Reproductive Endocrinology
OVARY, TESTES, AND IMPACT OF HORMONES ON METABOLIC FUNCTION

The Hepatokine Adropin Is Regulated by Estrogen

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Introduction: menopause is associated with weight gain, visceral adiposity and NAFLD. Rodent ovariectomy (OVX) is an accepted model for human menopause. Many OVX studies add high-fat diet or old-age to accentuate deranged phenotype. We have shown OVX alone induced weight gain and changes in liver transcriptome including downregulation of *Enho,* encoding for the hepatokine adropin (1). Here, we explore changes in VAT cytokine and adipokine genes, hepatic miRNA, and liver triglyceride content induced by OVX, in addition to estrogen's role in regulation of adropin. **Methods:** 9-week-old C57BL/6J female mice underwent OVX or sham surgery. Groups of 10 mice were sacrificed at 6- and 12-weeks post-surgery and tissues harvested including mesenteric adipose tissue representing VAT. Liver TG was quantified using Cayman colorimetric assay. In-vitro studies performed in the murine hepatic cell-line, BNL1.ME. Adropin was measured using ELISA. **Results:** OVX induced adverse inflammatory cytokine & adipokine gene expression in VAT at 6-weeks post-surgery (*Il18* 1.1 p=0.01, *Rares2* 2.9, p=0.003, *Retn* 5.5, p=0.002) and 12-weeks post-surgery (*Tnfa* 2.3 p<0.001, *Cxcl5* 1.9 p=0.04). In the liver, OVX induced an increase in TG content at 12 weeks post-surgery (realtive increase vs sham 2.0 p=0.05). Hepatic *Enho* expression showed a strong inverse coorelation with total body weight gain (r= -0.7 p<0.001) and liver TG content (r=-0.4, p=0.04). In-vitro, estrogen induced an increase in *Enho* (relative mRNA change vs. growing medium 2.6, p=0.004); though protein level was unchanged, a trend for increased adropin was found in supernatant (relative change vs control 2.2 P=0.09). In-silico analysis of data from OVX mice treated with estrogen showed up-regulation of *Enho* (relative change vs vehicle, 6 p<0.001). At 6-weeks post-surgery OVX induced changes in hepatic miRNA profile with 48 miRNAs differentially expressed vs SHAM (24 up & 24 down). Integrating data from same sample RNA-SEQ and miRNA-SEQ created a network of differently expressed miRNA with oppositely differently expressed known specific mRNA targets. mIR-29, a known regulator of *Enho* in liver, was not found to be correlated with *Enho* expression in this context. **Conclusions:** OVX alone is sufficient to induce adverse changes in VAT gene expression and liver TG. Hepatic adropin gene expression is regulated by estrogen and its downregulation was strongly correlated to phenotypes relevant to menopause induced metabolic dysfunction, weight gain and increased liver fat. Thus, adropin should be further explored as a novel therapeutic and/or biomarker for menopause induced metabolic dysfunction.

(1) Stokar, J., Gurt, I., Cohen-Kfir, E., Yakubovsky, O., Hanna, A., Assayag, E., & Dresner-Pollak, R. (2019). RNA-Seq Analysis of Ovariectomy-Induced Changes in Mouse Liver Reveals New Targets for Menopause-Associated Metabolic Derangement. Journal of the Endocrine Society, 3(Supplement_1), SUN-033.

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Upregulation of Aryl Hydrocarbon Receptor in Granulosa Cells by Endoplasmic Reticulum Stress Contributes to the PCOS Pathophysiology

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Studies have demonstrated that endocrine disrupting chemicals (EDC) are involved in the pathophysiology of PCOS, and aryl hydrocarbon receptor (AHR) mediates the cellular effect of EDC by inducing xenobiotic metabolizing enzymes including cytochrome P450 1B1 (CYP1B1). However, recent studies suggest the novel role of AHR in various diseases, including obesity and cancer progression, independent from the EDC metabolism. We previously demonstrated that endoplasmic reticulum (ER) stress, a newly recognized local factor, contributes to PCOS pathology by affecting diverse functions of granulosa cells. We hypothesized that ER stress induces the expression of AHR and activates its downstream signaling in granulosa cells, irrespective of the presence of EDCs, thereby promoting PCOS pathogenesis. At first, we determined the upregulation of AHR, AHR nuclear translocator (ARNT), and AHR target gene cytochrome P450 1B1 (CYP1B1) in the granulosa cells of PCOS patients and model mice by immunohistochemical staining and qPCR. We examined CYP1B1 as a representative AHR target gene. Treatment of cultured human granulosa-lutein cells (GLCs) with tunicamycin (ER stress inducer) upregulated the expression of AHR, ARNT and CYP1B1. Knockdown of AHR decreased the tunicamycin-induced expression and activity of CYP1B1, suggesting the intermediary role of AHR in upregulation of AHR activity by ER stress. To confirm the role of AHR *in vivo*, we administered the AHR antagonist CH223191 to PCOS model mice. The administration of the antagonist restored estrous cycling and decreased the number of atretic antral follicles, concomitant with downregulation of AHR and CYP1B1 in granulosa cells. Taken together, this study indicates that AHR and downstream signaling are activated by ER stress in GLCs of PCOS. Moreover, downregulation of local AHR expression and activation restores a normal reproductive phenotype in a PCOS mouse model. Our findings demonstrate that AHR activated by ER stress in the follicular microenvironment contributes to PCOS pathology, and that AHR represents a novel therapeutic target for PCOS.

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A Genetically Defined Male Counterpart of Polycystic Ovary Syndrome: Evidence for Ovarian-Independent Pathogenesis