we used female Sprague-Dawley rats focused on an animal model for fetal programming (Desai et al., 2007). On day 10 of gestation, the rats in the control group were provided an ad libitum diet while the rats in the maternal undernutrition (MUN) group had a 50% food-restricted diet that was quantitated off the normal intake of the controls. NPY was quantified using qRT-PCR. The results show that the control group in all cases had a higher mean fold change for NPY than those in the restricted group. Females in the control group had higher expression of NPY in the hindbrain while males had higher NPY expression in the hypothalamus. Our hypothesis was not supported by the data found.

INTRODUCTION

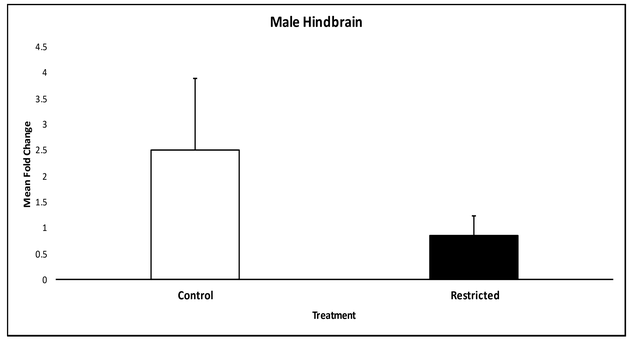
- **Obesity and Energy Balance**
  - The influence of gonadal steroids on appetite and body composition has a substantial influence on energy balance and fat distribution.
  - Leptin and ghrelin are metabolic regulators that act on the HPG axis to moderate reproduction and energy expenditures.
  - Energy deficits signal the gut to release ghrelin therefore; stimulating the rise of NPY.
- **Therefore, a disruption in energy balance should lead to metabolic dysfunction that results in a change in body composition.**

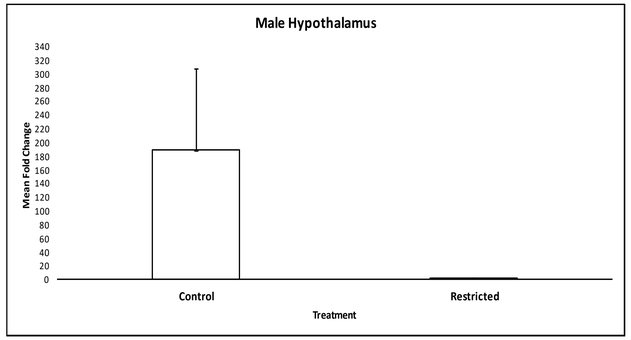
- **Obesity and NPY**
  - The influence of intrauterine malnutrition during critical periods affects the body's regulatory mechanisms.
  - The hypothalamus and hindbrain are critical in regulation of consumption and body mass using NPY.
  - NPY plays a significant role in metabolic activity and is linked to food intake and energy expenditure.
- **Therefore, nutrition restriction in utero should increase NPY expression in the hypothalamus and hindbrain.**

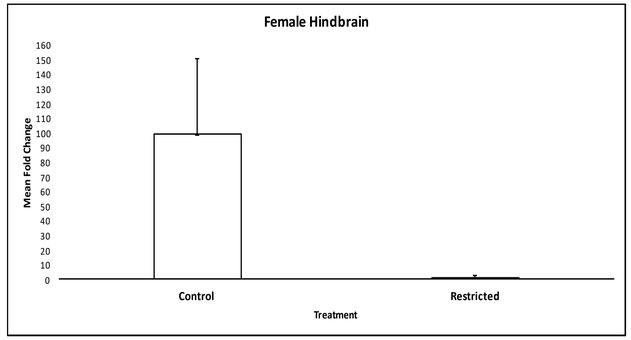
METHODS

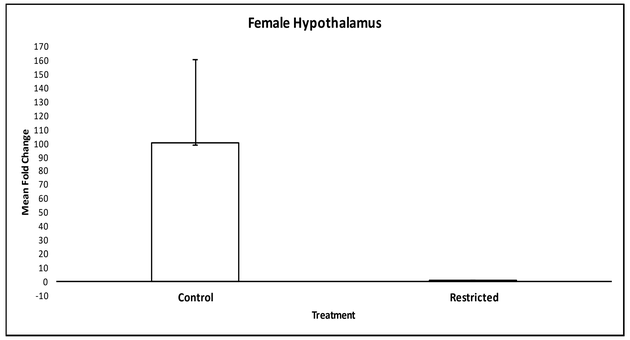
- **Subjects:** Food restricted, and controlled female and male Sprague-Dawley rat offspring were postnatally sacrificed at 30 or 60 days.
- **Tissue Harvest:** Brains were removed, frozen on dry ice, and stored at -80˚C for gene expression using qRT-PCR.
- **qRT-PCR for the hindbrain and hypothalamus:** mRNA was extracted, using an RNAeasy mini lipid kit (Qiagen) according and reverse transcribed using an iScript reverse transcription cDNA synthesis kit (Biorad) according to manufacturer's instructions. cDNA corresponding to 1 µg of mRNA was used as a template for the QPCR reactions which used the TaqMan FastStart Essential DNA Probes Master Mix (Sigma) according to manufacturer's instructions using PrimeTime Std qPCR assays (IDT technologies) .

GENE EXPRESSION RESULTS



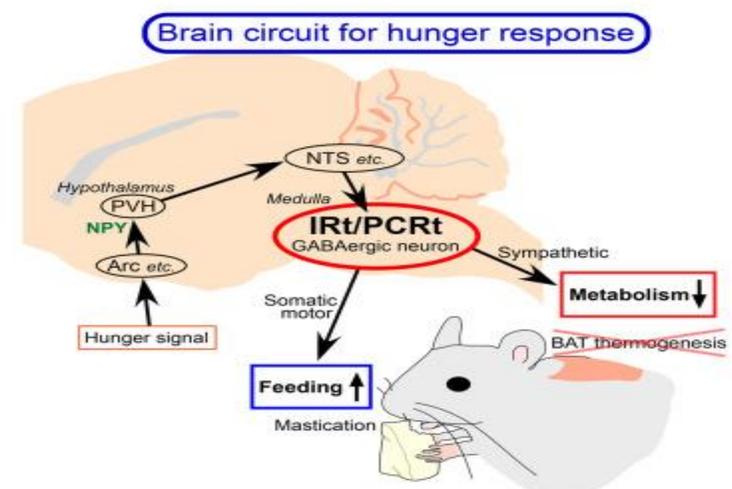






The nutritionally restricted group compared to the control group did not have an increase in NPY in both the hindbrain and hypothalamus of the male rats. Males in the control group had a higher expression of NPY in the hypothalamus compared to females.

The nutritionally restricted group compared to the control group did not have an increase in NPY in both the hindbrain and hypothalamus of the female rats. Females in the control group had a higher expression of NPY in the hindbrain compared to males.



The hypothalamus and hindbrain regions are responsible for the regulation of the hunger response using NPY. NPY is generated in the hypothalamus and is sent to the medulla to activate GABAergic neurons. These neurons increase mastication, while decreasing metabolism.

CONCLUSIONS

- NPY expression was not affected by the nutritional restriction in utero as the control groups had higher levels compared to the restricted.
- Unlike females, males had the highest expression of NPY in the hypothalamus even though previous research suggested that females would have a higher prevalence.
- Sex differences in nutritional restricted –induced effects on metabolic regulation are responsible for sexually dimorphic effects of NPY on obesity.

ACKNOWLEDGEMENTS

1.) Desai, M., Gayle, D., Babu, J., & Ross, M. G. (2007). The timing of nutrient restriction during rat pregnancy/lactation alters metabolic syndrome phenotype. American journal of obstetrics and gynecology, 196(6), 555.e1–555.e5557. https://doi.org/10.1016/j.ajog.2006.11.036
2.) Khorram, O., Keen-Rinehart, E., Chuang, T. D., Ross, M. G., & Desai, M. (2015). Maternal undernutrition induces premature reproductive senescence in adult female rat offspring. Fertility and sterility, 103(1), 291–8.e2.
3.) Rhinehart E. M. (2016). Mechanisms linking energy balance and reproduction: impact of prenatal environment. Hormone molecular biology and clinical investigation, 25(1), 29–43. https://doi.org/10.1515/hmbci-2016-0004