Long-term Air Pollution Exposure and Acceleration of Atherosclerosis and Vascular Inflammation in an Animal Model

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Context Recent studies have suggested a link between inhaled particulate matter exposure in urban areas and susceptibility to cardiovascular events; however, the precise mechanisms remain to be determined.

Objective To test the hypothesis that subchronic exposure to environmentally relevant particulate matter, even at low concentrations, potentiates atherosclerosis and alters vasomotor tone in a susceptible disease model.

Design, Setting, and Participants Between July 21, 2004, and January 12, 2005, 28 apolipoprotein E−/− (apoE−/−) mice were, based on randomized assignments, fed with normal chow or high-fat chow and exposed to concentrated ambient particles of less than 2.5 µm (PM2.5) or filtered air (FA) in Tuxedo, NY, for 6 hours per day, 5 days per week for a total of 6 months.

Main Outcome Measures Composite atherosclerotic plaque in the thoracic and abdominal aorta and vasomotor tone changes.

Results In the high-fat chow group, the mean (SD) composite plaque area of PM2.5 vs FA was 41.5% (9.8%) vs 26.2% (8.6%), respectively (P<.001); and in the normal chow group, the composite plaque area was 19.2% (13.1%) vs 13.2% (8.1%), respectively (P= .15). Lipid content in the aortic arch measured by oil red-O staining revealed a 1.5-fold increase in mice fed the high-fat chow and exposed to PM2.5 vs FA (30.0 [8.2] vs 20.0 [7.0]; 95% confidence interval [CI], 1.21-1.83; P=.02). Vasoconstrictor responses to phenylephrine and serotonin challenge in the thoracic aorta of mice fed high-fat chow and exposed to PM2.5 were exaggerated compared with exposure to FA (mean [SE], 134.2% [5.2%] vs 100.9% [2.9%], for phenylephrine, and 156.0% [5.6%] vs 125.1% [7.5%], for serotonin; both P=.03); relaxation to the endothelium-dependent agonist acetylcholine was attenuated (mean [SE] of half-maximal dose for dilation, 8.9 [0.2] vs 4.3 [0.1] × 10−8, respectively; P=.04). Mice fed high-fat chow and exposed to PM2.5 demonstrated marked increases in macrophage infiltration, expression of the inducible form of nitric oxide synthase, increased generation of reactive oxygen species, and greater immunostaining for the protein nitration product 3-nitrotyrosine (all P<.001).

Conclusion In an apoE−/− mouse model, long-term exposure to low concentration of PM2.5, altered vasomotor tone, induced vascular inflammation, and potentiated atherosclerosis.

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diovascular risk susceptibility remain unclear, although prior studies have demonstrated activation of inflammatory pathways, production of reactive oxygen species, and alterations in vasomotor tone.\textsuperscript{8-13} Although these pathways could all be potentially relevant, almost all studies to date have involved evidence from time-series analysis of human participants\textsuperscript{8,9} or in vitro studies, where cells were exposed to nonphysiologic and sometimes high concentrations of particulate matter.\textsuperscript{14,15} From experiments in animal models via routes that do not replicate true personal exposure\textsuperscript{16} or from studies that involve short-term exposure in humans and animals that may not reflect the situation between January 27 and February 28, 2005. Concentrations of ambient particulate matter in Manhattan (on the roof top of Hunter College, New York, NY) and Tuxedo (New York University School of Medicine, A. J. Lanza Laboratory) were monitored using an oscillating microbalance (Tapered-Element Oscillating Microbalance, Model 1400; Rupprecht and Patashnick, East Greenbush, NY). For the concentrated ambient particulate matter in the exposure chambers, samples were collected on Teflon filters (Gelman Tello, 37 mm, 0.2 mm pore; Gelman Sciences, Ann Arbor, Mich) and weighed before and after sampling in a temperature and humidity-controlled weighing room. The weight gains were used to calculate the exposure concentrations.

**METHODS**

**Animal Model**

Six-week-old apolipoprotein E\textsuperscript{−/−} (apoE\textsuperscript{−/−}) male mice (Taconic Europe, Denmark) were enrolled and housed 2 to a cage in an Association for Assessment and Accreditation of Laboratory Animal Care–accredited animal housing facility. They were fed either high-fat chow (n=12; Adjusted Calories Diet, TD 88137, Harlan, Indianapolis, Ind) or normal chow (n=16) for at least 10 weeks before exposure to PM\textsubscript{2.5} or filtered air. Assignments to high-fat chow vs normal chow and PM\textsubscript{2.5} vs filtered air were randomized. The Committees on Use and Care of Animals from New York University and Mount Sinai School of Medicine approved all experimental procedures.

**Exposure to PM\textsubscript{2.5}**

Animals were exposed to concentrated PM\textsubscript{2.5} composed of the northeastern regional background at A. J. Lanza Laboratory of New York University, located within Sterling Forest State Park in Tuxedo, NY, 40 miles northwest of Manhattan, where most of the PM\textsubscript{2.5} is attributed to long-range transport. The concentrated air particles were generated using a versatile aerosol concentration enrichment system developed by Sioutas et al\textsuperscript{16} and modified by Chen and Nadziejko.\textsuperscript{19} The mice were exposed to PM\textsubscript{2.5} at nominal 10\textsuperscript{\times} ambient concentrations for 6 hours per day, 5 days per week for a total of 6 months. The control mice in the experiment were exposed to an identical protocol with the exception of a high-efficiency particulate-air filter positioned in the inlet valve position to remove all of the PM\textsubscript{2.5} in the filtered air stream. Exposures began on July 21, 2004, and were stopped on January 12, 2005; all mice were killed between January 27 and February 28, 2005. Concentrations of ambient particulate matter in Manhattan (on the roof top of Hunter College, New York, NY) and Tuxedo (New York University School of Medicine, A. J. Lanza Laboratory) were monitored using an oscillating microbalance (Tapered-Element Oscillating Microbalance, Model 1400; Rupprecht and Patashnick, East Greenbush, NY). For the concentrated ambient particulate matter in the exposure chambers, samples were collected on Teflon filters (Gelman Tello, 37 mm, 0.2 mm pore; Gelman Sciences, Ann Arbor, Mich) and weighed before and after sampling in a temperature and humidity-controlled weighing room. The weight gains were used to calculate the exposure concentrations.

**Blood Lipid and Vascular Studies**

Mice were killed by injection of lethal doses of pentobarbital after blood collection directly from left ventricle puncture. The collected blood was centrifuged at 3000 rpm for 5 minutes, and serum was separated and collected. Total cholesterol and triglyceride levels were assayed using diagnostic kits (Thermo Electron, Louisville, Colo). The ascending aortas were removed and the 2-mm thoracic aortic rings were suspended in individual organ chambers filled with physiological salt solution buffer (sodium chloride, 130 mEq/L; potassium chloride, 4.7 mEq/L; calcium chloride, 1.6 mEq/L; magnesium sulfate, 1.17 mEq/L; potassium diphosphate, 1.18 mEq/L; sodium bicarbonate, 14.9 mEq/L; EDTA, 0.026 mEq/L; and glucose, 99.1 mg/dL [5.5 mmol/L]; pH, 7.4), aerated continuously with 5% carbon dioxide in oxygen at 37°C, as previously described.\textsuperscript{22} Briefly, for vasoconstrictor responses, vessels were allowed to equilibrate for at least 1 hour at a resting tension of 700 mg before being subjected to graded doses of serotonin (10\textsuperscript{−10} to 10\textsuperscript{−5} mEq/L) or phenylephrine (10\textsuperscript{−8} to 10\textsuperscript{−3} mEq/L). Responses were then expressed as a percentage of the peak response to 120 mEq/L of potassium.
chloride. The vessels were then washed thoroughly and allowed to equilibrate for 1 hour before beginning experiments with acetylcholine. After a stable contraction plateau was reached with serotonin, which was about 50% of peak tension generated with maximal dose potassium chloride, the rings were exposed to graded doses of the endothelium-dependent agonist acetylcholine ($10^{-10}$ to $10^{-5}$ mEq/L).

**Morphometric Analysis**

Segments of thoracic aorta were frozen in liquid nitrogen and embedded in Optimal Cutting Temperature compound (Tissue-Tek, Sakura Finetek USA Inc, Torrance, Calif) for oil red-O and confocal microscopy measurements of reactive oxygen species with the oxidatively active fluorescent dye 2',7'-dichlorodihydrofluorescein diacetate (Molecular Probes, Eugene, Ore). The abdominal aorta was fixed in 10% zinc formalin and embedded in paraffin for hematoxylin-eosin and immunohistochemical staining for anti-CD68, inducible nitric oxide synthase (NOS), and endothelial NOS. For estimation of atherosclerotic plaque size, 4 successive sections were collected on the same slide, and at least 10 sections from 3 consecutive slides per area per mouse (thoracic aorta and abdominal aorta) were examined. Each image was digitized with a digital camera and analyzed under a research microscope (Zeiss Axioskop with Spot I digital camera, Jena, Germany) with National Institutes of Health (NIH) Image software version 1.61 (Wayne Rasband, NIH, http://rsb.info.nih.gov/NIH-image). Plaque areas were adjusted for the cross-sectional vessel cavity area and expressed as a percentage value. All analyses were performed blindly without knowledge of the origin of the samples.

**Immunohistochemical Analysis**

Antibodies against CD68, inducible NOS, and endothelial NOS were purchased from Santa Cruz Biotechnology Incorporated (Santa Cruz, Calif). A polyclonal antinitrotyrosine antibody was obtained from Upstate Cell Signaling Solutions (Lake Placid, NY). Immunohistochemical staining was performed by using the primary antibodies (1:200 concentration) and a detection system (Immunoperoxidase Secondary Detection System; Chemicon International, Temecula, Calif), and quantified with software (NIH Image) after digitization of the images with a camera system (Zeiss Axioskop with Spot I digital camera). At least 10 sections were stained per mouse and quantification was also performed blindly. Data are expressed as the percentage of the lesion staining positive for the protein.

**Statistical Analyses**

Data are expressed as mean (SD) unless otherwise indicated. The half-maximal dose (either dilation or constriction) value for each experiment was obtained by logarithmic transformation. Vascular responses were compared using 1-way analysis of variance with half-maximal dose for dilation and peak responses as dependent variables. When significance was detected, a post hoc Newman-Keuls multiple comparison test was performed. Difference between 2 group observations was compared with t test. All P values are 2-tailed; P<.05 was considered significant. All statistical analyses were performed by using GraphPad Prism software version 3.02 (GraphPad Software Inc, San Diego, Calif).

**RESULTS**

There were no baseline differences in weight between the groups (Table 1); weights increased in all mice at the end of the study compared with the baseline (P<.001, in both normal chow and high-fat chow groups). At the end of the experimental period before mice were killed, total cholesterol level increased significantly in the mice exposed to filtered air (P<.001) and PM$_{2.5}$ (P=.007). However, changes in triglyceride levels were not significant in both filtered air (P=.80) and PM$_{2.5}$ groups (P=.15).

**PM$_{2.5}$ Concentrations During the Study Period**

The mean (SD) daily PM$_{2.5}$ concentration at the study site in Tuxedo, NY, was 10.6 (3.4) µg/m$^3$, although the PM$_{2.5}$ concentration in the borough of Manhattan, NY, during the study period was 14.8 (3.4) µg/m$^3$. The mean concentration of PM$_{2.5}$ in the exposure chamber was 85 µg/m$^3$ (approximately 8-fold concentration from ambient Tuxedo.

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**Table 1. Weight and Lipid Measurements of Mice Fed Normal vs High-Fat Chow and Exposed to PM$_{2.5}$ vs Filtered Air**

<table>
<thead>
<tr>
<th></th>
<th>Normal Chow, Mean (SD)</th>
<th>High-Fat Chow, Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Filtered Air (n=8)</td>
<td>PM$_{2.5}$ (n=8)</td>
</tr>
<tr>
<td>Weight, g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before exposure</td>
<td>20 (2)</td>
<td>20 (1)</td>
</tr>
<tr>
<td>After exposure</td>
<td>27 (3)</td>
<td>28 (2)</td>
</tr>
<tr>
<td>Lipids, mg/dL†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>850.6 (94.0)</td>
<td>783.4 (88.1)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>513.4 (208.8)</td>
<td>506.8 (251.9)</td>
</tr>
</tbody>
</table>

Abbreviation: PM$_{2.5}$, concentrated ambient particles of less than 2.5 µm.

SI conversions: To convert cholesterol to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113.

†After exposure to either PM$_{2.5}$ or filtered air in mice fed normal or high-fat chow.
levels). Because the mice were exposed for 6 hours a day, 5 days a week, the equivalent PM$_{2.5}$ concentration to which the mice were exposed in the chamber normalized over the 6-month period was 15.2 µg/m$^3$, which is close to the annual average PM$_{2.5}$ National Ambient Air Quality Standard of 15 µg/m$^3$.23

Vasomotor Function
Figure 1 depicts responsiveness to the vasoconstrictors serotonin, phenylephrine, and the endothelium-dependent agonist acetylcholine in thoracic aortic segments. Table 2 details the half-maximal doses (constriction and dilation), peak constrictor, and vasodilator responses of the mice in all 4 groups. The mice fed high-fat chow and exposed to PM$_{2.5}$ demonstrated an increase in the half-maximal dose for dilation to acetylcholine with no changes in peak relaxation compared with the mice exposed to filtered air and fed high-fat chow and normal chow.

Atherosclerosis Burden With PM$_{2.5}$
In vivo MRI imaging of atherosclerosis burden in the abdominal aorta revealed significantly increased plaque burden in the mice fed high-fat chow compared with the mice fed normal chow (mean [SD], 34 [7] vs 23 [4] units; $P<.001$). Mean (SD) plaque areas in the mice exposed to PM$_{2.5}$ and fed high-fat chow vs the mice exposed to filtered air and fed high-fat chow were 33 (10) vs 27 (13) units, respectively ($P=.10$), although plaque areas in the mice exposed to PM$_{2.5}$ and fed normal chow vs the mice exposed to filtered air and fed normal chow were 24 (14) vs 23 (13) units, respectively ($P=.60$).

Figure 2 provides representative sections from morphometric analysis of the aorta in the 4 groups and Table 3 provides composite plaque area by hematoxylin-eosin staining, lipid content by oil red-O staining, and macrophage infiltration by immunohistochemical staining in the aorta of the experimental groups. Macrophage infiltration was observed predominantly in the intimal and medial areas of the arterial wall and less so in the adventitial layers.

![Figure 1. Mean Vasoconstriction of Aortic Rings in Response to Serotonin and Phenylephrine, and Vasorelaxation in Response to Acetylcholine](image)

PM$_{2.5}$ indicates concentrated ambient particles of less than 2.5 µm. Error bars represent SE. Values represent responses to graded doses of serotonin or phenylephrine expressed as a percentage of the peak response to 120 mEq/L of potassium chloride solution, or responses to graded doses of acetylcholine expressed as a percentage of preconstricted tension in response to serotonin. For serotonin and phenylephrine, $P=.03$ for mice exposed to PM$_{2.5}$ and fed high-fat chow vs other 3 groups. For acetylcholine, $P=.04$ for half-maximal dose for dilation vs all other groups.

<table>
<thead>
<tr>
<th>Table 2. Effects of PM$_{2.5}$ and High-Fat Chow on Responses to Serotonin, Phenylephrine, and Acetylcholine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serotonin</strong></td>
</tr>
<tr>
<td><strong>Filtered Air</strong></td>
</tr>
<tr>
<td><strong>Constriction Response to Serotonin</strong></td>
</tr>
<tr>
<td><strong>EC$_{50}$, mean (SE) dose</strong></td>
</tr>
<tr>
<td><strong>Phenylephrine</strong></td>
</tr>
<tr>
<td><strong>EC$_{50}$, mean (SE) dose</strong></td>
</tr>
<tr>
<td><strong>Acetylcholine</strong></td>
</tr>
<tr>
<td><strong>ED$_{50}$, mean (SE) dose</strong></td>
</tr>
</tbody>
</table>

Abbreviations: PM$_{2.5}$, concentrated ambient particles of less than 2.5 µm; EC$_{50}$, half-maximal dose for constriction; ED$_{50}$, half-maximal dose for dilation.

*Comparison by 1-way analysis of variance between the group of mice fed high-fat chow and exposed to PM$_{2.5}$ vs the other 3 groups.
**PM$_{2.5}$ and Vascular Inflammation**

A 2.6-fold higher inducible NOS content was apparent in the mice exposed to PM$_{2.5}$ and fed high-fat chow compared with the mice exposed to filtered air and fed high-fat chow (mean [SD], 13.0 [3.6] vs 4.9 [1.1]; 95% confidence interval [CI], 1.54-3.12; $P < .001$) and a 4-fold increase in the mice exposed to PM$_{2.5}$ and fed normal chow compared with the mice exposed to filtered air and fed normal chow (3.2 [0.9] vs 0.8 [0.5]; $P < .001$)

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**Figure 2.** Representative Photomicrographs of Hematoxylin-Eosin Staining and CD68 Immunohistochemical Staining of Abdominal Aortic Sections, and Oil Red-O Staining of Aortic Arch Sections

<table>
<thead>
<tr>
<th>Filtered Air</th>
<th>PM$_{2.5}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Chow</td>
<td>13.2 (8.1)</td>
</tr>
<tr>
<td>High-Fat Chow</td>
<td>19.2 (13.1)</td>
</tr>
</tbody>
</table>

*Abbreviations: NOS, nitric oxide synthase; PM$_{2.5}$, concentrated ambient particles of less than 2.5 µm.*

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**Table 3.** Analysis of Plaque and Immunohistochemical Staining Parameters*

<table>
<thead>
<tr>
<th>Staining</th>
<th>Normal Chow, Mean (SD)</th>
<th>Filtered Air</th>
<th>PM$_{2.5}$</th>
<th>$P$ Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plaque area, %</td>
<td>13.2 (8.1)</td>
<td>19.2 (13.1)</td>
<td>.15</td>
<td></td>
</tr>
<tr>
<td>Oil red-O</td>
<td>10.0 (4.1)</td>
<td>15.3 (11.8)</td>
<td>.13</td>
<td></td>
</tr>
<tr>
<td>CD68</td>
<td>7.0 (2.2)</td>
<td>12.8 (3.7)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>3-Nitrotyrosine</td>
<td>1.1 (0.8)</td>
<td>4.4 (1.5)</td>
<td>&lt;.001</td>
<td></td>
</tr>
<tr>
<td>Endothelial NOS</td>
<td>0.6 (0.3)</td>
<td>1.1 (0.5)</td>
<td>.06</td>
<td></td>
</tr>
<tr>
<td>Inducible NOS</td>
<td>0.8 (0.5)</td>
<td>3.2 (0.9)</td>
<td>&lt;.001</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: NOS, nitric oxide synthase; PM$_{2.5}$, concentrated ambient particles of less than 2.5 µm.*

*Plaque area was analyzed from hematoxylin-eosin positive areas of aortic arch and oil red-O positive areas of abdominal aorta (composite score) and is expressed as a percentage. Oil red-O staining was from thoracic aorta, and CD68, 3-nitrotyrosine, and inducible NOS staining was from abdominal aorta. The average value for at least 10 sections from each location in each animal was determined (n = 6 for high-fat chow, n = 8 for normal chow). Data are expressed as percentage of positive staining.

†Compared using $t$ test.

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PM$_{2.5}$ indicates concentrated ambient particles of less than 2.5 µm; NOS, nitric oxide synthase. Original magnification $\times 100$. Brown chromogen was used for staining and hematoxylin was used for counterstaining.

95% CI, 2.22-5.31; $P<.001$) (Table 3, Figure 3, and Figure 4), whereas no significant difference was observed between the groups for endothelial NOS staining (Table 3). In parallel with increased inducible NOS expression, more 3-nitrotyrosine was detected in the plaque from mice exposed to PM$_{2.5}$ in both high-fat and normal chow groups (Table 3, Figure 3, and Figure 4). Lipid content in the aortic arch measured by oil red-O staining revealed a 1.5-fold increase in mice fed high-fat chow and exposed to PM$_{2.5}$ vs mice fed high-fat chow and exposed to filtered air (30.0 [8.2] vs 20.0 [7.0]; 95% CI, 1.21-1.83; $P=.02$). In situ detection of reactive oxygen species in aortic sections revealed markedly increased hydrogen peroxide generation in the aorta of mice exposed to PM$_{2.5}$ compared with mice exposed to filtered air. These radicals were abolished by preincubation of aortic sections with catalase, a superoxide dismutase mimetic 9-Mn[III] tetrakis-4-benzoic acid porphyrin chloride, or a hydroxyl radical scavenger (mercaptopropionyl glycine) (available from authors upon request).

**COMMENT**

In an animal model of apoE$^{-/-}$ mice, we found that exposure to environmentally relevant concentrations of regional northeastern PM$_{2.5}$ accelerates atherosclerosis. PM$_{2.5}$ exposure also attenuates responsiveness to an endothelium-dependent agonist and heightens vasoconstrictor responsiveness. Additionally, vascular inflammation and protein nitration are prominent aspects of PM$_{2.5}$-mediated effects on the vasculature. Our findings provide a potential biological basis for the association between atherosclerosis-related events noted in time-series analysis and prospective population cohort studies.2,3,24 Data from these studies and other studies have revealed that the relationship between cardiovascular risk and PM$_{2.5}$ is essentially linear across a large range of concentrations without a discernible lower safe threshold concentration.6

Our results suggest that even seemingly low concentrations of PM$_{2.5}$ exposure may have detrimental effects on the vasculature and bolster emerging data suggesting progression of carotid-intima media thickening, a commonly used surrogate for atherosclerosis.25 The concentration used in our study (although enriched) when normalized over a 24-hour/7-day period is well within the range of PM$_{2.5}$ concentrations that individuals living in urban areas such as New York City are exposed to, and thus has implications for the long-term impact of particulate matter exposure on urban populations.

Potentiation of atherosclerosis with PM$_{2.5}$ was noted in both the thoracic and abdominal aorta and was especially higher in response to high-fat feeding. The lack of an association between PM$_{2.5}$ and certain aortic measurements in mice fed normal chow may be due to type II
error. Furthermore, the percentage increase in plaque burden with PM2.5 precisely paralleled the increase in macrophage and fatty infiltration noted in aorta, suggesting that these processes might be related. Although results from MRI of the abdominal aorta in the mice fed high-fat chow did not reveal a significant difference between those exposed to PM2.5 vs those exposed to filtered air, the trends supported an effect of PM2.5 on progression and, consistent with this, the overall composite plaque burden measured by morphometry revealed a significant impact of PM2.5 on progression with high-fat feeding. The use of MRI to assess aortic plaque burden in our study serves to provide proof of concept, in designing future studies on the impact of particulate matter exposure on atherosclerosis.

Our study is in agreement with a prior study, performed in rabbits linking air pollutants to atherogenesis and extends these observations to a chronic model system that more closely mimics the human context. Our study, however, differs from the rabbit study in several important respects. First, the rabbit study involved intrapulmonary instillation of high concentrations of PM10 (particles <10 µm), twice a week for 4 weeks. In contrast, our study used an inhalation exposure to PM2.5 over a 6-month period that may be relevant to populations inhaling low levels of PM2.5 and is therefore akin to chronic exposure in humans (assuming an overall life span in mice of 2-3 years). Second, a marked systemic and pulmonary inflammatory response was noted in the rabbit study. This suggests exposure to very high levels of particulate matter that may not exactly mirror the in vivo clinical context. In contrast, the mean concentrations of PM2.5 in our study were 72% of the ambient concentrations measured in mid-town Manhattan, NY, during the same period, and therefore provide a real world context for the relevance of our findings to the whole northeastern region and not only to the urban cores.

Our study design and methods are different from an earlier study published by our group. In our previous study, which involved an entirely different animal cohort, we demonstrated a nonsignificant trend toward increase in aortic sinus plaque measurements in double knock-out mice (apoE−/− and low-density lipoprotein receptor−/−) but no differences in grossly discernible plaque in this model when mice were fed high-fat chow for 4 months. The complexity of the double knock-out model and the genotype interaction with PM2.5 and high-fat chow made definitive interpretation of these findings difficult, providing the basis for a simpler design using a commonly used animal model of atherosclerosis (apoE−/−) in our study.

Responses to the endothelium-dependent agonist acetylcholine were attenuated in the PM2.5 and high-fat chow group but not in the filtered air and high-fat chow or normal chow groups, highlighting a possible interaction of PM2.5 with high-fat intake. Increases in reactive oxygen species, such as superoxide in the vessel wall with PM2.5 exposure, may have influenced concentrations of bioavailable nitric oxide leading to diminished responses to agonists. The heightened responses to the vasoconstrictors phenylephrine and serotonin may potentially reflect alterations in the nitric oxide pathway in conjunction with up-regulation of other endogenous vasoconstrictors. Our experiments suggest the simultaneous generation of a number of radical species and is in agreement with prior studies demonstrating that PM2.5 pollution is a potent inducer of multiple free-radical species. Prior experiments in animal models have demonstrated that even brief (<2 hours) exposure to PM2.5 results in generation of intracellular reactive oxygen species and activation of proinflammatory pathways. Although our study did not assess the contribution of various reactive oxygen species generating sources in the vessel wall, the striking increase in macrophages in the PM2.5-exposed mice provides at least 1 putative pathway. Alternately, oxidant stress may be induced directly in the arterial wall in response to the constituents in PM2.5, such as transition metal elements or other elements that may translocate beyond the alveoli.

Finally, it is possible that an inflammatory response in the lung (cells and circulating mediators) may result in activation of inflammatory cascades in the vessel wall and potentiation of atherogenesis. Irrespective of the mechanism through which vascular inflammation is provoked by air pollutants, increases in macrophage-derived reactive oxygen species in conjunction with increased expression of the high output enzyme inducible NOS may set the stage for production of the highly toxic radical species peroxynitrite.
increased 3-nitrotyrosine residues, as noted in our study, represent the footprints of peroxynitrite generation and may lead to inactivation of a number of proteins that may be essential for maintenance of vascular homeostasis. The PM$_{2.5}$ concentrations during exposures in our study are environmentally relevant and are well within the range of concentrations attained in metropolitan areas. Importantly, the average exposure throughout the 24-hour period was well within the present-day National Ambient Air Quality Standards (<65 µg/m$^3$ and close to the annual average of 15 µg/m$^3$). These results suggest that repeated periods of short-term (eg, several hours) exposure to high particulate matter levels, such as those occurring during rush hour traffic, is potentially capable of promoting progression of atherosclerosis, although the mean daytime particulate matter exposure concentration is within national recommendations. This may potentially have implications for the relevance of both the 24-hour and annual average National Ambient Air Quality Standards.

In conclusion, exposure to particulate matter alters vasomotor tone and potentiates atherosclerosis and vascular inflammation. These findings support the need for targeted studies that help delineate the precise constituents in particulate matter that confer this risk and the molecular pathways involved, and provide a fundamental basis leading to human population studies.

**Author Contributions:** Dr Rajagopalan had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. **Study concept and design:** Sun, Lippmann, Chen, Rajagopalan. **Acquisition of data:** Sun, Wang, Jin, Natanzon, Duquaine, Aguinaldo, Fayad, Chen, Rajagopalan. **Analysis and interpretation of data:** Sun, Wang, Natanzon, Brook, Aguinaldo, Fuster, Rajagopalan. **Drafting of the manuscript:** Sun, Wang, Jin, Aguinaldo, Chen, Rajagopalan. **Critical revision of the manuscript for important intellectual content:** Sun, Natanzon, Duquaine, Brook, Fayad, Fuster, Lippmann, Chen, Rajagopalan. **Obtained funding:** Sun, Lippmann, Chen, Rajagopalan. **Administrative, technical, or material support:** Sun, Wang, Jin, Natanzon, Aguinaldo, Fayad, Fuster, Rajagopalan. **Study supervision:** Sun, Lippmann, Chen, Rajagopalan.

**Financial Disclosures:** None reported. **Funding/Support:** This study was supported by grants R827351 from the Environmental Protection Agency (Drs Lippmann and Chen), ES00260 from the National Institute of Environmental Health Sciences (Drs Lippmann and Chen), and R01ES013406-01 from the National Institutes of Health (Dr Rajagopalan). Confocal microscopy was performed at the Mount Sinai School of Medicine–Microscopy Shared Research Facility, supported with funding from National Institutes of Health–National Cancer Institute shared resources grant 1 R24 CA095823-01 and National Science Foundation major research instrumentation grant DMR-9724504. **Role of the Sponsors:** The funding organizations did not participate in the design and conduct of the study, in the collection, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

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27. Sun, Lippmann, Chen, Rajagopalan. **Drafting of the manuscript:** Natanzon, Brook, Aguinaldo, Fuster, Rajagopalan. **Obtained funding:** Sun, Lippmann, Chen, Rajagopalan. **Administrative, technical, or material support:** Sun, Wang, Jin, Natanzon, Aguinaldo, Fayad, Fuster, Rajagopalan. **Study supervision:** Sun, Lippmann, Chen, Rajagopalan.