A. OVERALL COVER PAGE

Project Title: Effects of WTC Dust Exposure on Cardiac and Cognitive Functions	
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Human Subjects: NA	**Vertebrate Animals:** NA
hESC: No	**Inventions/Patents:** No

B. OVERALL ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The proposed studies seek to gain a better understanding of the bases for the still-increasing incidence of cardiovascular (CV) and neurodegenerative anomalies reported in 9/11 First Responders (FR). These landmark studies will be performed in a rat model exposed to WTC dusts obtained in/unique to the critical first 72 hr after the buildings collapsed, using relevant exposure scenarios that mimic mouth-breathing exposures and dust levels faced by FR during that critical period. Results from these studies will allow us to: further clarify whether exposure to WTC dust exacerbates the CV and neurologic phenotype, i.e., effects that have led to changes in CV and brain health of exposed FR; ascertain if the dusts were themselves capable of inducing an Alzheimer Disease/PTSD phenotype in exposed hosts; obtain important biochemical/molecular clues to the etiologies for the increasingly evident CV/neurologic problems seen in these exposed FR.

Aim 1 will define effects of WTC dust exposures on cardiovascular (CV) function and A□ aggregate accumulation in the heart.
Aim 2 will assess effects of WTC dust exposures on the development of neurodegenerative disease.
Supplemental Aim will evaluate effects of WTC dust exposures on development of markers/behavioral changes associated with PTSD.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Not Applicable

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1U01OH012056-01
07-01-2020 - 06-30-2021 (Currently in NCE)

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List of Terms and Abbreviations

AD Alzheimer's disease
CVD Cardiovascular disease
FR First responders
LV Left Ventricle
RV Right Ventricle
SHR Spontaneously hypertensive rats
WTC World Trade Center

Abstract

Effects of WTC Dust Exposure on Cardiac and Cognitive Functions

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Data have revealed increases in cardiovascular (CVD) and neurodegenerative diseases in First Responders (FR) who were present at the Ground Zero over the 9/11-13/01 period. While it has yet not been shown if WTC (World Trade Center) dusts were causative for these pathologies in FR, our study of spontaneously hypertensive SHR rats exposed to WTC dusts noted previous reductions in airway ciliated cells and dust clearance from the lung. It is thus likely exposures to WTC dusts resulted in exaggerated responses in situ compared to that by other urban air pollutants, including particulate matter (PM). We know long-term exposure to ambient PM caused a heart failure phenotype in mice (decreased cardiac function and impaired cell function) and neurologic as well as Alzheimer disease (AD)-like changes. As both CVD and AD are age-related, share risk-factors, have overlapping bio-chemistries, and are characterized by aggregates of amyloid precursor protein (found in AD brains and CVD hearts), based on the “heart-to-head” pathogenesis paradigm, we hypothesize here that inhalation of Ground Zero dust particles likely led - in a manner exaggerating that caused by PM - to alterations in cardiac and cognitive function, so as to impart severe chronic impacts on FR health. As a model, SHR rats were exposed to WTC dust for two consecutive days (using dusts collected on-site 9/12-13/01). Both longitudinally and at fixed timepoints over a 3 month and 1-yr post-exposure period, data were obtained in support of two inter-related Aims. Aim 1 was to define effects of WTC dust exposures on CV function and Aβ aggregate accumulation in the heart. Aim 2 was to assess effects of the exposures on development of neurodegenerative disease. We found that there is minimal cardiac and pulmonary functional changes at up to 1 year follow-up, however we found clear retention of metals from the WTC dust and alterations in genes and proteins in the lungs and heart of these rats. The studies address NIOSH goals (CVD Cross-Sector Program, Public Safety Program [Priority 1: Reduce chronic illnesses among firefighters] and FFFFIPP [Goal 1: Reduce CV deaths among firefighters]), keep with major initiatives to reduce illness among firefighters/FR via NIOSH-generated knowledge, interventions, technologies, and fulfill the Zadroga Act research mandate.

Section 1

Significant or Key Findings

Our study was formed on the basis of two Specific Aims.

Aim 1: was to define effects of WTC dust exposures on cardiovascular (CV) function and Aβ aggregate accumulation in the heart. We found at up to one year of follow-up, rats exposed to WTC dust had no significant changes in cardiac function. We also measured pulmonary function and discovered minor changes at 3 months post-exposure that were diminished by 12 months post-exposure. Invasive cardiac measurements determined a clear (though not significant) reduction in contractility of the left ventricle, which may indicate the beginning stage of heart failure, which was not yet evident in these rats.

Aim 2: was to assess the effects of WTC dust exposures on the development of neurodegenerative disease. We have collected brain tissue from WTC-dust-exposed rats and have measured cognitive function from rats 9 months after exposure to WTC dust. We found no changes in anxiety-like behavior. We are collecting cognitive function from rats to measure memory function at later timepoints, as well has have a clear molecular picture of the changes in neural function using tissue collected from these studies.

Translation of Findings

Our current findings indicate that exposure to WTC by first responders (FR) during the WTC disaster may have a contribution to pulmonary and cardiac disease. These data gained here provides valuable general information about associations between exposures to building collapse-derived dusts/materials generated during rescue operations and toxic effects in the CV and nervous systems, it will also allow for a clearer understanding of mechanistic underpinnings for development of these effects in individuals who might face similar types of exposure scenarios (i.e., earthquake recovery, building collapses, etc.) in the future. These studies will expand the current knowledge of the pathophysiology encountered by repeated exposures to Ground Zero dusts and be translatable towards the well-being of FR impacted by cognitive/neurologic and CV dysfunction.

Research Outcomes/Impact

The proposed studies seek to gain a better understanding of the bases for the still-increasing incidence of cardiovascular (CV) and neurodegenerative anomalies reported in 9/11 FR. The outcomes of this work are all “potential outcomes” as they could lead to impact workplace risk if used. These landmark studies were performed in a rat model exposed to WTC dusts obtained in/unique to the critical first 72 hr after the buildings collapsed, using relevant exposure scenarios that mimic mouth breathing exposures and dust levels faced by FR during that critical period. Results and tissue collected from these studies will allow us to: further clarify whether exposure to WTC dust exacerbates the CV and neurologic phenotype, i.e., effects that have led to changes in CV and brain health of

exposed FR; ascertain if the dusts were themselves capable of inducing an Alzheimer Disease/PTSD phenotype in exposed hosts; obtain important biochemical/molecular clues to the etiologies for the increasingly evident CV/neurologic problems seen in these exposed FR.

This research will add to the growing knowledge about risk of CVD and neurologic/cognitive dysfunctions among WTC rescue and recovery workers (FR). On one hand, this will translate into an understanding of the presence\absence of an increased risk that may be related to WTC exposures. On the other, it could also lead to evidence-based surveillance programs. This will represent a direct benefit to participants in all WTC surveillance programs.

Section 2

Scientific Report

Background

First Responders (FR) arriving within hours of the WTC disaster on September 11th, 2001, continue to reveal drastic health problems in multiple body systems. Diseases affecting the cardiovascular (CV) system and cognition1,2 have become increasingly apparent in FR all these years later. Specific mechanisms underlying these chronic diseases remain unclear, but evidence attributes the repeated exposures of the FR to WTC dusts at Ground Zero. Development of health problems in FR is strongly correlated with arrival time/time spent at site and whether or not a respirator was used.3 In the early periods after the disaster, WTC dusts were not only encountered at high “doses” (estimates for over first 72 hr were at 100s of mg/m3, that only reduced to mg - hundreds of µg/m3 range after ≈ 3 wk), but they had unique characteristics including being primarily supercoarse (> 95% of mass 10-53 µm [MMAD ≈ 22 µm]) and being mostly alkaline.4,5 Because the dusts acted like alkaline agents, they caused damage to respiratory epithelium and reduced clearance in the lungs of exposed rats.6,7 Thus, it is possible that entrained WTC dust particles could have been similarly retained for very long periods in the lungs of exposed FR. This would mean WTC dust particles could impart long-term/chronic effects in the lungs in particular, but potentially systemically and in distal organs as well.6

Exposure to urban particulate matter (PM) from sources like traffic, industry, and woodburning is known to increase the risk of CVD in the forms of heart failure,8 atherosclerosis,9,10 and arrhythmia,11,12 as well as CVD-related deaths.8 In particular, PM2.5 is thought to be crucial for these health effects as it has the ability to deposit in alveoli and subsequently enter into the bloodstream and potentially cross the blood-brain barrier.13 These associations are strongest in individuals with long-term PM exposures due to living close to busy roads or in large cities. Interestingly, the repeated exposures of FR to WTC dusts has been linked with increased development of heart disease,14-16 CV-related hospitalizations,17 and circulating levels of CVD markers.18 Prolonged PM

exposure is also associated with alterations in cerebral blood hemodynamics,19 and cognitive function,20,21 as well as atrophy of the brains of older adults.22 Studies have also found a positive correlation between PM levels and development of Alzheimer disease (AD).23 If in fact WTC dusts, due to their prolonged retention in the lungs, can give rise to exaggerated responses compared to that from other urban air pollutants (including PM), this could provide a mechanism to begin to explain why there have been ongoing increases in the incidence of CVD and neurologic disorders among the FR repeatedly exposed to Ground Zero dusts in the earliest periods after the disaster. This could also be a way to explain the strong correlation with the occurrence in exposed FR of post-traumatic stress disorder (PTSD),24–26 a cluster of pathologies characterized by cognitive decline.27,28 Whether or not there is a similar correlation with regard to occurrence of AD in exposed FR is as-yet not known. However, as with increased risk for lung cancer, one cannot outright preclude the possibility of this specific pathology developing simply due to an absence of epidemiologic data to-date.

Both CVD and AD are strongly associated with aging and have shared etiologies reactive to PM exposure. That these both include inflammation and oxidative stress29,30 suggests systemic involvement in each case. This is evidenced by the fact that decreased CV function is a risk factor for AD, and that AD may play a causative role in CVD. Further, amyloid-β (Aβ), critical for plaque formation in brains in AD, has been found not only in the blood31 but also accumulating in the hearts of AD patients.32 This suggests both diseases could share overlap in pathology, irrespective of the agent(s) leading to their induction. Thus, these studies are significant as they will determine if the alkaline supercoarse WTC dusts to which FR were repeatedly exposed over the course of the first week post-disaster were capable of inducing systemic alterations that - in an exaggeration of effects from long-term exposure to PM - led to CV and cognitive decline in exposed FR as they grow older. These studies will also bring further evidence to the heart-head connection and how exposure to contaminants like PM and those encountered at Ground Zero might cause/exacerbate cardiac/neurologic declines over time.

Specific Aims

Our study was based upon the following two Aims:

Aim 1: will define effects of WTC dust exposures on cardiovascular (CV) function and Aβ aggregate accumulation in the heart.

Aim 2: will assess effects of WTC dust exposures on the development of neurodegenerative disease.

Methodology

Exposure to Ground Zero dust: Adult (≈ 3 mo) SHR rats were exposed on two consecutive days to Ground Zero dusts collected within the first 72 hr of the 9/11 disaster. The concentration used will model that likely experienced over a 4-hr period by a reference

FR (i.e., 33 mg/m3 in rat model = 250 mg dust/m3 FR exposure, total of 2 mg). Follow-up was completed up to one year post-exposure.

Echocardiography: Rats underwent serial echocardiography at 3 and 12 months post-exposure using a Vevo 3100 ultrasound machine. This allowed not only for us to ascertain how WTC dust affects cardiac function, but also how the dust affects progression of heart failure with aging. Rats will be placed under anesthesia (1-3% isoflurane in O_2) and placed on a heated stage controlled with a rectal thermostat. The chest was shaved and a 24 MHz transducer will be used to acquire LV and RV parameters. Ventricular wall thickness, chamber dimension, and ejection fraction was measured using M-mode ultrasound from both ventricles. Pulse-wave Doppler was acquired along the mitral valve, tricuspid valve, aortic valve and pulmonary valve to obtain relevant flow characteristics and cardiac output. RV function was measured with tricuspid annular plane systolic excursion (TAPSE). Additional B-mode images was acquired along the parasternal long and short axes to utilize speckle-tracking for strain analyses. These measurements were completed in one session and rats were lightly anesthetized for < 10 min/session. It was key to also obtain the described RV measurements as the RV has a connection with the lungs that may be affected by exposures to the WTC dusts.

Cardiac pressure-volume loops: At sacrifice (3 and 12-month post-exposure), subsets of rats underwent recording of LV pressure and volume to obtain load-independent measures of cardiac contractility using a Millar Mikro-tip pressure-volume catheter interfaced with an MPVS signal processing and analysis with Lab-chart Pro software. The catheter was calibrated in different volumes according to manufacturer protocols prior to each session. Rats were anesthetized with isoflurane and then intubated and connected to a small animal ventilator after anesthesia is confirmed via lack of pedal response. During the procedure, body temperature was maintained at 37°C. The catheter was extended carefully into the LV apically while observing the pressure-volume (PV) loop. Pressure used for data analysis was the average of at least 10 waveforms/rat. After baseline PV loops were obtained, end-systolic and end-diastolic pressure volume relations were obtained with aortic and inferior vena cava occlusion, respectively. These techniques allowed acquisition of ± dP/dT max, tau, ejection fraction (EF), and cardiac output. Additionally, RV and pulmonary artery pressure were obtained by insertion of the catheter into the apex.

Pulmonary functional assessment: Given the sustained alterations in pulmonary function seen in SHR rats exposed to WTC dust, it is hypothesized these alterations can drive immune cell infiltration into the airspace and inflammation, leading to changes in pulmonary function. To test this, airway function after dust exposure was measured as described in Aim 1. Airway function and responses to aerosolized methacholine was measured on anesthetized rats using a Flexivent system. A single compartment model was used to assess total respiratory system resistance (R), compliance (C), and pressure volume (PV) loops at baseline and after delivery of increasing doses of methacholine (0–50 mg/ml). Individual peak responses were determined at each dose for each rat. A Flexivent forced expiration extension was used to assess flow limitations to provide in vivo pulmonary function of direct clinical relevance.

Pulmonary histology and molecular assessment: After sacrifice, pulmonary tissue was collected for histology and molecular assessment. The lung bloc was removed, and the right lung was tied off and removed. This lobe was flash-frozen and later lysed in RIPA buffer for immunoblotting or used for RNA isolation. The remaining left lobe was inflation-fixed with neutral buffered formalin. The fixed lobe was processed and embedded in paraffin and cut and mounted on slides for routine histology and immunofluorescence.

Behavioral analyses: Several behavioral tests were done at 9 months post-exposure to assess anxiety-like behaviors. These included the light/dark test, the elevated plus maze, and the open field test.

Results and Discussion

WTC dust exposure maintained long-term retention of metals in pulmonary tissue. As shown in Fig. 1, metal analysis was completed using the pulmonary tissue obtained after sacrifice from rats 12 months post-exposure. Retention of metals found in the WTC dust, especially titanium, demonstrate that the exposure of these animals recapitulates previous findings demonstrating dust retention.

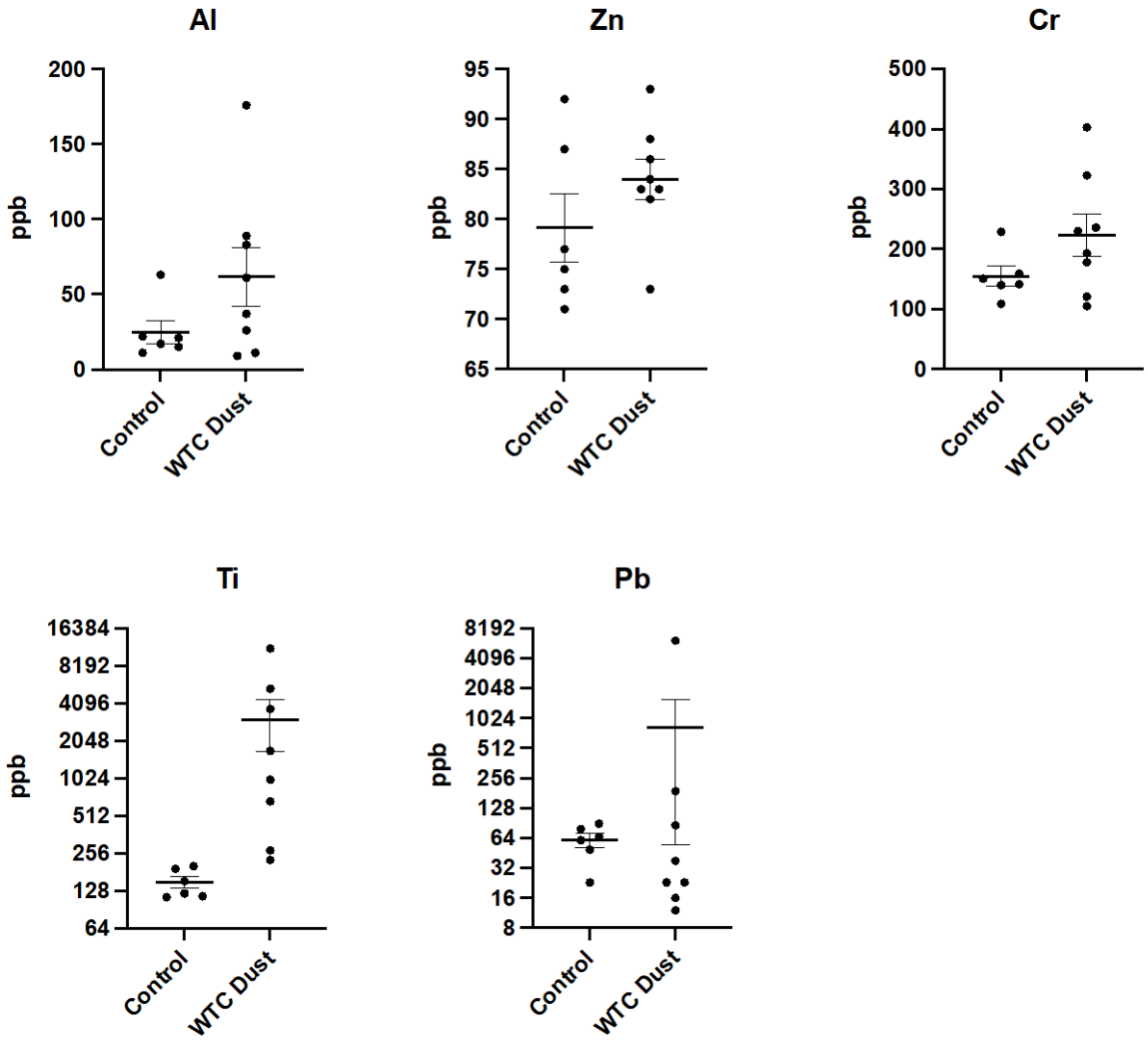
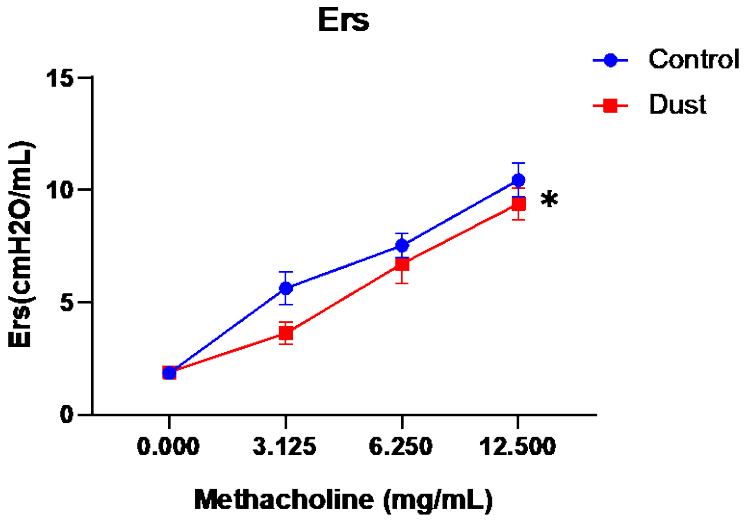


Figure 1: Metal concentrations detected in lungs of rats 12-months after exposure to WTC dusts.

Pulmonary function is decreased in rats exposed to WTC dust at 3 months, but not at 12 months follow-up. Utilizing the Flexivent, we measured pulmonary function at 3- and 12-months post-exposure to WTC dusts. Slight alterations can be found at 3 months in elastance (Ers), but there are no changes at 12 months (Fig. 2). Full Flexivent parameters are shown in Figs. 3 and 4 (3 month post-exposure) and Figs. 5 and 6 (12 month post-exposure). Overall, rats had very little evidence of pathological lung function at these timepoints.

3 months



12 months

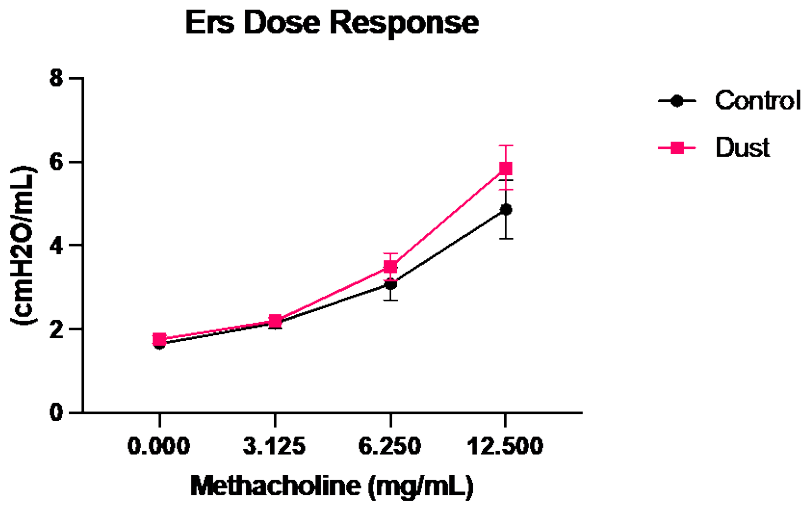


Figure 2. Elastance (Ers) with various doses of methacholine as measured with Flexivent at 3- and 12 months post-exposure to WTC dusts. * indicates *P*<0.05 via two-way ANOVA for control vs. dust.

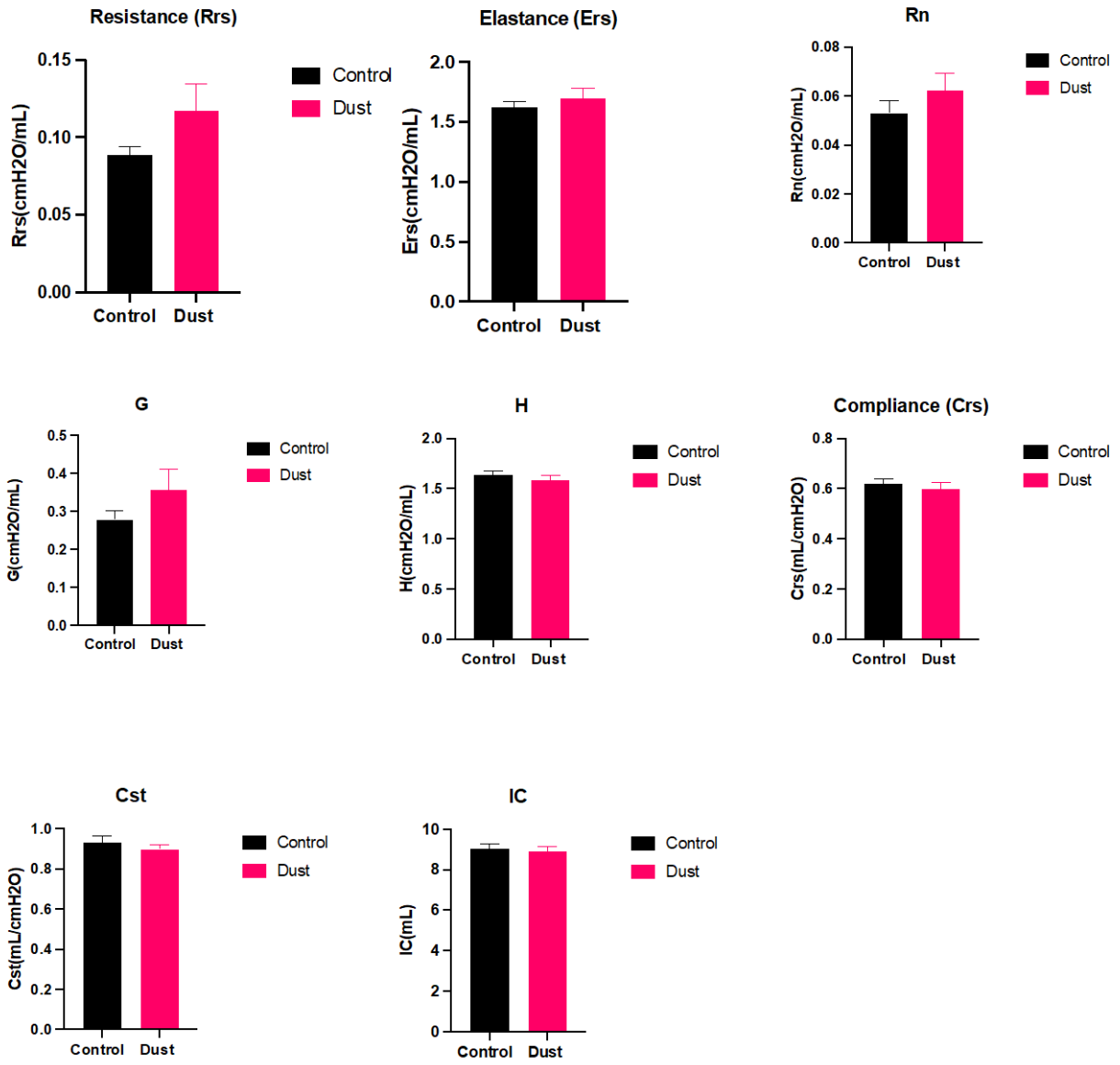


Figure 3. Baseline Flexivent measurements (without methacholine) 3 month post-exposure to WTC dusts. Rrs: Resistance; Ers: elastance; Crs: compliance; Rn: Newtonian resistance (resistance of conducting airways); G: tissue damping (resistance of alveoli); H: elastance (alveoli); Cst: quasi-static compliance; IC: ; FEV0.1: Forced expired volume in the first 0.1 seconds; FVC: forced vital capacity; PEF: peak expiratory flow.

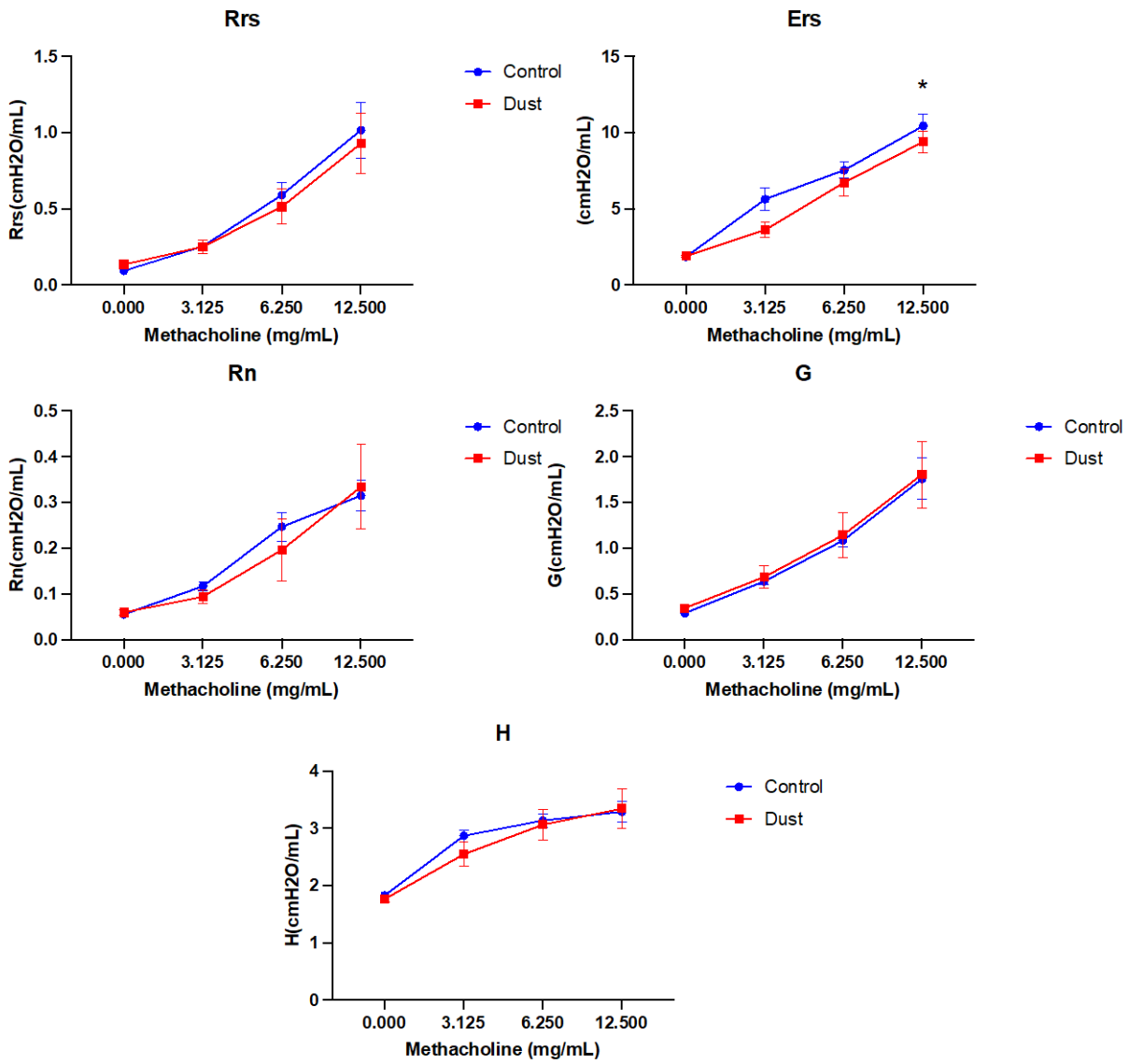


Figure 4. Flexivent measurements with methacholine challenge, 3 month post-exposure to WTC dusts. Rrs: Resistance; Ers: elastance; Rn: Newtonian resistance (resistance of conducting airways); G: tissue damping (resistance of alveoli); H: elastance (alveoli). * indicates *P*<0.05 via two-way ANOVA for control vs. dust.

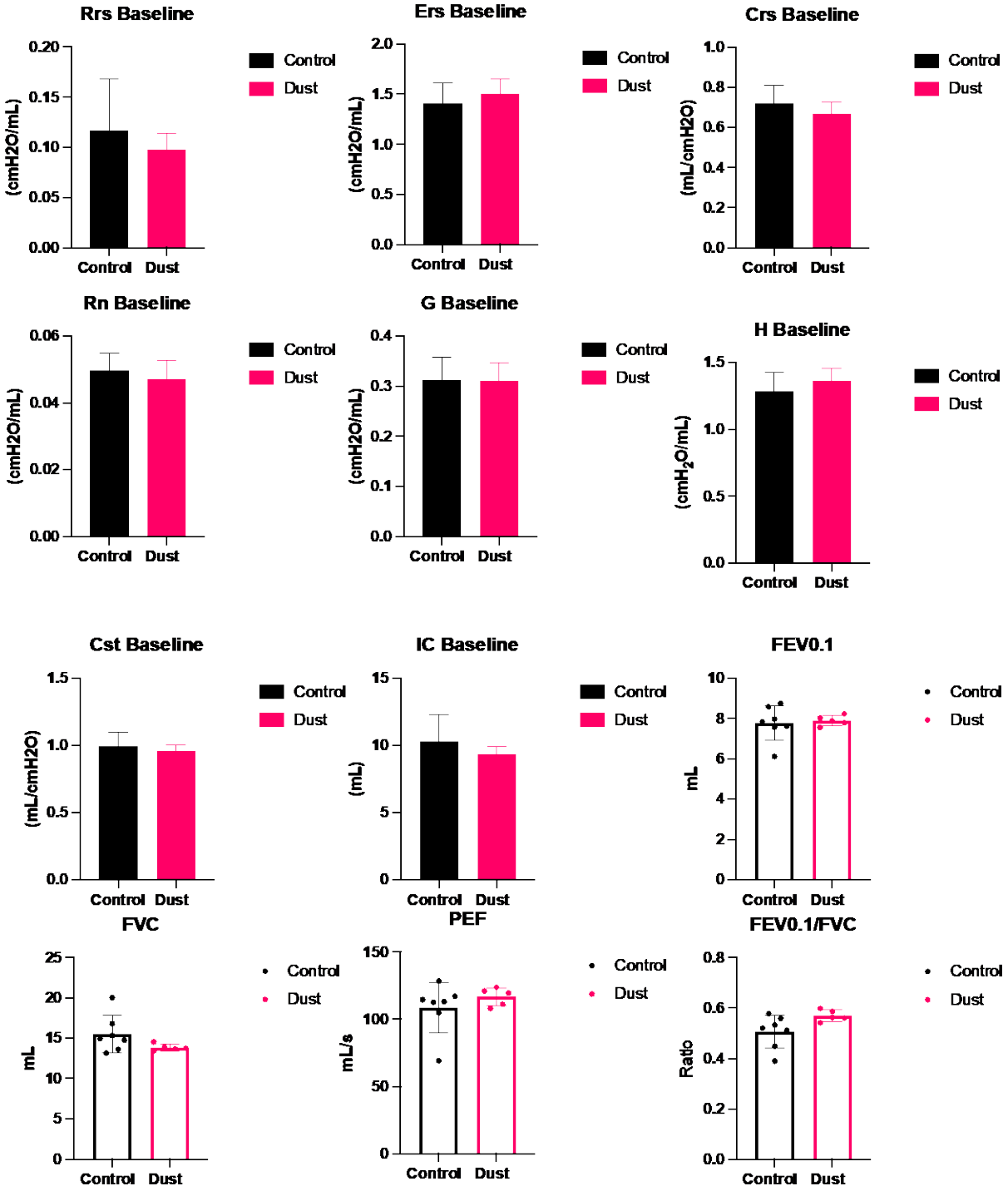


Figure 5. Baseline Flexivent measurements (without methacholine) 12 month post-exposure to WTC dusts. Rrs: Resistance; Ers: elastance; Crs: compliance; Rn: Newtonian resistance (resistance of conducting airways); G: tissue damping (resistance of alveoli); H: elastance (alveoli); Cst: quasi-static compliance; IC: ; FEV0.1: Forced expired volume in the first 0.1 seconds; FVC: forced vital capacity; PEF: peak expiratory flow.

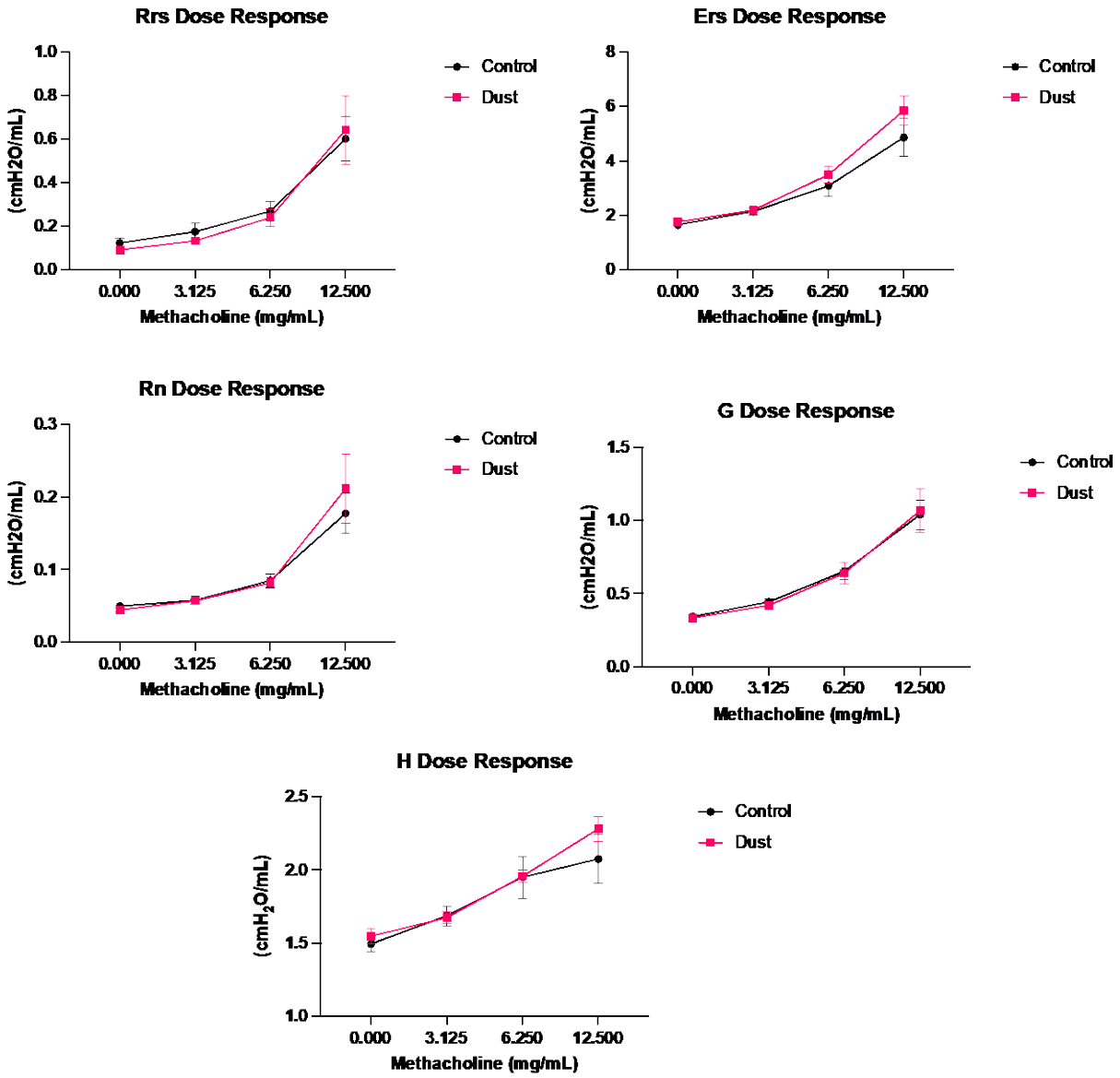


Figure 6. Flexivent measurements with methacholine challenge, 12 month post-exposure to WTC dusts. Rrs: Resistance; Ers: elastance; Rn: Newtonian resistance (resistance of conducting airways); G: tissue damping (resistance of alveoli); H: elastance (alveoli).

Pulmonary histology demonstrates mild alveolar remodeling 3 months after dust exposure. Lungs from SHR rats 3 months after exposure to WTC dust demonstrated a slight change increase in mean linear intercept (MLI, Fig. 7) (*P*=0.09). Increased MLI indicates larger alveolar size. More animals may determine if the effect is biologically relevant.

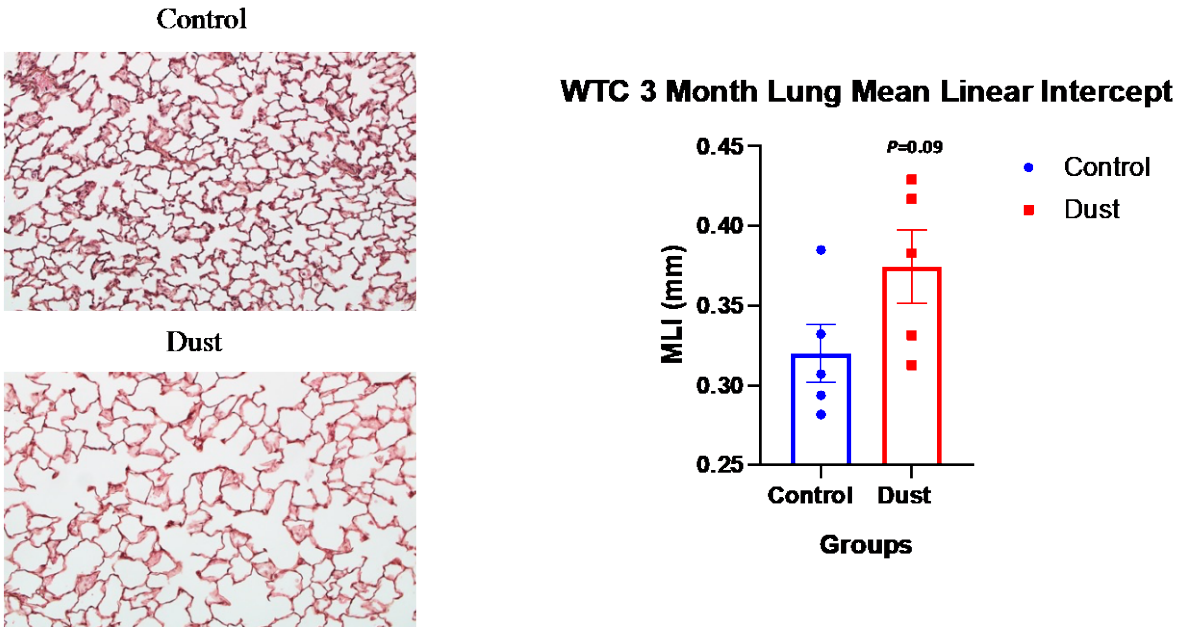


Figure 7. Representative hemotoxylin and eosin staining and calculated mean linear intercept from the lungs of rats exposed to WTC dusts 3 months prior to sacrifice. P value indicated as a result of Student's t-test.

Vascular staining in lungs indicates no vascular remodeling 3 months post-WTC dust exposure. Lungs from rats sacrificed 3 months after exposure to WTC dusts were sectioned and stained with α-smooth muscle actin. Vessels were measured that were stained positive. As shown in Fig. 8, there was no statistical difference in the wall thickness, wall area, external diameter, or resulting percentages in vessels in the lungs of dust-exposed rats compared to control rat lungs. This indicates that there is no pulmonary remodeling evident 3 months after exposure to WTC dusts in SHR rats.

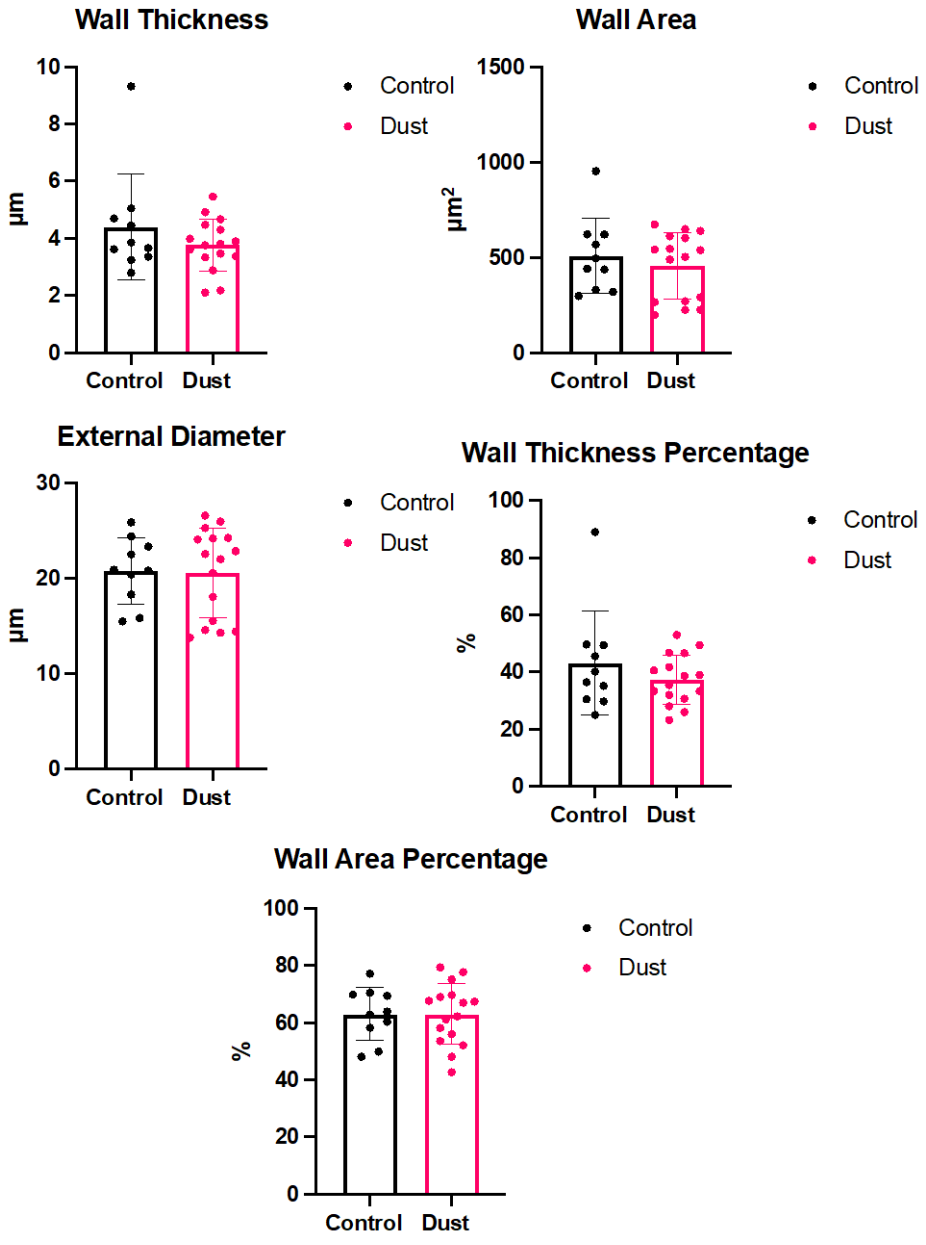


Figure 8. Vessel characterization using α-smooth muscle actin staining in pulmonary tissue from rats exposed to either WTC dust or control (no dust) 3 months prior.

Immunoblotting of COPD-related proteins 3 months post-WTC dust exposure demonstrated changes in some rats. Lungs from rats sacrificed 3 months after exposure to WTC dusts were used for immunoblots of moesin and cyto b245. These proteins have been implicated in COPD and asthma. Though there was no statistical significance via t-test, several of the dust-exposed rats had increased expression of these proteins (Fig. 9).

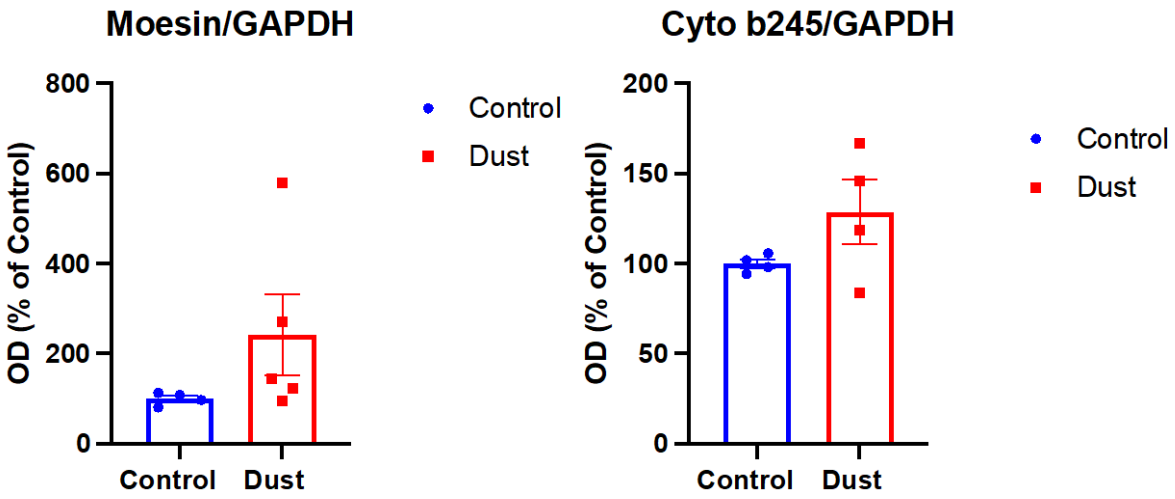


Figure 9. Immunoblotting of pulmonary tissue 3 months post-exposure to WTC dusts or control tissue.

Exposure to WTC dusts did not cause changes in systolic function or left ventricular morphology at 3 or 12 months post-exposure. Echocardiography revealed that at 3 months (Fig. 10) and 12 months (Fig. 11) after dust exposure, LV morphology and systolic function were unchanged compared to control rats. Thus, at these timepoints WTC dust has no impact on cardiac function. SHR rats at this timepoint have not begun to have cardiac dysfunction, thus waiting to a further timepoint (18 months) is merited to examine the "double-hit" of hypertension and dust exposure.

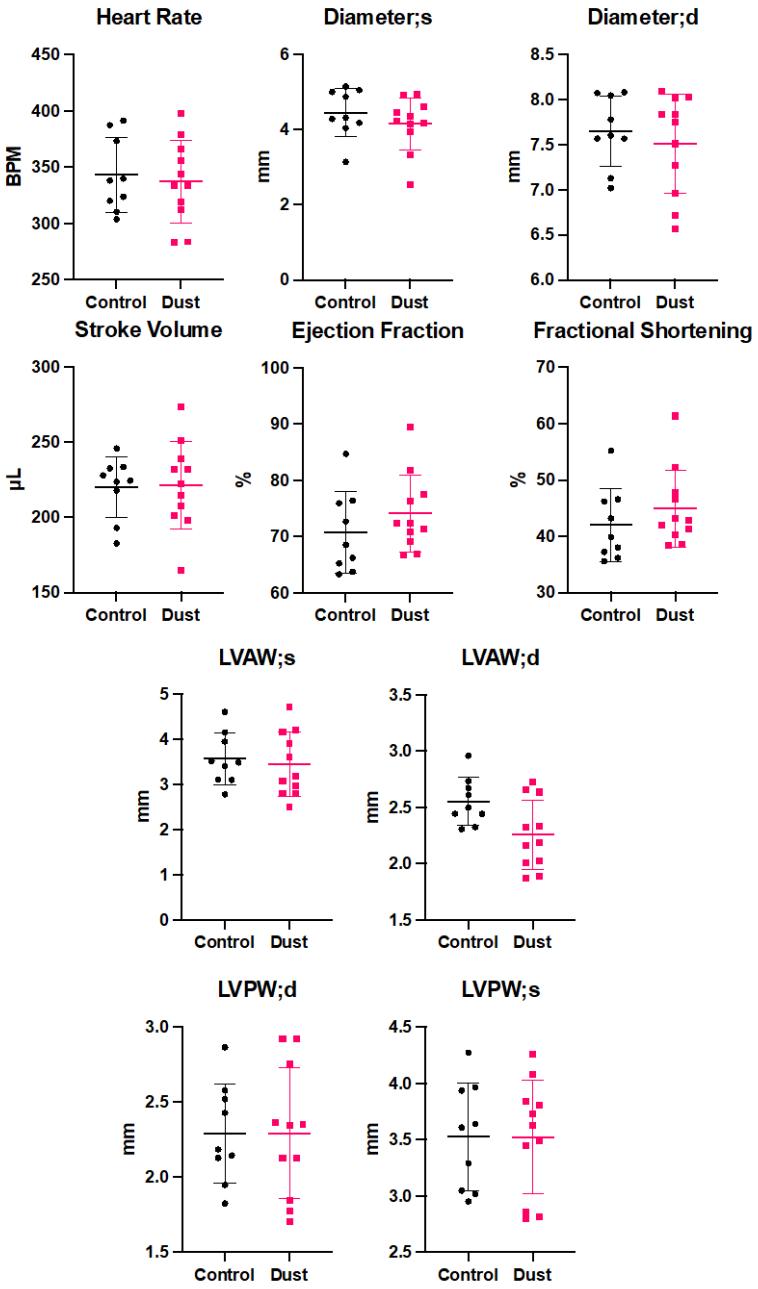


Figure 10. Systolic function and cardiac morphology as examined with echocardiography 3 months post dust exposure. s: systole, d: diastole; LVAW: left ventricle anterior wall; LVPW: left ventricle pulmonary wall.

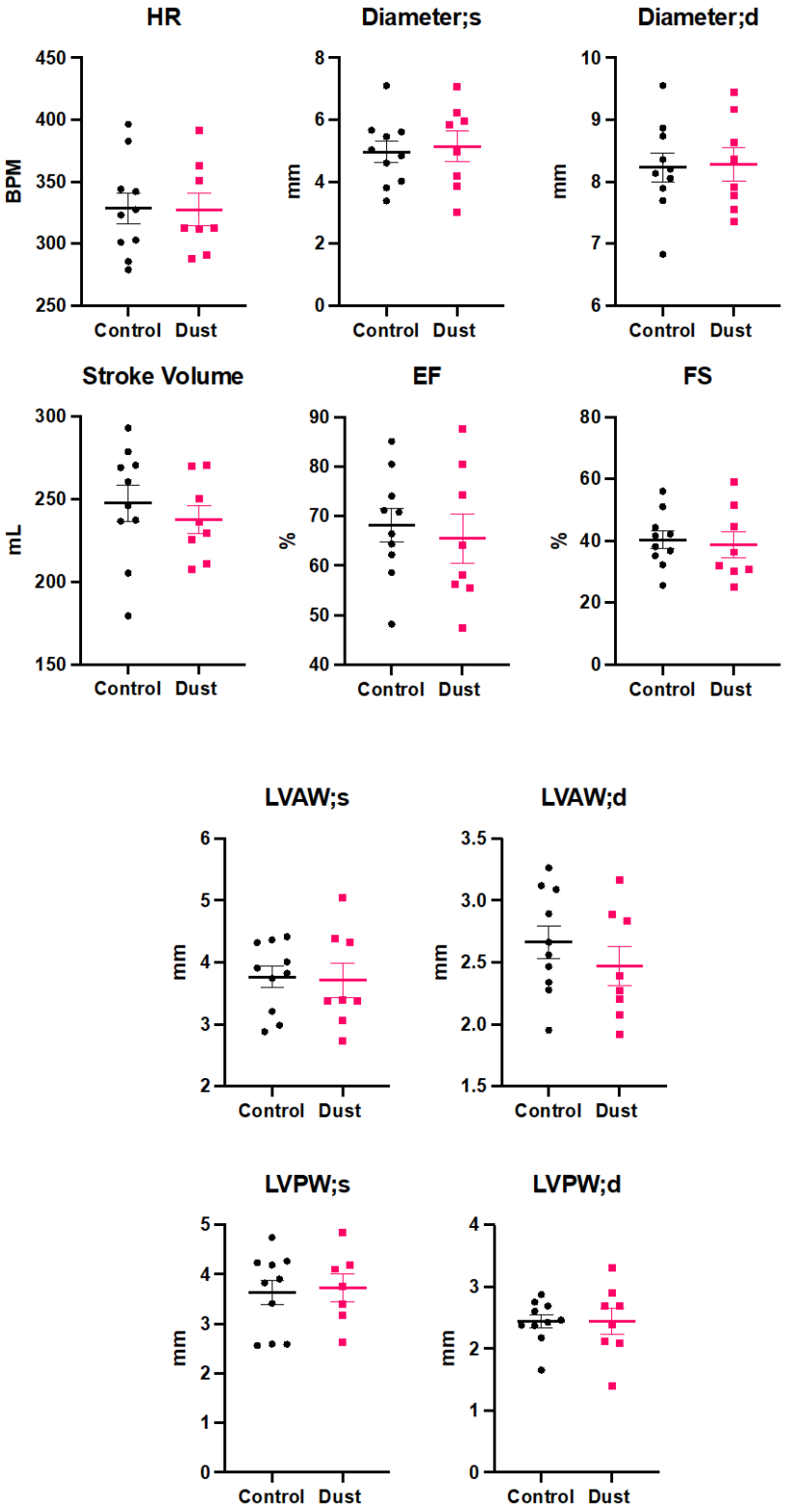


Figure 11. Systolic function and cardiac morphology as examined with echocardiography 3 months post dust exposure. s: systole, d: diastole; LVAW: left ventricle anterior wall; LVPW: left ventricle pulmonary wall.

Exposure to WTC dusts caused minor changes in diastolic function at 3 or 12 months post-exposure. Echocardiography revealed that at 3 months (Fig. 12) and 12 months (Fig. 13) after dust exposure, diastolic function was slightly altered compared to control rats. E/E', a measure of cardiac stiffness, was increased at 3 months, but this effect was not seen at the 1 year timepoint. This may be reflective of a small number of rats that were able to undergo this measure at the 12 month timepoint. SHR rats at this timepoint have not begun to have cardiac dysfunction, thus waiting to a further timepoint (18 months) is merited to examine the "double-hit" of hypertension and dust exposure.

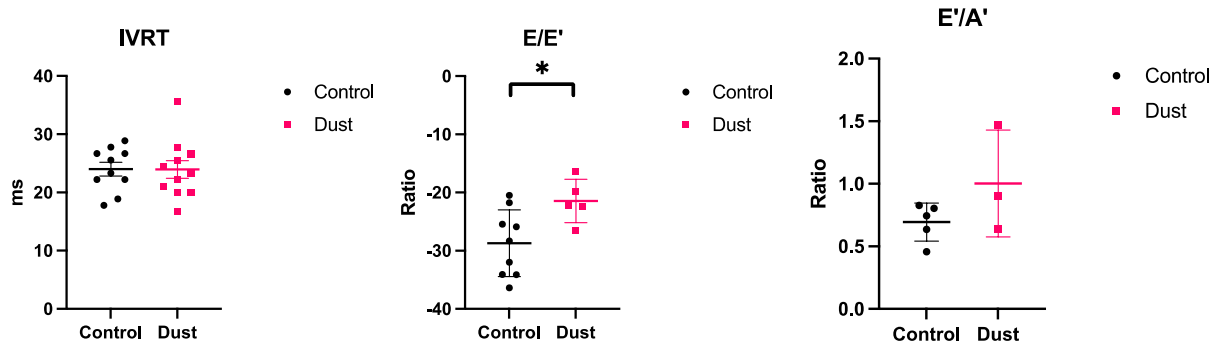


Figure 12. Diastolic function as examined with echocardiography 3 months post dust exposure. IVRT: isovolumic relaxation time; E/E': peak velocity of early filling as measured by pulse-wave doppler (E) and tissue doppler (E'); E'/A': early and active filling as measured by tissue doppler.

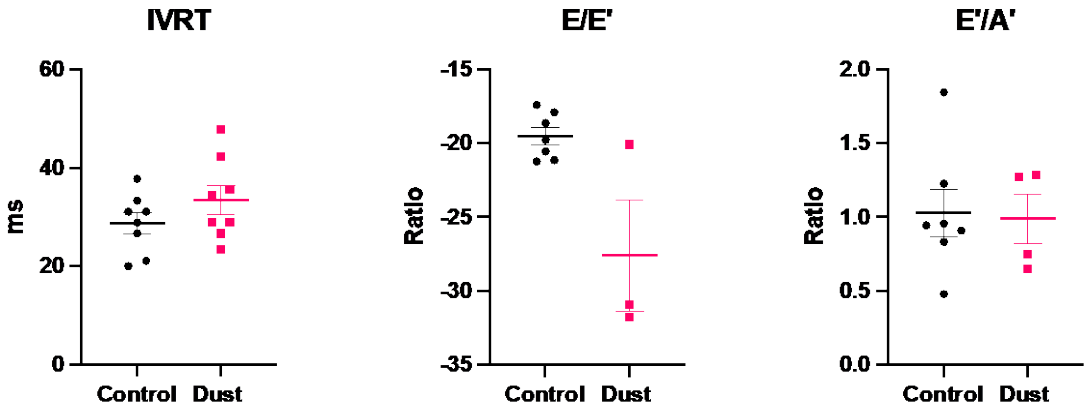


Figure 13. Diastolic function as examined with echocardiography 12 months post dust exposure. IVRT: isovolumic relaxation time; E/E': peak velocity of early filling as measured by pulse-wave doppler (E) and tissue doppler (E'); E'/A': early and active filling as measured by tissue doppler.

Exposure to WTC dusts did not cause changes in right ventricular (RV) function at 3 or 12 months post-exposure. Echocardiography revealed that at 3 months (Fig. 14) and 12 months (Fig. 15) after dust exposure, RV function were unchanged compared to control rats. Thus, at these timepoints WTC dust has no impact on cardiac function. SHR rats at this timepoint have not begun to have cardiac dysfunction, thus waiting to a further timepoint (18 months) is merited to examine the "double-hit" of hypertension and dust exposure, that may affect the RV.

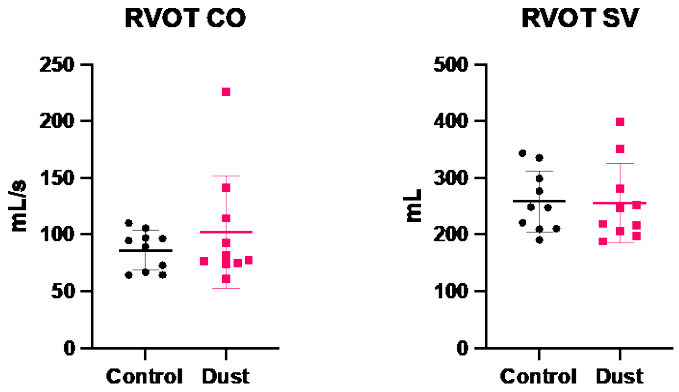


Figure 14. RV function as examined with echocardiography 3 months post dust exposure. RVOT: Right ventricular outflow tract; CO: cardiac output; SV: stroke volume.

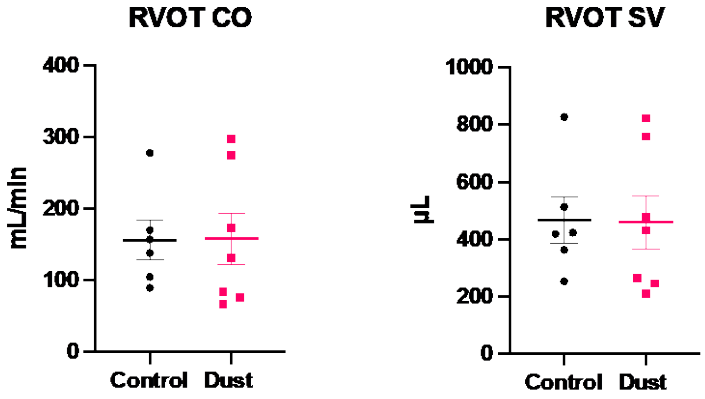


Figure 15. RV function as examined with echocardiography 12 months post dust exposure. RVOT: Right ventricular outflow tract; CO: cardiac output; SV: stroke volume.

Exposure to WTC dusts caused reduced contractility in the LV 12 months post-exposure. Pressure-volume loop analyses revealed no changes in systolic function from load-dependent measures, recapitulating what was found via echocardiography (Fig. 16). However, the load independent measure for contractility (Ees) was slightly decreased though not statistically significant (*P*=0.0748 via t-test, n=3 dust rats). SHR rats at this timepoint have not begun to have cardiac dysfunction, thus waiting to a further timepoint (18 months) is merited to examine the “double-hit” of hypertension and dust exposure.

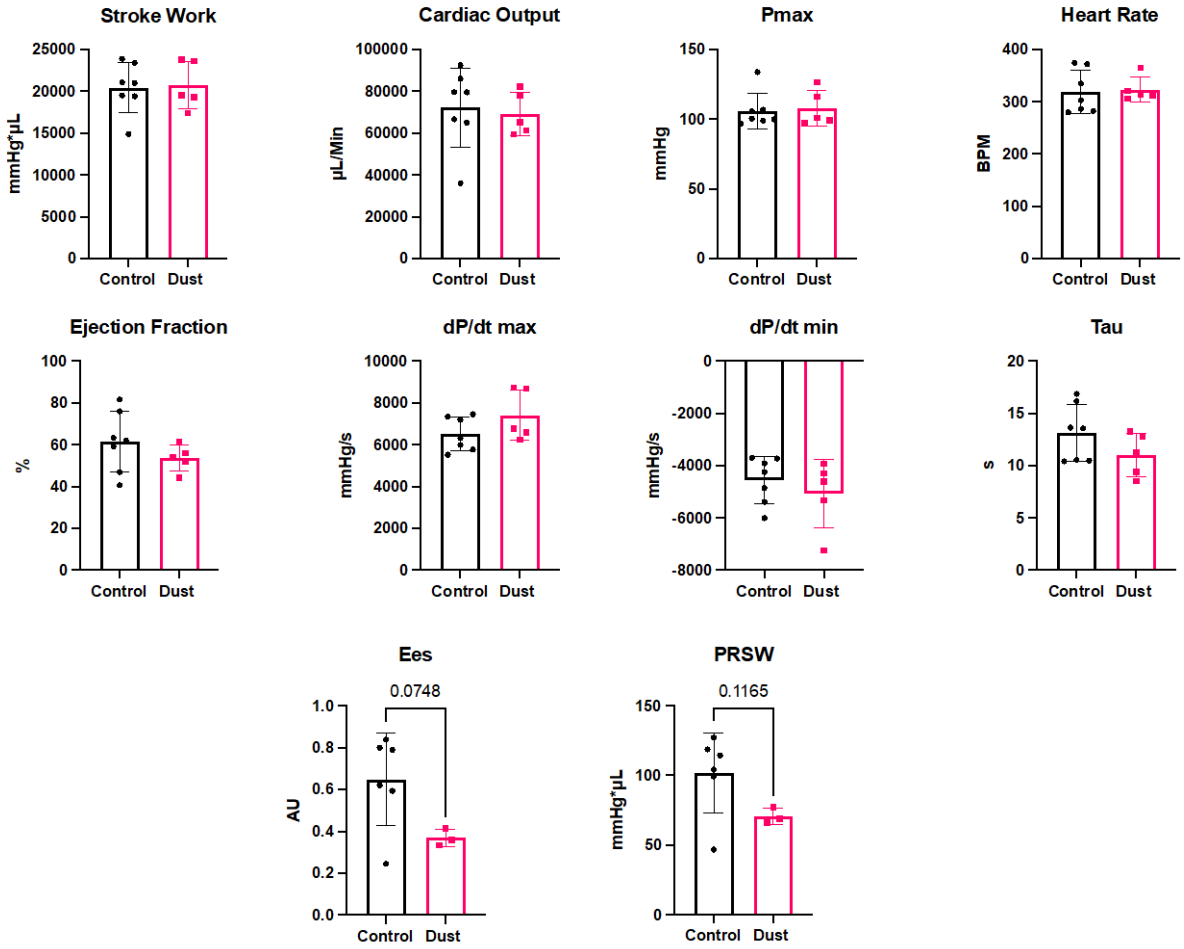


Figure 16. Pressure-volume loop physiology from the left ventricle of rats 12 months after exposure to dust or control. Numbers indicate *P* values from t-test. Pmax: max pressure; Ees: contractility; PRSW: preload-recruitable stroke work.

Exposure to WTC dusts caused no change in RV function via PV loops. Pressure-volume loop analyses revealed no changes in systolic function from load-dependent measures in the RV, recapitulating what was found via echocardiography (Fig. 17). SHR rats at this timepoint have not begun to have cardiac dysfunction, thus waiting to a further timepoint (18 months) is merited to examine the “double-hit” of hypertension and dust exposure, that may affect the RV.

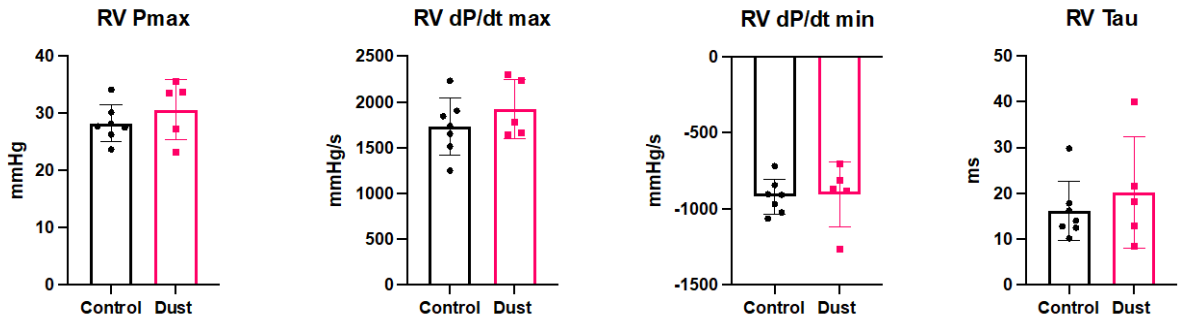


Figure 16. Pressure-volume loop physiology from the right ventricle of rats 12 months after exposure to dust or control. Pmax: max pressure.

Exposure to WTC dusts caused no change in anxiety behavior at 9 months post-exposure. Behavioral analyses of rats using the light/dark test, open field, and elevated plus maze found no changes at 9 months post-exposure to WTC dust compared to control (Fig. 17).

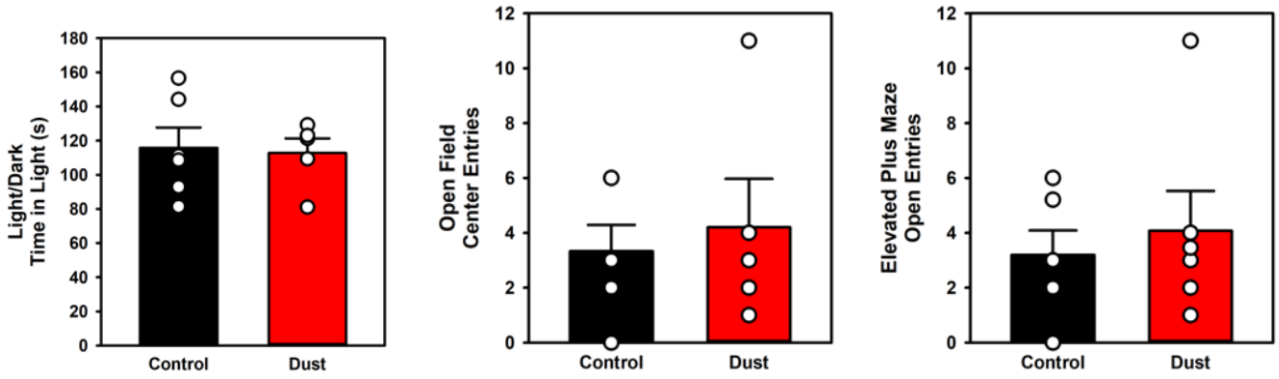


Figure 17. Behavioral analyses from rats 9-months after WTC dust exposure compared to control animals.

Conclusions

Studies from Aim 1 completed an extensive examination of the time course of cardiac function in the SHR hosts due to WTC dust exposure and produced an extensive body of data related to cardiac responses arising from the repeated dust exposures. Dust exposure may give rise to CV defects like diastolic dysfunction (evident in mitral flow E/A ratio changes from echocardiography, PV loop changes), or in more severe changes like systolic dysfunction and impaired contractility are only becoming to become evident at 12 months post-exposure. These both may become evident at later timepoints. It is anticipated these alterations in cardiac function will also manifest as changes in oxidative stress as well as at a structural level, possibly including an increased presence of Aβ deposits as found in human AD patients, but experiments need to be completed for this assessment at these and longer timepoints. While these changes have not as-yet been seen at up to 12 mo. post-exposure, since these animals do not develop heart failure until later in life, i.e., develop heart failure until around 20-mo-of-age, the longer proposed timeline in the future studies will clearly allow for a determination of whether the WTC dust exposures could have impacted on heart disease progression overall. There is, however, a potential that dust exposure does not cause a clear change in cardiac function.

Further, utilizing the same animals, we were able to assess pulmonary function, histology, and molecular changes in WTC dust-exposed rats. Though further work is required for 12 months post-exposure, we found minor changes in pulmonary function and markers for pulmonary disease. These may further develop as the metals we found are still retained in lung tissue, and follow-up at later timepoints is needed.

Studies for Aim 2 included a behavioral test conducted on rats 9 months post-exposure. There were no changes in anxiety like behaviors at this timepoint between dust and control rats. Further examination in future cohort for memory-like behavior, as well as brain histology is warranted.

Further, we have banked the tissue and serum from these animals and are continuing to assess that pathology with molecular assays. Future work should concentrate on co-variables/exposures as well as following the animals to further timepoints post-exposure.

Publications

Park S, Lu Y, Shao Y, Prophete C, Horton L, Sisco M, Lee H, Kluz T, Sun H, Costa M, Zelikoff J, Chen L, Gorr MW, Wold LE, Cohen MD: [2022] Longitudinal Impact of WTC Dust Inhalation on Rat Cardiac Tissue Transcriptomic Profiles. *International Journal of Environmental Research and Public Health* 19 (2): 919.

Mears MJ, Aslaner DM, Barson C, Cohen M, Gorr MW, Wold LE: [2021] Health Effects Following Exposure to Dust from the World Trade Center Disaster: An Update. *Life Sciences* 120147.

C. OVERALL PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
N/A: Not NIH Funded	Park SH, Lu Y, Shao Y, Prophete C, Horton L, Sisco M, Lee HW, Kluz T, Sun H, Costa M, Zelikoff J, Chen LC, Cohen MD. Longitudinal impact on rat cardiac tissue transcriptomic profiles due to acute intratracheal inhalation exposures to isoflurane. PloS one. 2021;16(10):e0257241. PubMed PMID: 34648499; PubMed Central PMCID: PMC8516213; DOI: 10.1371/journal.pone.0257241.
N/A: Not NIH Funded	Park SH, Lu Y, Shao Y, Prophete C, Horton L, Sisco M, Lee HW, Kluz T, Sun H, Costa M, Zelikoff J, Chen LC, Cohen MD. Longitudinal impact on rat cardiac tissue transcriptomic profiles due to acute intratracheal inhalation exposures to isoflurane. PloS one. 2021;16(10):e0257241. PubMed PMID: 34648499; PubMed Central PMCID: PMC8516213; DOI: 10.1371/journal.pone.0257241.
N/A: Not NIH Funded	Neczypor EW, Mears MJ, Ghosh A, Sassano MF, Gumina RJ, Wold LE, Tarran R. E-Cigarettes and Cardiopulmonary Health: Review for Clinicians. Circulation. 2022 January 18;145(3):219-232. PubMed PMID: 35041473; PubMed Central PMCID: PMC8820458; DOI: 10.1161/CIRCULATIONAHA.121.056777.
N/A: Not NIH Funded	Neczypor EW, Mears MJ, Ghosh A, Sassano MF, Gumina RJ, Wold LE, Tarran R. E-Cigarettes and Cardiopulmonary Health: Review for Clinicians. Circulation. 2022 January 18;145(3):219-232. PubMed PMID: 35041473; PubMed Central PMCID: PMC8820458; DOI: 10.1161/CIRCULATIONAHA.121.056777.
N/A: Not NIH Funded	Neczypor EW, Saldaña TA, Mears MJ, Aslaner DM, Escobar YH, Gorr MW, Wold LE. e-Cigarette Aerosol Reduces Left Ventricular Function in Adolescent Mice. Circulation. 2022 March 15;145(11):868-870. PubMed PMID: 35184570; PubMed Central PMCID: PMC8923958; DOI: 10.1161/CIRCULATIONAHA.121.057613.
N/A: Not NIH Funded	Neczypor EW, Saldaña TA, Mears MJ, Aslaner DM, Escobar YH, Gorr MW, Wold LE. e-Cigarette Aerosol Reduces Left Ventricular Function in Adolescent Mice. Circulation. 2022 March 15;145(11):868-870. PubMed PMID: 35184570; PubMed Central PMCID: PMC8923958; DOI: 10.1161/CIRCULATIONAHA.121.057613.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

NOTHING TO REPORT

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization? No

C.5 OTHER PRODUCTS AND RESOURCE SHARING

NOTHING TO REPORT

D. OVERALL PARTICIPANTS

D.1 WHAT INDIVIDUALS HAVE WORKED ON THE PROJECT?

Commons ID	S/K	Name	Degree(s)	Role	Cal	Aca	Sum	Foreign Org	Country	SS
LEWOLD	Y	Wold, Loren E	PHD	PD/PI	2.0	0.0	0.0			NA
COHENM2004	Y	COHEN, MITCHELL D	BS,MS,PHD	PD/PI	0.1	0.0	0.0			NA
HUFNAGLE.4	N	Mackos, Amy R	BS,PHD	Faculty	1.0	0.0	0.0			NA
GORR01	Y	Gorr, Matthew William	MS,PHD,BS	Co-Investigator	5.0	0.0	0.0			NA

Glossary of acronyms:
S/K - Senior/Key
Cal - Person Months (Calendar)
Aca - Person Months (Academic)
Sum - Person Months (Summer)
Foreign Org - Foreign Organization Affiliation
SS - Supplement Support
RS - Reentry Supplement
DS - Diversity Supplement
OT - Other
NA - Not Applicable

D.2 PERSONNEL UPDATES

D.2.a Level of Effort

Not Applicable

D.2.b New Senior/Key Personnel

Not Applicable

D.2.c Changes in Other Support

Not Applicable

D.2.d New Other Significant Contributors

Not Applicable

D.2.e Multi-PI (MPI) Leadership Plan

Not Applicable

E. OVERALL IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

NOTHING TO REPORT

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

Not Applicable

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?

NOTHING TO REPORT

G. OVERALL SPECIAL REPORTING REQUIREMENTS SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

NOTHING TO REPORT

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

Not Applicable

G.4.b Inclusion Enrollment Data

NOTHING TO REPORT

G.4.c ClinicalTrials.gov

Does this project include one or more applicable clinical trials that must be registered in ClinicalTrials.gov under FDAAA?

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

NOT APPLICABLE

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT
No foreign component
G.10 ESTIMATED UNOBLIGATED BALANCE
Not Applicable
G.11 PROGRAM INCOME
Not Applicable
G.12 F&A COSTS
Not Applicable

I. OVERALL OUTCOMES

I.1 What were the outcomes of the award?

Please see the detailed attachment that incorporates all Outcomes of Award in the Accomplishments section.