Study of the effects of chronic cadmium exposure on the pathogenesis of pulmonary arterial hypertension.

Dakotah Dominique Cathey

University of Louisville

Follow this and additional works at: https://ir.library.louisville.edu/etd

Part of the Pharmacology, Toxicology and Environmental Health Commons

Recommended Citation

This Master's Thesis is brought to you for free and open access by ThinkIR: The University of Louisville's Institutional Repository. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of ThinkIR: The University of Louisville's Institutional Repository. This title appears here courtesy of the author, who has retained all other copyrights. For more information, please contact thinkir@louisville.edu.
STUDY OF THE EFFECTS OF CHRONIC CADMIUM EXPOSURE ON THE PATHOGENESIS OF PULMONARY ARTERIAL HYPERTENSION

By
Dakotah Dominique Cathey
B.S., University of Louisville, 2020

A Thesis
Submitted to the Faculty of the
School of Medicine at the University of Louisville
In Partial Fulfillment of the Requirements
For the degree of

Master of Science in Pharmacology and Toxicology

Department of Pharmacology and Toxicology
University of Louisville
Louisville, Kentucky

December 2023
STUDY OF THE EFFECTS OF CHRONIC CADMIUM EXPOSURE ON THE PATHOGENESIS OF PULMONARY ARTERIAL HYPERTENSION

By
Dakotah Dominique Cathey
B.S., University of Louisville, 2020

Thesis Approved on
August 31, 2023

By the Following Thesis Committee:

Jiapeng Huang, M.D. Ph.D.

Lu Cai, M.D. Ph.D.

J. Calvin Kouckam, Ph.D.

Jamie Young, Ph.D.

Robert Buchanan, Ph.D.
DEDICATION

This thesis is dedicated to my mother, Stacy, along with my aunts, Tracy and Mary who have cheered me on through this journey with their love and guidance.
ACKNOWLEDGEMENTS

I would like to personally thanks my mentors Dr. Huang and Dr. Cai for continuing to push me past my personal limitations as well as their mentorship, kindness and understanding. I would also like to thank my other committee members, Dr. Kouokam and Dr. Buchanan for guiding me into continuing with my education after graduating with my bachelor’s degree, as well as Dr. Young for your generous friendship and counsel. Additionally, I would like to thank Dr. Jun Cai, Dr. Tan, Dr. Gozal, Dr. Monreal, Dr. Pantalos, and Dr. Rane for their support and guidance during this educational period. Lastly, I would like to thank Wendy, Zoe, Chunjie, Sandy, and Ahmed for their generous friendship and teaching me the multiple methods needed to complete this study as well as the University of Louisville Center for Integrative Environmental Health Sciences for funding this project.
We reported pulmonary artery hypertension (PAH patients had elevated levels of Cd in both blood and urine, therefore, this study tested whether Cd directly induces PAH or facilitates PAH pathogenesis in mouse models. C57/6J mice were initially exposed to drinking water with or without 5ppm Cd for 8 weeks. Then, half the mice in both control and Cd groups were given SU5416 and hypoxia (SuHx for 4 weeks to induce PAH, resulting in 4 subgroups: Control, Cd, PAH, and Cd+PAH. Diastolic and systolic functions of the left and right ventricles (LV, RV were examined with echocardiography before and after PAH. Eight-week Cd exposure did not significantly change RV structure or systolic function. However, 12-week Cd exposure significantly increased RV systolic function, exacerbated PAH-associated RV dysfunction, and significantly increased structural remodeling in LV, RV, and lungs, although did not further exacerbate RV and lung remodeling in the setting of PAH.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEDICATIONS</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>v</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>viii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>4</td>
</tr>
<tr>
<td>RESULTS</td>
<td>17</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>45</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>51</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>54</td>
</tr>
<tr>
<td>ABBREVIATIONS</td>
<td>56</td>
</tr>
<tr>
<td>CURRICULUM VITAE</td>
<td>58</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Overall Schematic of Project Protocol</td>
<td>05</td>
</tr>
<tr>
<td>2. Injection Station and Hypoxia Chamber</td>
<td>06</td>
</tr>
<tr>
<td>3. Vevo 2100 Machine and Mouse Supine Position</td>
<td>08</td>
</tr>
<tr>
<td>4. Echo Views</td>
<td>09</td>
</tr>
<tr>
<td>5. RVSP Catheter Schematic</td>
<td>10</td>
</tr>
<tr>
<td>6. Anatomy of a Pulmonary Artery</td>
<td>13</td>
</tr>
<tr>
<td>7. Nonmuscular vs Partial Muscular vs Fully Muscular</td>
<td>14</td>
</tr>
<tr>
<td>8. Medial Thickness and Wall Area</td>
<td>15</td>
</tr>
<tr>
<td>9. Effects of Cd exposure with or without PAH on body weight</td>
<td>18</td>
</tr>
<tr>
<td>10. Effect of 8 vs 12 weeks of Cd exposure with or without PAH on systolic function</td>
<td>20</td>
</tr>
<tr>
<td>11. Effects of 8 vs 12 weeks of Cd exposure with or without PAH on cardiac output</td>
<td>22</td>
</tr>
<tr>
<td>12. Effects of 8 vs 12 weeks of Cd exposure with or without PAH on RV morphology</td>
<td>24</td>
</tr>
<tr>
<td>13. 12 weeks of Cd exposure with or without PAH on RVSP</td>
<td>26</td>
</tr>
<tr>
<td>14. Effects of 12 weeks of Cd exposure on organ toxicity</td>
<td>28</td>
</tr>
<tr>
<td>15. H&amp;E staining of LV (A) and RV (B) myocardium</td>
<td>30</td>
</tr>
<tr>
<td>16. SR Staining of LV (A) and RV (B) myocardium. Quantification of CPA%</td>
<td>32-33</td>
</tr>
</tbody>
</table>
17. WGA Staining of LV (A) and RV (B) myocardium. Quantification of Cardiomyocyte Area (C) .................................................................35-36
18. H&E Staining of Lung Tissue.................................................................38
19. SR of Lung Tissue and Quantification of CPA%............................................40
20. IHC-αSMA Staining on Lung Tissue..........................................................42
21. Muscularization content, Medial Thickness, and Wall Area Percentage based on αSMA Quantification.........................................................43
INTRODUCTION

Pulmonary arterial hypertension (PAH) is a cardiovascular disease (CVD) defined as chronic high-pressure present within the pulmonary vasculature of the lungs [1-3]. This disease is mainly characterized by two distinct features, the muscularization of the pulmonary arteries, and right ventricle (RV) dysfunction [1-3]. Initially, the pulmonary arteries undergo vasoconstriction and reduce the amount of blood undergoing gas exchange in the lungs [4]. As a result, the RV will undergo initial hypertrophy to mediate the increase in blood pressure and push more blood into the lungs [5]. Over time however, the pulmonary arteries undergo vascular muscularization, a process in which the walls of the pulmonary arteries become stiff, i.e., muscularized, preventing expansion and contraction in response to blood pressure [1-5]. This muscularization process causes entire right heart to work harder to push more blood through the pulmonary artery and the lungs [5]. This increase in work will eventually lead to cor pulmonale, or right heart failure, due to the ventricle having trouble in keeping up demand [5]. Since the distinct characteristics of PAH are caused through a combination of mitochondrial dysfunction-mediated oxidative stress, inflammation, and chronic fibrosis, pinpointing the exact generating factor of this disease is difficult for both researchers and clinicians, as the characteristics listed above are hard to treat with the current medications [1-5].

According to the National Organization of Rare Diseases (NORD), an estimated one to two people per 1 million are diagnosed with PAH each year in the United States [6]. Once diagnosed, patients are generally given a combination of different medications including calcium channel blockers, diuretics, vasodilators, and in some extreme cases
l lung transplantation [5, 6]. For research and clinical purposes, patients are divided into three groups, low-, intermediate-, and high-risk based on clinical test results from the World Health Organization’s (WHO) classification system [7]. This includes directly measuring pulmonary arterial pressure (mPAP), determining a 6-minute walk distance (6MWD), and cardiac index (CI) to determine right heart function. We tend to see a difference in 1- to 3-year survival rates for each group differ significantly [7]. From the Journal of the American Heart Association (JAHA), the 1- and 3- year survival rates for low- intermediate- and high-risk patients range from 1%-7%, 7%-20%, and 12%-55%, respectively [8]. For that reason, we can see that even with current therapeutics, the overall survival rate of PAH patients remains low. PAH is currently thought to be induced through either genetic, epigenetic, or environmental factors. However, to determine overall PAH pathogenesis would be intricate and complicated. Therefore, we shifted our focus to preventable measures instead of overall disease treatment (RV dysfunction and vascular muscularization). This will ultimately be done through determining the potential relationship between environmental toxicity and PAH pathogenesis, specifically with heavy metal Cadmium (Cd).

Cd is a natural earth element prominently found in soils and rocks [9]. While usually extracted as a byproduct of other metals, Cd itself is commonly used for common everyday items like batteries, pigments, and coatings [9]. Due to common occupational practices like mining and refining, Cd can be released into the environment through water runoff and air pollutants [9, 10]. Human exposure to Cd can occur through a myriad of different ways including ingestion of a Cd contaminated diet or continuous inhalation near mining facilities [9]. Because of this, chronic exposure to Cd has been shown to cause irregularities within multiple organs, mainly the kidneys, liver, lungs, heart, and brain [11, 12].
Past research by the Centers of Disease Control and Prevention (CDC) have shown that blood and urine Cd usually reflects recent exposures, rather than the exposure level [13]. While the exposure could be either acute or chronic, chronic exposure is more likely the case [13]. Furthermore, the agency reports that both blood and urine Cd levels within humans increase linearly with age, therefore it should be biomonitored more strictly by physicians and public health officials as it could cause hypertension, pneumonitis, and cancers [13, 14]. Since human exposure to Cd has been increasing in prevalence, knowing the potential harm that the heavy metal causes in generating adverse health effects is crucial in potential treatment. Previous clinical studies from our laboratory have shown that patients diagnosed with PAH had increased levels of Cd in both blood and urine samples. Therefore, this study aims to study the effects of Cd in the pathogenesis of PAH and RV dysfunction.
MATERIALS AND METHODS

Animal Model:

A total of 40 male C57/6J black mice at 8 weeks of age were purchased from the Jackson Laboratory (Bar Harbor, Maine). For two weeks, all mice were initially accommodated at the University of Louisville Donald E. Baxter I Research Center, housed at a continuous 22°C with a 12-hour:12-hour light-dark cycle, where the mice had access to standard rodent chow and tap water. After initial accommodation, the mice were then randomly separated into two groups, those who received tap water (n=20) and those who received 5ppm Cd water (n=20) (Figure 1). This setup continued for approximately 8 weeks until an initial transthoracic echocardiography (echo) was performed (Week 0) [2, 3].

After initial echo (Week 0), PAH was induced in 10 randomized control and 10 Cd treated mice, making the final group total to four: CTRL (n=10), Cd CTRL (n=10), SuHx (n=10), and Cd-SuHx (n=10). PAH induction was conducted through a combination of a weekly subcutaneous (s.c.) injection of Semaxinib [Sugen 5416 (SU5416)], a vascular endothelial growth factor receptor (VEGFR) inhibitor that prevents the process of angiogenesis, accompanied by a 4-week exposure of hypoxia (10% oxygen) in a commercially designed chamber (Oxycycler model A4XO, Biospherix, Redfield, NY, USA). Non-PAH induced mice had a vehicle s.c. injection and placed in a normoxia chamber respectively (Figure 2). This setup continued for four weeks, with echo being performed on Week 4. After week 4, 28 mice (n=7/group) were euthanized, and heart and lung
tissue was collected. For week 5, 12 mice (n=3/group) had invasive right ventricular systolic pressure measurements performed on them using a pressure-tipped catheter. After mice were euthanized, heart and lung tissue were collected and used for histological and biochemical analyses (Figures 1 and 2).

Figure 1

**Figure 1: Overall Protocol Schematics.** Adult C57/6J Black Mice were initially separated into two groups, those with regular tap water (n=20) and those with 5ppm Cd water (n=20) for 8 weeks before transthoracic echocardiography (Week 0). Ten mice from both control and Cd groups had PAH induced creating 4 groups, CTRL, Cd CTRL, SuHx, and Cd-SuHx. Echo was performed again at Week 4, where after last echo 28 mice (n=7/group) were euthanized and the remaining 12 mice (n=3/group) underwent RV invasive pressure measurements.
Figure 2

Figure 2: Injection Station for SU5416 or Vehicle and Hypoxia Chamber. The above photos showed the platforms where injections of either SU5416 or the vehicle was performed (A, B) and the normoxia/hypoxia chambers respectively (C). Mice were anesthetized with a mixture of isoflurane and oxygen and then placed into the nose cone and containment platform. Mice were then given 20mg/kg SU5416 or vehicle injection to their respective body weights subcutaneously. After injection, mice were then placed into the normoxia/hypoxia chamber for the rest of the duration of the study.
Transthoracic Echocardiography:

To measure heart function, transthoracic echocardiography (echo) was performed. By using the VEVO 2100 Imaging System FUJIFILM Visual Sonics (Toronto, ON, Canada), multiple heart images like the parasternal long axis (PLAX), parasternal short axis (PAS), aortic valve short axis (AVSAX), and apical short chamber (A4C) were obtained to determine both diastolic and systolic function [2, 3] (Figures 3 and 4). During the echo process, mice were anesthetized with a combination of isoflurane and oxygen (0.5-1.5% isoflurane in oxygen at a rate of 1L/min) and placed in supine position on a platform with their paws placed down such that the machine can measure heart rate, breathing rate, and obtain an EKG for proper monitorization. Body weight was obtained and the hair over the chest was then removed using the hair removal cream Nair (Ewing, New Jersey). Echo was performed by two trained echocardiographers averaging about 15-20 minutes per mouse to limit the exposure of anesthetics. Echo was initially performed before PAH induction for baseline measurements after 8 weeks of Cd exposure (week 0), then performed again 4 weeks (week 4) post PAH induction [2, 3] (Figure 3).
Figure 3: VEVO 2100 Visual Sonics Imaging Transthoracic Echocardiography

Machine (left) and Mouse Position on Platform (right). Ultrasound machine that is used to perform transthoracic echocardiography to measure cardiac function of the mice in real time. Transducers on the left have gel placed on them and then placed on the hairless chests of anesthetized mice (right) to view images that pop up on the screen. These images help determine the extent of both diastolic and systolic function.
Figure 4: Echo Views of Parasternal Long Axis, Parasternal Short Axis, and Apical 4 Chamber. These 4 views were used to determine common PAH measurements such as cardiac output, systolic function, and RVFW thickness. All 40 mice had echo measurements performed for week 0 and week 4.
RV Invasive Pressure Measurement:

The pressure of the right ventricle was measured at week 5 to determine not only whether the PAH induction model succeeded, but to evaluate any changes associated with Cd exposure with and without PAH. Mice were anesthetized with a mixture of isoflurane and oxygen and placed into a supine position. Assuming there is no pulmonic stenosis, RVSP is equal to pulmonary arterial pressure. The hair was removed from their chests using Nair, and then a small incision was made over the right jugular vein. The right jugular vein is then isolated and ligated, where the pressurized-tipped Scisense catheter by Transonic (London, ON, Canada) is placed into the right atrium and the RV (Figure 5). Once in the RV, the catheter was zeroed to atmospheric pressure and RV pressure was then measured for around 30 measurements at steady state. LabChart Pro Software (AD Instruments, Colorado Springs, CO) was used to quantify RVSP [2, 3].

Figure 5

Figure 5: A schematic of the Transonic Pressurized-Tip Catheter measuring RV pressure. Image made on the Biorender website.
**Histology:**

Portions of both heart and lung tissue were initially fixed with 10% formalin for 72hrs before dehydration using increasing gradients of alcohol and xylene (Citrisolve, Thermo Fisher Scientific, Waltham, MA), and paraffin embedded. Embedded sections were cut at 5µm, mounted on glass slides, and placed in a tissue oven overnight. When staining was performed, sections were initially deparaffinized through two 5-minute soaks in xylene and rehydrated through washes of decreasing grades of ethanol. After general staining, the slides were then dehydrated using increasing grades of ethanol washes, placed in two different soaks of xylene for 5-minutes each, then mounted with mounting solution (SurgiPath Sub-X Mounting Medium, Leica, Richmond, IL) and cover slipped (Hematoxylin & Eosin, Sirius Red, and Immunohistochemistry). The other processing method had the slides washed with PBS, then mounted with 4', 6-diamidino-2-phenylindole (DAPI) nuclear staining and then cover slipped (Wheat Germ Agglutinin) due to the fluorescent nature of the stain.

A. Hematoxylin and Eosin (H&E) Staining was used for determining basic structural differences between the heart and lung tissue of the four groups respectively [2].

B. Picrosirius Red (SR) (CP Lab Chemicals, Novato, CA) Staining was used for determining the collagen content of tissue samples. Multiple images of 50µm scale were produced for both heart and lung tissues. Images were analyzed using ImageJ Software (National Institutes of Health, Bethesda, MD) where an upper and lower threshold containing the total tissue area and just the positive collagen-stained area was obtained. After performing this task for all obtained tissue images, the sum of the total stained area and the sum of the positive collagen-stained area was calculated [15]. To
determine the collagen proportional area (CPA%) the following equation was used:

\[
CPA\% = \frac{\sum \text{Collagen Stained Area}}{\sum \text{Total Stained Area}} \times 100\%
\]

C. Wheat Germ Agglutinin (WGA) (Thermo Fischer Scientific, Waltham, MA) Staining was used to determine cardiomyocyte area. WGA is a lectin that specifically binds to the glycoproteins of the sarcolemma within the cell membranes of both skeletal and cardiac muscle. For this purpose, WGA can determine whether cardiomyocytes undergo hypertrophy. Multiple images of 20µm scale were obtained of both the LV and RV of the heart. Next, images were uploaded to ImageJ software and the scale was globalized for all respective images, such that the area of the cells were normalized to scale. Then using the freehand tool on the ImageJ software, the outer cell membrane of the cardiomyocytes was traced and measured for their respective areas. After measuring >10 random cells for each image, the areas were then averaged out for each LV and RV respectively [16, 17]. The overall fold of control was determined for all subsequent groups, to determine the change between the control group and the others respectively [2].

D. Immunohistochemistry (IHC) Staining was used to understand changes within the vascular system of pulmonary arteries (PA). All blood vessels contain three specific layers, the tunica intima (TI), the tunica media (TM), and the tunica adventitia (TA) (Figure 6). The tunica intima consists of vascular endothelial cells that line the lumen of the blood vessel. The tunica media, consists of α-smooth muscle cells which provides archaeal support and allows the blood vessel to undergo vasoconstriction or
vasodilation in response to blood pressure changes. Finally, the tunica adventitia consists of connective tissue which gives overall basal support to the blood vessels. Vascular muscularization is a process where the pulmonary artery can’t respond to changes in lung pressure due to the increased stiffness of the pulmonary artery. Therefore, to determine the effects of Cd toxicity on PAH, α smooth muscle cells of the tunica media which is the main portion that undergo the process of muscularization in PAH, was stained with via α-Smooth Muscle Actin (αSMA) (Abcam AB5694, Waltham, MA).

**Figure 6**

*Figure 6: Anatomy of a Pulmonary Artery.*
Image made with Biorender.
I. Pulmonary Vascular Muscularization was determined by locating and identifying PAs. PAs were placed into either one of three categories, nonmuscular (NM), partially muscular (PM), or fully muscular (FM) [18] (Figure 7). NM PA were defined as having very little to no positive αSMA staining, meaning that no remodeling occurred within the TM of the PA. PM PA were defined as having some positive αSMA staining, meaning that the PA is beginning to undergo the process of muscularization [18] (Figure 7). Finally, FM PA were defined as having full αSMA staining, meaning that the entire TM is positively stained, making gas exchange harder task perform [18] (Figure 7). Once classified, the sum of each NM, PM, and FM PA per mouse was totaled and compared to determine the changes in muscularity.

Figure 7

Figure 7: Classification of nonmuscular (NM), partially muscular (PM) and fully muscularized (FM) pulmonary arteries. Image made with Biorender.
II. Pulmonary Vascular Assessment was determined through the following two calculations:

i. Medial Thickness: The measurement of the thickness of the tunica intima and tunica media (Figure 8A). As stated above, both the tunica intima and the tunica media undergo vascular muscularization, thus, to determine how much muscularization occurs, the thickness should be measured using the following equation in both the x and y directions then taking the average of the two measurements [18, 19]:

\[
\text{Medial Thickness (MT\%)} = \frac{(\text{External diameter} - \text{Inner Diameter})}{\text{Outer Diameter}} \times 100\%
\]

ii. Wall Area: The measurement of the TI and TM area relative to the overall arterial area [18, 19] (Figure 8B).

\[
\text{Wall Area (WA\%)} = \frac{\text{Medial Wall Area}}{\text{Total Arterial Area}} \times 100\%
\]

Figure 8: Measurements of medial thickness (A) and wall area (B). Image made in Biorender.
Statistics:

Experiments in this study were conducted with 12 mice for echo assays, 3 mice for RVSP, and up to 7 mice for all histopathological studies. Experimental results were given at mean ± SD. Analyses was performed using GraphPad Prism 8 Software for Windows. Normality was checked: and when non-normal, the data was either log transformed, or the nonparametric equivalent test was performed. For echo analysis, a 2-Way ANOVA was performed with a Tukey correction for significance. For staining analysis, a One-Way ANOVA with Tukey comparison was performed to compare all groups, followed by an unpaired student t-test. Statistical significance was defined as a p-value ≤ 0.05.
RESULTS

Effect of Cd on body weight with or without PAH.

Over the duration of the study, the body weights of the animals were recorded weekly. Their body weights shifted based on their exposure to Cd and whether PAH was induced. Both the CTRL and Cd CTRL groups showed a steady increase in body weight, with the Cd exposed mice weighing significantly more than the CTRL mice respectively. However, the SuHx mice lost weight following the first week of disease induction which stayed constant for the rest of the study (Figure 9). There were no significant differences between the PAH and Cd-PAH groups (Figure 9).
Figure 9: Effects of Cd with or without PAH on Body Weight. Body weight of the Cd and CTRL mice steadily increased over the course of the study, while the SuHx and Cd-SuHx groups lost weight at week 1 which stayed constant (n=10 for all groups totaling 40). *, **, and *** Indicates a significant difference (p<0.05, p≤0.001, and p≤0.0001 respectively) between the control. +, ++, and +++ indicate a significant difference (p<0.05, p≤0.001, and p≤0.0001 respectively) between the Cd CTRL. #, ##, and #### indicates a significant difference (p<0.05, p≤0.001, and p≤0.0001 respectively) between the SuHx. &, &&, &&& indicates a significant difference (p<0.05, p≤0.001, and p≤0.0001 respectively) between the Cd-SuHx. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
Effect of Cd on RV systolic dysfunction with and without PAH.

The Tricuspid Annular Plane Systolic Excursion (TAPSE) is an echo measurement of how much the tricuspid annulus moves during systole [20]. This measurement was done to determine RV systolic function, which is responsible for pushing blood to the lungs [20]. Eight weeks of chronic Cd exposure had no significant effect on the TAPSE values; however, 12 weeks of chronic Cd exposure caused a significant increase (p=0.0287) in TAPSE value when compared to controls (Figure 10). For both SuHx and Cd-SuHx groups, TAPSE values decreased when compared to the CTRL (p=0.0018 and p=0.0027, respectively), however, Cd did not further worsen TAPSE in PAH mice (Figure 10).
**Figure 10:** Effects of Cd on RV Systolic Function with or without PAH. 8-weeks of chronic Cd exposure did not affect RV systolic function, however, 12-weeks of chronic Cd exposure and SuHx cause significantly increased TAPSE values. Cd exposure alone caused significantly increased TAPSE values. TAPSE values significantly decreased with PAH induction and Cd exposure did not further worsen TAPSE in the context of PAH group (n=6 per group at Week 0 and n=3 per group at Week 4). * and ** Indicate a significant difference (p<0.05 and p≤0.001 respectively) between the control, ns indicates that there was no significant difference between the non-Cd vs Cd exposed week 0 groups and the SuHx vs Cd-SuHx week 4 groups respectively. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
The Right Ventricular Outflow Tract Velocity Time Interval (RVOT VTI) is an echo measurement that denotes the distance that blood travels after one heartbeat from the RV, through the pulmonary artery, to the lungs [20]. Cardiac output, or the distance of blood that is pushed out of the heart per one beat, is a common measurement of how effectively the RV is beating [20]. Neither 8 nor 12 weeks of chronic Cd exposure alone significantly effected cardiac output when compared to the controls (Figure 11). Both SuHx and Cd-SuHx both had decreased RVOT VTI when compared to the CTRL with p-values of 0.0046 and 0.0012 respectively (Figure 11). Furthermore, Cd caused worse RV cardiac output in the setting of PAH as the Cd-SuHx group had lower RV cardiac output when compared to the SuHx group with a significance of p=0.016 (Figure 11).
**Figure 11:** Effects of Cd on cardiac output with or without PAH. Neither 8 nor 12 weeks of chronic Cd exposure alone affected RV cardiac output significantly when compared to the controls. Both SuHx and Cd-SuHx had decreased RVOT VTI when compared to the CTRL. Cd caused worse RV cardiac output in the setting of PAH as the Cd-SuHx group had lower RV cardiac output when compared to the SuHx group (n=6 per group at week 0 and n=3 per group at week 4). ** Indicates a significant difference (p<0.05 and p≤0.001 respectively) between the control. # indicates a significant difference (p<0.05) between SuHx vs Cd-SuHx. ns indicates that there was no significant difference between the non-Cd vs Cd exposed week 0 groups. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
Effects of Cd on RV hypertrophy with or without PAH.

The RV undergoes hypertrophy and remodeling due to the increase of pulmonary arterial pressures. Right Ventricle Free Wall (RVFW) Thickness is an echo measurement used to measure the thickness of the RV [2, 3, 20]. Neither 8 or 12 weeks of chronic Cd exposure caused any significant differences in RV hypertrophy when compared to the CTRL, whilst SuHx significantly caused RV hypertrophy when compared to the controls (p=0.0039 for SuHx and p=0.0006 for Cd-SuHx) (Figure 12). However, there was no significant difference between the SuHx and Cd-SuHx groups indicating Cd exposure did not worsen RV hypertrophy in the setting of PAH, respectively (Figure 12).
Figure 12: Cd exposure did not cause RV hypertrophy and did not worsen RV hypertrophy in PAH. Neither 8 nor 12 weeks of chronic Cd exposure alone affected RVFW thickness significantly when compared to the control. Both SuHx and Cd-SuHx had increased RVFW thickness when compared to the CTRL. Cd did not cause exacerbate RVFW thickness when compared to the SuHx group (n=6 per group at week 0 and n=3 per group at week 4). ** and ***Indicates a significant difference (p<0.01 and p≤0.001 respectively) between the control. ns indicates that there was no significant difference between the non-Cd vs Cd exposed week 0 groups. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
Effects of Cd on Right Ventricular Pressure with or without PAH.

Right Ventricular Systolic Pressure (RVSP) is a measurement of the RV to determine whether mice developed pulmonary arterial pressure like PAH mice. After 12 weeks of chronic Cd exposure, there was no significant difference in RV pressure when compared to the CTRL (Figure 13). The SuHx mice had an increase in RVSP (p=0.034) when compared to the CTRL, indicating the SuHx mice developed an increase in RV pressure and PAH (Figure 13). The Cd-SuHx group had significantly decreased RVSP when compared to the SuHx group (p=0.034), indicative of heart failure (Figure 13).
Figure 13: Effects of on RVSP Cd with or without PAH. Chronic Cd exposure did not affect RVSP when compared to the CTRL. SuHx mice developed PAH as indicated by the significant increase of RVSP whilst Cd exposure significantly decreased RVSP in the setting of PAH (n=3 per group). * Indicates a significant difference (p<0.05) with the control. ### indicates a significant difference (p<0.001) with SuHx. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
**Effects of Cd on Organ Toxicity with or without PAH.**

The organ weight-to-body weight ratio is a common technique used to determine organ toxicity within the body of animal studies. After euthanizing 7 mice per group, the heart and lungs of each mouse was weighed. Twelve weeks of Cd alone did not affect heart or lung weights (Figure 14). However, SuHx induction caused significantly increased heart and lung weights, indicating changes occurring within the body potentially on a macro and micro level (Figure 14). Cd-SuHx did not cause any significant changes in heart and lung weights when compared to SuHx (Figure 14).
Figure 14: Cd exposure did not further increase heart/lung weight-to-body weight ratio in the setting of PAH. Cd exposure alone did not increase heart and lung weights. PAH induction significantly increased the heart/lung weight-to-body-weight ratio (n=7 per group). ** and *** Indicates a significance (p-value<0.01 and p<0.0001) compared to the control. ns indicate no significance between the CTRL vs Cd CTRL and SuHx vs Cd-SuHx respectively. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
Effects of Cd on cardiac morphology with or without PAH: LV and RV

After confirming through the echo data that there were changes to heart function through Cd exposure and SuHx, determining morphological changes through a H&E stain was the next step in this study. For the LV, cardiomyocyte disorganization appears in the Cd exposed groups (Cd CTRL and Cd-SuHx) while the non-Cd groups which didn’t contain any cardiomyocyte disorganization (CTRL and SuHx) (n=3 per group) (Figure 15A). For the RV, 12 weeks of chronic Cd (n=3) significantly increased cardiomyocyte disorganization of the RV. Furthermore, the Cd-SuHx (n=3) mice had more cardiomyocyte disorganization (Figure 15B) when compared to the SuHx, indicating that Cd may exacerbate RV remodeling.
Figure 15: Hematoxylin and Eosin (H&E) stain on the LV (A) and RV (B). Effects of chronic Cd exposure with and without PAH on both the morphology of the LV and RV. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
**Effects of Cd on cardiac collagen content with or without PAH: LV and RV**

After determining that chronic Cd exposure increased disorganization of both the LV and RV, we studied potential remodeling and fibrosis. Collagen is an indicator of tissue remodeling; thus, a SR staining was performed [15]. Results show an increase in collagen fibers present within the LV of the Cd exposed groups (Cd CTRL and Cd-SuHx, n=3 per group), which wasn’t the case with the non-exposed groups (CTRL and SuHx n=3 per group) respectively (Figure 16A). For the RV, Cd with or without SuHx, along with the SuHx group, had increased positively stained collagen fibers when compared to the CTRL (n=3 per group) (Figure 16B).

Quantification of the SR staining via the collagen proportional area percentage (CPA%) corroborated these results. Cd exposed groups (Cd CTRL and Cd-SuHx) had significantly increased collagen content within both the LV (p=0.0013 and p=0.01) and RV (p=0.0043 and p=0.0016) when compared to the CTRL respectively (Figure 16C). However, SuHx alone did not increase collagen content within the LV but did increase collagen content within the RV when compared to the CTRL (p=0.0062), representative of the RV inflammation and dysfunction of the disease without LV impact in these PAH mice (Figure 16C). There was no difference between the RV of the SuHx and Cd-SuHx group in term of collagen content, however, the LV was significant (p=0.0028).
Figure 16: Picrosirius red images of the LV (A), RV (B), and quantification (C) of collagen content. The representative images showed an increase in collagen content within the LV of Cd exposed groups (A), an increase in collagen content of the RV within both Cd-exposed groups and SuHx mice (B), and the quantification of those images (C) (n=3 per group). ** and *** Indicates a significance (p-value<0.01 and p<0.001) compared to the control. # indicates significance (p-value<0.05) compared to SuHx. ns indicate no significance between the CTRL vs Cd CTRL and SuHx vs Cd-SuHx respectively. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
Effects of Cd on cardiac hypertrophy with or without PAH: LV and RV

Since collagen increase is a fibrotic response due to chronic inflammation, determining whether there was any hypertrophy within the heart from Cd and PAH would be beneficial [2, 3, 21]. WGA staining was performed, where cardiomyocytes were fluorescently outlined and measured to determine the extent of hypertrophy. In relation to the CTRL, Cd exposure alone caused significantly increased LV cardiomyocyte area (p=0.0466 Figure 17A), while not affecting the RV (Figure 17B). SuHx group demonstrated significant RV hypertrophy when compared to controls (Figure 17B), while Cd exposure in addition to PAH did not further exacerbate RV hypertrophy (Figure 17B). There was a non-statistically significant trend of decreased LV and RV hypertrophy with Cd in the setting of PAH (Figure 17C). This could indicate maladaptive heart failure in these mice.
Figure 17

A

CTRL

Cd CTRL

SuHx

Cd-SuHx

B

CTRL

Cd CTRL

SuHx

Cd-SuHx
Figure 17: Representative WGA staining of the LV (A), RV (B), and quantification (C). Cd increased the cardiomyocyte area of the LV but did not affect the RV cardiomyocyte area. SuHx groups demonstrated significant RV hypertrophy when compared to the controls, while Cd exposure in addition to PAH induction did not further worsen RV hypertrophy (n=3-6). ** and *** Indicates a significance (p-value<0.01 and p<0.001) compared to the control. # indicates significance (p-value<0.05) compared to SuHx. ns indicate no significance between the CTRL vs Cd CTRL and SuHx vs Cd-SuHx respectively. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
Effects of Cd on lung morphology with or without PAH.

As stated in the introduction, in PAH the heart reacts to the changes within the PAs within the lungs. Since it was concluded that collagen content and hypertrophy increased due to Cd exposure, it would be reasonable to determine the extent of lung morphological changes with Cd exposure. Bronchiole complexes are structures within the lungs which contain three main components of gas exchange: bronchioles, pulmonary arteries and arterioles, and alveolar sacs [18, 22, 23]. Therefore, focusing on these aspects of lung biology was crucial in determining the extent of morphological changes that was occurring within the lungs. By performing an H&E stain, results showed that the size of the alveolar sacs, PA lumens, and the appearance of cell clusters begin to appear over the course of the experiments. In the CTRL group, we see large, open alveolar sacs, large open PA’s, and very few cell clusters (Figure 18). This changed with the Cd CTRL group, where clustering appears, as well as the reduction of alveolar sacs and PA lumens (Figure 18). The SuHx groups had even less spaces within the alveolar sacs, as well as narrowing of the PA lumens and multiple clusters appearing (Figure 18). These are indications that inflammation and remodeling is occurring in the lungs.
Figure 18

**Figure 18: H&E staining of lung tissues focusing on bronchiole complexes.** The representative images showed lung tissues stained with H&E as per the Materials and Methods Section. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
**Effect of Cd on collagen content with or without PAH: Lungs**

With the results from the H&E staining hinting at inflammation and remodeling, next was to determine the fibrotic effects via measuring the collagen contents of the lungs with SR staining. The amount of positively stained collagen content surrounding the bronchiole and PA was minimal in the CTRL group. However, the amount of positively stained collagen increased around the PA in the Cd CTRL, SuHx, and Cd-SuHx groups (Figure 19A). Interestingly, more positively stained collagen was present around the bronchioles themselves in the SuHx groups, hinting that remodeling was not only occurring at the PAs but also at the bronchioles (Figure 19A).

After imaging, quantification was performed to determine the CPA%. Cd exposure increased the amount of collagen content within the lungs by almost 20% in relation to the CTRL group (p=0.0055), while the SuHx and Cd-SuHx group increased almost 30% (p=0.0013) and 20% (p=0.004) respectively (Figure 19B). Interestingly, the Cd-SuHx group contained 10% less collagen compared to the SuHx group (p=0.05) (Figure 19B).
Figure 19: Representative images of Sirius Red Staining of Lung Tissue (A) and its quantification via the Collagen Proportional Area Percentage (CPA%) (B). The representative image showed the collagen content for each representative group (A) and the quantification of the collagen content (B) (n=3-6). ** and *** Indicates a significance (p<0.01 and p<0.001) compared to the control. # indicates significance (p-value<0.05) compared to SuHx. ns indicate no significance between the CTRL vs Cd CTRL and SuHx vs Cd-SuHx respectively.
Effects of Cd on pulmonary vascular muscularization with or without PAH.

To determine whether the pulmonary vasculature was affected by Cd toxicity and SuHx, PA muscularity, medial thickness, and wall area were quantified. To determine the extent of vascular muscularization IHC staining with αSMA was performed. After performing the initial staining, any changes within the PAs were studied. The CTRL having minimal muscularized PA’s, however Cd showed partially muscularized PAs (Figure 20). SuHx increased partial and full muscularization of PAs, and Cd-SuHx also demonstrated increased PA abnormalities (Figure 20).

After obtaining the images, PAs were classified as nonmuscular (NM), partial(ly) muscular (PM), or fully muscular (FM) [18], and measured to determine their medial thickness’ and wall area’s [18, 19, 24]. The CTRL group had about 60-80% of NM, 10-15% PM, and 5-10% FM PA’s, with low medial thickness and wall area at around 20% (Figure 21). When mice were exposed to Cd, the muscularization process increased, with NM PA’s decreasing from the CTRL by approximately 40%, increased PM PAs to 20-70%, and FM counts to 5-35% (Figure 21). Both the medial thickness and wall areas of the Cd CTRL group increased significantly in relation to the CTRL group, to approximately 70% for each with a p<0.0001 for the medial thickness and p=0.0049 for wall area respectively (Figure 21). SuHx mice had NM, PM, and FM PAs ranging from 20-40% each, with increased medial thickness ranging from 50-60% (p=0.0043) and wall area ranging from 60-70% (p<0.001) relative to the CTRL group (Figure 21). Cd-SuHx caused more PAs to undergo muscularization with less NM and PM PAs at 20% and 30% respectively, while significantly increasing the number of FM PA’s at almost 50% (Figure 21). While the medial thickness and wall area of Cd-SuHx mice increased significantly relative to the CTRL (p<0.001 and p=0.004), Cd-SuHx did not cause any additional significant
changes in neither medial thickness or wall area when compared to SuHx with a similar range of 40-50% for medial thickness and 50-60% for wall area (Figure 21).

**Figure 20**

*Figure 20: Representative images of bronchiole complexes with positively stained* α*-Smooth Muscle Actin within pulmonary arteries.* Effects of Cd on vascular muscularization with or without PAH. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.*
Figure 21: Muscularization severities based on nonmuscular (NM), partially muscular (PM), or fully muscular (FM) pulmonary arteries and the quantification of medial thickness and wall area of pulmonary arteries in bronchiole complexes reflective of Cd toxicity and PAH induction. Cd exposure and PAH induction significantly decreased the number of normal PAs and increased medial thickness and wall area (n=4-6). *, **, and *** Indicates a significance (p<0.05, p<0.01, and p<0.001) compared to the control. # indicates significance (p-value<0.05) compared to SuHx. ns
indicate no significance between SuHx vs Cd-SuHx respectively. CTRL=control, Cd CTRL = Cd control, SuHx = SU5416+Hypoxia, and Cd-SuHx = Cd+SU5416+Hypoxia.
DISCUSSION

The pathogenesis of PAH is largely unknown and possibly linked to a multitude of biological factors including genetics, epigenetics, environmental exposure, mitochondrial dysfunction-mediated oxidative stress, essential metals dyshomeostasis, chronic inflammation, and fibrosis [2, 5, 6, 25]. Although there are therapeutics on the market to lessen the symptoms of PAH such as calcium channel blockers and vasodilators, preventing the disease is the best course of action since the mortality of PAH continues to be high [6]. Thus, the need of finding novel targets for therapeutics to increase the survival rate of PAH patients is of utmost importance.

Currently, factors like chronic exposure to heavy metals are not understood as much as they should be. Continuous exposure to heavy metals such as Cd, is becoming a more frequent problem worldwide [11, 12, 26]. Due to the onset of global climate change and jobs within the construction industry, Cd is entering the environment at an alarming rate [11, 12, 26]. While those who work these types of jobs have some personal protective equipment (PPE) to protect themselves from the harmful effects of Cd and other heavy metals, the runoff from those jobs is entering waterways and soil, where plants and animals become exposed to and absorb these metals [27].

Previous studies have shown that there is a link between Cd toxicity and CVDs [28, 29]. Young et al found that a combination of obesity and Cd exposure disrupts essential metal homeostasis potentially leading to diabetic and obesity related cardiovascular pathologies [28]. A review article published about Cd exposure and CVDs by Messner and Bernhard reference publications that proved Cd exposure specifically targets
endothelial cells of the vascular system and promote foam cell formation, initiating atherosclerosis [29]. Since heavy metal toxicity has been known to cause increased oxidative stress and essential metal dyshomeostasis, alongside Cd itself being known to target the endothelial cells within the vascular system and even cause hypertension, it isn’t so far fetched to think that it could be a potential environmental agent causing the pathogenesis of PAH.

Clinical studies performed in our laboratory have showed that PAH patients had increased levels of Cd in both blood and urine samples [28-30]. Results from these studies concluded that Cd levels were approximately 5 times higher in whole blood samples and nearly 9 times higher in urine levels. Thus, with these findings we decided to investigate whether (1) chronic Cd exposure can induce pulmonary and RV changes that mimic PAH and that (2) whether chronic Cd exposure can worsen PAH when PAH is induced.

All mice survived the overall duration of the experiment. After 8 weeks of Cd exposure, the mice had increased body weights compared to the CTRL group. Once PAH was induced in Week 1, the body weights of those mice significantly decreased in relation to the CTRL group which continued throughout the duration of the study for SuHx groups. While previous literature has shown that Cd toxicity causes a decrease in body weight [31], other studies have claimed that Cd and other heavy metals could cause weight gain based on the different dosages in humans [32]. PAH induction causing decreased body weight corroborates previously published studies [2, 3] hinting the first signs of disease.

In general, 8 weeks of Cd exposure did not cause any significant changes to RV function or morphology such as systolic function, cardiac output, or RVFW thickness. Twelve weeks Cd exposure didn’t cause any significant differences in cardiac output or
RVFW thickness, nor did 12 weeks Cd exposure alone cause any significant differences in RVSP in relation to the CTRL. However, 12 weeks of Cd exposure did cause increased RV systolic function (TAPSE), indicating that Cd could potentially cause systolic stimulation. These results could indicate that 12 weeks of 5ppm Cd exposure might not be enough to cause significant LV or RV functional changes, and it could potentially take a longer exposure time or higher dosage for differences to further initiate.

PAH induction caused decreased systolic function, cardiac output, RVFW thickness, and RVSP in relation to the CTRL group as expected. All these factors are common patterns of the disease, proving that the SU5416+10% hypoxia model worked and mimicked disease conditions within the mice as past literature noted [2, 3].

Interestingly, 12 weeks of Cd exposure in the setting of PAH resulted in further reduction of RV cardiac output and RVSP when compared to PAH alone. In response to PAH, the RV usually develops adaptive responses like initial hypertrophy to pump more effectively. Over time, the RV is not able to adapt to PAH and develops maladaptive responses to PAH by showing reduced cardiac output and lower RVSP due to the inability to generate high PA pressures anymore. Our results indicated Cd exposure in the setting of PAH induction could exacerbate or fasten the development of maladaptive responses and heart failure. While the general literature regarding Cd’s effect on heart function are controversial, several published studies have demonstrated that Cd could impede heart functionality [33].

Since echo is a physiological assessment of heart function, Cd toxicity could potentially be occurring on a biochemical level instead of a physiological level. Therefore, we measured the weights of the hearts and lungs to estimate the effects of Cd on the organs.
Changes in organ weight can indicate toxicity due to factors such as proliferation, cell death, and fibrosis. Cd alone didn’t cause significant differences in organ weights when compared to the CTRL. However, the SuHx groups showed a significant increase in the lung/heart weight-to-body weight suggesting that the weights of both the hearts and lungs were increased due to disease progression. There was no significant difference between the SuHx and the Cd-SuHx groups respectively.

Cd alone and in the setting of PAH caused LV cardiomyocytes to become disordered which was not seen in the CTRL or SuHx mice. For the RV, PAH induction caused cardiomyocyte disorganization as reflective of the disease [2]. Cd alone and in the setting of PAH also caused RV cardiomyocyte disorganization indicating the Cd might cause the RV to undergo remodeling. While past publications have specifically focused on the LV [29, 34], these results are new in showing the effect that Cd exposure alone and in the setting of PAH has on the entire heart.

An increase in collagen is an indicator of fibrosis, remodeling, and hypertrophy [21]. Within the Cd exposed groups, there were significant increases in collagen content in both the LV and RV consistent with prior studies that Cd exposure can cause both inflammation and fibrosis within the heart [34]. As expected, SuHx mice had only increased collagen content within the RV, not the LV, as PAH does not impact the LV. Hence, Cd exposure alone increased collagen content within both the LV and RV, indicating that Cd might cause some remodeling and fibrosis within both ventricles, which past publications have not focused on [29, 34-35]. However, Cd didn’t exacerbate the amount of collagens present within the RV in the setting of PAH.

As Cd exposure caused an increase in the collagen contents of both the LV and the RV, we then aimed to determine whether cardiomyocyte hypertrophy was present. Cd exposure significantly increased the LV cardiomyocyte area relative to the CTRL
similarly to previously published studies [35] but did not increase RV cardiomyocyte area. Cd exposure in the setting of PAH did not further increase LV or RV hypertrophy when compared to PAH alone. While previous literature has shown that Cd exposure can cause increased cardiomyocyte area [34, 35], our results indicated the impact of Cd on RV hypertrophy might be different from the LV. Since PAH is a disease primarily of lung vasculatures, we then studied impacts of Cd on lungs with and without PAH.

Cd exposure alone caused the narrowing of the lumens of the PAs and alveolar sacs, an increase in collagen content, and an increase in cell clustering like PAH [36, 37]. This could indicate Cd alone might cause PAH-esque pathological changes in mice. Furthermore, there was an increase in positively stained collagens around the bronchioles in the SuHx groups, hinting that PAH caused not only fibrotic vasculature changes, but bronchiole changes as well [18, 36]. Quantification analyses showed that Cd exposure alone increased the amount of collagen within the lungs almost 20% in relative to the CTRL group, while the SuHx and Cd-SuHx group increased almost 30% and 20% respectively [35-37, 39, 40]. Cd-SuHx had 10% less collagen when compared to the SuHx mice which could be that Cd-SuHx induced late stage of RV pathologies when inflammation and fibrosis subside and scaring dominates.

Since the lungs of Cd exposed mice demonstrated fibrosis, determining the morphological changes in the pulmonary vasculature is important to investigate. Relative to the CTRL, 12 weeks of Cd exposure alone caused significant decrease of NM PA and increased the amount of PM and FM PA’s. Interestingly, Cd-SuHx had significantly more FM PAs compared to SuHx [18]. This may indicate that Cd and other PAH pathogenic factors synergistically induce the muscularization process in PAH. It has been well established in literature that SuHx causes increased number of muscularized PAs [18-19], however, the effects of Cd on pulmonary vascular muscularization are novel in
determining pathogenesis of PAH. 12 weeks of Cd exposure alone caused an increase in both medial thickness and wall area, like key characteristics of PAH. However, Cd in the setting of PAH did not cause further significant changes in medial thickness or wall area in relation to PAH alone [18-19]. This could indicate that PAH has already induced extensive damages of PAs and further insults from Cd could not affect the thickness of PAs.

Therefore, 12 weeks of Cd exposure increased collagen content, caused more PAs to begin the muscularization process, and increased wall area and medial thickness. Twelve weeks of Cd in the setting of PAH did not further increase collagen content, wall area, or medial thickness, however, Cd in the setting of PAH did increase the number of FM PAs when compared to SuHx. Overall, these results might indicate that Cd exposure with or without PAH causes significant morphological changes within the lungs and might worsen the muscularization process in the setting of PAH.
OVERALL CONCLUSIONS, LIMITATIONS, AND FUTURE WORKS

SUMMARY OF TRANSTHORACIC ECHOCARDIOGRAPHY AND BODY WEIGHT

Eight weeks of Cd exposure did not affect RV function or morphology, however, 12 weeks of Cd exposure increased RV systolic function, indicative of systolic stimulation. Twelve weeks of Cd in the setting of PAH did not affect systolic function or cardiac output when compared to PAH alone. However, Cd significantly reduced RV systolic pressure in the setting of PAH when compared to PAH alone, indicating heart failure.

SUMMARY OF HEART HISTOLOGY

Twelve weeks of Cd exposure alone caused cardiomyocyte disorganization, significantly increased the collagen content of both the LV and RV, and increased LV hypertrophy. Twelve weeks of Cd exposure in the setting of PAH did not further increase RV collagen content or RV hypertrophy when compared to PAH alone.

SUMMARY OF LUNG HISTOLOGY

Twelve weeks of Cd exposure alone significantly increased the amount of collagen within the lung, caused more PAs to begin the vascular muscularization process, caused PAs to have larger wall areas and medial thickness when compared to
the control. Twelve weeks of Cd exposure in the setting of PAH significantly reduced the amount of collagen content and increased the number of FM PAs within the lungs when compared to the PAH only group.

GENERAL CONCLUSIONS

These results indicate that 12 weeks of Cd exposure caused significant lung, LV, and RV functional and morphological disturbances. Twelve weeks of Cd in the setting of PAH worsened RV function compared to PAH only mice but had less effects on the lungs and RV histology when compared to the PAH only mice. When Cd is combined with PAH induction, Cd promoted heart failure developments. Overall, these results demonstrate that chronic Cd exposure alone or in the setting of PAH induction might cause or worsen pathological features that mimic PAH.

CLINICAL RELEVANCE, LIMITATIONS, AND FURTHER RESEARCH OPPORTUNITIES

Human exposure to Cd is becoming a more common occurrence as climate change and environmental exposure drastically increases. Our results suggest that chronic Cd exposure could cause or worsen PAH symptoms. Increased Cd concentrations within blood and urine samples during clinical testing could help physicians to perform preventative measures to avoid further exposure or use chelation therapy to remove Cd from the body [28, 30]. Heavy metal testing could be added to 6-minute walk distance and echocardiography to begin preventative measures to stop PAH from developing.
There are limitations of this study. One, duration of exposure might play important roles in LV, RV, and lungs pathology from Cd. Two, dosage of Cd might affect the LV, RV, lungs differently. Third, we only studied male mice and whether these effects are the same in female mice is unknown. We plan to study dose, exposure duration and sex impact in our models in the future. Third, whether agents targeting Cd could reverse LV, RV, and lungs pathologies from Cd is unknown. Fourth, molecular mechanisms underlying Cd effects on PAH and RV function needs further study.

Further research in exploring molecular mechanisms such as inflammation, fibrosis, and oxidative stress regarding the lungs, LV, and RV will further help explain how Cd could potentially cause or worsen PAH.
REFERENCES

19.


<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmonary Arterial Hypertension</td>
<td>PAH</td>
</tr>
<tr>
<td>Pulmonary Artery</td>
<td>PA</td>
</tr>
<tr>
<td>Centers for Disease Control and Prevention</td>
<td>CDC</td>
</tr>
<tr>
<td>National Institute of Occupational Health and Safety</td>
<td>NIOSH</td>
</tr>
<tr>
<td>World Health Organization</td>
<td>WHO</td>
</tr>
<tr>
<td>National Organization of Rare Diseases</td>
<td>NORD</td>
</tr>
<tr>
<td>Journal of the American Heart Association</td>
<td>JAHA</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Cd</td>
</tr>
<tr>
<td>Transthoracic Echocardiography</td>
<td>Echo</td>
</tr>
<tr>
<td>Right Ventricular Systolic Pressure</td>
<td>RVSP</td>
</tr>
<tr>
<td>Hematoxylin &amp; Eosin</td>
<td>H&amp;E</td>
</tr>
<tr>
<td>Picrosirius Red</td>
<td>SR</td>
</tr>
<tr>
<td>Wheat Germ Agglutinin</td>
<td>WGA</td>
</tr>
<tr>
<td>Nonmuscular</td>
<td>NM</td>
</tr>
<tr>
<td>Partially Muscular</td>
<td>PM</td>
</tr>
<tr>
<td>Fully Muscular</td>
<td>FM</td>
</tr>
</tbody>
</table>
CURRICULUM VITAE
Dakotah Dominique Cathey

Mailing Address: 2706 Chickasaw Avenue Apartment #25 Louisville, KY 40206
Email: DDCATH01@louisville.edu
Phone: (502) 724-8884

Education
2012-2015 University of Kentucky [Transferred to University of Louisville]
2017-2020 B.S. in Chemistry; University of Louisville

Professional Experience
2023-Present Pre-doctoral Student
Department of Pharmacology and Toxicology
University of Louisville, Louisville KY

2021-2023 Master’s Student
Department of Pharmacology and Toxicology
University of Louisville, Louisville KY

2021-2021 Research Technician II, Laboratory of Dr. Jiapeng Huang at
Cardiovascular Innovation Institute
Department of Anesthesiology and Perioperative Medicine
University of Louisville, Louisville KY

2019-2021 Phlebotomist, University of Louisville Jewish Hospital
Louisville, KY

2019-2020 Undergraduate Research Student, Joseph Calvin Kouokam
Laboratory, Department of Pharmacology and Toxicology
University of Louisville, Louisville
Seminars

2023 Seminar. “Effects of Cadmium on Pulmonary Arterial Hypertension”. University of Louisville, School of Medicine, Department of Pharmacology and Toxicology Seminar Series, Louisville, KY. May 2023.

2022 Seminar. “Sulforaphane Does Not Protect Right Ventricular Systolic and Diastolic Functions in NRF2 Knockout Pulmonary Arterial Hypertension Mice”. University of Louisville, School of Medicine, Department of Pharmacology and Toxicology Seminar Series, Louisville, KY. April 2022.

Honors and Awards

2023 T32 Pre-Doctoral Fellowship in Environmental Health Sciences
University of Louisville Graduate Student Union Student Travel Award

2021 University of Louisville Diversity Scholarship

2020 American Chemical Society Student Excellence Award
Dean’s List at the University of Louisville

Society Memberships

National Healthcareer Association
Society of Toxicology
American Chemical Society

Meetings Attended

2023 62nd Annual Meeting of the Society of Toxicology
Ohio Valley Chapter of the Society of Toxicology Virtual Trainee Summer Meeting

2022 Research Louisville! University of Louisville

2019 Kentucky Academy of Science Annual Meeting

Publications

Journals