Exploratory Analysis of Estrogen-Mediated Gene Expression in Central Ghrelin Signaling Pathways

CENTER FOR HEALTH SCIENCES
OKLAHOMA STATE UNIVERSITY

Katie Burch, B.S; Dusti Sloan, PhD

School of Biomedical Sciences

INTRODUCTION

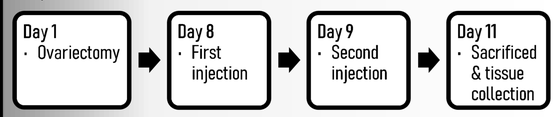
As of 2020, 67% of Americans are overweight or obese. Particularly concerning is the greater incidence of obesity in post-menopausal women. Previous studies have shown that estrogen decreases food intake and body weight; however, it is not yet clear how estrogen affects central pathways that regulate feeding.

OBJECTIVE

To address this, we used a microarray to compare estrogen-mediated changes in gene expression within metabolic signaling pathways. For this poster, I focus on the ghrelin pathway. Ghrelin is the hormone responsible for creating the sense of hunger. This pathway includes the brain areas of the arcuate nucleus of the hypothalamus (ARC), the paraventricular nucleus of the hypothalamus (PVN), and the lateral hypothalamus area (LHA).

METHODS

Animals: Adult, female Sprague-Dawley rats (n=6) were bilaterally ovariectomized and allowed 7 days for recovery. They were then given either oil vehicle (oil; 0.1mL, n=3) or estrogen (E; 10ug, 0.1mL, n=3) injections on days 8 and 9 and sacrificed on day 11. Body weights were recorded pre-operatively and on days 3-5 and 8-11.



Tissue Preparation: Brains and other tissues were collected at sacrifice. Brain punches (1 x 3mm) were taken from ARC, PVN, and the nucleus of the solitary tract (NTS).

RNA Isolation: RNA was isolated from each of the selected brain regions, using a Biorad Aurum Total RNA Mini kit per manufacturer's instructions. The isolated RNA was then sent for microarray analysis at Thermo Fisher Scientific–Microarray Research Service Lab using the Rat Clariom S Assay. Results were analyzed using the Transcriptome Analysis Console (TAC) software 4.0.

RESULTS

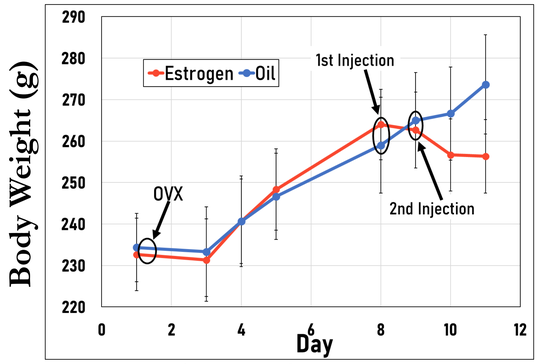
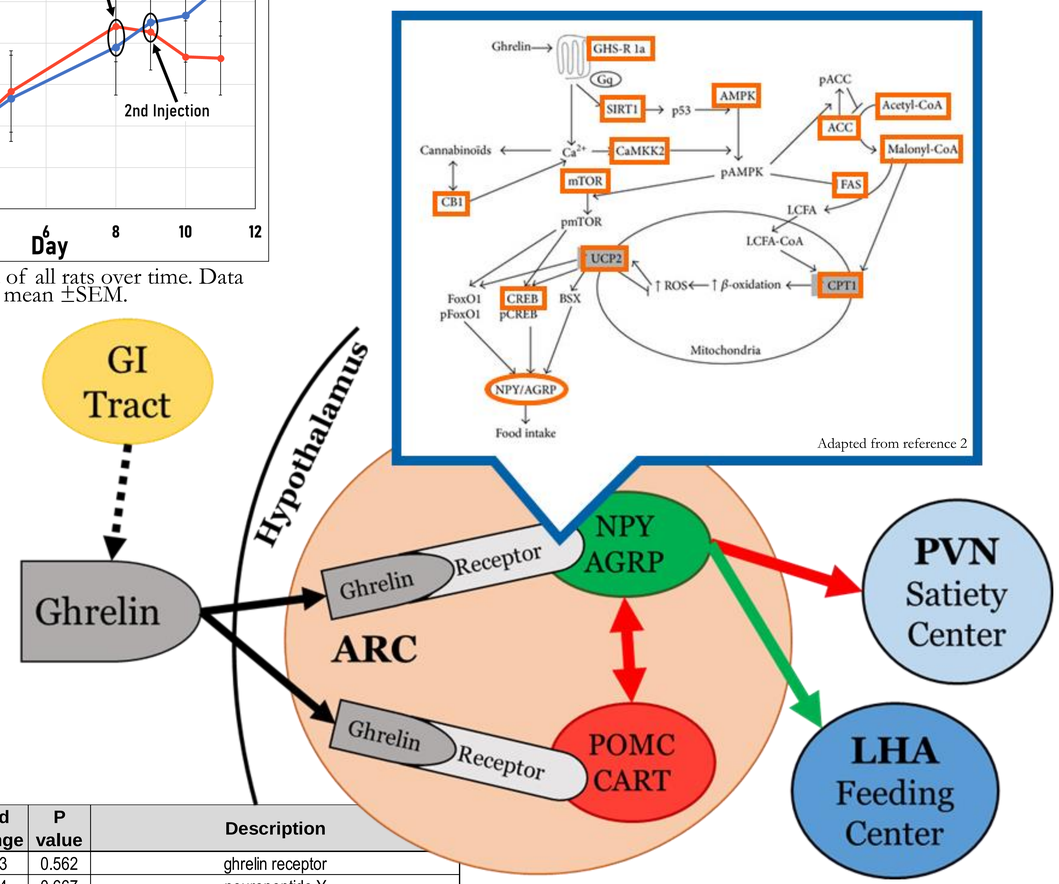


Figure 1: Body weight of all rats over time. Data shown as mean ±SEM.



Acronym	E Avg.	Oil Avg.	Fold Change	P value	Description
GHS-R 1a	9.32	9.63	-1.23	0.562	ghrelin receptor
NPY	13.44	13.63	-1.14	0.667	neuropeptide Y
AGRP	6.86	6.84	1.01	0.299	agouti related neuropeptide
POMC	9.84	11.50	-3.17	0.337	proopiomelanocortin
CART	15.12	15.20	-1.05	0.870	CART prepropeptide
MC3R	8.51	8.73	-1.16	0.389	melanocortin 3 receptor
NPY1R	9.70	9.99	-1.22	0.796	ARC neuropeptide Y receptor Y1
Intracellular Signaling					
SIRT1	12.78	12.76	1.01	0.916	SIRT 1
CB1	11.09	11.62	-1.44	0.248	cannabinoid receptor 1
CaMMK2	9.02	9.24	-1.17	0.356	calcium/calmodulin-dependent protein kinase kinase 2
AMPK	12.50	12.51	-1.01	0.818	AMP-activated protein kinase
ACC	11.24	11.06	1.13	0.375	acetyl-Coenzyme A carboxylase
Malonyl CoA	8.81	8.89	-1.06	0.766	malonyl Coenzyme A
FAS	13.73	13.83	-1.07	0.574	fatty acid synthase
mTOR	11.00	10.89	1.08	0.524	mechanistic target of rapamycin
CPT1	11.47	11.26	1.16	0.155	carnitine palmitoyltransferase
CREB	11.58	11.80	-1.16	0.593	cAMP responsive element binding protein 1
UCP2	13.68	13.58	1.07	0.450	uncoupling protein 2

CONCLUSION

The changes in body weights confirmed the effects of estrogen. For the ghrelin pathway within the ARC, gene expression was not largely affected by estrogen. However, the POMC had a notable difference, higher in the oil treated rats. This result was surprising, as POMC is known as anorexigenic and goes on to activate the satiety center.
These results could be due to it being only day 4 of treatment, or because the ghrelin pathway is not the source of metabolic dysregulation for rats lacking estrogen.

FUTURE DIRECTIONS

Since there are estrogen receptors located in these same areas of the brain, this demonstrates a potential mechanism by which estrogen affects central pathways to decrease food intake and body weight. Future studies will examine estrogen-mediated intracellular signaling within the PVN. Additionally, estrogen-mediated signaling pathways within other brain areas such as the lateral hypothalamus area(LHA) and dorsal vagal complex (DVC) may be investigated.

REFERENCES

1. Center for Disease Control and Prevention. (2022). Adult Obesity Facts. https://www.cdc.gov/obesity/data/adult.html

2. Delporte C. (2013). Structure and physiological actions of ghrelin. Scientifica, 2013, 518909. https://doi.org/10.1155/2013/518909

ACKNOWLEDGEMENTS

Funding provided by OCAST HR18-089 (KSC)