Exposure to diesel exhaust particulates induces cardiac dysfunction and remodeling

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Bradley JM, Cryar KA, El Hajj MC, El Hajj EC, Gardner JD. Exposure to diesel exhaust particulates induces cardiac dysfunction and remodeling. J Appl Physiol 115: 1099–1106, 2013. First published July 25, 2013; doi:10.1152/japplphysiol.00343.2013.—Chronic exposure to diesel exhaust particulates (DEP) increases the risk of cardiovascular disease in urban residents, predisposing them to the development of several cardiovascular stresses, including myocardial infarctions, arrhythmias, thrombosis, and heart failure. DEP contain a high level of polycyclic aromatic hydrocarbons, which activate the aryl hydrocarbon receptor (AHR). We hypothesize that exposure to DEP elicits ventricular remodeling through the activation of the AHR pathway, leading to ventricular dilation and dysfunction. Male Sprague-Dawley rats were exposed by nose-only nebulization to DEP (31). Under aerobic conditions, HIF-1α, the competitive pathway to the AHR, and vascular endothelial growth factor, a downstream mediator of hypoxia inducible factor-1α (26 and 47% decrease vs. vehicle, respectively). These findings indicate that exposure to DEP induced left ventricular dilation by loss of collagen through an AHR-dependent mechanism.

IN THE UNITED STATES, APPROXIMATELY 60,000 deaths annually are attributed to particulate air pollution (41). Acute and chronic exposure to particulate matter (PM) is linked to several cardiac diseases, including myocardial infarctions, arrhythmias, thrombosis, and heart failure; however, the mechanisms whereby particles produce cardiac toxicity are largely unknown (5). Several researchers have demonstrated that exposure to PM can produce cardiac dysfunction, but the changes observed differ according to the type of particle, route of exposure, and duration of exposure, leaving much controversy as to how exposure to particulates impacts the heart (12, 28, 35, 42). Moreover, it is not known how exposure to PM impacts the heart’s structural extracellular matrix (ECM), which is a critical component of cardiac function.

The myocardial ECM plays a pivotal role in maintaining the structure of the heart. The ECM is complex and composed of a fibrillar collagen network, basement membrane proteins, proteoglycans, glycosaminoglycans, and bioactive signaling molecules (27). Cardiac structure is primarily regulated through the accumulation and degradation of collagen (6, 27). Under normal physiological conditions, collagen turnover is tightly regulated; however, pathological conditions can disrupt this balance (10, 21, 34). In the chronic pressure overloaded heart, e.g., during hypertension, there is an increased deposition of extracellular collagen, causing stiffness of the ventricular wall (2, 22). Conversely, in the case of chronic volume overload, a condition observed with valve regurgitation, collagen turnover is shifted toward degradation, resulting in a loss of extracellular collagen, causing dilation of the chamber and weakening of the ventricular wall (10, 17, 22, 40). In either case, dysregulation of this balance, either toward accumulation or degradation, results in a change of cardiac structure and function.

Diesel exhaust particulates (DEP) are the most abundant outdoor source of airborne PM (9). DEP is composed of a heterogenous mixture of carbon, inorganic compounds, and polyaromatic hydrocarbons (PAH), ranging from fine (2.5–0.1 μm diameter) to ultrafine (<0.1 μm diameter) particles (9). The PAHs found in DEP are known ligands of the aryl hydrocarbon receptor (AHR) (14, 19, 30, 31). AHR signaling plays an important role in ECM remodeling (1, 25, 29, 38). AHR activation promotes collagen loss in various tissues, including human periodontal ligaments and Zebra fish fins (1, 38).

The role of the AHR is to mitigate the toxic effects induced by halogenated aryl hydrocarbons (31). Due to their hydrophilic nature, hydrocarbons diffuse across the cell membrane to initiate the AHR signaling cascade (14, 19, 30, 31). Binding of the hydrocarbon ligand stimulates the dissociation of AHR-ARNT heterodimer binds to the xenobiotic response element in the promoter region of genes involved in the expression of various biological and toxicological responses, including cytochrome P-450 (Cyp) enzymes, specifically Cyp 1A1 (14, 19, 30, 31).

The ARNT can also bind with hypoxia-inducible factor (HIF)-1α to regulate gene expression during low oxygen stress (31). Under aerobic conditions, HIF-1α is continuously synthesized and degraded. During hypoxia, HIF-1α degradation is inhibited, and translocation to the nucleus is triggered. Upon translocation, HIF-1α competitively dimerizes with ARNT and binds the hypoxic response element in the promoter region,
leading to loss of collagen, and ultimately causing ventricular hypertrophy, a phenotype consistent with pressure overload. Conversely, in the AHR+/− mice, the overexpression of AHR led to a volume overload phenotype with LV dilation (36). Although controversy remains regarding the regulation of these two pathways, activation of either the AHR or HIF-1α may be dependent on the concentration of the AHR’s ligand. Chronic exposure to the aryl hydrocarbons found in DEP leads to prolonged activation of the AHR, causing attenuation of the HIF-1α pathway (31). We hypothesize that, through the activation of the AHR, exposure to DEP induces ECM remodeling by shifting the collagenous ECM balance toward degradation, leading to loss of collagen, and ultimately causing ventricular dilation and dysfunction.

MATERIALS AND METHODS

Studies were performed using 9-wk-old male Sprague-Dawley rats. Rats were housed under standard environmental conditions and maintained on rat chow and tap water ad libitum. All experimental procedures were performed according to the principles outlined in the National Institutes of Health Guidelines for the Care and Use of Experimental Animals and approved by Louisiana State University Health Sciences Center’s Institutional Animal Care and Use Committee.

DEP exposure protocol. After arrival and a brief (1 wk) acclimation to rodent restrainers, rats were randomly divided into two groups: vehicle (0.9% saline + 0.02% Tween 80; n = 7) and DEP (SRM 2975, 0.2 mg/ml in 0.9% saline + 0.02% Tween 80; n = 8). SRM 2975 is a standard DEP derived from forklift exhaust. The National Institute of Standards and Technologies extensively characterized this particulate for content of PAHs and particle size distribution. This particulate is readily available from the National Institute of Standards and Technologies and can be acquired by other investigators for future comparative studies. The concentration used was based on literature dosing of intratracheal instillation (26). To avoid particle aggregation, all samples were prepared fresh before exposure, vortexed for 20 s, and sonicated for 15 min. Rats were exposed for a total of 20 min/day to either DEP or vehicle for a 5-wk period. The total volume nebulized was 10 ml of either DEP or vehicle. Particles were aerosolized using a PARI nebulizer (PARI Respiratory Equipment) at a flow rate of 8 l/min.

Taking into account the flow rate through our exposure system and the mass of the particles before nebulization, the maximum concentration of exposure was 8.75 mg/m³. This exposure concentration is well above ambient air levels, but represents a physiological high level of exposure. Furthermore, this concentration is comparable to other inhalation studies (23, 24). Previous studies have shown that a 20-min daily exposure to particulates is sufficient to cause LV dysfunction (28). The amount of particulate distributed to the animal during a 20 min exposure is ~3% of the particle concentration in the nebulized solution, or ~0.26 mg/m³, which falls within the hazardous range by air quality index (28). The study duration was based on a cigarette smoke exposure study by our laboratory, which found significant changes in cardiac function by 5 wk (4).

### Table 1. Necropsy evaluation

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<thead>
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<th>Vehicle</th>
<th>DEP</th>
<th>P Value</th>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td>0.13</td>
</tr>
<tr>
<td>LV/BW. mg/kg</td>
<td>2.3 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>0.24</td>
</tr>
<tr>
<td>RV/BW. mg/kg</td>
<td>0.6 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.20</td>
</tr>
<tr>
<td>Lung/BW. mg/kg</td>
<td>6.2 ± 1.0</td>
<td>7.1 ± 0.3</td>
<td>0.15</td>
</tr>
<tr>
<td>BW. kg</td>
<td>0.3 ± 0.9</td>
<td>0.3 ± 0.6</td>
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Values are means ± SD; n, no. of animals. DEP, diesel exhaust particulates; LV, left ventricle; RV, right ventricle; BW, body weight.

inducing the transcription of various genes, including vascular endothelial growth factor (VEGF) (19, 31).

Both the AHR and HIF-1α signaling pathways can alter ventricular structure. Thackaberry and colleagues (36) demonstrated that AHR−/− null mice, with increased cardiac expression of HIF-1α and VEGF, exhibited left ventricular (LV) hypertrophy, a phenotype consistent with pressure overload. Conversely, in the AHR+/− mice, the overexpression of AHR led to a volume overload phenotype with LV dilation (36). Although controversy remains regarding the regulation of these two pathways, activation of either the AHR or HIF-1α may be dependent on the concentration of the AHR’s ligand.

Chronic exposure to the aryl hydrocarbons found in DEP leads to prolonged activation of the AHR, causing attenuation of the HIF-1α pathway (31). We hypothesize that, through the activation of the AHR, exposure to DEP induces ECM remodeling by shifting the collagenous ECM balance toward degradation, leading to loss of collagen, and ultimately causing ventricular dilation and dysfunction.

Fig. 1. Diesel exhaust particulates (DEP) induced eccentric left ventricular (LV) dilation. Temporal echocardiogram data demonstrate that exposure to DEP induced LV dilation [LV internal diameter at diastole (LVIDd); A] and LV posterior wall thinning [posterior wall thickness at diastole (LVPWd); B] and reduced eccentric index (2 x LVPWd/LVIDd; C). There was no significant change in fractional shortening (FS) between groups. Values are means ± SE; n = 8 DEP and 7 vehicle. *P < 0.05 vs. vehicle. **P < 0.001 vs. vehicle.
Echocardiographic analysis. Cardiac ventricular dimensions and function were assessed in sedated rats by echocardiography (isoflurane 1.5%; VIVO 770; Visualsonics) 1 day before the start of the exposure protocol, and every week thereafter during the 5-wk protocol. The LV short-axis view was used to obtain B-mode two-dimensional images and M-mode tracings of the ventral (anterior) and dorsal (posterior) LV wall using a two-dimensional reference sector. Echocardiography provided measurements of LV internal diameter (LVID) and posterior wall thickness (LVPW) at diastole (d) and systole (s). LV systolic function was measured by fractional shortening (LVIDd – LVIDs/LVIDd). Eccentric index was evaluated by the relative wall thickness ratio 2 × LVPWd/LVIDd. All measurements were performed on three different cardiac cycles and averaged for each time point.

Pressure-volume loop analysis. At the end of the fifth week, LV function was assessed using pressure-volume loop analysis. Each rat was weighed, anesthetized with 3.5% isoflurane, and ventilated. The left jugular vein was cannulated. A Scisense pressure-volume catheter (FTS-1912B-9018) was introduced into the right carotid artery and advanced into the LV. Following a 10-min equilibration period; pressure and volume signals were continuously recorded at a sampling rate of 1,000 Hz using the Advantage PV System (model FY897B, Scisense, Ontario, CA). Data were acquired using an iWorx 308T data acquisition system with Labscribe software (iWorx) to calculate hemodynamic parameters of heart rate, LV end-systolic pressure, end-diastolic pressure (EDP), end-systolic volume, end-diastolic volume (EDV), stroke volume (SV), cardiac output (CO), ejection fraction, and inverse measure of relaxation rate (τ). Load-independent parameters of systolic function [end systolic pressure-volume relationship (ESPVR)], diastolic function [end-diastolic pressure-volume relationship (EDPVR)], prerecruitable stroke work, and maximum change in pressure over time (dP/dtmax) vs. EDV were obtained following partial occlusion of the abdominal vena cava to vary preload. All measures of cardiac function were evaluated from a minimum of 10 consecutive pressure-volume loops. For volume calibration, 0.1 mL of 15% saline was introduced into the jugular vein of the anesthetized animal, as previously described by Pacher and colleagues (32). After functional assessment, the heart was quickly removed and rinsed with ice-cold phosphate-buffered saline (PBS), and the LV, including septum and right ventricle were separated and weighed. A portion of the mid-LV region was fixed with 4% paraformaldehyde, and the remainder snap frozen in liquid nitrogen and stored at −80°C.

Analysis of the collagen matrix. Interstitial collagen volume fraction (CVF) was calculated from mid-LV sections at the 5-wk end point, as previously described (4, 11, 40). Briefly, LV sections were fixed in 4% paraformaldehyde and embedded in paraffin. Two sections (5 μm) were cut from each heart, attached to slides, and stained with collagen-specific Picrosirius red. Fluorescent images were captured (×20) using a Nikon Eclipse model no. TE2000-U fluorescence microscope and processed with NIS Elements software. The CVF for each section was determined by analyzing 10 random interstitial samples, taking care to exclude perivascular collagen (20 total samples/heart). The mean CVF for each heart was expressed as percent total area and then group averaged.

Western blot analysis. Cardiac protein expression was determined using Western blot analysis, as previously described (4). Briefly, LV tissue was homogenized with lysis buffer (RIPA buffer with protease inhibitors). Protein concentrations were determined using a Bradford protein assay (Bio-Rad), and an equal amount of protein (40 μg) was loaded into each lane. Proteins were separated using electrophoresis and then transferred onto nitrocellulose membranes (Bio-Rad). Membranes were blocked with 5% nonfat milk in Tris-buffered saline and 0.01% Tween for 1 h at room temperature, then incubated with the desired primary antibody overnight at 4°C. Antibodies used were as follows: AHR NB100-2289, Novus; HIF-1α sc-10790 and GAPDH sc-47724, Santa Cruz; transforming growth factor-β 3711, Cell Signaling; matrix metalloproteinase (MMP)-2 ab19015 and MMP-9 AB19016, Millipore; and MMP-8 AB81286 and MMP-13 AB39012, Abcam. Following incubation with secondary antibody, membranes were washed, and enhanced chemiluminescence (Pierce) used to detect protein bands.

Fig. 2. DEP reduced myocardial interstitial collagen. Representative collagen staining of LV sections by Picrosirius red (PSR) at the 5-wk end point is shown for vehicle (A) and DEP exposure (B). C, group averaged collagen volume fraction (CVF) calculated from PSR stained mid-LV sections. Values are means ± SE; n = 8 DEP and 7 vehicle. *P < 0.05 vs. vehicle.
LV extracts by Western blot analysis (H11005/B/H9251, and expressed as percent vehicle controls. Expression of the AHR, HIF-1 increased hypoxia inducible factor (HIF)-1 increased. After 3 × 5 min washes with PBS, the slides were incubated for 1 h with a 1:1,000 horseradish peroxidase-Goat Anti-Rabbit IgG (H+L) (Invitrogen 65-6120), diluted in 1% bovine serum albumin/PBS. After another 3 × 5 min PBS wash, the slides were incubated with 3,3′-diaminobenzidine (Sigma D3939) for 5 min. All slides were washed vigorously with ddH2O counterstained with hematoxylin, dehydrated through 70–100% ethanol and xylene, and mounted in tolune-based media for viewing. Images were taking using a Nikon Eclipse microscope with Nikon Elements analysis software.

**Growth factors and cytokines.** Cardiac growth factors and cytokines were measured in LV homogenates using the Milliplex Map Kit Rat Cardiovascular Disease Panel 1 following the manufacturer’s instructions (Millipore). LV tissue was homogenized with lysis buffer. The Lumines system utilizes the Lumines xMAP technology based on fluorescent-coded beads conjugated with specific monoclonal antibodies. A Bio-Plex array reader and Bio-Plex Manager software were used to analyze the samples. Tissue interleukin (IL)-1β levels were measured using a commercially available ELISA immunoassay kit (R&D Systems). LV tissue was homogenized (10 mg tissue to 0.1 ml RIPA). Protein concentrations were determined using a Bradford protein assay (Bio-Rad) for normalization of growth factors and cytokines to milligram of total protein.

**Statistical analysis.** Statistical analyses were performed using analysis software (Graphpad 5.0; Prism). Echocardiographic grouped data comparisons were made using two-way ANOVA. When significant F

**Immunohistochemistry.** LV sections were fixed in 4% paraformaldehyde and embedded in paraffin. Two sections (5 μm) were cut from each heart, attached to slides, and rehydrated through 100 and 95% ethanol and deionized water. Endogenous peroxidase activity was quenched by incubating the slides in 3% hydrogen peroxide, followed by a rinse in PBS. Sections were blocked with 4% bovine serum albumin for 30 min and incubated over night with 1:4,000 diluted polyclonal rabbit anti-rat CYP 1A1 antibody (Millipore AB1247). After 3 × 5 min washes with PBS, the slides were incubated for 1 h with a 1:1,000 horseradish peroxidase-Goat Anti-Rabbit IgG (H+L) (Invitrogen 65-6120), diluted in 1% bovine serum albumin/PBS. After another 3 × 5 min PBS wash, the slides were incubated with 3,3′-diaminobenzidine (Sigma D3939) for 5 min. All slides were washed vigorously with ddH2O counterstained with hematoxylin, dehydrated through 70–100% ethanol and xylene, and mounted in tolune-based media for viewing. Images were taking using a Nikon Eclipse microscope with Nikon Elements analysis software.

**Table 2. Cardiac functional parameters using pressure-volume catheter**

<table>
<thead>
<tr>
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<th>Vehicle</th>
<th>DEP</th>
<th>P Value</th>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>350 ± 8</td>
<td>381 ± 10*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CO, ml/min</td>
<td>40 ± 6</td>
<td>52 ± 8</td>
<td>0.092</td>
</tr>
<tr>
<td>ESP, mmHg</td>
<td>108 ± 6</td>
<td>121 ± 10</td>
<td>0.15</td>
</tr>
<tr>
<td>EDP, mmHg</td>
<td>8 ± 1</td>
<td>13 ± 2*</td>
<td>0.031</td>
</tr>
<tr>
<td>ESV, μl</td>
<td>278 ± 54</td>
<td>307 ± 48</td>
<td>0.35</td>
</tr>
<tr>
<td>EDV, μl</td>
<td>387 ± 61</td>
<td>452 ± 59</td>
<td>0.23</td>
</tr>
<tr>
<td>SV, μl/beat</td>
<td>108 ± 17</td>
<td>145 ± 18</td>
<td>0.081</td>
</tr>
<tr>
<td>EF, %</td>
<td>30 ± 4</td>
<td>34 ± 4</td>
<td>0.28</td>
</tr>
<tr>
<td>τ, ms</td>
<td>16 ± 1</td>
<td>14 ± 1*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ESPVR</td>
<td>0.7 ± 0.5</td>
<td>0.3 ± 0.2*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EDPRV</td>
<td>0.1 ± 0.2</td>
<td>0.03 ± 0.01</td>
<td>0.096</td>
</tr>
<tr>
<td>PRSW, mmHg</td>
<td>58 ± 6</td>
<td>41 ± 10</td>
<td>0.085</td>
</tr>
<tr>
<td>dP/dt vs. EDV, mmHg·s l−1, μl−1</td>
<td>22 ± 4</td>
<td>11 ± 4*</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. Animals were exposed to either vehicle or DEP for 20 min/day for 5 wk. Parameters of LV function were measured 90 min after final exposure. HR, heart rate; CO, cardiac output; ESP, end-systolic pressure; EDP, end-diastolic pressure; ESV, end-systolic volume; EDV, end-diastolic volume; SV, stroke volume; EF, ejection fraction; τ, inverse measure of relaxation rate; ESPVR, end-systolic pressure-volume relationship; EDPRV, end-diastolic pressure-volume relationship; PRSW, preload recruitable stroke work; dP/dt, change in pressure over time. *Statistical significance: P < 0.05 vs. vehicle.

**Fig. 3. DEP increased cardiac expression of aryl hydrocarbon receptor (AHR, A) and decreased hypoxia inducible factor (HIF)-1α (B).** Expression of the AHR, HIF-1α, and transforming growth factor (TGF)-β (C) in LV extracts by Western blot analysis (n = 5–6/group) is shown. Exposure to DEP for 5 wk induced a significant increase in the expression of AHR (A) and decrease in HIF-1α (B) relative to vehicle (P < 0.05). There was no change in TGF-β expression. Values are means ± SE.
Ding (Fig. 1). There was no change in fractional shortening by 3 wk and continuing to the 5-wk end point (8% increase; Table 2). There were no significant differences in EDV or end-systolic volume between the groups. Exposure to DEP significantly decreased \( \tau \) (19% decrease). SV, CO, and end-systolic pressure were not significantly different between vehicle and DEP-exposed rats. Heart rate was significantly increased in the DEP rats compared with vehicle (8% increase).

Load-independent parameters of contractility, ESPVR, and \( \frac{dP}{dt_{\text{max}}} \) vs. EDV were significantly decreased in response to DEP exposure (57 and 48% decrease, respectively; Table 2). There were no significant differences in EDPVR (\( P = 0.09 \)) or prer recruitable stroke work (\( P = 0.08 \)) between the groups.

**Exposure to DEP activates the AHR pathway.** Exposure to DEP significantly increased the cardiac expression of AHR (19% increase; 90 kDa; Fig. 3A). Immunohistochemistry revealed increased CYP 1A1 expression in LV sections of rats treated with DEP (Fig. 4).

**Exposure to DEP attenuates the HIF-1\( \alpha \) pathway.** DEP-exposed rats had a significant decrease in the cardiac expression of HIF-1\( \alpha \) (26% decrease; ~120 kDa; Fig. 3B); however, there was no difference in the cardiac expression of transforming growth factor-\( \beta \) (~12.5 kDa; Fig. 3C). Assessment of cardiovascular disease markers using a Luminex multiplex array demonstrated that exposure to DEP significantly decreased cardiac VEGF (47% decrease; Table 3). These rats also exhibited significantly increased troponin T in the heart (16% increase; Table 3). There were no significant differences in IL-1\( \beta \), IL-6, myeloperoxidase, plasminogen activator inhibitor-1, tissue inhibitor of MMP (TIMP)-1, or von Willebrand factor between groups.

**Exposure to DEP did not alter MMP expression.** There were no significant differences in the expression of MMP-9 (85 kDa), MMP-2 (63 kDa), MMP-8 (64 kDa), or MMP-13 (60 kDa) between the vehicle- and DEP-exposed rat hearts (Fig. 5).

### Table 3. Cardiovascular disease markers

<table>
<thead>
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<th>Vehicle</th>
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<th>( P ) Value</th>
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<tbody>
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<td>n</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>IL-1( \beta )</td>
<td>4.7 ± 0.7</td>
<td>5.6 ± 1.4</td>
<td>0.30</td>
</tr>
<tr>
<td>IL-6</td>
<td>25.9 ± 3.1</td>
<td>27.2 ± 3.8</td>
<td>0.40</td>
</tr>
<tr>
<td>MPO</td>
<td>3.3 ± 0.2</td>
<td>3.2 ± 0.2</td>
<td>0.31</td>
</tr>
<tr>
<td>PAI-1</td>
<td>372.9 ± 14.1</td>
<td>352.8 ± 6.4</td>
<td>0.12</td>
</tr>
<tr>
<td>TIMP-1</td>
<td>269.6 ± 31.9</td>
<td>319.7 ± 22.2</td>
<td>0.077</td>
</tr>
<tr>
<td>Troponin T</td>
<td>53.120 ± 2.199</td>
<td>61.960 ± 3.067*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>VEGF</td>
<td>108.7 ± 17.0</td>
<td>63.8 ± 3.3*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>vWF</td>
<td>2.4 ± 0.2</td>
<td>3.3 ± 0.7</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Values are means ± SE in pg/mg protein; \( n \), no. of animals. IL-1\( \beta \), interleukin-1\( \beta \); IL-6, interleukin-6; MPO, myeloperoxidase; PAI-1, plasminogen activator inhibitor-1; TIMP-1, tissue inhibitor of matrix metalloprotei nase-1; VEGF, vascular endothelial growth factor; vWF, von Willebrand factor. *Statistical significance: \( P < 0.05 \) vs. vehicle.
DISCUSSION

It is well documented that exposure to PM can produce cardiac dysfunction; however, the mechanisms by which this occurs is unknown (12, 28, 35, 42). Very few have examined how heart function changes in response to particle exposure, with fewer examining the impact of particles on the structural components of the heart (35, 42). Therefore, the goal of our study was to assess the impact of DEP on cardiac function and the ventricular ECM. We found that DEP-exposed animals developed cardiac dysfunction after 5 wk exposure, which was associated with significant alterations in LV interstitial collagen.

Compared with vehicle exposure, rats exposed to DEP exhibited significant LV chamber dilation, accompanied by a decrease in posterior wall thickness. By LaPlace’s Law, ventricular wall stress is directly related to cavity pressure times the radius and inversely related to the wall thickness. A disproportional change in any of these factors, such as an increased chamber radius or decreased wall thickness, can lead to increased stress on the ventricular wall, ultimately causing cardiac dysfunction (22). Hearts of DEP rats exhibited chamber dilation associated with wall thinning, as indicated by a decrease in the eccentric index (2 × LV wall thickness/LV chamber diameter), confirming eccentric dilation, a condition observed during decompensated volume overload heart failure (22). These findings are consistent with a study by Tankersley and colleagues (35) that demonstrated exposure to carbon black, a particle that has a carbon core analogous to that of DEP, resulted in similar eccentric LV dilation in senescent mice; however, there were no structural changes in middle-aged mice. Carbon black, unlike DEP, does not contain cardiotoxic PAHs (3, 37). This difference in the chemical composition of the particles may explain why chronic exposure to DEP degraded ventricular structure and function in our relatively healthy young animals.

The DEP-mediated alterations in structural integrity of the LV were accompanied by altered diastolic function, indicated by an increased EDP and decreased \( \frac{dP}{dt_{\text{max}}} \). Although there was no significant change in EDPVR (\( P = 0.09 \)), a measure of ventricular compliance, rats exposed to DEP trended toward increased LV compliance. Exposure to DEP significantly reduced ESPVR and \( \frac{dP}{dt_{\text{max}}} \) vs. EDV, indicating that contractility was impaired in the DEP-exposed group. Gordon et al. (12) also demonstrated reduced contractility after 4 wk of exposure to DEP, as measured by increased QA intervals via radiotelemetry. Despite decreased contractility and diastolic dysfunction, CO and SV were not decreased and trended to increase. One possible explanation for the maintenance of CO and SV is that 5 wk of DEP exposure may have caused changes in the heart similar to those found in the early, compensated stages of cardiac remodeling (17). DEP exposure resulted in increased heart rate, which has several influences on cardiac function. Heart rate is a component of CO; therefore, the increase in heart rate may explain why DEP-treated animals maintained CO. In addition, heart rate affects relaxation rate, such that the increased heart rate accelerated relaxation, resulting in a decreased \( \tau \). These findings support a compensatory response to DEP exposure by heightened sympathetic drive. We speculate that prolonged exposure to
DEP would lead to progressive LV eccentric dilation and further degradation of cardiac function.

Because DEP exposure resulted in eccentric LV dilation, we postulated that DEP disrupted myocardial collagen. The myocardial ECM plays a pivotal role in maintaining the structure of the heart. Cardiac structure is primarily regulated through the accumulation and degradation of collagen within the ECM (6, 27). Collagen turnover is tightly regulated under normal conditions; however, pathological stimuli can disrupt this regulation, resulting in a change of cardiac structure and ultimately function (10, 21, 34). Our study found that 5 wk of exposure to DEP caused a significant loss of myocardial collagen, providing a potential mechanism of the observed LV dilation and trend for increased LV compliance.

Exposure to DEP significantly increased the expression of AHR in the heart. Furthermore, DEP elicited increased expression of cardiac CYP 1A1, a biomarker for AHR activation (16). A prolonged activation of the AHR signaling pathway attenuates HIF-1α expression and its downstream signals, including VEGF (14, 19, 30, 31). Our data are in accord with these findings, as DEP-treated rats had significantly reduced cardiac HIF-1α and VEGF. Thackaberry and colleagues (36) demonstrated that both AHR and HIF-1α can induce changes in ventricular diameter and wall thickness, suggesting that these two pathways have a great impact on ventricular structure. Elevations in cardiac HIF-1α and VEGF are correlated with increased cardioprotection in several cardiac disorders, including pressure overload and myocardial infarction (7, 20, 33). Furthermore, blockade of HIF-1α and VEGF results in loss of protection, promoting heart failure (7, 20, 33). AHR activation induces a loss of collagen in various tissues (1, 25, 29, 38). Andreasen and colleagues (1) demonstrated that AHR activation significantly decreases collagen content during Zebrafish fin regeneration. Conversely, Corpechot and coworkers (8) demonstrated that HIF-1α promotes collagen production in hepatic stellate cells. However, the involvement of these pathways on myocardial collagen turnover is largely unknown.

AHR activation is also associated with increased expression of MMPs (1, 15, 18, 39). MMPs are ECM regulatory proteins that, upon activation, degrade collagen and other ECM components (34). Alterations in myocardial collagen are largely correlated to the balance between MMPs and their inhibitors (TIMPs), which can determine net collagen accumulation or degradation (34). In many cardiac diseases, increased MMP expression, including MMP-2, -9, -8, and -13, promote collagen degradation, leading to weakening of the ventricular wall and dilation (13, 34). Our studies showed no significant changes in MMPs or TIMP-1 at the 5-wk end point. However, in the DEP-treated animals, the expression of MMP-2 (P = 0.09) and MMP-13 (P = 0.07) tended to increase. In animal models of dilated cardiomyopathy, such as volume overload, MMP activity increases during the early compensatory stage, returns to normal levels, and is then further increased during decompensated heart failure (34). We predict that, although there was no change at this 5-wk time point, a longer exposure (weeks to months) may induce a significant increase in expression compared with vehicle. It is also possible that the loss of collagen observed in our studies was a result of an alternative pathway not observed in other cardiac diseases.

Wold and colleagues (42) demonstrated that exposure to concentrated PM 2.5, which contains particles from various sources, including traffic-related PM, resulted in alterations of the ventricular structure. In contrast to our findings of ventricular collagen loss, they found that PM exposure led to cardiac fibrosis. This divergence in our results may be due to differences in particle composition (traffic mixture PM vs. DEP), time and duration of exposure (6 h/day for 9 mo vs. 20 min/day for 5 wk), or species (mouse vs. rat). Most importantly, both studies demonstrated that PM exposure had a detrimental effect on cardiac structure and function, inducing remodeling similar to that observed in heart failure.

Our findings led us to conclude that exposure to DEP elicits eccentric LV dilation with systolic and diastolic dysfunction. In addition, exposure to DEP caused significantly increased cardiac AHR expression and attenuation of the HIF-1α pathway. Our data provide evidence that exposure to DEP induces ECM remodeling by impairing the accumulation of collagen, promoting increased degradation, which leads to a reduction in myocardial collagen, LV dilation, and dysfunction. Our data provide evidence that the AHR signaling pathway may play a critical role in this DEP-induced cardiac remodeling and dysfunction. Future studies could focus on the dose dependence of the cardiac injury and extend the protocol to assess the effects of chronic particulate exposure.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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