Dihydrotestosterone differentially regulates predictors of T cell function in visceral and subcutaneous adipose tissue of female mice

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Introduction

The incidence of polycystic ovary syndrome (PCOS), an oligo-ovulatory disorder best defined by high androgen levels and menstrual irregularity, has risen in tandem with the U.S. obesity rate in women (1). Obese PCOS patients have excessively high androgen levels and are severely insulin resistant (2). Importantly, insulin resistance is much more severe in women with abundant visceral white adipose tissue (VWAT), a phenotype that characterizes PCOS (3). Studies in female non-human primates have shown that androgens more potently suppress lipolysis in subcutaneous (SC) WAT as opposed to VWAT (4). However, the mechanisms by which androgens suppress lipolysis in adipocytes from females are *unknown*.

While low-grade chronic inflammation, microRNAs (miR155, miR21, miR29a, and miR146a), and hyperandrogenemia have all been linked to simple obesity, interactions between these factors have not been studied in PCOS. The hyperandrogenemia of PCOS is the driving factor behind the metabolic perturbations seen in these patients: hyperinsulinemia, increased VWAT mass, and dyslipidemia (1). Therefore, our group explored the effects of the potent, non-aromatizable androgen, dihydrotestosterone (DHT) on WAT inflammatory markers and microRNAs in female mice as a model for PCOS patients.

Hypothesis and Aims

Hypothesis: Short-term DHT administration to sexually mature, lean female mice will cause increased inflammation and modulation of miRs known to be related to obesity in SCWAT.

Aims:

1) Assess the effect of short-term DHT administration in sexually mature, lean female C57Bl mice on WAT cytokines and immune cell populations.

2) Examine the effect short-term DHT administration in sexually mature, lean female C57Bl mice on WAT miRs associated with obesity and insulin resistance.

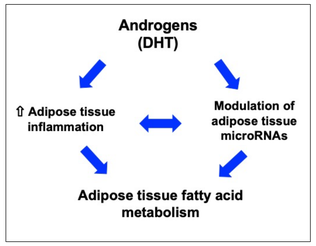


Figure 1. Model of the effects of DHT on WAT inflammation and miRs in control of WAT fatty acid metabolism.

Materials and Methods

DHT given post-natally to mice creates a PCOS-like metabolic phenotype (5).

Twenty 12 week old female C57Bl mice had vaginal swabs taken daily for a week. When animals were in the estrus phase of the cycle, daily SC injections of oil (control) or DHT were begun and continued for three estrous cycles (~12-15 days) (**Fig. 2**).

Mice were euthanized for blood and tissue collection during estrus of their 4th cycle. DHT mice that cycled irregularly and were not coming into the estrus were euthanized at day 14 of the study which was the median of treatment length for control mice.

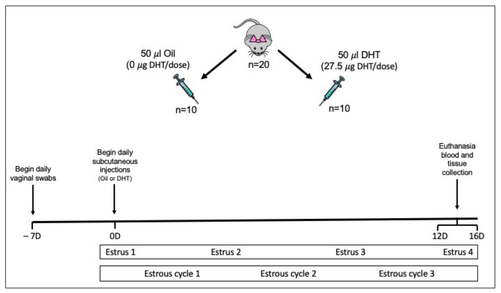


Figure 2. Timeline of animal experiments

Results

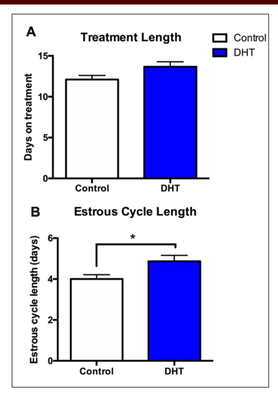


Figure 3. Treatment and estrous cycle length in control and DHT-treated mice * $p = 0.05$

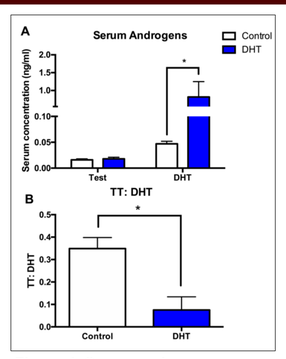
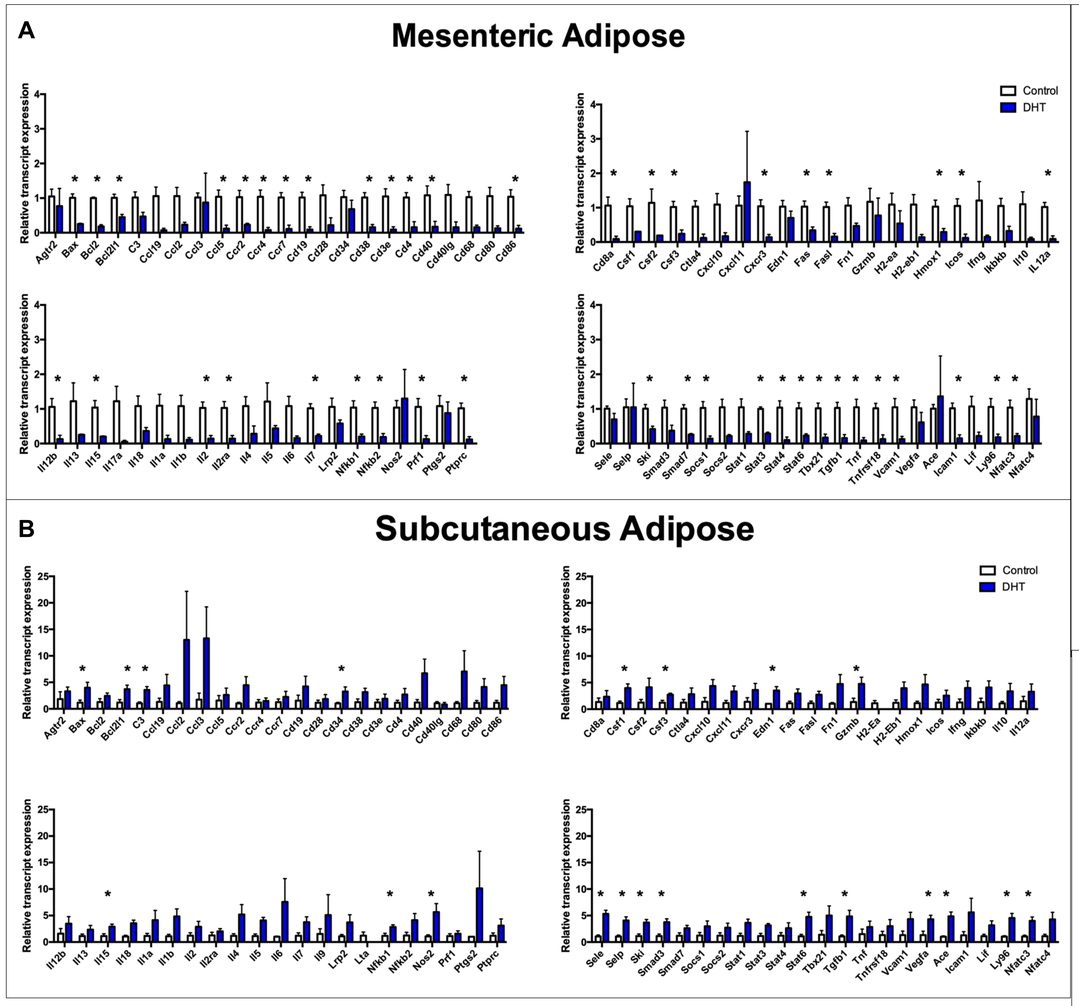


Figure 4. Serum androgen concentrations in control and DHT-treated mice. Serum testosterone and DHT were quantified by LC-MS/MS. * $p < 0.05$

Results



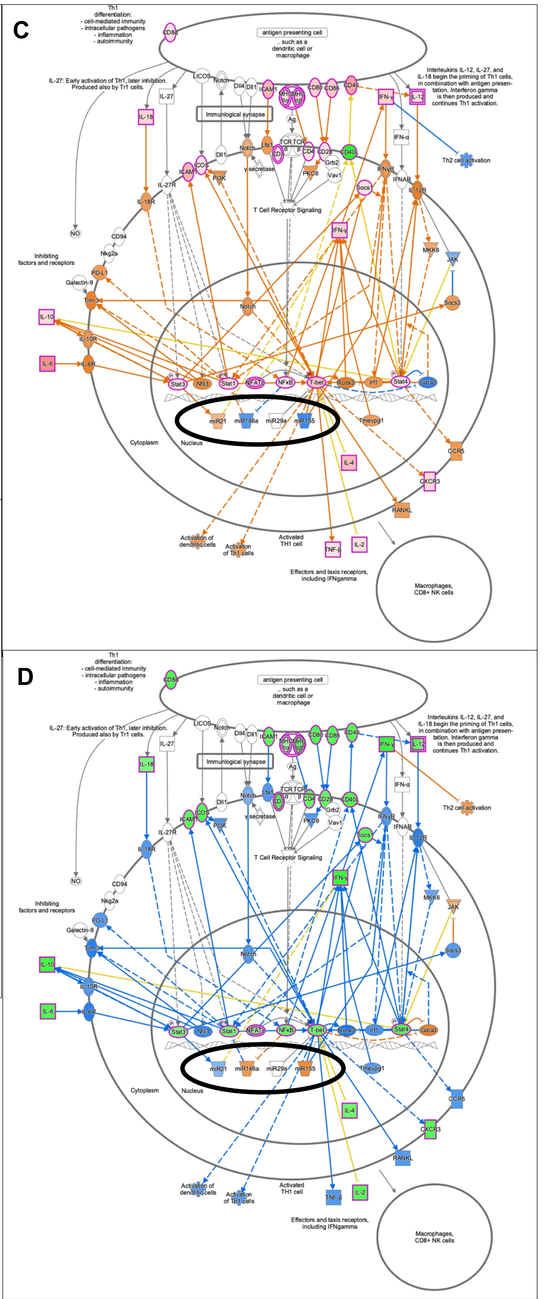


Figure 5. Relative transcript abundance of cytokines in adipose tissue of control and DHT-treated mice. A) mesenteric adipose B) subcutaneous adipose C) pathway analysis diagram of Th1 activation in mesenteric adipose D) pathway analysis diagram of Th1 activation in subcutaneous adipose. Pathway analysis was performed using Qiagen IPA software. DHT treatment caused decreased abundance of cytokine transcripts in mesenteric WAT and increased abundance of cytokine transcripts in subcutaneous WAT. Other pathways that were significantly downregulated in VWAT and upregulated in SCWAT by DHT treatment included: Th2 activation pathway, T helper cell differentiation pathway, and B cell to T cell communication. In VWAT, miR146a and miR155 are predicted to be activated and miR21 is predicted to be inhibited in Th1 cells (C). In SCWAT, the opposite finding occurred (D).
red: up-regulated green: down-regulated orange: predicted activation blue: predicted inhibition Dark black circle: highlights miRs in Th1 cells * $p < 0.05$

Conclusions & On-going research

Short-term treatment with DHT causes:

- Elongated estrous cycles and increased serum DHT concentrations (0.82 ± 0.43 ng/ml) which are half of those concentrations in the DHT PCOS mouse model (1.64 ± 0.32 ng/ml) (6)
- Down-regulation of cytokines in mesenteric and up-regulation of cytokines in subcutaneous white adipose tissue that affect Th1 and Th2 activation pathways, T helper cell differentiation, and B cell to T cell communication.

On-going research:

- Measurement of relative transcript levels of the following miRs in WAT: miR21, miR29a, miR146a, miR155
- Assessment of T and B cell abundance in WAT via immunofluorescence
- Tabulation of animal weight and fat pad weights (mesenteric, gonadal, retroperitoneal, subcutaneous)
- WAT T cell functionality assays

References

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