Female Mice are Protected from Chronic Inhaled Nicotine-Induced Pulmonary Hypertension via a Sex Hormone-Independent Mechanism

Idanis Z. Garcia[3], Nicholas D. Fried[1], Anna K. Whitehead[1], Eric Lazartigues[2], Xinping Yue[1], & Jason D. Gardner[1]

1. Dept of Physiology, LSUHSC – New Orleans; 2. Dept of Pharmacology & Experimental Therapeutics, LSUHSC – New Orleans;
3. Ponce Health Sciences University, PHSU – Ponce, Puerto Rico

National Heart, Lung, and Blood Institute

Introduction

Cigarette smoking has reached an all-time low in the United States, but the use of e-cigarettes increased by 46.2% in young adults and 77.8% in high school students between 2017-2018. Little is known about the long-term cardiopulmonary implications of these nicotine-delivery devices. Our group previously demonstrated that chronic inhaled nicotine induces pulmonary hypertension (PH) and right ventricular (RV) remodeling in male mice but not female mice, suggesting a sex difference. This led us to hypothesize that *female mice are protected against the nicotine-induced PH by a sex hormone-dependent mechanism*. We studied ovariectomized (OVX) and intact female mice to assess the role of female sex hormones in nicotine-induced PH.

Materials & Methods

- **Animals:** Female C57BL/6 mice aged 8-12 weeks.
- **Nicotine exposure:** Mice were exposed to intermittent nicotine vapor during the dark cycle for 12 hours/day for 8 weeks.
- **Cardiac functional assessment:** Echocardiography was performed using a Vevo 3100 Imaging System (VisualSonics, Toronto, Canada). Right ventricular systolic pressure (RVSP) was assessed in anesthetized mice using cardiac catheterization (Millar, Houston, Texas).
- **Ovariectomy:** Mouse ovaries were surgically removed one week prior to initiation of nicotine treatment.
- **Statistics:** Data are presented as mean±SEM. Statistical analyses were performed using 2-way ANOVA with Tukey's multiple comparison test. * = p<0.05; ** = p<0.01; *** = p<0.001.

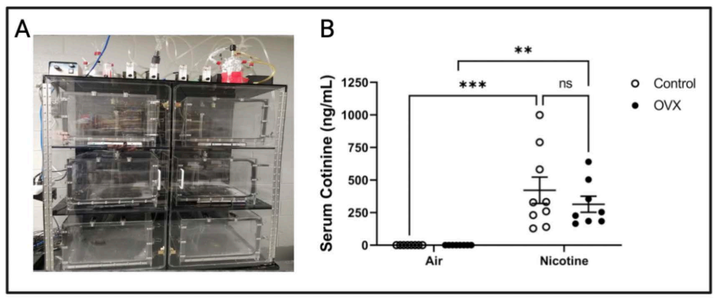

Figure 1. A. Nicotine exposure chamber from La Jolla Alcohol Research, Inc. (La Jolla, California). **B.** ELISA of serum cotinine levels in air- and nicotine-exposed mice following 12-hour exposure period. (**p<0.01, ***p< 0.001).

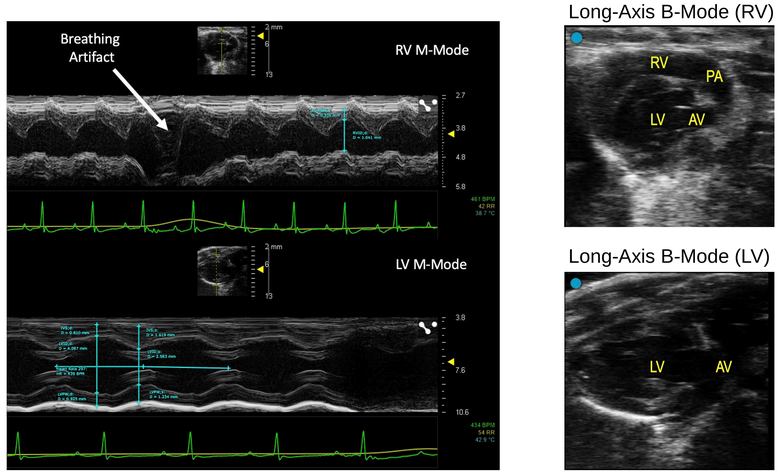

Figure 2. Example echocardiography imaging and measurements.

Results

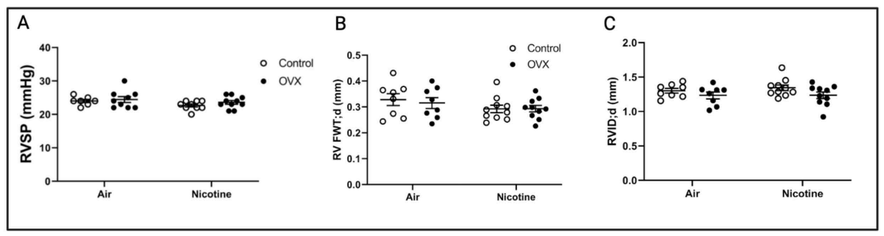

Figure 3. A. Chronic nicotine inhalation does not lead to increased RVSP (22.7±0.4 mmHg, n=9) versus air exposure (24.0±0.5 mmHg, n=7) in female mice. RVSP was unaffected by OVX of air-exposed mice (24.4±0.9mmHg, n=9) and OVX of nicotine-exposed mice (23.6±0.6 mmHg, n=10). **B.** RV free wall thickness during diastole (FWT;d) was not significantly different between air-control mice (0.328±0.023 mm, n=8), nicotine-control mice (0.292±0.015 mm, n=10), air-OVX mice (0.315±0.021 mm, n=8), and nicotine-OVX mice (0.293±0.013 mm, n=10). **C.** RV internal diameter during diastole (RVID;d) was, likewise, not significantly different between air-control (1.30±0.03 mm, n=8), nicotine-control (1.34±0.04 mm, n=10), air-OVX (1.23±0.05 mm, n=8), and nicotine-OVX (1.24±0.05 mm, n=10).

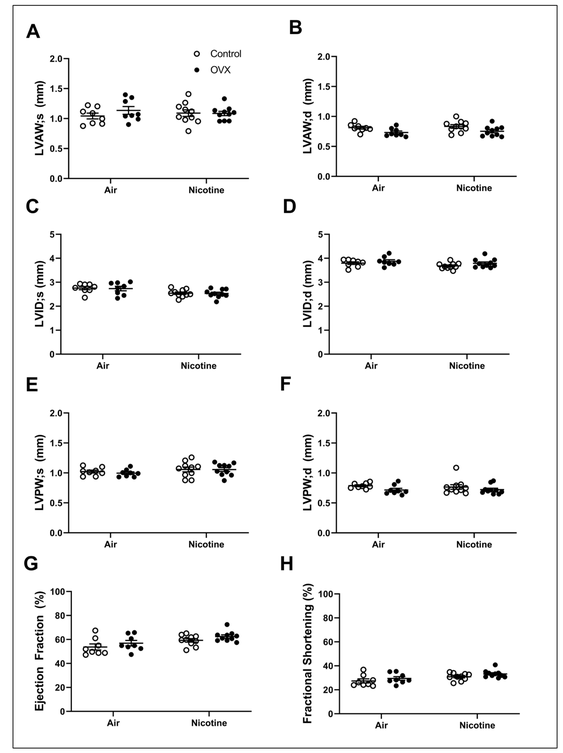

Figure 4. Nicotine and OVX do not affect left ventricular (LV) structure or function measured by echocardiography. **A-B.** LV anterior wall thickness at systole (LVAW;s) and diastole (LVAW;d). **C-D.** LV Internal diameter at systole (LVID;s) and diastole (LVID;d). **E-F.** LV posterior wall thickness at systole (LVPW;s) and diastole (LVPW;d). **G.** Ejection fraction (EF). **H.** Fractional shortening (FS).

Conclusions

- Chronic nicotine inhalation does not promote development of pulmonary hypertension (elevated RVSP) in intact or ovariectomized female mice.
- Female cardiopulmonary protection against nicotine-induced pulmonary hypertension and RV remodeling is not ovarian hormone mediated.

This work was funded by NIH NHLBI Grant #R01HL135635 (JG, XY, EL) and LSU Health Sciences Center, School of Medicine

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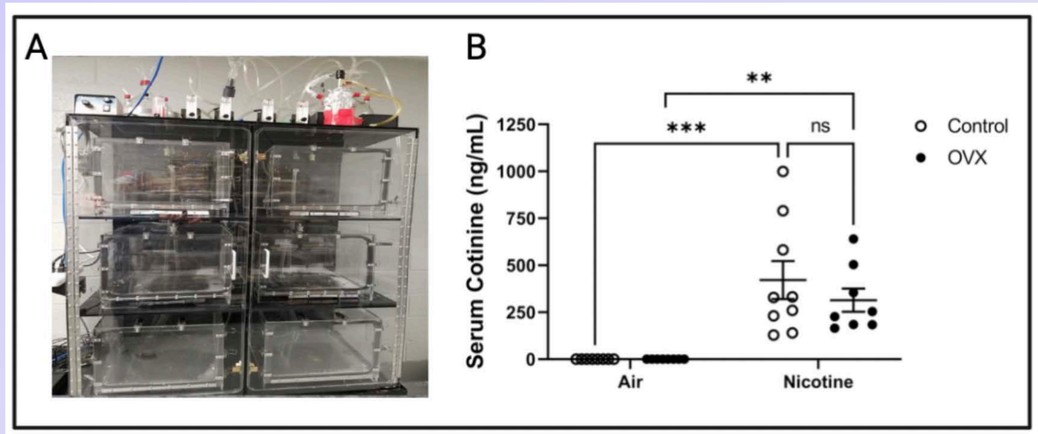

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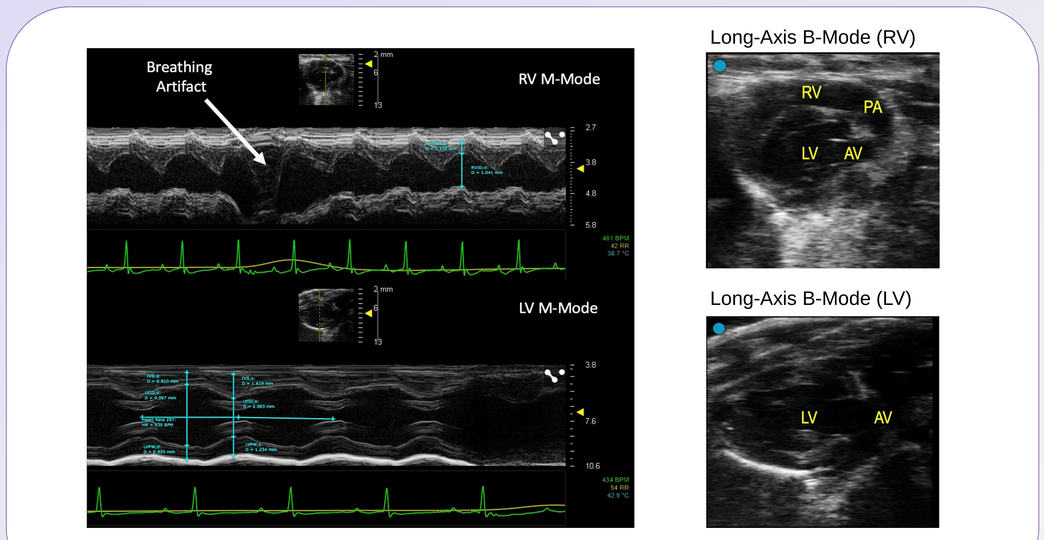

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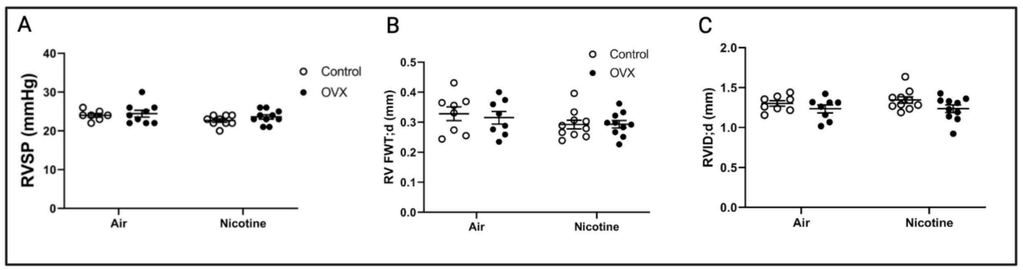

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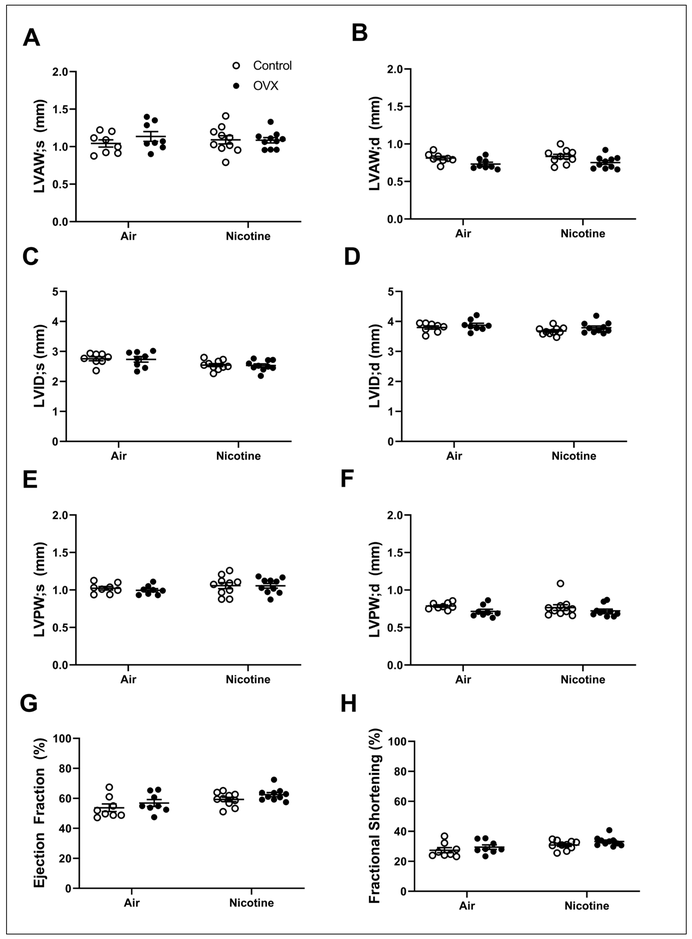

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