Cardiovascular Remodeling in Response to Long-Term Exposure to Fine Particulate Matter Air Pollution

Wold et al: Air Pollution-Induced Cardiomyopathy

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Abstract

Background—Air pollution is a pervasive environmental health hazard that occurs over a lifetime of exposure in individuals from many industrialized societies. However, studies have focused primarily on exposure durations that correspond to only a portion of the lifespan. We therefore tested the hypothesis that exposure over a considerable portion of the lifespan would induce maladaptive cardiovascular responses.

Methods and Results—C57BL/6 male mice were exposed to concentrated ambient particles <2.5 μm (PM2.5) or filtered air (FA), six hours/day, five days/week for nine months. Assessment of cardiac contractile function, coronary arterial flow reserve, isolated cardiomyocyte function, expression of hypertrophic markers, calcium handling proteins, and cardiac fibrosis were then performed. Mean daily concentrations of PM2.5 in the exposure chamber vs. ambient daily PM2.5 concentration at the study site were 85.3 vs. 10.6 μg/m³ (7.8-fold concentration), respectively. PM2.5 exposure resulted in increased hypertrophic markers leading to adverse ventricular remodeling characterized by MHC isoform switch and fibrosis, decreased fractional shortening (39.8±1.4 FA vs. 27.9±1.3 PM, FS%), and mitral inflow patterns consistent with diastolic dysfunction (1.95±0.05 FA vs. 1.52±0.07 PM, E/A ratio). Contractile reserve to dobutamine was depressed (62.3±0.9 FA vs. 49.2±1.5 PM, FS%) in response to PM2.5 without significant alterations in maximal vasodilator flow reserve. In vitro cardiomyocyte function revealed depressed peak shortening (8.7±0.6 FA vs. 7.0±0.4 PM, %PS) and increased time-to-90% shortening (72.5±3.2 FA vs. 82.8±3.2 PM, ms) and relengthening (253.1±7.9 FA vs. 282.8±9.3 PM, ms), which were associated with upregulation of pro-fibrotic markers and decreased total antioxidant capacity. Whole heart SERCA2a levels and the ratio of α/β-MHC were both significantly decreased (p<0.05) in PM2.5-exposed animals, suggesting a switch to fetal programming.

Conclusions—Long-term exposure to environmentally relevant concentrations of PM2.5 resulted in a cardiac phenotype consistent with incipient heart failure.

Key Words: air pollution, diesel exhaust, particulate matter, cardiovascular, cardiac, remodeling
Epidemiologic evidence supports an important association between air pollution exposure and cardiovascular risk. Current evidence suggests that long-term exposure increases cardiovascular risk to an even greater extent than short-term exposure.\(^1\) An acute (days) 10 μg/m\(^3\) increase in PM\(_{2.5}\) elevates cardiovascular mortality by 1%, whereas long-term exposure with levels chronically elevated by the same degree increases this risk by ≥ 10%. This supports the concept that aggregate exposure over time may be an even more important determinant of eventual risk than short-term exposure. Cumulative exposure has the potential of not only influencing adverse events, but could also play a fundamental role in organ-specific pathology.

Air pollution exposure occurs over a lifetime in many individuals, and an important consideration is the effect of long term exposure, or at the very least, over a substantial portion of an individual’s lifespan. Numerous investigations have elucidated potential biological mechanisms whereby exposure to PM\(_{2.5}\) may modulate disease susceptibility, including progression of atherosclerosis, inflammation and hypertension.\(^2\)-\(^6\) Most of these studies, although considered chronic (~10-20 weeks), have not explored the effects of exposure over longer periods. Long-term exposure in animal models may provide valuable insights into the resultant phenotype and serve as a foundation to better understand interactive effects of air pollution with other pervasive risk factors, including elevated LDL cholesterol or diabetes. In the present study, we focused on the long-term effects of air pollution exposure over more than half of a rodent’s (mouse) life-span. We hypothesized that air pollution itself, in the absence of other risk factors, may exert discernible effects on the cardiovascular phenotype that are indistinguishable from the protracted effects of other conventional risk factors such as
hypertension. This cardiovascular phenotype may explain the propensity to develop complications.

**Methods**

**Animals and Exposure:** C57BL/6 male adult mice were exposed for nine months, starting at eight weeks of age, to concentrated PM$_{2.5}$ from the Columbus, Ohio region. Concentrated PM$_{2.5}$ was generated using a versatile aerosol concentration enrichment system modified for long-term exposures (OASIS-1) and has been previously described$^2,7-9$ and characterized$^{10,11}$. The ambient mean daily PM$_{2.5}$ concentration at the study site was 10.6 $\mu$g/m$^3$. Mean daily concentration of PM$_{2.5}$ in the exposure chamber was 85.3 $\mu$g/m$^3$ (7.8-fold concentration from the ambient levels), with the control mice exposed to an identical treatment except for a high-efficiency particulate air filter (FA, Pall Life Sciences, East Hills, New York) that was positioned in the inlet valve to the exposure system to remove all of the particles from the airstream. Since the mice were exposed for six hours/day, five days/week, the equivalent PM$_{2.5}$ concentration to which the mice were exposed in the chamber normalized over the exposure period (9 months) was 15.2 $\pm$ 0.91 $\mu$g/m$^3$, which is close to the annual average PM$_{2.5}$ National Ambient Air Quality Standard (NAAQS) of 15 $\mu$g/m$^3$.

**Blood Pressure:** Systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial blood pressure (MAP) were measured using a computerized noninvasive tail-cuff manometry system (Visitech IITC model 129 System, Visitech Systems, Apex, North Carolina). To avoid anxiety due to the procedure, mice were trained for five consecutive days prior to the experimental procedure. To minimize procedure-associated effects on blood pressure, the first 10
of 20 blood pressure (BP) values recorded during each measurement were not used, and the remaining 10 values were averaged for analyses of the data from each mouse. BP was recorded daily for three days, and the average BP values are presented.

**In Vivo Cardiac Assessment:** *In vivo cardiac* function was determined using a VisualSonics Vevo 2100 with a 40 MHz transducer (Visualsonics, Toronto, Ontario). Mice were anesthetized in a sealed chamber with 2% isoflurane. Mice were then moved to a heated procedure board and anesthesia was sustained with 1.5% isoflurane (delivered in 100% O₂) supplied through a nose cone. The limbs were gently taped to ECG electrodes coated with electrode cream (Signa Gel, Parker Labs, Fairfield, New Jersey) and a rectal thermometer was inserted for maintenance of normothermia (37°C internal temperature). Nair hair removal lotion (Church & Dwight Co., Princeton, New Jersey) was applied to the chest in order to remove fur from the imaging location. Next, prewarmed ultrasound gel (Aquasonic, Parker Labs, Fairfield, New Jersey) was placed on the chest and a 15 MHz probe (optimized and dedicated to rodent studies) was placed in the parasternal, short axis orientation. LV systolic and diastolic internal dimensions (LVEsd and LVEDd) and systolic and diastolic posterior wall thickness (PWTs and PWTd) were recorded. Three loops of M-mode data were captured for each animal and data were averaged from at least five beat cycles/loop. Parameters were detected using the American Society for Echocardiography leading-edge technique by an investigator blinded to group assignment.¹² These parameters allow the determination of LV % fractional shortening (%FS) by the equation: 

\[ \%FS = \frac{(LVEDd - LVEDs)}{LVIDd} \times 100 \]

The echo probe was then moved to the subcostal orientation and a four chamber apical view was obtained and the mitral valve annulus was visualized. The sample volume was placed at the level of the mitral valve leaflets and pulsed
wave Doppler was used to capture the E and A peaks representing passive and active mitral flow, respectively. The relative ratio of the E/A wave from the transmitral valve flow waveform was used to evaluate LV diastolic function.

We adapted the method of Hartley et al.\textsuperscript{13} for evaluation of coronary flow and coronary flow reserve. From the apical four-chamber view, the probe was moved closer to the surface of the chest (maintaining the same angle as used for the mitral valve flow). Using color flow Doppler, we visualized the left coronary artery (LCA). The isoflurane concentration was adjusted to 1.0% and the animal was allowed to equilibrate for five minutes after which time the sample volume (0.5 mm gate size) was placed on the main LCA branch. Baseline coronary flow data was acquired and three traces were analyzed and averaged. Next, adenosine (140 µg/kg/min) was infused into the tail vein in order to maximally dilate the coronary vasculature. Maximal hyperemia was obtained within one minute. Three traces were analyzed and averaged to calculate the hyperemic coronary flow. Coronary flow velocity reserve (CFVR) was calculated using the following equation:

\[
\text{CFVR} = \frac{\text{CFV}_{\text{hyperemia}}}{\text{CFV}_{\text{baseline}}} \quad \text{14}
\]

All echocardiographic measurements were performed by an investigator blinded to group assignment and analyzed by a second investigator who was also unaware of group assignment.

\textit{In Vitro Cardiomyocyte Function:} Myocytes were isolated using retrograde perfusion through the aorta with liberase and cultured until the time of experiment as described previously.\textsuperscript{15-19} Myocyte mechanics (twitches) and intracellular Ca\textsuperscript{2+} transients were assessed using video-based sarcomere-detection coupled to a fluorescent system (IonOptix, Milton, Massachusetts).\textsuperscript{15-19} Myocytes were plated in small, glass-bottom inserts (Cell MicroControls, Norfolk, Virginia) and placed into a flow chamber attached to the stage of an inverted Olympus IX-71 microscope. The
cells were observed using a 40X objective and superfused with contractile buffer (CB) at 1 ml/min at ~37°C with a Warner in-line heater and an automatic temperature controller (Warner Instruments, Hamden, Connecticut). The constituents of the CB included (in mM, pH 7.4): 131 NaCl, 4 KCl, 10 HEPES, 1 CaCl2, 1 MgCl2 and 10 Glucose. The cells were field-stimulated at 1 Hz with 3 ms duration using a Myopacer Field-Stimulator system (IonOptix, Milton, Massachusetts), which uses a setup containing two platinum wires on either end of the chamber insert. To measure functional properties of the cells, the Sarcolen Sarcomere Length Acquisition Module (IonOptix, Milton, Massachusetts) was applied. Using the IonOptix video imaging system, sarcomere length was recorded using a Myocam-S Digital CCD camera. The following five parameters were recorded: sarcomere peak shortening normalized to baseline length (PS, the maximal % change of the sarcomere length from the resting state), sarcomere time-to-90% peak shortening (TPS90, time to 90% of cell shortening), time-to-90% relengthening (TR90, time to 90% of cell relaxation), and sarcomere departure and return velocities (±dL/dt, the maximal velocities of cell shortening and relengthening).

**Histological Analyses:** Frozen and paraffin-embedded hearts were cut from base to apex. The sections below the papillary muscles were identified and 4 μm cross sections were stained with hematoxylin and eosin for morphometric and Picrosirius-red (SR) for collagen analyses. Quantitative planimetric analyses were performed on four successive sections per slide, and at least 10 sections from three consecutive slides per mouse were examined. Each image was digitized using a digital camera and analyzed under a research microscope (Zeiss Axioscope with Spot I digital camera, Jena, Germany) using NIH Image software (version 1.61). Nuclear and cytosolic areas were quantified, and the results are expressed as ratios of nuclear to cytosolic.
areas (μm/μm). SR-positive stained area was quantified as a percentage of the total myocardial area (% area). All analyses were performed by an investigator blinded to group assignment.

**Western Immunoblotting:** Frozen hearts were homogenized in ice-cold 25 mM HEPES, pH 7.4, 50 mM NaCl, 1 mM MgCl₂, 2 mM EGTA, 0.1% TritonX-100, 0.1% sodium deoxycholate, 10 mM sodium pyrophosphate, 10 mM sodium fluoride, 1 mM sodium orthovanadate, 0.5 mM phenylmethanesulfonyl fluoride, 10 mg/ml aprotinin, 10 mg/ml leupeptin (~100 mg tissue/ml lysis buffer) and proteins were separated on SDS-PAGE gels and transferred to PVDF membranes. Membranes were probed with antibodies to SERCA2a ATPase (Biomol, SA-209, Plymouth Meeting, Pennsylvania), α-MHC (Genway, 20-272-191956, San Diego, California), β-MHC (Santa Cruz, sc71575, Santa Cruz, California), collagen I (Abcam, ab292, Cambridge, Massachusetts) and collagen III (EMD Millipore, 234189, Billerica, Massachusetts). Blots were developed using enhanced chemiluminescence (ECL Western Blotting Detection, GE Healthcare, United Kingdom) and expression levels were quantified using Image Quant software, version 5.0 (Molecular Dynamics, Sunnydale, California). The band density of the protein of interest was normalized to β-actin (Abcam, ab292, Cambridge, Massachusetts) or GAPDH (Abcam, ab9485, Cambridge, Massachusetts).

**Quantitative Real-Time PCR:** Total RNA was prepared from heart tissue using an RNeasy Mini kit (Qiagen, Valencia, California). cDNA was synthesized using a Transcriptor First-Strand cDNA Synthesis Kit (Roche, Indianapolis, Indianapolis). Quantitative real-time PCR was performed using the iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules,
California). Sequences of primers used for real-time PCR in this study have previously been described.\textsuperscript{10}

**Total Antioxidant Capacity:** The Cayman Chemical Antioxidant Assay Kit (Cayman Chemical, \#709001) was used to measure total antioxidant capacity of mouse plasma, per the manufacturer’s instructions. The methodology of this assay depends on the ability of antioxidants in the sample to inhibit ABTS\textsuperscript{®} oxidation (2,2’-azino-di-[3-ethylbenzthiazoline sulphonate]) to ABTS\textsuperscript{®} • + by metmyoglobin. The ability of antioxidants in the sample to prevent the oxidation of ABTS\textsuperscript{®} is compared with Trolox, a water-soluble analogue of tocopherol, and is quantified as molar Trolox equivalents.

**Statistical Analyses:** Data are expressed as mean ± standard error of the mean (SEM). Comparison of continuous variables was performed using a Student’s t-test. \(p< 0.05\) was considered statistically significant. Statistical analyses were performed using GraphPad PRISM 5 (La Jolla, California).

**Results**

**Long-term PM\textsubscript{2.5} exposure alters blood pressure and cardiac morphology:** Body weights were not different in PM\textsubscript{2.5} exposed mice after nine months compared to FA controls (exposure averages for each week are shown in Table 1); post-mortem analyses confirmed that absolute heart weight and the heart weight/body weight ratio of PM\textsubscript{2.5} exposed mice was significantly increased by 8.8\% compared to FA mice. Long-term exposure to PM\textsubscript{2.5} caused a significant increase in heart rate, systolic blood pressure, diastolic blood pressure, and mean arterial blood
pressure of C57BL/6 mice. However, pulse pressure was decreased in PM$_{2.5}$ compared to FA-exposed mice (Table 2).

**Long-term PM$_{2.5}$ exposure is associated with LV remodeling:** Male mice exposed to PM$_{2.5}$ had considerable cardiac remodeling that was characterized by an increase in both LVES$_d$ and LVED$_d$ compared to FA exposed controls. Posterior wall thickness was not different in diastole (PWT$_d$), but systolic posterior wall thickness (PWT$_s$) was decreased in PM$_{2.5}$ exposed mice. These changes were associated with reduced systolic function as evidenced by decreased % fractional shortening in the PM$_{2.5}$ compared to FA exposed mice. Mitral valve E/A ratio was lower in the PM$_{2.5}$ compared to FA exposed mice (Table 3).

**Contractile Reserve in mice exposed to long-term PM$_{2.5}$:** Chronic systolic dysfunction is often associated with diminished sensitivity of the $\beta$-receptor due to receptor desensitization. Thus we investigated the cardiac response to a $\beta$1-adrenergic agonist to assess chronotropic and contractile reserve. Both groups responded to dobutamine (3.65 $\mu$g/kg) with a significantly increased HR. However, examination of fractional shortening revealed a reduced contractile response to dobutamine in mice exposed to PM compared to FA controls (Table 3).

**Coronary flow-reserve is preserved in mice exposed long-term to PM$_{2.5}$:** Cardiac hypertrophy and remodeling is frequently linked to changes in coronary flow reserve. Upon stimulation with the vasodilator adenosine (150 $\mu$g/kg/min), both the PM$_{2.5}$- and FA-exposed groups had increased coronary flow to a similar degree, indicating preserved coronary flow reserve in PM$_{2.5}$ exposed mice (Table 3).
Long-term PM$_{2.5}$ exposure causes cardiomyocyte dysfunction *in vitro*: We performed functional assessment of isolated single cardiomyocyte function as an additional validation of cardiac-specific PM$_{2.5}$ effects (representative twitch from FA and PA-exposed shown in Figure 1A). Isolated myocytes from PM$_{2.5}$ exposed mice showed a significant decrease in % peak shortening (%PS; Figure 1B), along with an increase in time-to-90% peak shortening (Figure 1C; myocyte systolic dysfunction) and time-to-90% relengthening (Figure 1D; myocyte diastolic dysfunction). These results suggest *in vivo* cardiac dysfunction is also evident at the myocyte level (cardiomyocyte dysfunction), further confirming the results obtained by echocardiography.

Cardiomyocyte nucleus/cytoplasm ratio was markedly decreased in PM$_{2.5}$ exposed mice by 20.9% compared to FA mice (mean [SD], 0.0378 [0.00601] to 0.0299 [0.00281]; p<0.05; Figures 2A & 2B). Atrial natriuretic peptide (ANP) mRNA expression was increased and α-tubulin mRNA was not significantly different following long term PM$_{2.5}$ exposure (Figure 3A & B).

Long-term PM$_{2.5}$ exposure is associated with increased cardiac collagen deposition: Picrosirius-red staining (Figures 4A & B) indicated an increase in cardiac collagen deposition in mice exposed to PM$_{2.5}$ (166%). Real-time PCR revealed increased transforming growth factor (TGF)-β and collagen I, the major structural collagen in the myocardium, suggesting that PM$_{2.5}$ exposure altered gene expression that is consistent with a pro-fibrotic phenotype (Figure 3C & 3E). Western blot analyses confirmed increased collagen I in PM$_{2.5}$ exposed mice (Figure 5A), while neither collagen III nor osteopontin were altered (Figures 3D, 3F, & 5B).
Long-term PM$_{2.5}$ exposure decreases Ca$^{2+}$ reuptake into the sarcoplasmic reticulum and total antioxidant capacity: $\alpha$-MHC protein levels were not different (Figure 5C), however $\beta$-MHC protein levels were increased and the $\alpha$/\$beta$-MHC ratio was decreased in PM$_{2.5}$ exposed mice (Figure 5D & 5E), a pattern consistent with human and animal models of heart failure. Protein isolated from whole heart homogenates of PM$_{2.5}$ mice had significantly decreased levels of SERCA-2a (Figure 5F), suggesting reduced mechanisms to promote Ca$^{2+}$ reuptake into the sarcoplasmic reticulum in response to PM$_{2.5}$ exposure. Total antioxidant capacity in the plasma was significantly decreased in the plasma of PM$_{2.5}$ mice, suggesting that an imbalance in ROS/antioxidant production occurs following PM$_{2.5}$ exposure (Figure 6).

Discussion

Cardiac dysfunction and pathophysiologic adaptations consistent with dysfunctional myocardium were observed in mice exposed to environmentally-relevant concentrations of PM$_{2.5}$ over the course of a substantive portion of the lifespan. At the molecular level, these changes were typified by cardiac hypertrophy, cardiac muscle isoform switch, upregulation of profibrotic gene expression, and reduction in Ca$^{2+}$ reuptake mechanisms, all consistent with an incipient heart failure phenotype.

Epidemiological studies have now confirmed an association of PM$_{2.5}$ with cardiovascular morbidity and mortality. Short-term elevations over a period of a few hours to days can trigger sudden cardiac death, myocardial infarctions, and heart failure exacerbations.$^{20-25}$ However, exposure to a pervasive pollutant such as air pollution likely occurs over a lifetime, often at high levels for many individuals living in industrialized countries, including India and China.$^{26-30}$ Consistent with this concept of integrated exposure over several years, the best available
epidemiologic data appear to support the idea that the longer the chronicity of exposure, the higher the risk.\textsuperscript{1} In the Women’s Health Study which involved exposure over a few years, there was an even more marked increase in this risk (odd ratio for events 1.76).\textsuperscript{22} Conversely, regulation of air pollution through mandated controls has been shown to improve life expectancy.\textsuperscript{31}

In the present study, hearts from PM\textsubscript{2.5} exposed mice displayed increased systolic, diastolic, and mean arterial blood pressure and decreased pulse pressure, all changes that were consistent with a phenotype of incipient heart dysfunction. Such changes typically follow chronic left ventricular hypertrophy and diastolic dysfunction, which if left unchecked may eventually lead to systolic dysfunction and loss of contractile reserve. We did note evidence of systolic and diastolic dysfunction accompanied by fibrosis of the left ventricle and an increase in gene expression of the major structural collagen types in the myocardium, a finding that has previously been described in conjunction with pro-hypertensive stimuli.\textsuperscript{10} Resting LV systolic and diastolic dysfunction were also confirmed at the myocyte level, where abnormal contractile function and relaxation responses were present.

At a molecular level, heart failure is characterized by numerous changes in contractile pathways and is often characterized by a switch to a fetal programming. The myocardium was characterized by abnormally increased β-myosin heavy chain (β-MHC) following long-term PM\textsubscript{2.5} exposure. Expression of α- and β-MHC is tightly regulated by both developmental and hormonal factors.\textsuperscript{32} Expression of these isoforms is often altered in disease states, including cardiac failure or hypertrophy.\textsuperscript{33} In the failing adult mouse heart, a shift is observed from α-MHC to β-MHC.\textsuperscript{34} Up-regulation of β-MHC transcription is also seen as an early marker of cardiac hypertrophy. Although the adult human myocardium is normally comprised of β-MHC, it is
believed that minor changes in the expression of MHC isoforms significantly alter power output at the cellular power level.\textsuperscript{35} β-MHC contains lower adenosine triphosphatase activity and lower filament sliding velocity, however cross-bridge force is generated with a greater efficiency of energy consumption than α-MHC,\textsuperscript{36} suggesting that a shift from α- to β-MHC could be an adaptive response to preserve energy. Alternatively, contractile dysfunction can promote the progression of various heart diseases. Therefore, contractile dysfunction due to increased β-MHC levels could outweigh the benefits of improved clinical outcomes. At a molecular level, the downregulation of SERCA2a is indicative of abnormal calcium cycling.\textsuperscript{37} Decreased SERCA2a expression and activity is found in heart failure regardless of the etiology of the disease.\textsuperscript{38,39}

Mechanistic studies in animals have previously demonstrated direct acute effects of PM\textsubscript{2.5} on the myocardium that include generation of reactive oxygen species, spontaneous arrhythmias, alterations in myocardial blood flow and coronary vascular resistance (in the presence of coronary ischemia).\textsuperscript{40-43} PM\textsubscript{2.5} is also well known to increase blood pressure with several studies demonstrating that acute/sub-acute air pollution exposure can raise BP.\textsuperscript{44} Longer-term PM\textsubscript{2.5} exposure (over weeks) has been shown to sensitize the vasculature to vasoconstrictive mediators (angiotensin II), likely via reductions in nitric oxide and enhancement of calcium sensitization pathways including Rho/ROCK.\textsuperscript{5,10} Similarly, blockade of the Rho/ROCK pathway with Fasudil can obviate the exaggerated increase in BP induced by longer durations of PM\textsubscript{2.5} exposure in the presence of angiotensin II and decreased cardiac hypertrophy and collagen deposition.\textsuperscript{10} While mediators of increased blood pressure likely differ between acute and longer-term exposure, a sustained increase in diastolic and mean arterial blood pressure, as we have demonstrated in this study, may have considerable significance. Interestingly, these effects are similar to those that have been demonstrated to occur in humans where increases in diastolic blood pressure have
been noted.\textsuperscript{45-47} The effects on blood pressure may make it difficult for conclusions on the relative contribution of direct effects on the myocardium versus blood pressure-mediated effects. Nevertheless, the effects on both systolic and diastolic function at the whole organ and myocyte level were striking and certainly make a case for blood pressure-independent effects that may have stimulated the progression to an incipient heart-failure state.

Although increased life expectancy has occurred following a reduction in air pollution levels, particulate air pollution remains a significant problem in the US and the mechanisms by which air pollution directly affects the cardiovascular system are not completely defined.\textsuperscript{31} An association with heart failure and episodes of air pollution has been previously recognized, although the precise mechanisms remain unclear.\textsuperscript{23, 24} A number of pathways have been hypothesized and include sympathetic activation, increases in blood pressure and arrhythmogenesis, all of which are well known to occur following acute PM\textsubscript{2.5} exposure.\textsuperscript{44, 48} Our results suggest that long-term exposure has direct effects on the myocardium which may have immediate relevance to episodes of heart failure hospitalizations that have been reported following air pollution exposure.\textsuperscript{23, 49} Recurrent heart failure hospitalizations in vulnerable patients is a major cause of morbidity in the United States and worldwide and understanding factors that may play a role at the population level would allow refinement of our current understanding and better inform policy and guidelines.

The concentrations used in this experiment are lower than levels that humans are exposed to in many parts of the world. Daily and annual PM\textsubscript{2.5} levels among cities in Latin America, China and India can average 100-150 $\mu$g/m\textsuperscript{3}, approximately 10-15 fold higher than concentrations common to major U.S. cities.\textsuperscript{50-52} This order of magnitude higher exposure encountered routinely in urban regions of developing countries can only be attained in the
U.S. in laboratory environments. Therefore, our results provide additional understanding of the effects of this high level of PM$_{2.5}$ exposure on functional variables of etiological/pathogenic/prognostic importance. The pervasive nature of air pollution defines this as a risk factor with considerable population attributable risk. Thus, even small effects are very significant from a societal perspective.$^{53}$

Our study has several limitations that must be acknowledged. The exposures were not continuous, whereby the animals were not exposed during the weekends. An additional limitation is that we did not record the temporal response of hemodynamic changes over the duration of exposure. Therefore, we cannot comment on the timing of blood pressure increases in response to PM$_{2.5}$ exposure. Subsets of animals were used for different studies (in vivo vs. in vitro), therefore a potential bias could exist regarding the animals chosen for each study. Also, multiple tests were performed on the same animals, however we consistently observed the same phenotype at the whole organ and cellular levels, therefore it is likely that the constellation of outcomes is responding in concert.

In summary, chronic exposure to PM$_{2.5}$ over a consideration portion of the mouse lifespan results in adverse cardiac remodeling consistent with an incipient heart failure phenotype. These findings have implications for air pollution as an independent risk factor for the development of cardiovascular disease.

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Disclosures

None.

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Table 1. Weekly variation in Ambient and PM\textsubscript{2.5} concentrations.

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<tr>
<td>26</td>
<td>12.62</td>
<td>128.79</td>
</tr>
<tr>
<td>27</td>
<td>5.54</td>
<td>27.00</td>
</tr>
<tr>
<td>28</td>
<td>6.06</td>
<td>39.41</td>
</tr>
<tr>
<td>29</td>
<td>9.18</td>
<td>39.41</td>
</tr>
<tr>
<td>30</td>
<td>8.46</td>
<td>42.93</td>
</tr>
<tr>
<td>31</td>
<td>10.31</td>
<td>62.97</td>
</tr>
<tr>
<td>32</td>
<td>10.28</td>
<td>60.20</td>
</tr>
<tr>
<td>33</td>
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<td>72.19</td>
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<tr>
<td>34</td>
<td>9.67</td>
<td>66.49</td>
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<tr>
<td>35</td>
<td>16.34</td>
<td>138.60</td>
</tr>
<tr>
<td>36</td>
<td>2.63</td>
<td>20.37</td>
</tr>
<tr>
<td>37</td>
<td>20.38</td>
<td>266.37</td>
</tr>
<tr>
<td>38</td>
<td>9.79</td>
<td>86.86</td>
</tr>
<tr>
<td>39</td>
<td>18.91</td>
<td>142.12</td>
</tr>
</tbody>
</table>

The PM\textsubscript{2.5} data are weekly means for each group (Ambient or PM\textsubscript{2.5}), while the concentration ratios were affected by multiple factors, particularly ambient humidity.
Table 2. Body weight and blood pressure parameters of long-term PM$_{2.5}$ exposed mice.

<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>PM</th>
<th>p</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (g)</strong></td>
<td>30.6 ±0.3</td>
<td>29.7 ±0.4</td>
<td>0.103</td>
<td>FA=10;PM=9</td>
</tr>
<tr>
<td><strong>Heart weight (mg)</strong></td>
<td>124.0 ±2.4</td>
<td>138. ±3.7</td>
<td>0.014</td>
<td>FA=5;PM=5</td>
</tr>
<tr>
<td><strong>Heart/BODY weight (mg/g)</strong></td>
<td>4.54 ±0.11</td>
<td>4.16 ±0.16</td>
<td>0.037</td>
<td>FA=5;PM=4</td>
</tr>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td>352.5 ±4.4</td>
<td>379.4 ±6.3</td>
<td>0.008</td>
<td>FA=5;PM=5</td>
</tr>
<tr>
<td><strong>Systolic blood pressure (mmHg)</strong></td>
<td>103.6 ±1.4</td>
<td>112.2 ±1.9</td>
<td>0.0015</td>
<td>FA=10;PM=9</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mmHg)</strong></td>
<td>76.17 ±0.5</td>
<td>86.9 ±1.8</td>
<td>&lt;0.0001</td>
<td>FA=10;PM=9</td>
</tr>
<tr>
<td><strong>Mean arterial pressure (mmHg)</strong></td>
<td>86.6 ±0.6</td>
<td>96.3 ±1.4</td>
<td>&lt;0.0001</td>
<td>FA=10;PM=9</td>
</tr>
<tr>
<td><strong>Pulse pressure (mmHg)</strong></td>
<td>29.9 ±1.5</td>
<td>24.1 ±1.3</td>
<td>0.0095</td>
<td>FA=10;PM=9</td>
</tr>
</tbody>
</table>

Body weight (g), heart weight (mg), ratio heart weight to body weight (mg/g), heart rate (HR) beats per minute (BPM), systolic/diastolic blood pressure, mean arterial pressure (MAP), and pulse pressure of male adult C57BL6 mice exposed to PM$_{2.5}$ or FA for nine months. Data are expressed as mean ± SEM and analyzed using a Student’s t-test.
<table>
<thead>
<tr>
<th></th>
<th>FA</th>
<th>PM</th>
<th>p</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVES_d (mm)</td>
<td>2.56 (±0.10)</td>
<td>2.85 (±0.1)</td>
<td>0.038</td>
<td>FA=10;PM=9</td>
</tr>
<tr>
<td>LVED_d (mm)</td>
<td>4.25 (±0.10)</td>
<td>3.95 (±0.06)</td>
<td>0.0319</td>
<td>FA=10 ; PM=9</td>
</tr>
<tr>
<td>PWT_d (mm)</td>
<td>0.73 (±0.01)</td>
<td>0.69 (±0.03)</td>
<td>0.2494</td>
<td>FA=10 ; PM=9</td>
</tr>
<tr>
<td>PWT_s (mm)</td>
<td>1.28 (±0.02)</td>
<td>1.00 (±0.04)</td>
<td>&lt;0.0001</td>
<td>FA=10 ; PM=9</td>
</tr>
<tr>
<td>FS (%)</td>
<td>39.83 (±1.37)</td>
<td>27.92 (±1.30)</td>
<td>&lt;0.0001</td>
<td>FA=10 ; PM=9</td>
</tr>
<tr>
<td>Mitral E/A (ratio)</td>
<td>1.95 (±0.05)</td>
<td>1.52 (±0.07)</td>
<td>0.0001</td>
<td>FA=9 ; PM=8</td>
</tr>
<tr>
<td>Dobutamine HR (bpm)</td>
<td>583.8 (±8.1)</td>
<td>596.6 (±5.6)</td>
<td>0.231</td>
<td>FA=5 ; PM=5</td>
</tr>
<tr>
<td>Dobutamine FS (%)</td>
<td>62.32 (±0.85)</td>
<td>49.23 (±1.45)</td>
<td>≤0.0001</td>
<td>FA=9 ; PM=8</td>
</tr>
<tr>
<td>Coronary flow velocity (mm/s)</td>
<td>342.9 (±32.6)</td>
<td>255.6 (±12.5)</td>
<td>0.0705</td>
<td>FA=6 ; PM=4</td>
</tr>
<tr>
<td>Coronary flow velocity reserve (mm/s)</td>
<td>800.4 (±41.5)</td>
<td>745.5 (±21.8)</td>
<td>0.3771</td>
<td>FA=5 ; PM=3</td>
</tr>
</tbody>
</table>

Left ventricular end systolic diameter (LVES_d), left ventricular end diastolic diameter (LVED_d), diastolic posterior wall thickness (PWT_d), systolic posterior wall thickness (PWT_s), fractional shortening (FS), mitral flow E to A ratio (mitral E/A), heart rate after Dobutamine challenge (Dobutamine HR), and fractional shortening following dobutamine challenge, coronary flow velocity and coronary flow velocity reserve are expressed as mean ± SEM and analyzed using a Student’s t-test.
Figure Legends

**Figure 1.** Isolated myocyte function from mice exposed to FA or PM for nine months in the OASIS-1 trailer.  A. Representative shortening of FA and PM myocyte contraction.  B. Percent peak shortening (%PS) normalized to sarcomere length (SL). B. Time-to-90% peak shortening (TPS90) and C. Time-to-90% relengthening (TR90).  n=20-30 myocytes from 3-4 mice/group. Data are expressed as mean ± SEM and analyzed using a Student’s t-test. p< 0.05 was considered statistically significant.

**Figure 2.** Histological assessments of FA and PM2.5-exposed hearts.  A. Representative heart tissue sections.  B. Quantitative analyses of nucleus/cytoplasm ratio. Data are expressed as mean ± SEM (n=5) and analyzed using a Student’s t-test. p< 0.05 was considered statistically significant.

**Figure 3.** Quantitative cardiac mRNA expression of A. ANP, B. α-Tubulin, C. TGFβ, D. Osteopontin, E. Collagen I, and F. Collagen III in FA and PM2.5-exposed hearts.  Data are expressed as mean ± SEM (n=5) and analyzed using a Student’s t-test. p< 0.05 was considered statistically significant.

**Figure 4.** Collagen assessment of FA and PM2.5-exposed hearts.  A. Representative heart tissue sections stained with Picrosirius-red.  B. Quantitative assessment of area positive stained for collagen. Data are expressed as mean ± SEM (n=5) and analyzed using a Student’s t-test. p< 0.05 was considered statistically significant.
**Figure 5.** Protein analyses and representative western immune blots from whole heart homogenates of FA and PM$_{2.5}$-exposed mice. A. Collagen I, B. Collagen III, C α-MHC, D. β-MHC, E. Ratio α-/β-MHC, F. SERCA2a. Data are expressed as mean ± SEM (n=5) and analyzed using a Student’s t-test. p< 0.05 was considered statistically significant.

**Figure 6.** Total plasma antioxidant capacity in FA and PM$_{2.5}$-exposed mice. Data are expressed as mean ± SEM (n=6) and analyzed using a Student’s t-test. p< 0.05 was considered statistically significant.
Figure 1

A

B

C

D

FA

PM

FA

PM

Circulation
Heart Failure
JOURNAL OF THE AMERICAN HEART ASSOCIATION

TPS 90 (sec)

TR 90 (sec)
Figure 2

A

B

FA

PM

Nucleus/Cytoplasm Ratio

FA

PM

0.020

0.025

0.030

0.035

0.040

0.045

0.045

0.040

0.035

0.030

0.025

0.020

FA

PM

*
Figure 3

A

ANP mRNA

(1/2ΔCT)

FA PM

FA PM

B

α-Tubulin mRNA

(1/2ΔCT)

FA PM

FA PM

C

TGFβ mRNA

(1/2ΔCT)

FA PM

FA PM

D

Osteopontin mRNA

(1/2ΔCT)

FA PM

FA PM

E

Collagen I mRNA

(1/2ΔCT)

FA PM

FA PM

F

Collagen III mRNA

(1/2ΔCT)

FA PM

FA PM
Figure 4

A

B
Figure 5

A

Collagen I Expression (Arbitrary Units)

FA PM

Collagen I

β actin

FA PM

140 kDa

42 kDa

B

Collagen III expression (Arbitrary Units)

FA PM

Collagen III

β actin

FA PM

138 kDa

42 kDa

C

α MHC expression (Arbitrary Units)

FA PM

α MHC

β actin

FA PM

223 kDa

42 kDa

D

β MHC expression (Arbitrary Units)

FA PM

β MHC

β actin

FA PM

223 kDa

42 kDa

E

Ratio α/β MHC expression (Arbitrary Units)

FA PM

SERCA-2 expression (Arbitrary Units)

FA PM

SERCA-2

β actin

FA PM

110 kDa

42 kDa