Early life exposure to air pollution induces adult cardiac dysfunction

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^1College of Medicine and the Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio; ^2Department of Anesthesiology and Intensive Care Medicine, Rheinische Friedrich-Wilhelms-University, University Medical Center, Bonn, Germany; ^3College of Public Health, The Ohio State University, Columbus, Ohio; ^4College of Nursing, The Ohio State University, Columbus, Ohio

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Gorr MW, Velten M, Nelin TD, Youtz DJ, Sun Q, Wold LE. Early life exposure to air pollution induces adult cardiac dysfunction. Am J Physiol Heart Circ Physiol 307: H1353–H1360, 2014. First published August 29, 2014; doi:10.1152/ajpheart.00526.2014.—Exposure to ambient air pollution contributes to the progression of cardiovascular disease, particularly in susceptible populations. The objective of the present study was to determine whether early life exposure to air pollution causes persistent cardiovascular consequences measured at adulthood. Pregnant FVB mice were exposed to filtered (FA) or concentrated ambient particulate matter (PM<sub>2.5</sub>) during gestation and nursing. Mice were exposed to PM<sub>2.5</sub> at an average concentration of 51.69 μg/m<sup>3</sup> from the Columbus, OH region for 6 h/day, 7 days/wk in utero using weaning at 3 wk of age. Birth weight was reduced in PM<sub>2.5</sub>-exposed mice compared with FA (1.36 ± 0.12 g FA, <i>n</i> = 42 mice; 1.30 ± 0.15 g PM<sub>2.5</sub>, <i>n</i> = 67 <i>P</i> = 0.012). At adulthood, mice exposed to perinatal PM<sub>2.5</sub> had reduced left ventricular fractional shortening compared with FA-exposed mice (43.6 ± 2.1% FA, 33.2 ± 1.6% PM<sub>2.5</sub>, <i>P</i> = 0.001) with greater left ventricular end systolic diameter. Pressure-volume loops showed reduced ejection fraction (79.1 ± 3.5% FA, 35.5 ± 9.5% PM<sub>2.5</sub>, <i>P</i> = 0.005), increased end-systolic volume (10.4 ± 2.5 μl FA, 39.5 ± 3.8 μl PM<sub>2.5</sub>, <i>P</i> = 0.001), and reduced dP/dt maximum (11,605 ± 200 μl/s FA, 9,569 ± 800 μl/s PM<sub>2.5</sub>, <i>P</i> = 0.05) and minimum (<i>−</i>9,203 ± 235 μl/s FA, <i>−</i>7,045 ± 189 μl/s PM<sub>2.5</sub>, <i>P</i> = 0.0005) in PM<sub>2.5</sub>-exposed mice. Isolated cardiomyocytes from the hearts of PM<sub>2.5</sub>-exposed mice had reduced peak shortening (%FS, 8.53 ± 2.82% FA, 6.82 ± 2.04% PM<sub>2.5</sub>, <i>P</i> = 0.003), slower calcium reuptake (τ, 0.22 ± 0.09 s FA, 0.26 ± 0.07 s PM<sub>2.5</sub>, <i>P</i> = 0.048), and reduced response to β-adrenergic stimulation compared with cardiomyocytes isolated from mice that were exposed to FA. Histological analyses revealed greater picro-sirius red-positive-stained areas in the PM<sub>2.5</sub> vs. FA group, indicative of increased collagen deposition. We concluded that these data demonstrate the detrimental role of early life exposure to ambient particulate air pollution in programming of adult cardiovascular diseases and the potential for PM<sub>2.5</sub> to induce persistent cardiac dysfunction at adulthood.

maternal exposure; air pollution; cardiovascular development; inflammation

IT HAS BECOME INCREASINGLY EVIDENT that the environment plays a major role in the development of human systemic diseases. Current studies have indicated that inhalation of ambient particulate matter (PM) ranks as the ninth cause of overall disease (21). Well-established evidence that exposure to air pollution is linked with heart failure, myocardial infarction, lung dysfunction, lung cancer, and increased morbidity (5, 6, 14, 16, 20, 22, 24, 31) has caused a worldwide need for policy change. Further research regarding air pollution exposure in susceptible populations, including those with obesity, existing heart disease, cystic fibrosis, and more sensitive states is needed, particularly concerning exposure during development.

The sensitive nature of the developing fetus, termed “plasticity” (1), allows the fetus to adapt to an adverse intrauterine environment, e.g., nutritional deficits, maternal inflammation, or stress. The results of these adaptations can be evident in adulthood and have been linked to the development of various metabolic, neurological, pulmonary, and more recently, cardiovascular diseases. This “developmental origin of adult disease” could provide yet another explanation for the detrimental effects of maternal exposure to air pollution during fetal development on the hearts and lungs of offspring.

The consequences of exposure to air pollution during development are not well defined. Evidence from epidemiological studies indicates a strong association between ambient concentrations of air pollutants, including PM, and poor developmental outcomes, such as low birth weight (3, 19, 23) preterm birth (34, 37), and intrauterine growth restriction (10, 36). Animal studies have confirmed these outcomes (25, 38, 40, 44) and shown genetic and epigenetic changes in animals maternally exposed to air pollution (32). Consequences can be seen manifested as poor neurological outcomes (39, 49), increased airway inflammation, and respiratory dysfunction (18). The combination of these factors leads to the hypothesis that air pollution causes an adverse intrauterine environment, altering regular development, which increases the likelihood of subsequent adult diseases.

In the present study, we exposed mice to ambient PM with diameter less than 2.5 μm (PM<sub>2.5</sub>) during perinatal development, from gestation until weaning. Mice were subsequently assessed at adulthood for functional and structural deficits of the cardiovascular system compared with mice exposed to filtered air (FA) alone. This exposure paradigm allows us to examine the consequences at adulthood of early life exposure to relevant concentrations of ambient PM<sub>2.5</sub>.

MATERIALS AND METHODS

Animals and exposure. All animals were handled according to NIH guidelines under Institutional Animal Care and Use Committee (IACUC) protocols approved at both The Research Institute at Nationwide Children’s Hospital and The Ohio State University, Columbus, Ohio. FVB male and female mice were housed for at least 1 wk in our facility before breeding. Pregnancy was confirmed and time-dated with the presence of a vaginal plug. Dams were exposed to concentrated PM<sub>2.5</sub> from the Columbus, OH region using the OASIS-1 aerosol concentration system, which has
been previously described (47). Dams were placed into the exposure system for 6 h a day, 7 days a wk, separated into either PM_{2.5} exposure or air that has been filtered by a high-efficiency air filter (FA). The average PM_{2.5} concentration that the animals were exposed to in this study was 51.69 μg/m³. Because the exposure time represents one-quarter of the day, this is equivalent to a 24-h average of 12.92 μg/m³, below the national air quality standard of 15 μg/m³. Dams were paired overnight, and exposure was begun the day after a plug was observed. Exposures were continued until weaning at 3 wk of age. Following weaning, both groups of mice were returned to room air until they reached 3 mo of age, and male offspring were subjected to analyses as described below.

**Echocardiography.** Cardiac function was analyzed by echocardiography (40 MHz transducer, Vevo 2100; Visualsonics, Toronto, Ontario, Canada). Internal temperature was maintained at 37°C, as mice were continuously sedated with 1% isoflurane (in 100% O₂) during assessment. Prewarmed ultrasound gel (Aquasonic; Parker Laboratories, Fairfield, NJ) was used on the chest with a 15-MHz probe optimized for mice placed in the parasternal, short-axis orientation. Data were averaged from at least three analyses per mouse. Left ventricular (LV) dimensions (LV end-systolic and end-diastolic diameters, LVEDd, and LVESd) and posterior wall thickness at systole (PWTs) and diastole (PWTd) were assessed, using the leading-edge technique according to the American Society for Echocardiography. Fractional shortening (FS) was calculated using the equation: %FS = ([LVEDd - LVESd]/LVESd * 100]. Additional images were acquired to obtain aortic and pulmonary dimensions, and pulse-wave Doppler imaging was used to obtain aortic and pulmonary velocities. Stroke volume (SV) was estimated utilizing the velocity-time integral trace multiplied by the cross-sectional area of the vessel, and this was used to calculate cardiac output (CO) as the product of SV and heart rate (HR).

**Pressure-volume measurements.** LV hemodynamic parameters were assessed in vivo using a pressure-volume (PV) catheter according to the manufacturer’s instructions (Transonic Scisense, London, Ontario, Canada). Mice were anesthetized and intubated, and a 1.2-French pressure-conductance catheter was retrogradely inserted through the right carotid artery into the LV. Following positioning of the catheter in the LV, mice were allowed to stabilize while anesthesia was maintained at 0.8 vol% isoflurane. The following systolic and diastolic hemodynamic parameters were acquired using 8–10 consecutive loops: HR, CO, LV end-diastolic volume (EDV), LV end-systolic volume (ESV), ejection fraction (EF, EDV – ESV)/EDV * 100], maximum dP/dt (+dP/dt), minimum dP/dt (−dP/dt), LV end-diastolic pressure, LV end-systolic pressure, arterial elastance, and relaxation constant (r Weiss).

**Cardiomyocyte isolation and measurement of function and intracellular Ca^{2+} transients.** Cardiomyocytes were isolated as previously described (33, 42, 43, 46, 47). Briefly, hearts were removed and retrogradely perfused through the aorta with Liberase and trypsin until cardiac tissue was dispersed. Left ventricular tissue was washed, gently minced, and passed through a 50-μm mesh. Cellular suspension was placed on laminin-coated glass-bottom inserts (Cell MicroControls, Norfolk, VA) for functional analyses. Glass-bottom inserts were perfused with warm contractile buffer (27, 33, 42, 43, 46, 47) within a flow chamber attached to an Olympus IX-71 microscope. Cells were stimulated (1 Hz, 3-ms duration) with a Myopacer Field-Stimulator system (IonOptix, Milton, MA), and functional properties of the cells were evaluated with the Sarclen Sarcomere Length Acquisition Module using the MyoCam-S Digital CCD camera video imaging system (IonOptix). Analyses of 40–45 cells from five mice per treatment provided the parameters of sarcomere percent peak shortening (normalized to baseline length, %PS; cellular equivalent of %FS), sarcomere maximal departure, return velocities (± dL/dt), and sarcomere time-to-90% PS and relengthening (TPS_{90} and TR_{90}, respectively). For Ca^{2+} measurements, cardiomyocytes in glass-bottom dishes were loaded with fura-2 AM (0.5 μM) for 20 min at 25°C and then washed and treated with normal culture media for 20 min at 25°C. Fluorescence was recorded in stimulated cardiomyocytes using a dual-excitation, single-emission system (IonOptix). Transients were analyzed for values of calcium release (TPS_{90}) and reuptake (τ, TR_{90}). To measure the effect of β-adrenergic stimulation on contractility, contractility was measured in cells treated with 10^{-7} M isoproterenol for 10 min.

**Tissue analyses.** Mice were euthanized, and heart tissue was either fixed in 4% paraformaldehyde or snap frozen using liquid nitrogen. Fixed tissue was transferred to 70% ethanol after 24 h, paraffin embedded, and cut into 5-μm sections. Paraffin-embedded hearts were cut from the base to the apex. Five-micron-thick slides taken at the level below the papillary muscles were stained with picro-sirius red (12). Images were taken from the septum, anterior, posterior, and free wall, and quantitative planimetric analyses were performed. Percentage of picro-sirius red-positive-stained area was quantified as percentage of total myocardial area.

**Statistical analyses.** All data are reported as means ± SE with n = 3–5 mice from at least five different litters. Cardiomyocyte data are reported with 40–45 cells from five mice for each value. Data were analyzed using a Student’s t-test (two-tailed) with Prism 6 (GraphPad, San Diego, CA), and differences were considered statistically significant if P < 0.05.

**RESULTS**

**PM_{2.5} exposure during perinatal development results in lower birth weight but greater body weight at adulthood.** To evaluate the consequences of PM_{2.5} exposure during fetal development, we assessed maternal weight gain and fetal birth weight. Maternal body weight was not different in PM_{2.5}-exposed dams after 20 days of pregnancy, and there was no difference in weight gain compared with FA controls (data not shown). However, birth weights were significantly lower in pups that were born to PM_{2.5}-exposed dams compared with pups born to FA-exposed control mice. At 3 mo of age, body weights were significantly greater in mice that were PM_{2.5}-exposed during perinatal development compared with FA-exposed controls. Absolute heart weight and heart weight normalized to tibia length were increased in pups that were exposed to PM_{2.5} during perinatal development compared with FA-exposed control mice (Table 1).

**Perinatal PM_{2.5} exposure results in LV remodeling.** Three-month-old mice exposed to PM_{2.5} during perinatal development showed substantial cardiac remodeling as illustrated by increased LVEDd and LVEDs dimensions and reduced PWTs (Fig. 1, A–E). Morphological alterations were associated with lower systolic function, as indicated by reduced %FS in PM_{2.5}-exposed mice compared with FA controls (Fig. 1F).

**Perinatal PM_{2.5} exposure causes LV systolic dysfunction.** Transthoracic echocardiographic analyses revealed reduced systolic function in mice that were PM_{2.5} exposed during perinatal development (Fig. 1F). We performed PV analyses evaluating morphological changes and LV function (Fig. 2, A

**Table 1. Parameters from mice exposed to FA or PM_{2.5} during development**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FA</th>
<th>PM_{2.5}</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g</td>
<td>1.36 ± 0.12</td>
<td>1.30 ± 0.15</td>
<td>0.012</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>22.4 ± 0.5</td>
<td>24.0 ± 0.3</td>
<td>0.013</td>
</tr>
<tr>
<td>Heart weight, mg</td>
<td>107.6 ± 3.1</td>
<td>119.4 ± 2.7</td>
<td>0.014</td>
</tr>
<tr>
<td>Heart weight/tibia length</td>
<td>60.2 ± 1.5</td>
<td>66.9 ± 1.5</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Values are means ± SE, FA, filtered air; PM_{2.5}, particulate matter with diameter less than 2.5 μm.
Perinatal PM2.5 exposure is associated with increased cardiac collagen deposition. Morphometrical analyses of picrosirius red-stained histological sections indicated an increase in collagen deposition in 3-mo-old mice hearts that were exposed to PM2.5 during perinatal development (Fig. 5). Quantitative real-time PCR analyses revealed no differences in inflamma-

and B). LV ESV and EDV were both increased in 3-mo-old mice that were exposed to PM2.5 compared with FA-exposed controls, confirming the increased dimension observed by echocardiography (Fig. 2, C and D). Also consistent with echocardiographic findings, these structural alterations were associated with reduced LV contractility in mice exposed to PM2.5, as evident by reduced EF and dP/dtmax compared with FA-exposed controls (Fig. 2, E and F). HRs during PV analyses were not different between groups (501 ± 11 beats/min FA vs. 504 ± 6 beats/min PM2.5).

LV diastolic dysfunction is evident in mice exposed to PM2.5 during perinatal development. LV relaxation was decreased in mice that were PM2.5 exposed during perinatal development, as indicated by reduced dP/dtamin (Fig. 2G), which is common in other models of heart failure. Arterial load was increased in PM2.5-exposed mice compared with FA controls, as indicated by increased arterial elastance (Fig. 2H).

Perinatal exposure to PM2.5 results in in vitro cardiomyocyte dysfunction. Perinatal PM2.5 exposure resulted in in vivo systolic and diastolic dysfunction in 3-mo-old mice. To further evaluate potential cell-specific mechanisms, we performed functional analyses in isolated cardiomyocytes. Cardiomyocytes isolated from mice that were exposed to PM2.5 during perinatal development showed alterations in sarcomere function evident at the cellular level, as indicated by a reduction in %PS (Fig. 3, A and B). TPS90, TR90, +dl/dt, and −dl/dt were not different between groups (Fig. 3, C and D).

In vitro β-adrenergic response is suppressed in PM2.5-exposed mice. Sarcomere shortening was altered in response to β-adrenergic stimulation in cardiomyocytes isolated from 3-mo-old mice that were exposed to PM2.5 during fetal development and nursing (Fig. 3E). Absolute sarcomeric contraction was not different following β-adrenergic stimulation with isoproterenol, as indicated by %PS of cardiomyocytes isolated from 3-mo-old mice that were exposed to PM2.5 or FA during fetal development and nursing (Fig. 3F). However, in contrast to nonstimulated cardiomyocytes, duration of contraction was increased in response to β-adrenergic stimulation in 3-mo-old mice that were exposed to PM2.5 during perinatal development, as indicated by reduced +dl/dt and −dl/dt compared with FA-exposed mice (Fig. 3, G and H).

Perinatal exposure to PM2.5 results in delayed calcium reuptake. Calcium transient amplitude (%PS FURA) was not different in 3-mo-old mice that were exposed to PM2.5 or FA during perinatal development (Fig. 4A). However, calcium reuptake was delayed in mice from the PM2.5 group, as indicated by an increase in τ duration compared with the FA group (Fig. 4B).
tory cytokines, chemokines, or fibrotic genes in 3-mo-old mice from either group (data not shown).

DISCUSSION

In the current study, we found that exposure to environmentally relevant PM2.5 concentrations during early life can cause significant cardiovascular dysfunction at adulthood. Contractility defects in the perinatally exposed mice measured in isolated cardiomyocytes confirmed this observation and further established functional consequences on the calcium-handling system and contractile machinery of the heart, with impaired calcium reuptake and response to β-adrenergic stimulation. A profibrotic response was indicated by increased collagen deposition in the myocardium, but analyses at the molecular level did not indicate increased inflammatory or fibrotic markers.

This study is the first to show an incipient heart failure phenotype in mice exposed to PM2.5 during development both in vivo and in vitro. A recent study where mice were exposed to higher particulate concentrations of diesel exhaust (~300 µg/m³) for 5 days a week until E17.5 also revealed increased collagen deposition, but a reduction in cardiovascular function was not observed in adult mice. However, this study revealed
reduced blood pressure as measured using a tail cuff and an impaired response to transaortic constriction (45). Myocardial oxidative stress from animals maternally exposed to urban air pollution has also been shown (9), which could play a role in the cardiovascular dysfunction observed in the adult mice in our study.

Cardiac inflammation resulting in matrix remodeling and fibrosis is a hallmark of various cardiac diseases in humans and experimental models (12, 13, 41). Inflammation during development may be sufficient to induce cardiovascular dysfunction observed in the adult mice in our study.

Cardiac inflammation resulting in matrix remodeling and fibrosis is a hallmark of various cardiac diseases in humans and experimental models (12, 13, 41). Inflammation during development may be sufficient to induce cardiovascular dysfunction observed in the adult mice in our study. In particular, rodent models investigating cardiac ischemia or pressure overload have reported a transient increase in inflammatory and fibrotic markers in response to the initial insult (12, 41). However, this insult results in a persistent collagen deposition, leading to cardiac fibrosis with sustained physiological consequences, including reduced contractility and relaxation.

The observed collagen deposition in our study is notably lower compared with other disease models. In a model of maternal inflammation induced by lipopolysaccharide injection, a cardiovascular phenotype was observed that was similar to what we found in mice exposed to both perinatal and in utero...
PM$_{2.5}$ exposure (42, 43). Additionally, recent studies examining the placenta of pregnant mice exposed to PM$_{2.5}$ have shown altered morphology, such as reduced maternal blood space area as well as recruitment of inflammatory cells to the placental vasculature (44). Neuroinflammation has also been observed in mice exposed to diesel exhaust during the prenatal period, evident by increased microglial activation and brain cytokine levels (4). Taken together, these studies suggest that exposure to PM$_{2.5}$ causes maternal inflammation contributing to adulthood dysfunction in the resultant offspring; however, the mechanism(s) of how inflammation contributes to the development of cardiovascular dysfunction remain unclear.

Exposure to other contaminants during gestation has led to similar detrimental effects. In a study where exposure to polycyclic aromatic hydrocarbons (PAHs, a contaminant found in air pollution) was monitored in pregnant women, increased PAH exposure during the first trimester increased the risk for fetal growth ratio reduction (7). This was confirmed in controlled animal studies where exposure to PAH altered placental vasculature, increased apoptotic markers during gestation, and reduced survival of resultant adult mice (11). Prenatal exposure to environmental sidestream tobacco smoke has also been shown to increase atherogenesis, superoxide dismutase activity, and mitochondrial damage in the heart tissue of mice (48), as well as increase blood pressure reactivity in humans (8).

Another mechanism that may contribute to the adulthood consequences of developmental exposure to PM$_{2.5}$ is the translocation of PM into the developing fetus. Although this has not been shown, the possibility that PM could translocate into the bloodstream of a pregnant mother through the lungs, and then enter the fetus through the placental vasculature is validated by several studies. In particular, radiolabeled particles can pass into the circulation from the lungs in both humans and rats, including iridium particles less than 100 nm (17, 28, 29). However, this research remains controversial, with conflicting research showing no translocation into the bloodstream when technicium-labeled 100-nm particles were inhaled following a single exposure (26). Regardless, PAHs and metals as components of PM can enter the bloodstream via inhalation and affect the developing fetus.

Lowered birth weights of mice exposed in utero to PM$_{2.5}$ compared with FA is in concurrence with epidemiological
studies linking higher PM exposure to decreases in birth weight (2, 19, 30, 35). This is confirmed in a similar study where mice were exposed to diesel exhaust during a portion of development (15). However, in another study where mice were exposed to high concentrations of diesel exhaust during gestation, body weight was not significantly reduced in 2-day-old pups (38). This disagreement could be due to the strain of mice used.

Further research is needed to identify a critical window in development in which exposure to ambient air pollution has the greatest effect. Research is also needed that will elucidate particular mechanisms that cause improper development or injury of the cardiovascular system during development. These studies will help to validate PM$_{2.5}$ exposure as a unique and relevant model to evaluate the Barker hypothesis of the developmental programming of adult diseases.

**GRANTS**

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**

9. Damaceno-Rodrigues NR, Vasconcelos MM, Negri EM, Zanchi AC, Cademuro CR, Saldiva PH, Dolhnikoff M, Caldini EG. Effects of postnatal lung injury of the cardiovascular system during development. These studies linking higher PM exposure to decreases in birth weight (2, 19, 30, 35). This is confirmed in a similar study where mice were exposed to diesel exhaust during a portion of development (15). However, in another study where mice were exposed to high concentrations of diesel exhaust during gestation, body weight was not significantly reduced in 2-day-old pups (38). This disagreement could be due to the strain of mice used.

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