Coronary and Cerebral Pathophysiological Adaptations in Intact vs. Ovariectomized Pigs with Heart Failure

Veterinary Research Scholars Program
University of Missouri

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NEXTGEN PRECISION HEALTH

Abstract

Postmenopausal women pose a higher risk of developing heart failure with preserved ejection fraction (HFpEF) and related cognitive decline. Previous data suggest pathological effects of HFpEF on both heart and brain are amplified with a loss of female sex hormones. The goal of this study was to determine the role of female sex hormones on coronary and cerebral arteriole pathological transcriptomic and functional adaptations in a clinically relevant porcine model of HFpEF. Ossabaw swine were fed a Western diet and underwent aortic-banding (AB, n=9) or AB plus ovariectomy (AB-OVX, n=3). Coronary and cerebral vessels were isolated, pressurized, and exposed to increasing doses of sodium nitroprusside (SNP, nitric oxide mimetic), or U46619 (thromboxane 2A agonist) to assess endothelial-independent vascular function. % SNP-induced dilation was reduced in AB-OVX compared to AB (coronary 87.6 v. 97.8; cerebral 26.9 v. 83.6) and % U46619-induced constriction was also reduced in AB-OVX compared to AB (cerebral 38.0 v. 50.7). RNA seq identified 286 differentially expressed genes (DEGs) in cerebral arterioles and 17 DEGs in coronary arterioles. Ingenuity pathway analysis (IPA) on cerebral arterioles predicted inhibition of BDNF (z-score > -2), a gene regulated by estradiol. BDNF inhibition was associated with decreased cognition and increased peripheral vascular disease. IPA in the coronary arterioles revealed decreased expression of upstream regulator DUSP1, a signaling effector involved in MAPK activity and apoptosis. These findings indicate distinct pathological transcriptomic and functional adaptations in HFpEF with the loss of female sex hormones and suggest genes associated with BDNF and DUSP1 are potential therapeutic targets.

Objectives

1. **Determine the impact of ovariectomy on coronary and cerebral vascular function in an obese swine model of heart failure with preserved ejection fraction (HFpEF)**
2. **Identify gene candidates affected by the loss of female sex hormones for future therapeutic targeting**

Methods & Materials

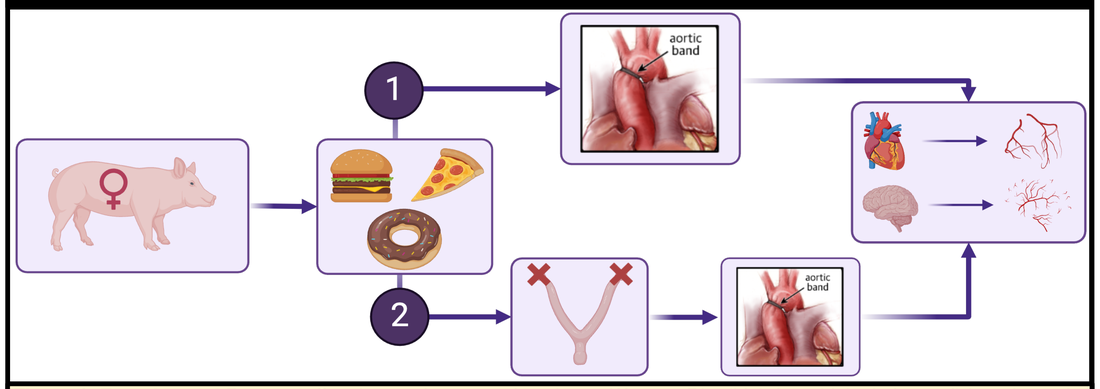


Figure 1. Experimental Protocol. Ossabaw swine were placed on a Western Diet (high fat + high cholesterol) for ~4 months and either aortic banded (AB, group 1, n=4) or ovariectomized 1 month prior to aortic banding (AB-OVX, group 2, n=3). Coronary and cerebral arterioles were collected 6 months following aortic banding procedure for *in vitro* experiments.

Vessel Function Experiments

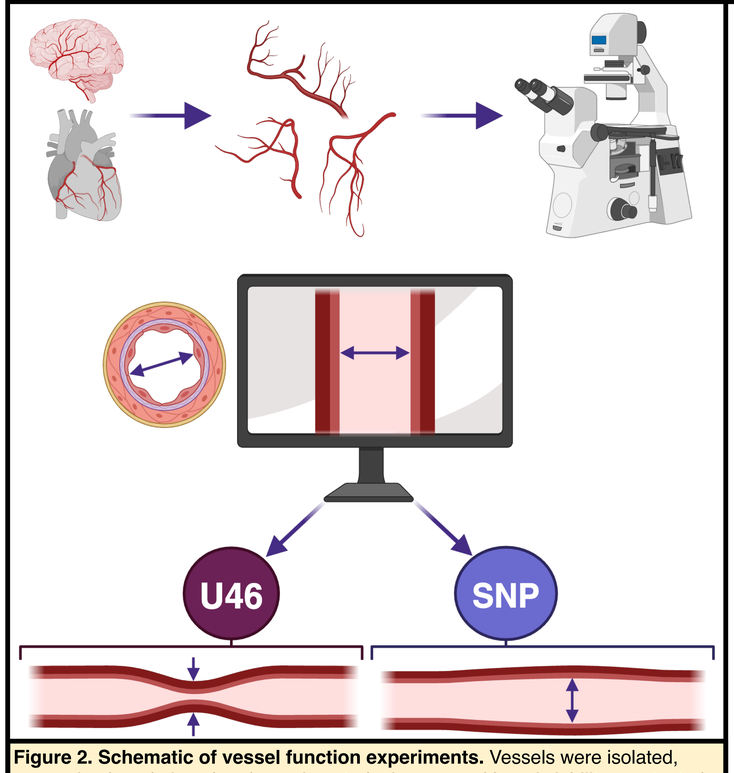


Figure 2. Schematic of vessel function experiments. Vessels were isolated, pressurized, and placed under an inverted microscope. Vessel viability was tested using 80k approximately 30 minutes after warm-up. Vessels which did not respond to 80k were eliminated and replaced. At 1 hour, vessel tone was assessed to ensure the requirement of 60-80% of max diameter was met prior to beginning dose curves. If less than 60%, vessels were pre-constricted using U46. Dose curves were administered in increments of 3 minutes with increasing concentrations of either U46 or SNP. LabChart software was utilized to track changes in vessel diameter and for initial data analysis.

RNA-seq Analysis

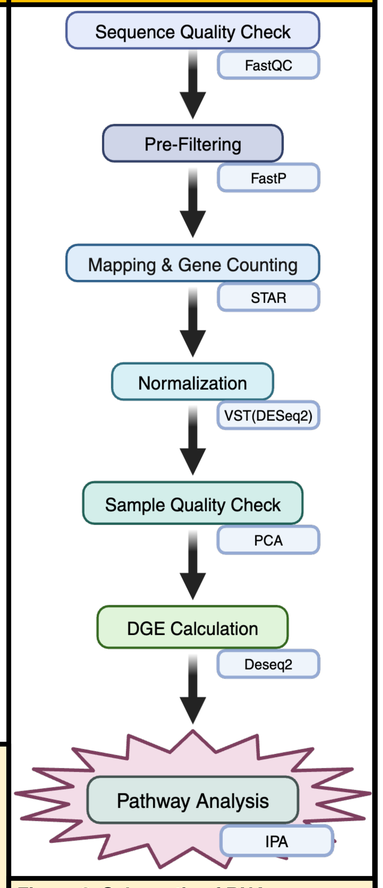
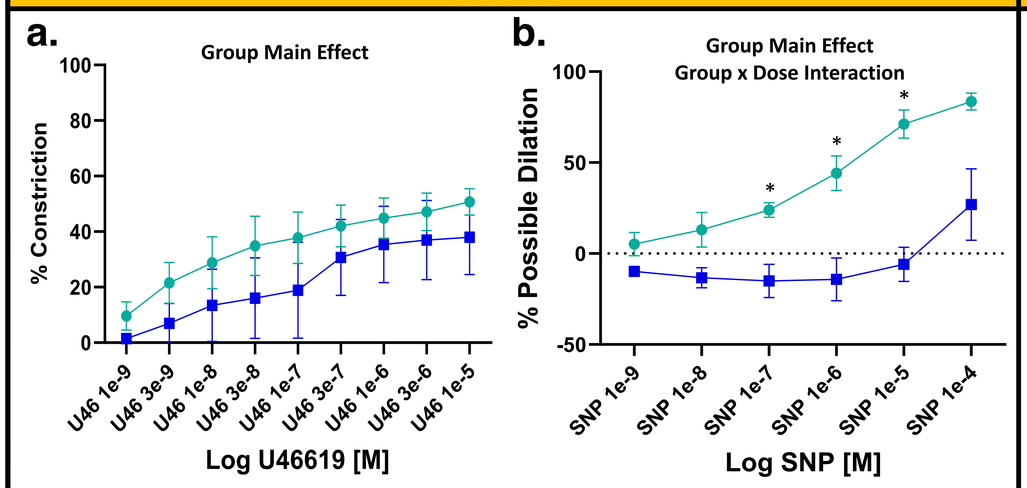


Figure 3. Schematic of RNA-seq Analysis pipeline utilized to obtain gene map data.

Vessel Function Results

Cerebral



KEY:
- AB Untreated
- AB+OVX Untreated

Figure 6a. Cerebral Arteriole Function. A) U46619-induced constriction was significantly reduced in AB-OVX (36.95%, blue squares) compared to AB (50.67%, green circles), two-way ANOVA Group Main Effect. **B)** SNP-induced dilation was reduced in AB-OVX (26.90%, blue squares) compared to AB (83.56%, green circles), two-way ANOVA Group Main Effect and Group x Dose Interaction. Interestingly, AB-OVX vessels responded abnormally to SNP by constricting rather than dilating to five of the six doses (**b, blue squares**).

Coronary

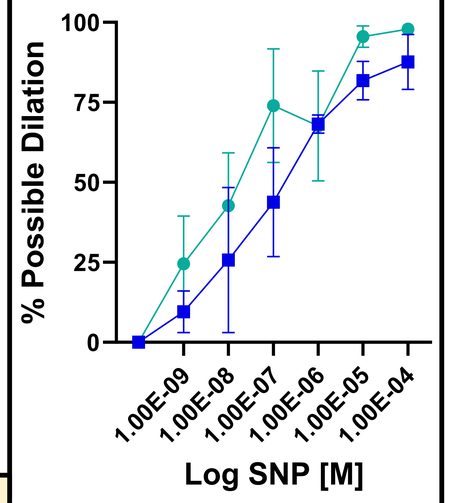


Figure 7. Coronary Arteriole Function. SNP-induced dilation was not statistically different in AB-OVX (87.58%, blue squares) compared to AB (97.84%, green circles), although we did notice a trend for reduction in the AB-OVX group.

RNA Sequencing Results

Cerebral

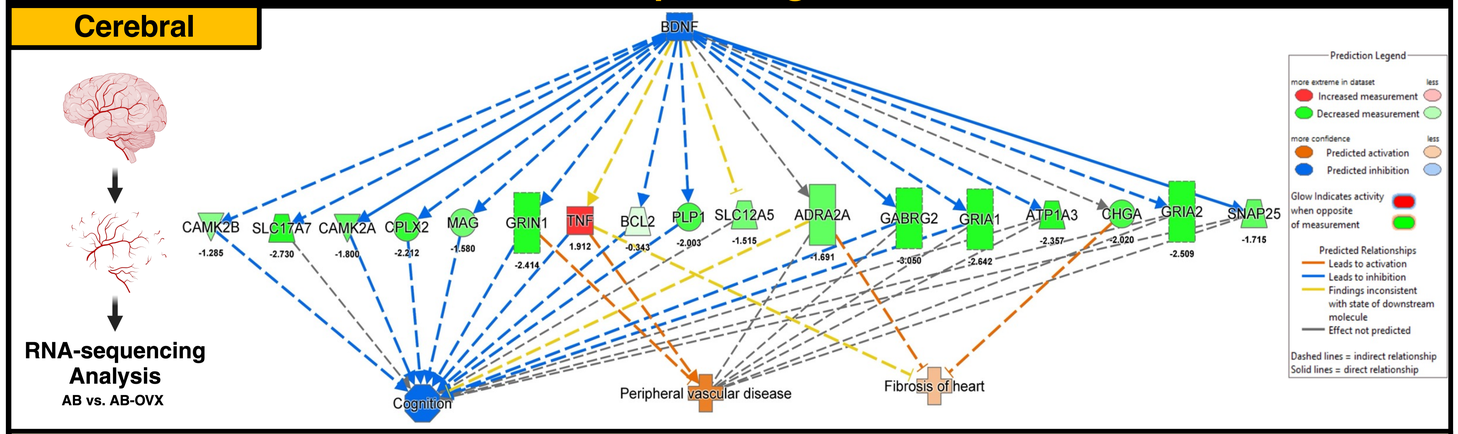


Figure 4. Ingenuity Pathway Analysis (IPA) in Cerebral Arterioles. RNA-sequencing identified a total of 286 differentially expressed genes (DEG's). IPA indicated expression of BDNF was significantly decreased (adjusted p-value of < 0.05) in AB-OVX with downstream affects including decreased cognition, increased peripheral vascular disease, and increased fibrosis of the heart those models. BDNF is a potent neurotrophic factor supporting neuron survival and integration. BDNF appears to play a role in proper heart development and function with receptors expressed in cardiac monocytes, endothelial cells, and vascular smooth muscle cells. Estradiol is considered a primary regulator of BNDF.

Coronary

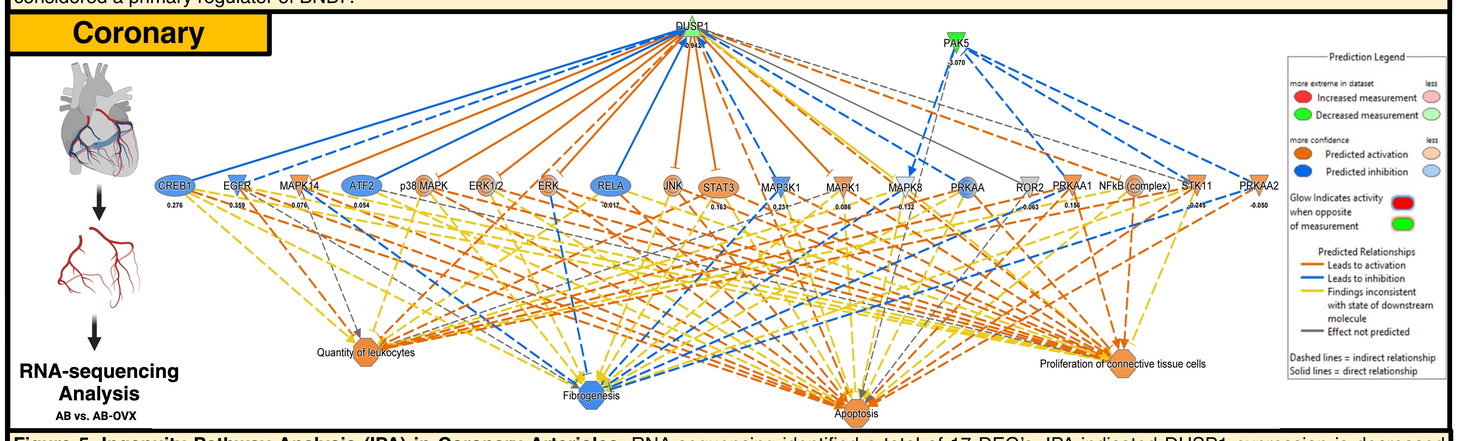


Figure 5. Ingenuity Pathway Analysis (IPA) in Coronary Arterioles. RNA sequencing identified a total of 17 DEG's. IPA indicated DUSP1 expression is decreased (adjusted p-value of < 0.05) in AB-OVX with downstream implications of increased leukocyte quantity, increased fibrogenesis, increased apoptosis, and increased proliferation of connective tissue cells. DUSP1 works to regulate p38 Mitogen-Activated Protein Kinase (MAPK) activity and is likely a critical signaling effector of the heart. MAPK/ERK2 pathways are heavily involved in vascular cell proliferation, differentiation, migration, senescence, and apoptosis. MAPK's also play a critical role in regulating cardiac hypertrophy and remodeling of the heart in response to increased workload or pathological insult.

Conclusions

- **Ovariectomy significantly reduced cerebral vessel functional capacity in aortic banded obese swine**
- **RNA sequencing data suggest genes associated with BDNF, DUSP1, and PAK5 as potential therapeutic targets for future studies**

Acknowledgements

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Schematics created using BioRender. Top upstream regulators and functions generated by Ingenuity pathway Analysis (Qiagen). Data collected and analyzed through LabChart. Graphs created using GraphPad Prism.