The Effects of Removing esr1 from Dopamine Specific Brain Regions

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Introduction

- Previous work in our laboratory has shown that loss of ovarian hormones via ovariectomy (OVX) significantly reduces voluntary wheel running). Our lab showed that the nucleus accumbens brain region (i.e., the major "reward" pathway region) is significantly affected by OVX, and that changes in genes associated with dopamine signaling in this brain region strongly associate with the reduction in voluntary wheel running, implicating gene expression changes in this brain region as being mechanistically responsible for the physical inactivity associated with hormone loss

- This was particularly interesting because adipose tissue metabolism is also adversely affected in the aromatase KO mouse, and estrogen loss, either by OVX or system-wide estrogen receptor (ER) deletion, also both adversely affect adipose tissue lipid metabolism via yet unknown mechanisms. It's hypothesized that estrogen loss impairs adipose tissue metabolism partially by reduced ER signaling in the nucleus accumbens brain region

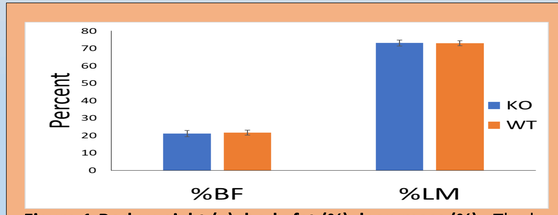
- To test this hypothesis, a novel mutant was created that lacks the estrogen receptor alpha (ERa) selectively in dopamine receptor-positive brain regions (i.e., the D1mouseERKO mouse model). Here, we will compare adipose tissue metabolic phenotypes between the D1ERKO and WT mice.

Purpose of Study and Hypothesis

- Determine how deletion of Esr1 (i.e., the gene that encodes ER alpha) from dopamine receptor 1 (i.e., DRD1)-specific brain regions affects white and brown adipose tissue metabolism in association with their behavioral/systemic metabolic phenotype. The overarching hypothesis is that loss of estrogen signaling from dopamine-positive brain regions will impair adipose tissue metabolism, which will associate with physical inactivity.

- Aim 1. Compare WT and D1ERKO mice for: spontaneous physical activity (SPA) and energy expenditure (EE) (i.e., assessed via metabolic chambers); glucose tolerance (glucose AUC, fasting glucose and insulin); and body composition (% body fat and lean mass, fat distribution via fat pad weights).

- Aim 2. Compare BAT and WAT samples from WT and D1ERKO mice for: cell size and subjective characterization of browning phenotype; UCP1 gene and protein expression; gene and/or protein expression of mitochondrial markers (OX PHOS subunits, PGC1a); and gene and protein expression of insulin signaling and lipolytic proteins (e.g., AKT, GLUT4, ATGL, B3AR).



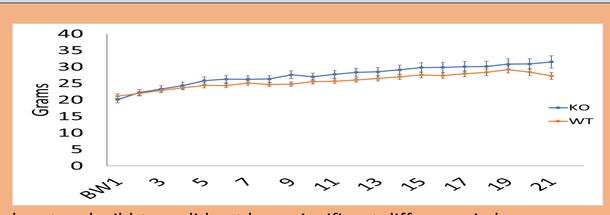
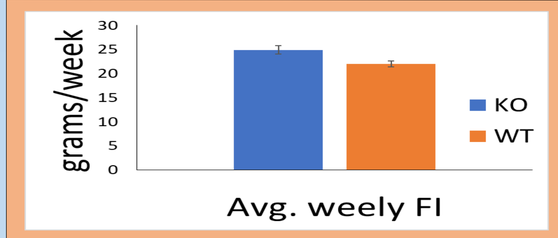


Figure 1:Body weight (g), body fat (%), lean mass (%) : The knock-out and wild-type did not have significant difference in lean mass percent or Body Fat percent. There was a slight difference in body weight in grams, the knock-out mouse tended to have a higher body weight.



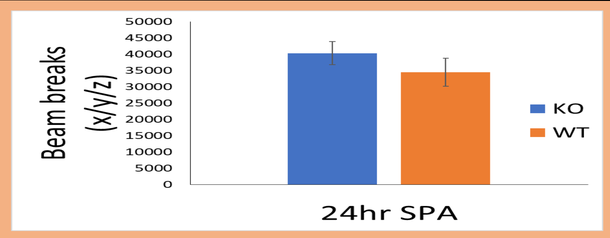
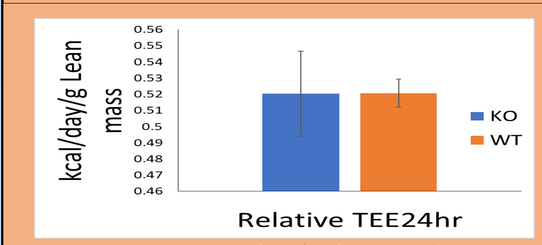


Figure 2 Food intake (g total over a couple of reliable weeks represented as kcal/day), SPA (x, y, z) in light and dark cycle: FI: When comparing weekly food intake averages, they resulted in variable significance across the 20 weeks. Each week had a different conclusion. The knock-out mouse ate more in weeks 5, 11, 15, and 18.
SPA: The knock-out mouse tended to have higher spontaneous physical activity than the wild-type mouse.



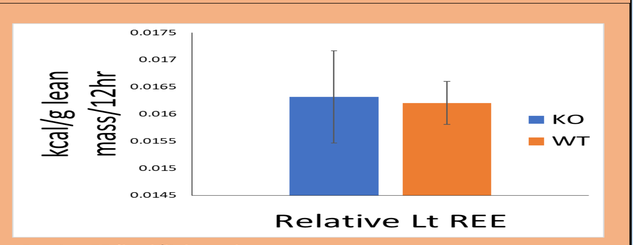
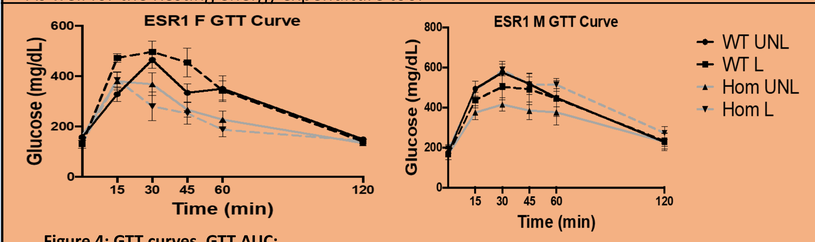


Figure 3: Relative Total EE (kcal/day) co-varied for BW, Relative Resting EE (kcal/light cycle)
Total energy expenditure has shown that there is trending significance suggesting that the knock-out mouse has higher energy expenditure than the wild type mouse.
As well for the Resting energy expenditure too.



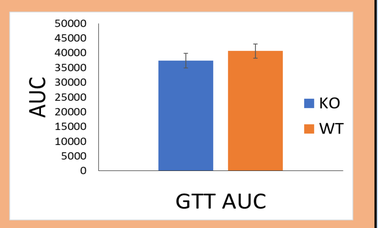
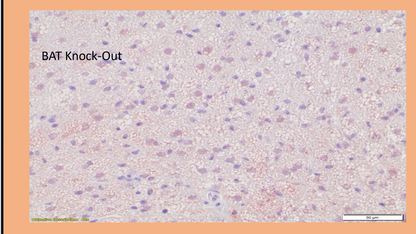
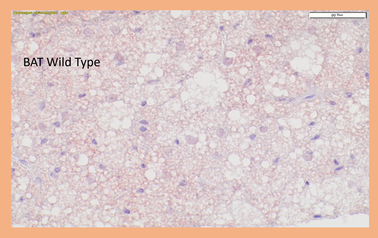


Figure 4: GTT curves, GTT AUC:
The average area under the curve shows that the wild type mouse had higher glucose tolerance compared to the knock-out mouse.





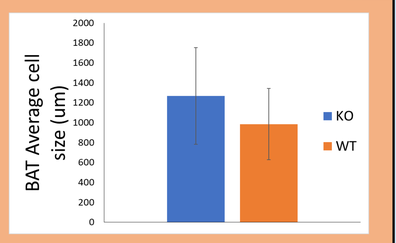
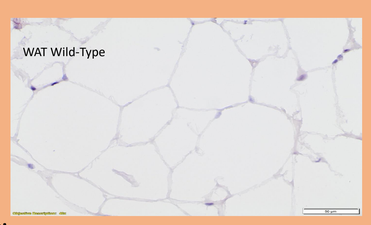


Figure 5. BAT rep images, BAT mean cell size:
The brown adipose tissue knock out mouse had overall a larger cell size whereas in the brown adipose tissue wild type mouse there were some random large cells that increased the average cell size.



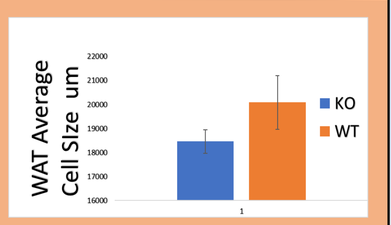


Figure 6: WAT rep images, WAT mean cell size:
The white adipose tissue knock out mouse tended to be slightly smaller than the white adipose tissue wild type mouse. The Wild type mouse cell sizes as shown in the representative images are just a tad bit larger in almost all images assessed.

Methods

- ***Adipose tissue collection and histology***

At the end of our study, adipose tissue samples were extracted and the tissue weights were recorded. The interscapular brown adipose tissue (BAT), subcutaneous inguinal (SQ) white adipose tissue (WAT), and visceral (perigonadal) WAT (PGAT) samples were fixed in formalin through paraffin embedment. -Methods of this here- They will be stained for uncoupling protein 1 (UCP1) for 30 min with a heat-induced epitope using DAKO brand citrate in a decloaking chamber to prep for histology samples. The histology samples will be then sectioned and evaluated via an Olympus BX34 photomicroscope (Olympus, Melville, NY). Images will be taken via an Olympus SC30 Optical Microscope Accessory CMOS color camera. After we have the images I will be able to pull them up in there different groups by using a software called "ImageJ," I will then circle at least 50 adipocytes in an image to see mean cell size and distribution, indication of browning via evidence of multilocular phenotype.

- **Adipose tissue gene expression via qPCR**

BAT, SQ, and PGAT samples will be homogenized in TRIzol solution using a tissue homogenizer. Total RNA is to be isolated according to the Qiagen's RNeasy lipid tissue protocol and assayed using a Nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE, United States) First strand cDNA will be synthesized from RNA to asses purity, using the cDNA reverse transcription kit. Quantitative real time PCR will be done using the ABI Step OnePlus sequence detection system. The following genes will be measured and analyzed, normalized to the housekeeping gene and expressed relative to WT: leptin, adiponectin, UCP1

References

1. Deborah Clegg, Sex Hormones and Cardiometabolic Health: Role of Estrogen and Estrogen Receptors, 2017, p. 1-11.
2. Dusti A. Shay, Changes in Nucleus Accumbens Gene Expression Accompany Sex-Specific Suppression of Spontaneous Physical Activity in Aromatase Knockout Mice, 2020, p. 5-13.
3. Stepahnie L. Clookey, Beta 3 Adrenergic Receptor Activation Rescues Metabolic Dysfunction in Female Estrogen Receptor Alpha-Null Mice, 2019, p. 1-4.
4. Young-Min Park, Effects of intrinsic aerobic capacity and ovariectomy on voluntary wheel running and nucleus accumbens dopamine receptor gene expression, 2016, p. 383-389.

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