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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RECIPROCAL EFFECTS OF OVARIAN AND METABOLIC DYSFUNCTION

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

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Background: Polycystic ovary syndrome (PCOS) is a heterogeneous condition that affects 6-10% of women of reproductive age. PCOS is often characterized by a triad of ovulatory dysfunction, hyperandrogenism, and cardiometabolic dysfunction. Both ovarian-related and ovarian-independent factors have been implicated in the pathogenesis of PCOS, but it remains to be determined which are the inciting events and which are the secondary consequences. Studies of male relatives of women with PCOS have proposed a male counterpart of PCOS, which suggests that PCOS is not always a primary disorder of female reproduction, but rather can be, at least in part, a condition of cardiometabolic dysregulation and hyperandrogenism, with ovarian dysfunction as a secondary consequence.
Methods: To investigate a genetically defined male counterpart of PCOS, we optimized a polygenic risk score (PRS) algorithm for predicting PCOS based on 206,851 unrelated women of European ancestry in the UK Biobank, then used this algorithm to calculate PCOS PRS for 176,360 men in the UK Biobank. We used logistic regression to calculate odds ratios for dichotomous outcomes by comparing men with high and low PRS (testing a variety of percentile cutoffs) and ANCOVA to compare continuous outcomes across deciles of PRS. All analyses were adjusted for age, age^2, assessment center, genotyping array, and the first 10 principal genetic components to account for ancestry.
Results: Men who carried a high PCOS PRS (top 20%) had a 17% increased risk of obesity defined as BMI ≥30 kg/m^2 (OR 1.17, 95% confidence interval [CI] 1.14-1.20, p=1.3x10^{-30}), 15% increased risk of type 2 diabetes mellitus (OR 1.15, 95% CI 1.09-1.20, p=5.3x10^{-8}), 5% increased risk of coronary artery disease (OR 1.05, 95% CI 1.01-1.09, p=0.03), and 5% increased risk for androgenic alopecia (OR 1.05, 95% CI 1.01-1.08, p=0.01). BMI, hemoglobin A1c, triglycerides, and the free androgen index all increased across deciles of the PRS, while HDL and SHBG decreased across PRS deciles (p all <0.001). The relationship between the PCOS PRS and coronary artery disease, HDL, and triglycerides appeared to be mediated by BMI. In contrast, the associations between the PCOS PRS and type 2 diabetes mellitus and hemoglobin A1c remained significant after adjusting for BMI, suggesting independent mechanisms of pathogenesis.
Conclusions: By demonstrating associations between PCOS genetic risk factors and cardiometabolic dysfunction and androgenic conditions in men, we have shown that these genetic risk factors can act independently of ovarian function. Thus, at least in some cases, the reproductive dysfunction of PCOS in women may arise secondarily from disruption of biological pathways common to both men and women. Future dissection of these biological pathways will further inform efforts to identify pathological mechanisms underlying PCOS.

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Effect of Experimentally Induced Sleep Fragmentation and Hypoestrogenism on Fasting Nutrient Utilization in Pre-Menopausal Women

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Background: Both sleep disturbance and menopause have independently been associated with weight gain in women. Possible mechanisms contributing to this weight gain may be changes in resting energy expenditure (REE) and/or nutrient utilization. Therefore, in the current study we aimed to examine the effects of experimentally induced sleep fragmentation and pharmacologic estradiol (E2) withdrawal on REE and nutrient utilization in the fasted state. *Design:* We studied pre-menopausal women during 5-night inpatient studies repeated in the mid-to-late follicular phase (high-E2; n=21) and following leuprolide-induced hypoestrogenism (low-E2; n=9 completed second visit). During each admission there were two nights of unfragmented sleep [8-h time in bed (TIB)] and three nights of fragmented sleep [9-h TIB]. Sleep was fragmented using an auditory stimulus delivered every 15 minutes that sustained wake for 2 minutes, producing 1 hour of wake after sleep onset. Study diets consisted of 3 meals and a snack each day and were iso-caloric across the two visits. REE and nutrient utilization were assessed in the fasted state via indirect calorimetry and compared between E2 states following unfragmented and fragmented sleep using linear mixed models. **Results:** Sleep fragmentation in the high-E2 state increased the respiratory quotient (RQ; +3%; p=0.03) with an accompanying increase in carbohydrate oxidation (+20%; p=0.02) and decrease in fat oxidation (-16%; p=0.03). The same effect was observed in response to E2-withdrawl during unfragmented sleep [increased RQ (+5%; p=0.01) and carbohydrate oxidation (+33%; p=0.01), and decreased fat oxidation (-26%; p=0.01)]. There was no additive effect of sleep fragmentation on nutrient utilization in the low-E2 state suggesting a possible ceiling (RQ and carbohydrate oxidation) and floor (fat oxidation) effect. There was no effect of sleep fragmentation or E2 state on REE. **Conclusion:** Both sleep fragmentation and hypoestrogenism were shown to alter fasting nutrient utilization, but not REE, in a manner that may contribute to weight gain in menopausal women. These findings are important for understanding weight gain during menopause, which is characterized by estrogen withdrawal and often accompanied by sleep disturbances.

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Heterozygous Eif4nif1 Stop Gain Mice Replicate the Primary Ovarian Insufficiency Phenotype