Title: The Role of Estrogen Signaling in Sexually Dimorphic Adipose Tissue Accumulation

Motivation and Background: Mammalian white adipose tissue (WAT) distribution and expansion is sex-dependent, with males preferentially accumulating visceral WAT (VWAT) and females exhibiting a subcutaneous WAT (SWAT) accumulation bias. Interestingly, females switch to a male-like pattern of WAT distribution after menopause when estrogen levels decline, indicating sex hormones play a role in the distribution of subcutaneous and visceral WAT mass, yet the molecular mechanisms governing these processes *in vivo* are not well understood. WAT distribution is strongly correlated with the development of pathologies related to obesity, with accumulation of VWAT being more detrimental for metabolic health than accumulation of SWAT, which may confer protection against these pathologies. Our lab has shown that there is a sexually dimorphic pattern of adipocyte precursor (AP) activation in mice in response to high fat diet, with males having robust AP activation in the VWAT but not SWAT and females having activation in both VWAT and SWAT.[1] Once APs are activated they commit to differentiating into mature adipocytes and thus contribute to WAT mass. Interestingly, the sex-specific AP activation pattern observed occurs in an estrogen-dependent manner. Therefore, estrogen levels appear to be crucial for AP activation and expansion of SWAT but not VWAT. Herein I propose to identify **the role of estrogen signaling in sexually dimorphic WAT expansion and elucidate the mechanisms controlling differential AP activation in male and female mice. Hypothesis:** Estrogen receptor alpha (ERα) is required for AP activation and expansion of SWAT and there are distinct molecular mechanisms driving WAT expansion in VWAT and SWAT, with VWAT expansion being independent of ERα activity.

Aim1: Characterize the requirement of ERα in the activation of adipocyte precursors in SWAT. For this aim, we will knockout the *Esr1* gene in APs using an inducible Cre-recombinase system driven by the AP-specific promoter *PdgfRα* (Figure 1).[2] This will enable us to ablate ERα expression postnatally to avoid any developmental phenotypes. We will then test the proliferation of APs via incorporation of BrdU, a nucleoside analog, in these *ERα-APKO* mice upon high-fat diet (HFD) or standard diet feeding (SD). After the HFD-induced AP proliferation phase, incorporation of BrdU will be assessed in APs via flow cytometry. If ERα is required for AP proliferation in female SWAT, we expect to see a decrease in BrdU positive cells when challenged with HFD only in this depot. If ERα is also important for AP proliferation in male SWAT, we expect to see an even lower percentage of BrdU+ cells than wildtype (WT) littermates. We do not expect to see an impairment in AP proliferation in visceral fat in males or females.

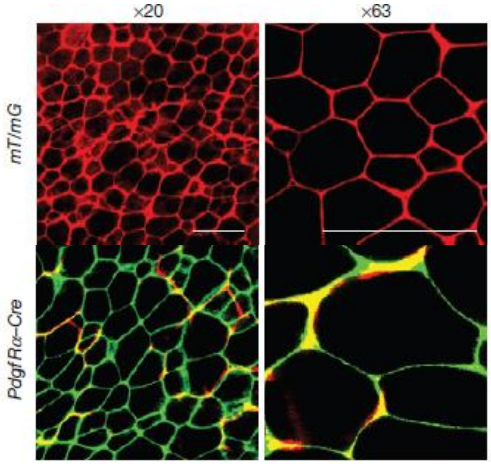

Figure 1. Adipocytes are derived from PdgfRa+ precursor cells. Fat from mouse strain with fluorescent-membrane dTomato/ membrane eGFP (mT/mG) Cre reporter. Cre excision is marked by a switch from tdTomato expression to eGFP expression. PdgfRa-Cre labels all mature adipocytes in WAT but PdgfRa is not expressed in mature adipocytes, thus the GFP expression observed in adipocytes of PdgfRa-Cre:mT/mG mice is due to lineage tracing.[2]

Aim2. Identify differences in WAT depot estrogen levels. Even though WAT can produce estrogen locally, our findings suggest that circulating levels of estrogen are required for SWAT AP activation but not VWAT AP activation in both males and females.[1] Therefore, I hypothesize that circulating levels of estrogen influence estrogen levels in SWAT but not VWAT to drive adipogenesis upon periods of HFD. To test this, I will

measure estradiol levels in the WAT depots of WT female and male mice on days 1, 3, and 5 of HFD or SD. Our lab has shown that activation of APs initiates on day 1 of HFD, with a peak on day 3, and returns to SD levels by day 5.[3] Hormone extraction from WAT will be performed and levels of estradiol and estrone will be quantified by liquid chromatography tandem-mass spectrometry. I expect to see increased levels of estrogen in SWAT of WT female mice on day 3 of HFD compared to VWAT. Because WT males do not have significant circulating levels of estrogen, I do not expect to see a difference in VWAT and SWAT levels. I can also perform the same experiment in ovariectomized (Ovx) females and estrogen-treated males, where circulating levels of estrogen are diminished/increased respectively as compared to WT mice. If I see a decrease in estrogen levels in SWAT of Ovx females and an increase in SWAT of estrogen-treated males on day 3 of HFD as compared to WT, then circulating levels of estrogen influence SWAT levels of estrogen upon HFD and promote WAT expansion through estrogen signaling in this depot.

Aim3: Elucidate distinct molecular mechanisms of adipogenesis in VWAT and SWAT. Our lab recently identified FOXM1, a nuclear fork box protein, as an important gene in male visceral AP activation (unpublished). Interestingly, FOXM1 has been shown to work with ERα to promote gene expression in a breast cancer model.[5] Furthermore, when in the presence of activated ERα, FOXM1 drives the expression of a different gene program than when in absence of ERα.[5] Therefore, we hypothesize that FOXM1 is important in adipocyte hyperplasia in both males and females but it acts through distinct molecular mechanisms depending on the presence of estrogen. To test this, we will perform RNAseq on isolated APs from both fat depots under HFD and SD conditions on WT and Ovx female mice. If FOXM1 is working with ERα to promote AP activation in SWAT, we expect to see an increase in gene expression in FOXM1-ERα targets only in subcutaneous fat of WT females. If we do not see this same pattern in the subcutaneous fat of Ovx females, but we do find increased gene expression of FOXM1 targets in the visceral fat, we can conclude that in the presence of estrogen, FOXM1 and ERα promote the activation of APs and expansion of subcutaneous WAT and in the absence of estrogen, FOXM1 alone promotes activation of APs and expansion of visceral WAT. To further confirm this, I will perform co-immunoprecipitation (co-IP) on isolated APs from both depots from SD and HFD-fed mice to assess if FOXM1 partners with ERα in SWAT but not VWAT of WT females.

Intellectual Merit and Broader Impact: This study will clarify for the first time the mechanistic role of estrogen signaling in WAT and will significantly impact the field of adipose tissue biology. We will also set precedent on elucidating distinct sex-dependent molecular pathways governing WAT mass expansion. As a hispanic woman in science, my goal is to inspire others to pursue careers in science and become advocates for minorities in STEM. By sharing my research findings in activities coordinated by Yale organizations and minority-focused science conferences I plan to motivate not only undergraduate women and minorities to pursue careers in science, but I will also educate the greater community and general public about the importance of science education in order to advance knowledge beyond an academic environment.

IACUC Approval: We have clearances and training for all handling and proposed mouse procedure (Yale IACUC protocol 2012-11249). The University's Assurance number with the Office of Laboratory Animal Welfare is #A3230-01, approval through 5/31/19. IACUC oversees the University's centralized, AAALAC-accredited animal resource, the Yale Animal Resources Center (YARC).

References: [1]Jeffery, E., et. al. (2016). **Cell Metabolism**, 24(1):142-50. [2]Berry, R., Rodeheffer, M. (2013). **Nature Cell Biology**, 15(3): 302-308. [3]Jeffery, E., et. al. (2015). **Nature Cell Biology**, 17(4): 376-385. [4]Falk, R. T., et. al. (2008). **Cancer Epidemiology, Biomarkers, and Prevention**, 17(8): 1891–1895. [5]Sanders, D., et. al. (2013). **Genome Biology**, 14:R6.