Long Term Estrogen Loss Worsens Heart Function in Aged Female Mice

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ABSTRACT

Heart disease is the leading cause of death worldwide and according to the American Heart Association, the risk of HD in aging menopausal women doubles compared to men of the same age. Excessive contractility of blood vessels is a common feature in heart disease.
Clinical and animal studies further support that estrogen loss worsen the contractility in the heart but the details remain unclear. Thus, the overall goal of this work was to examine how the timing and duration of estrogen loss affects heart failure.

Our hypothesis is that long-term estrogen loss following heart failure worsens cardiac function of the aged female heart.

To mimic menopause, we surgically removed the ovaries from female mice at 2.5 months of age, waited 5 or 12 months for estrogen loss, and induced heart failure using a drug that increases the contractility of the heart.
Our results show that estrogen loss at 12 months caused a greater impairment on the heart's response in increased heart contractility.

Understanding the effects of estrogen loss and HD is crucial to improving and finding alternative treatments for aging menopausal women. with heart disease.

INTRODUCTION

Cardiovascular Disease is responsible for 17.7 million deaths in the world, and 655,000 in the United States alone according to the Center of Disease Control. Risk factors such as high blood pressure, high cholesterol, age and sex hormones (estrogen deficiency) can increase the risk of heart disease. A common feature of cardiac failure is increased chronic beta-adrenergic stimulation.

Chronic sympathetic stimulation results in disturbed homeostasis, heart failure and increased contractility of the cardiomyocyte. Systolic dysfunction occurs as a result from mitochondrial dysfunction, cardiac remodeling inflammation, ventricular hypertrophy, and impaired G protein-coupled receptors.

This result is due to the stimulation of beta-adrenergic receptors (β-AR's), part of the G-coupled receptors. Both clinical and animal studies show that estrogen loss and age exacerbate cardiac β-AR signaling and contractile function is mediated by G protein-coupled receptor kinases (GPRK2). Dysfunction in myocardial β-AR dysregulate the contractility of AC-PKA pathway activation, increased epinephrine and norepinephrine. Cardiac sympathetic stimulation via heart failure contractility is increased due to GRK2 phosphorylating β-AR's and regulating AC-PKA.

METHODOLOGY

Table 1: 5-and 12-Month Mice Treatment Groups: The four treatment groups include SHAM, OVX, ISO and OVX+ISO (n=5, n=5, n=7, and n=5, respectively).

Treatment Group	SHAM	OVX	ISO	ISO+OVX
Sample Size 5-MOS	5	5	7	5
Sample Size 12-MOS	5	5	7	5

i. Bilateral ovariectomy (OVX) or SHAM surgery was performed in female mice at 2.5 months of age.
ii. Mice were infused with Isoproterenol (ISO; 400µg/kg/h) at 5 months (5M) post OVX for 3 days or 12 months (12M) post OVX for 3 days via mini osmotic pumps to induce chronic sympathetic stimulation. SHAM and OVX groups were treated with saline.
iii. Transthoracic M-Mode echocardiography was used to observe cardiac function in the form of fractional shortening (%FS) and ejection fraction (EF).

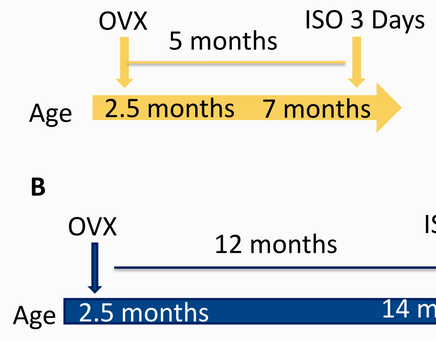

Figure 1: Timeline of Experimental Methods: A) 5M models were infused with saline or ISO at 7.5 months for 3 days. The B) 12M models were infused with saline or ISO at 14.5 months for 3 days.

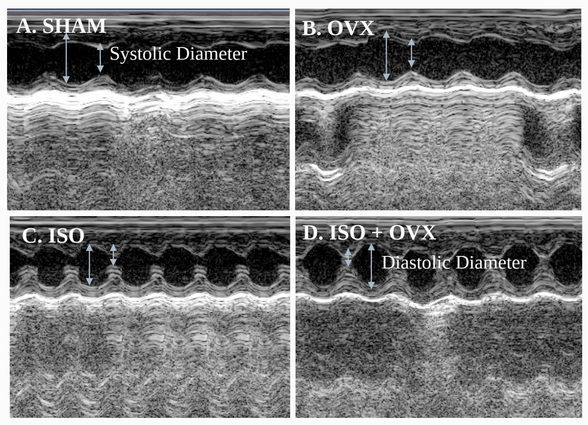

Figure 2A-D: 12M echocardiography M-Mode tracings of left-ventricular diastolic and systolic diameter for A) SHAM, B) OVX, C) ISO, and D) ISO +OVX treatment groups.

RESULTS

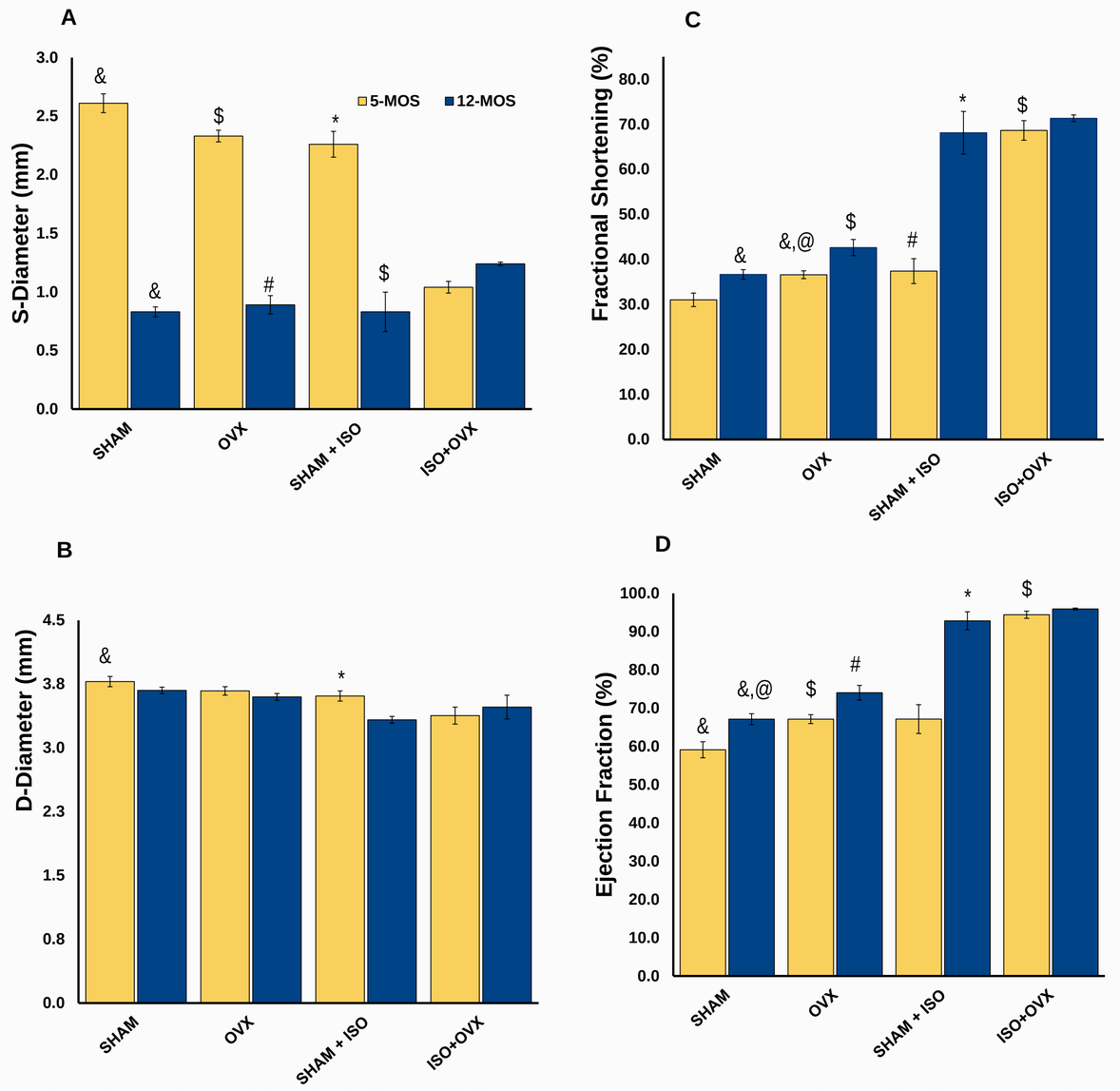

Figure 2: Echocardiographic calculations of left ventricular structure and function A) Systolic diameter B) Diastolic Diameter C) Fractional Shortening D) Ejection Fraction. Statistical analysis was performed using student t-test, n=5-7; #P<0.05 vs. 5-MOS OVX;
*P<0.001 vs. 5-MOS-SHAM+ISO, &P<0.05 vs SHAM+ISO within time group, @P<0.05 vs. OVX within time group, $P<0.05 vs. ISO+OVX within time group, %P<0.05 vs. ISO+OVX within time group

RESULTS

i. OVX,ISO, and ISO+OVX significantly decreased in S-Diameter in 5M and 12M.
ii. Largest decrease in S-Diameter was in ISO+OVX group for 5M and 12M. 12M had a greater decrease in S-Diameter than 5M.
iii. SHAM compared to SHAM +ISO decreased in D-Diameter at only at 12M. D-Diameter significantly decreased between 5M and 12M.

Ovariectomy (prolonged estrogen deficiency):

i. OVX group increased %FS and EF between 5M and 12M time models.
ii. SHAM compared to OVX group increased %FS and EF within groups at 5M and 12M models.
iii. SHAM compared to OVX had higher %FS and EF at 12M.

Isoproterenol (Chronic Sympathetic Stimulation):

i. SHAM +ISO compared to ISO+OVX group increased %FS and EF within groups in the 5M and 12M models.
ii. SHAM compared to SHAM+ISO group resulted in a 1.2-fold increase of %FS and EF in the 5M model.
iii. SHAM and SHAM+ISO compared to the OVX+ISO group resulted in a greater fold increase in %FS and EF in the 12M model than the 5M.
iv. OVX compared to OVX+ISO group resulted in a 1.8-fold increase of %FS and EF at 5M.

CONCLUSIONS

The results presented here show that estrogen loss impairs left ventricular cardiac response to β-AR stimulation, and that prolonged estrogen loss may blunt the sympathetic response in the heart. These results highlight the importance of studying the long-term effects of estrogen loss during menopause in the treatment and management of heart disease.

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BIBLIOGRAPHY

Bontaş E., Radu-Ioniţă F., Munteanu A., Mocanu I. (2018) Basic Aspects of Cardiac Remodelling. In: Dumitrescu S., Ţintoiu I., Underwood M. (eds) Right Heart Pathology. Springer, Cham.
Kathryn, D. (2020). Assessing menopausal female susceptibility to heart disease : a focus on ampk ' s ability to mitigate cardiac remodeling through estrogen-dependent gene programs.
Lucia, C. de, Eguchi, A., & Koch, W. J. (2018). New Insights in Cardiac β-Adrenergic Signaling During Heart Failure and Aging. Frontiers in Pharmacology, 0(AUG), 904.
Ventura-Clapier, R., Dworatzek, E., Seeland, U., Kararigas, G., Et al. (2017). Sex in basic research: Concepts in the cardiovascular field. Cardiovascular Research, 113(7), 711–724.