Exposure to Fine Particulate Air Pollution Is Associated With Endothelial Injury and Systemic Inflammation

C. Arden Pope III, Aruni Bhatnagar, James P. McCracken, Wesley Abplanalp, Daniel J. Conklin, Timothy O'Toole

Rationale: Epidemiological evidence indicates that exposures to fine particulate matter air pollution ($PM_{2.5}$) contribute to global burden of disease, primarily as a result of increased risk of cardiovascular morbidity and mortality. However, mechanisms by which $PM_{2.5}$ exposure induces cardiovascular injury remain unclear. $PM_{2.5}$-induced endothelial dysfunction and systemic inflammation have been implicated, but direct evidence is lacking.

Objective: To examine whether acute exposure to $PM_{2.5}$ is associated with endothelial injury and systemic inflammation.

Methods and Results: Blood was collected from healthy, nonsmoking, young adults during 3 study periods that included episodes of elevated $PM_{2.5}$ levels. Microparticles and immune cells in blood were measured by flow cytometry, and plasma cytokine/growth factors were measured using multiplexing laser beads. $PM_{2.5}$ exposure was associated with the elevated levels of endothelial microparticles (annexin V^+/$CD41^-$/$CD31^+$), including subtypes expressing arterial-, venous-, and lung-specific markers, but not microparticles expressing $CD62^+$. These changes were accompanied by suppressed circulating levels of proangiogenic growth factors (EGF [epidermal growth factor], sCD40L [soluble CD40 ligand], PDGF [platelet-derived growth factor], RANTES [regulated on activation, normal T-cell–expressed and secreted], GROα [growth-regulated protein α], and VEGF [vascular endothelial growth factor]), and an increase in the levels of antiangiogenic (TNFα [tumor necrosis factor α], IP-10 [interferon γ-induced protein 10]), and proinflammatory cytokines (MCP-1 [monocyte chemoattractant protein 1], MIP-1α/β [macrophage inflammatory protein 1α/β], IL-6 [interleukin 6], and IL-1β [interleukin 1β]), and markers of endothelial adhesion (sICAM-1 [soluble intercellular adhesion molecule 1] and sVCAM-1 [soluble vascular cellular adhesion molecule 1]). $PM_{2.5}$ exposure was also associated with an inflammatory response characterized by elevated levels of circulating $CD14^+$, $CD16^+$, $CD4^+$, and $CD8^+$, but not $CD19^+$ cells.

Conclusions: Episodic $PM_{2.5}$ exposures are associated with increased endothelial cell apoptosis, an antiangiogenic plasma profile, and elevated levels of circulating monocytes and T, but not B, lymphocytes. These changes could contribute to the pathogenic sequelae of atherogenesis and acute coronary events. *(Circ Res.* 2016;119:1204-1214. DOI: 10.1161/CIRCRESAHA.116.309279.)

Key Words: air pollution ■ cardiovascular disease ■ inflammation ■ particulate matter ■ vascular disease

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Exposure to fine particulate matter air pollution (≤2.5 μm in aerodynamic diameter, $PM_{2.5}$) increases the risk of developing cardiovascular disease (CVD) and premature cardiovascular mortality.[1,2] Prospective cohort studies[3–6] indicate that chronic exposure to $PM_{2.5}$ may contribute to the initiation and progression of atherosclerosis, hypertension, and type 2 diabetes mellitus[7] and is associated with increased risk of adverse cardiovascular outcomes,[5,6,8,9] resulting in reduced life expectancy.[10] In addition, short-term exposures to $PM_{2.5}$ are associated with increased daily mortality,[11] acute coronary events,[12] and ischemic stroke,[13] especially in individuals with pre-existing atherosclerotic disease.[14–16] A recent assessment of factors that contribute to the global burden of disease estimates that ambient and household air pollution are among the top 10 contributors to premature mortality worldwide—largely because of the estimated effect of $PM_{2.5}$ on ischemic heart disease.[17]

Despite extensive investigations, it is unclear how inhaled $PM_{2.5}$, a pulmonary insult, can initiate adverse cardiovascular responses. Studies investigating the underlying processes have implicated the generation or exacerbation of a dysfunctional endothelium because of systemic inflammation.[1] The endothelium plays a key role in regulating blood pressure,

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From the Department of Economics, Brigham Young University, Provo, UT (C.A.P.); and Diabetes and Obesity Center, Division of Cardiovascular Medicine, Department of Medicine, University of Louisville, KY (A.B., J.P.M., W.A., D.J.C., T.O.).

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Correspondence to C. Arden Pope III, PhD, 142 FOB, Brigham Young University, Provo, UT 84602, E-mail cap3@byu.edu

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Nonstandard Abbreviations and Acronyms	
CD40L	CD40 ligand
EGF	epidermal growth factor
GROα	growth-regulated protein α
IL-6	interleukin 6
IL-8	interleukin 8
IL-1β	interleukin 1β
IP-10	interferon γ-induced protein 10
MCP-1	monocyte chemoattractant protein 1
MIP-1α	macrophage inflammatory protein 1α
$PM_{2.5}$	fine particulate matter < 2.5 μm in aerodynamic diameter
RANTES	regulated on activation, normal T-cell–expressed and secreted
sICAM-1	soluble intercellular adhesion molecule 1
sVCAM-1	soluble vascular cellular adhesion molecule 1
TNF-α	tumor necrosis factor α
VEGF	vascular endothelial growth factor

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atherogenesis, and thrombosis, and therefore endothelial dysfunction could, in part, contribute to cardiovascular morbidity and mortality associated with $PM_{2.5}$ exposure. However, previous data showing associations between ambient $PM_{2.5}$ and endothelial dysfunction[18,19] or systemic inflammation[20–22] are from individuals with moderate to high CVD risk,[19,22] diabetes mellitus,[18,20] or ischemic heart disease.[21] As a result, it remains unclear whether endothelial dysfunction reflects direct $PM_{2.5}$-induced injury or is secondary to disease exacerbation because of $PM_{2.5}$ inhalation. Because endothelial dysfunction and systemic inflammation are strongly associated with atherosclerosis[23,24] and diabetes mellitus,[25,26] an increase in the severity of these diseases would affect both the immune system and the endothelial function, even if these processes were not direct targets of $PM_{2.5}$. Therefore, it is difficult to conclude that inhaling $PM_{2.5}$ leads to systemic inflammation or endothelial dysfunction or to determine how these changes may be related to the pathogenesis of atherosclerotic lesion formation and acute coronary events.

Hence, to determine whether $PM_{2.5}$ directly affects the endothelium, we examined biomarkers of subtle endothelial injury and mild systemic inflammation in a cohort of young healthy individuals with low CVD risk. It has been previously reported that the acute inhalation of $PM_{2.5}$ does not induce frank endothelial dysfunction in healthy individuals[18]; therefore, to identify subclinical, endothelial injury, we measured circulating levels of endothelium-derived microparticles. Microparticles are submicron vesicular structures that are shed from activated or apoptotic cells.[27–29] Elevated circulating levels of such microparticles reflect endothelial damage.[30,31] To evaluate systemic inflammatory responses, we measured changes in immune cells, cytokines, growth factors, and vascular adhesion molecules. We found robust evidence of endothelial injury and systemic inflammation with exposure to increased $PM_{2.5}$ levels even in young healthy individuals. These findings lend support to the view that $PM_{2.5}$ causes endothelial injury, potentially by establishing a mild inflammatory state. Our results are also consistent with the possibility that aberrant immune responses and endothelial injury may be early causes of endothelial dysfunction and CVD attributable to $PM_{2.5}$ exposure in susceptible individuals.

Methods

Study Population

Research subjects included 3 groups of 24 persons (Table 1) who were recruited for each of 3 consecutive winter/spring study periods, including January to March 2013, January to March 2014, and December 2014 to April 2015. All study participants were healthy, young adults recruited from Provo, UT. All subjects were nonsmokers without exposure to second-hand smoke at home, work, or school. Air pollution episodes in Utah Valley occur under predictable conditions that include a combination of snow cover, relatively high barometric pressure, and stagnant atmospheric conditions. For each of the study periods, subjects were further divided into 2 draw groups of 12. At prearranged times (between 11:00 AM and 12:00 PM on Tuesdays or Thursdays) and during times of variable air pollution, the subjects in each draw group had their blood drawn (Figure 1) and completed a questionnaire about current health status, level of exercise, beginning date of the last menstrual period, and supplement use.

In the first study period, only males were enrolled, whereas in the other periods, both sexes were enrolled. For the second and third study periods, subjects were provided with dietary supplements and asked to consume 2 640 mg soft gel tablets in the morning and evening with food. Consumption of these supplements began 3 weeks before the first blood draw and continued until the final blood draw. Subjects were randomly (stratified by sex and group) assigned capsules of omega-3 fatty acids (a total daily consumption of 2560 mg, within the upper range of recommended dose) or identically sized placebo capsules of soybean oil (Pro Omega softgel/lemon capsules and Placebo Soybean Oil softgel/lemon, Nordic Naturals, Inc, Watsonville, CA). Informed consent was obtained from all research subjects. The study was approved by the Institutional Review Board for Human Subjects at Brigham Young University, and procedures followed were in accordance with institutional guidelines.

Blood Collection and Processing

For the analysis of microparticles and immune cell populations, 8 mL of blood was collected in sodium citrate–containing cell preparation tubes (CPT Vacutainer; Becton Dickinson). These tubes were centrifuged at 1700*g* for 30 minutes at room temperature and shipped overnight to the University of Louisville for analysis. On arrival, the tubes were centrifuged again, and the upper layer containing mononuclear cells and plasma was collected. This material was diluted with an equal volume of PBS and centrifuged at 500*g* for 10 minutes. Aliquots of the supernatant were used for the analysis of microparticles, whereas the cell pellet was washed once with PBS and centrifuged. The final cell

Table 1. Summary of Research Subject Characteristics*

	All	2013	2014	2015
No. of subjects	72	24	24	24
No. of blood draws	384	144	120	120
Age, y, mean (SD)	23 (2)	23 (2)	23 (2)	23 (2)
Race (% white/other)	88/13	92/8	83/17	88/12
Male, %	65	100	46	50
BMI, mean (SD)	24 (3)	24 (3)	23 (3)	24 (3)
Systolic blood pressure (SD)	126 (14)	130 (12)	130 (12)	118 (16)
Diastolic blood pressure (SD)	74 (8)	70 (9)	76 (6)	76 (9)

BMI indicates body mass index.

*Subject characteristics data, including biometric data, were collected at the time of enrollment or at the time of initial blood draw.

pellet was resuspended in a volume of 300 µL PBS+2% FCS, 150 µL of which was used for the analysis of immune cell populations.

For the analysis of platelet–monocyte aggregates, 3 mL of blood was collected in an acid-citrate dextrose tube (ACD Vacutainer; Becton-Dickinson), and then 1 mL of aliquots was diluted with 3 mL of PBS and fixed with 1.3 mL of 4% paraformaldehyde for 30 minutes on ice. Red blood cells were lysed by the addition of 24 mL of water, the samples centrifuged at 400*g* for 10 minutes, and the cell pellet resuspended in 1 mL of Tyrode buffer and shipped to the University of Louisville as above.

Inflammatory cytokines were measured in frozen plasma aliquots by analytic services at Eve Technologies (Calgary, Alberta, Canada). An array of 42 human cytokines (Human Cytokine Array/Chemokine Array 42-Plex, Discovery Assay) and an array that included soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cellular adhesion molecule (sVCAM-1; Human Neurodegenerative Disease Array 2-Plex, Discovery Assay) were analyzed using multiplexing laser bead technology.

Flow Cytometry

To measure microparticles, plasma aliquots were centrifuged for 2 minutes at 10 000*g* to pellet residual cells and debris. The supernatant was collected and then centrifuged for 45 minutes at 17 000*g*. The resulting microparticle pellet was resuspended in PBS containing 1% FCS and 2.5 mmol/L Ca^{2+} and incubated with Fc block for 10 minutes at 4°C. Then a cocktail of fluorescently conjugated antibodies including Pacific blue-Annexin V (Life Technologies), APC-anti-CD34 (Becton Dickinson), PECy7-anti-CD41 (Becton Dickinson), PE-anti-CD31 (eBioscience), PECy5.5-anti-CD62E (Becton Dickinson), FITC–anti-EphB4 (R&D Systems), and APC-anti CD143 (Biolegend) was added. In addition, 1 antibody (anti-Ephrin B2; Santa Cruz) was labeled in the laboratory (Zenon Alexa 488 labeling kit; Life Technologies) and also added to the staining cocktail. After 30 minutes at room temperature, 25 000 volumetric counting beads were added and 10 000 events were collected on a multilaser flow cytometer (Becton Dickinson, BD™ LSR II). An identical sample with no antibodies was used as a gating control. Microparticle numbers were quantified in gated populations <1 µm in size and positive for Annexin V staining using the FlowJo software package and normalized to the number of counting beads (volume) collected. Specific populations were defined by phenotype as documented in Table 2 and illustrated in Online Figure I.

To quantify immune cell populations, resuspended cell pellets from above were incubated with Fc block (Miltneyi) for 10 minutes at 4°C, followed by the addition of fluorescently tagged antibodies (eBioscience) recognizing CD14 (650NC), CD16 (fluorescein isothiocyanate [FITC]), CD19 (Alexa 700), CD8 (APC-e780), and CD4 (PECy7) for 30 minutes at 4°C. The cells were washed once, resuspended in 350 µL PBS+2% FCS and 50 000 volumetric counting beads (Accucount Particles; Spherotech) were added before analysis. Samples were collected for 2 minutes on the multilaser flow cytometer (Becton Dickinson). Cell numbers were quantified using the FlowJo software package and normalized to the number of counting beads (volume) collected.

To quantify platelet–monocyte aggregates, cell pellets from above were washed once in Tyrode solution and then incubated with Fc block (Miltenyi) for 10 minutes on ice. The samples were then incubated with an FITC-conjugated anti-CD41 antibody (eBioscience) and an APC-conjugated anti-CD45 (eBioscience) antibody for 30 minutes on ice, washed, resuspended in Tyrode solution, and 10 000 events were collected on the multilaser flow cytometer. A sample with isotype control antibodies was used as a control. The percent of double-positive events was determined using FloJo software.

Measurement of $PM_{2.5}$ Air Pollution

Daily ambient concentrations of $PM_{2.5}$ in Utah Valley were collected from 3 monitoring sites: Lindon (located at the northern part of the valley), North Provo (approximately centrally located), and Spanish Fork (located at southern end of valley). At the Lindon and North Provo sites, hourly concentrations of $PM_{2.5}$, based on continuous ambient monitors, were also collected and used to estimate average $PM_{2.5}$ concentrations for the 24-hour period from noon to noon for each day—the approximate 24-hour time period before each blood draw. These data were obtained from the Utah Department of Environmental Quality (Salt Lake City, UT). During the past 2 study periods, supplemental continuous ambient monitoring of $PM_{2.5}$ (using a Thermo Scientific tapered element oscillating microbalance TEOM monitor) was conducted outside of the building, where the blood draws were conducted and indoor monitoring (using a Thermo Scientific portable DataRAM4 monitor) was conducted inside the blood-draw room. Average $PM_{2.5}$ concentrations for the 24-hour period from noon to noon for each day were calculated. Monitored $PM_{2.5}$ concentrations were nearly identical for all ambient monitors (Figure 1). The indoor measured concentrations of $PM_{2.5}$ were much lower. $PM_{2.5}$ concentrations from the North Provo monitor were used as the primary exposure measure.

Statistical Analysis

Associations with $PM_{2.5}$ exposures and microparticles and markers of inflammation were evaluated by estimating 2 similar regression approaches: fixed-effects regression models controlling for subject-specific differences and a subject-mean–adjusted regression that accounts for subject-specific differences by subtracting out subject-level means. The fixed-effects model controls for subject-level differences by estimating subject-specific fixed-effects as part of the model, whereas the subject-mean–adjusted model controls for subject-level differences by first subtracting out the subject-level means, and regressing deviations from these means on $PM_{2.5}$ (expanded statistical methods in the Online Data Supplement).

Plots of subject-specific differences over pollution concentrations along with regression plots were generated. To explore the sensitivity of the results, models were estimated that excluded observations from participants who reported any acute illness at the time of the blood draw, models that controlled for time exercised on the day of the draw and the day before the draw, models that controlled for menstruation (as indicated by blood-draw date being <6 days since beginning date of the last menstrual period), and models that excluded observations for days with $PM_{2.5}$ concentrations >100 µg/m^3. In addition, rather than using $PM_{2.5}$ concentrations 24 hours before the blood draws, models that used $PM_{2.5}$ concentrations 12 and 48 hours before the blood draws, respectively, were estimated. Finally, using data for the final 2 winter/spring time periods, models that included interaction terms for sex and $PM_{2.5}$, and fish oil (versus placebo) and $PM_{2.5}$ were estimated to test for the effect modification by sex and by fish oil supplement use.

For analysis of the multiple inflammatory cytokines and adhesion molecules, we estimated the fixed-effects and subject-mean–adjusted models. The percent change (and 95% confidence intervals) for each analyte per 10 µg/m^3 increase in $PM_{2.5}$ relative to its mean value was calculated and plotted in order based on *t* values. This approach comprehensively evaluates all measured analytes, allows for direct comparisons and evaluation of the strength of the statistical associations, and mitigates concerns about multiple testing and selective reporting. All statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Inc, Cary, NC).

Results

A summary of selected characteristics of research subjects is provided in Table 1. $PM_{2.5}$ concentrations and timing of blood draws for the 3 study periods are illustrated in Figure 1.

$PM_{2.5}$, Microparticles, and Immune Cells

Table 2 presents descriptions and summary statistics of microparticle and immune cell measurements and subject-mean–adjusted regression results. Online Table I reports regression results for the fixed-effects and subject-mean regressions. Delayed shipment of blood because of weather, broken blood collection tubes during centrifugation, and instrument malfunction during analysis resulted in some missing data. $PM_{2.5}$ concentrations were significantly (P<0.001) associated with elevated endothelial microparticles and all endothelial subgroups (venous, arterial, lung, nonlung, lung arterial, and lung venous).

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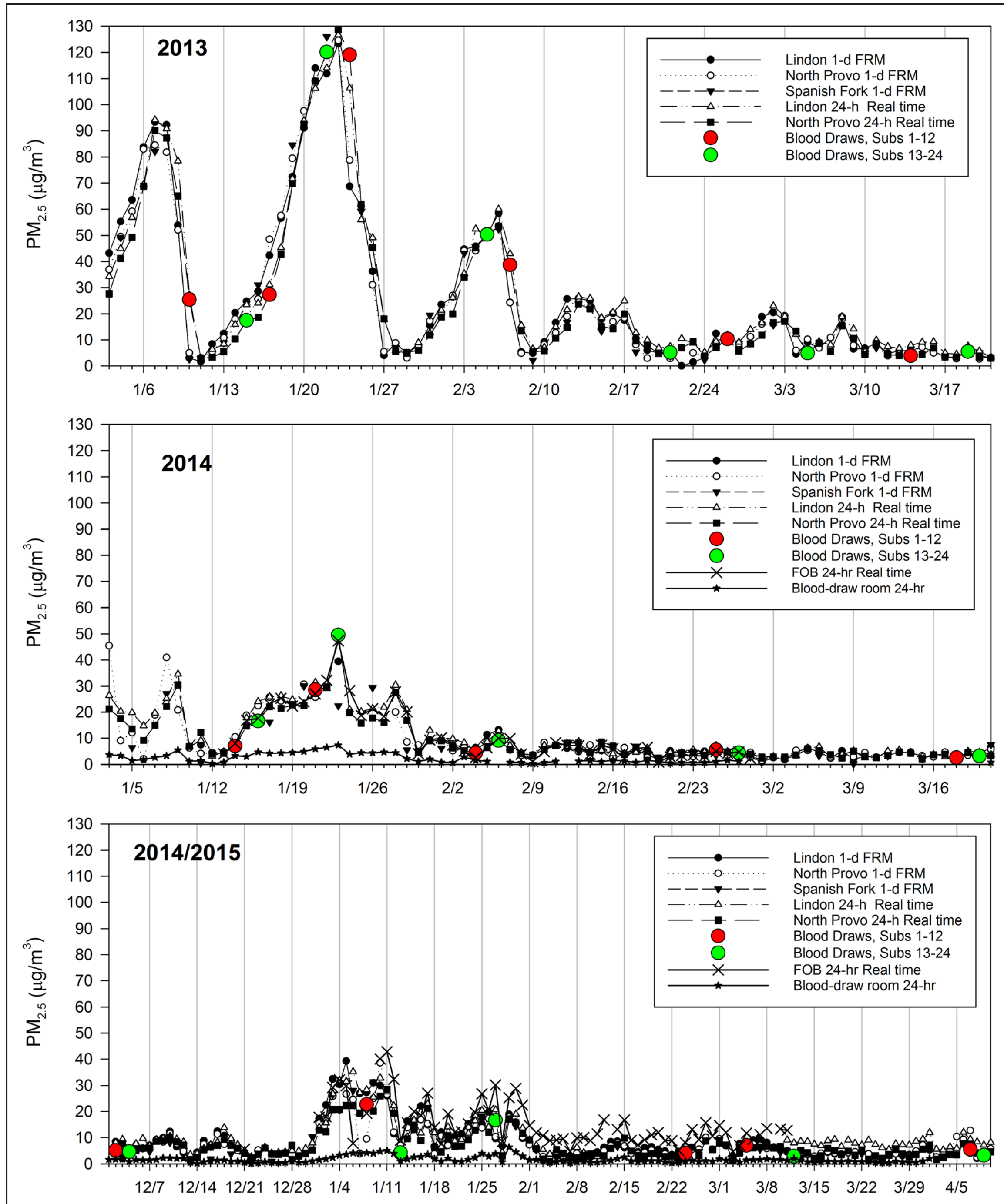

Figure 1. Fine particulate matter <2.5 µm in aerodynamic diameter ($PM_{2.5}$) concentrations and blood-draw dates plotted during study periods. Line plots indicate ambient $PM_{2.5}$ concentrations for various sites and methods where 1-d FRM indicates daily concentrations based on the Federal Reference Method and 24-h Real time indicates 24-h average concentrations based on continuous monitors from the Department of Environmental Quality sites (Lindon, North Provo, and Spanish Fork). FOB 24-h Real time indicates 24-h concentrations from monitor located adjacent to blood-draw building and Blood draw room 24-h indicates 24-h concentrations from monitor inside the blood-draw room. Dots indicate the times of and $PM_{2.5}$ concentrations at blood draws for each 12-subject blood-draw group.

The associations were significant even after using a Bonferroni correction for multiple testing (P<0.05/15). Microparticles derived from endothelial progenitor cells, platelets, and activated endothelial cells ($CD62^+$) were not positively associated with $PM_{2.5}$. $PM_{2.5}$ concentrations were also significantly associated with elevated immune cell levels, including monocytes, natural killer cells, helper T cells, and killer T cells. $PM_{2.5}$ concentrations were not associated with B cells, but were weakly associated with platelet–monocyte aggregates.

The associations between $PM_{2.5}$ and subject-mean–adjusted values for endothelial microparticles and monocytes are illustrated in Figure 2A and 2B. Associations for lung, nonlung, venous, lung venous, arterial, and lung arterial endothelial microparticles are similar to that illustrated in Figure 2A.

Table 2. Description and Summary Statistics of Microparticles and Immune Cells and Regression Coefficients for $PM_{2.5}$ From the Subject Mean–Adjusted Regression Models

Outcome Variables	Phenotype	No. of obs.	Mean*	SD	Coefficient (×10; SE)	*P* Value	R^2
Microparticles							
MP, EPC	$CD34^+/CD31^+$	332	22.03	21.68	−0.09 (0.34)	0.796	0.00
MP, Platelet	$CD41^+$	332	37.37	34.94	−1.33 (0.55)	0.017	0.02
MP, Endothelial	$CD31^+/CD41^-$	332	6.76	10.14	1.00 (0.16)	<0.001	0.11
MP, Lung endothelial	$CD31^+/CD41^-/CD143^+$	331	2.82	4.48	0.42 (0.07)	<0.001	0.10
MP, Nonlung endothelial	$CD31^+/CD41^-/CD143^-$	331	3.88	6.40	0.56 (0.10)	<0.001	0.09
MP, Venous endothelial	$CD31^+/CD41^-/EphB4^+$	329	2.55	5.54	0.48 (0.09)	<0.001	0.09
MP, Lung venous endothelial	$CD31^+/CD41^-/EphB4^+/CD143^+$	329	2.06	4.57	0.39 (0.07)	<0.001	0.08
MP, Arterial endothelial	$CD31^+/CD41^-/EphrinB2^+$	331	3.68	5.03	0.37 (0.07)	<0.001	0.07
MP, Lung arterial endothelial	$CD31^+/CD41^-/EphrinB2^+/CD143^+$	331	3.27	4.56	0.31 (0.07)	<0.001	0.06
MP, Activated endothelial	$CD62^+$	332	17.91	16.11	−0.63 (0.26)	0.014	0.02
MP, Lung-activated endothelial	$CD62^+/CD143^+$	332	3.93	4.25	0.005 (0.06)	0.943	0.00
MP, Venous-activated endothelial	$CD62^+/EphB4^+$	332	4.40	6.67	−0.02 (0.10)	0.876	0.00
MP, Lung venous–activated endothelial	$CD62^+/EphB4^+/CD143^+$	329	3.57	3.80	−0.002 (0.06)	0.980	0.00
MP, Arterial-activated endothelial	$CD62^+$/ $EphrinB2^+$	330	5.17	5.03	0.03 (0.08)	0.702	0.00
MP, Lung arterial–activated endothelial	$CD62^+$/ $EphrinB2^+/CD143^+$	330	4.52	4.47	0.05 (0.07)	0.518	0.00
Immune cells							
Monocytes	$CD14^+$	365	22503	15535	863.99 (185.95)	<0.001	0.06
Natural killer cells	$CD16^+$	365	17530	16784	660.22 (182.24)	<0.001	0.03
Helper T cells	$CD4^+$	365	72604	42633	2151.75 (504.36)	<0.001	0.05
Killer T cells	$CD8^+$	365	39259	24421	1038.21 (323.52)	0.001	0.03
B cells	$CD19^+$	365	18242	22527	−310.72 (304.33)	0.308	0.00
Platelet–monocyte aggregates	$CD45^+/CD41^+$	368	4.71	5.62	0.20 (0.09)	0.020	0.01

EPC indicates endothelial progenitor cells; MP, microparticles; and PM, fine particulate matter <2.5 μm in aerodynamic diameter.
*Per volume of the analytic tube. All microparticle subpopulations were <1 μm and Annexin V^+.

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Indeed, relative effects of $PM_{2.5}$ exposures were similar across all endothelial subgroups. Expressed as a percent increase relative to the mean level of microparticles, a 10 μg/m^3 increase in $PM_{2.5}$ was associated with a 15% increase in all endothelial microparticles and a 15%, 14%, 10%, 9%, 19% and 19% increase in lung, nonlung, arterial, lung arterial, venous, and lung venous endothelial microparticles, respectively. With regards to the immune cell responses, a 10 μg/m^3 increase in $PM_{2.5}$ was associated with a 4%, 4%, 3%, and 3% increase in the levels of monocytes, natural killer cells, helper T cells, and killer T cells, respectively.

$PM_{2.5}$, Inflammatory Cytokines, and Adhesion Molecules

Figure 3 presents the estimated associations between $PM_{2.5}$ and all 42 measured growth factors and cytokines and 2 soluble adhesion molecules. The estimated associations are presented as percent change (and 95% confidence intervals) in each analyte per 10 μg/m^3 increase in $PM_{2.5}$ relative to the mean. Estimated associations are ordered from left to right based on *t* values. $PM_{2.5}$ was associated with changes in several circulating growth factors and cytokines involved in systemic inflammation, including tumor necrosis factor α (TNF-α), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α) and MIP-1β, interferon γ-induced protein 10 (IP-10), and interleukin 8 (IL-8), IL-6, and IL-1β, as well as the soluble adhesion proteins sICAM-1 and sVCAM-1.

For TNF-α, MCP-1, IL-8, MIP-1α, MIP-1β, and IP-10, the associations were highly statistically significant with a 10 μg/m^3 incremental increase in $PM_{2.5}$ associated with a 1.25 (SE=0.19, P<0.0001), 5.22 (SE=1.09, P<0.0001), 2.96 (SE=0.70, P<0.0001), 0.86 (SE=0.21, P<0.0001), 1.65 (SE=0.52, P=0.002), and 4.05 (SE=1.34, P=0.003) pg/mL increase in each cytokine, respectively. The associations with TNF-α, MCP-1, IL-8, MIP-1α, and sICAM-1 were statistically significant even when using the Bonferroni correction for multiple testing of 44 analytes (P<0.05/44). For IL-6, IL-10, and IL-1β, the associations were also observed, but they were marginally statistically significant with a 10 μg/m^3 increase in $PM_{2.5}$ associated with a 0.09 (SE=0.05, P=0.05), 0.11 (SE=0.06, P=0.05), and 0.29 (SE=0.15, P=0.06) pg/mL increase in each cytokine, respectively.

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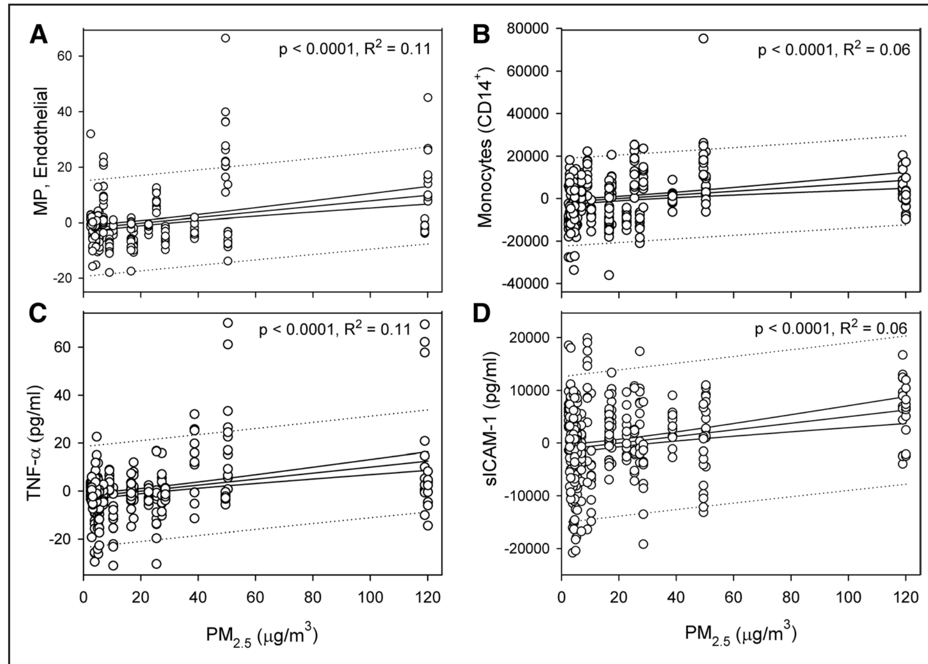

Figure 2. Subject-mean–adjusted values for endothelial microparticles (MP; A), monocytes (B), tumor necrosis factor α (TNF-α; C), and soluble intercellular adhesion molecule 1 (sICAM-1; D) plotted over fine particulate matter <2.5 µm in aerodynamic diameter ($PM_{2.5}$) concentrations, with fitted regression lines, 95% confidence limits, and 95% prediction limits.

Elevated $PM_{2.5}$ exposures were associated with significant reductions in the epidermal growth factor (EGF) and platelet-derived growth factor, as well as sCD40L (soluble CD40 ligand), growth-regulated protein α (GROα), and RANTES (regulated on activation, normal T-cell–expressed and secreted; Figure 3).

Elevated $PM_{2.5}$ exposures were also significantly associated with the 2 soluble adhesion molecules, sICAM-1 and sVCAM-1, with a 10 µg/m^3 increase in $PM_{2.5}$ associated with a 628.43 (SE=125.19, P<0.0001) and 2288.44 (SE=1030.08, P=0.03) pg/mL increase, respectively. The associations between subject-mean–adjusted values for TNF-α and sICAM-1

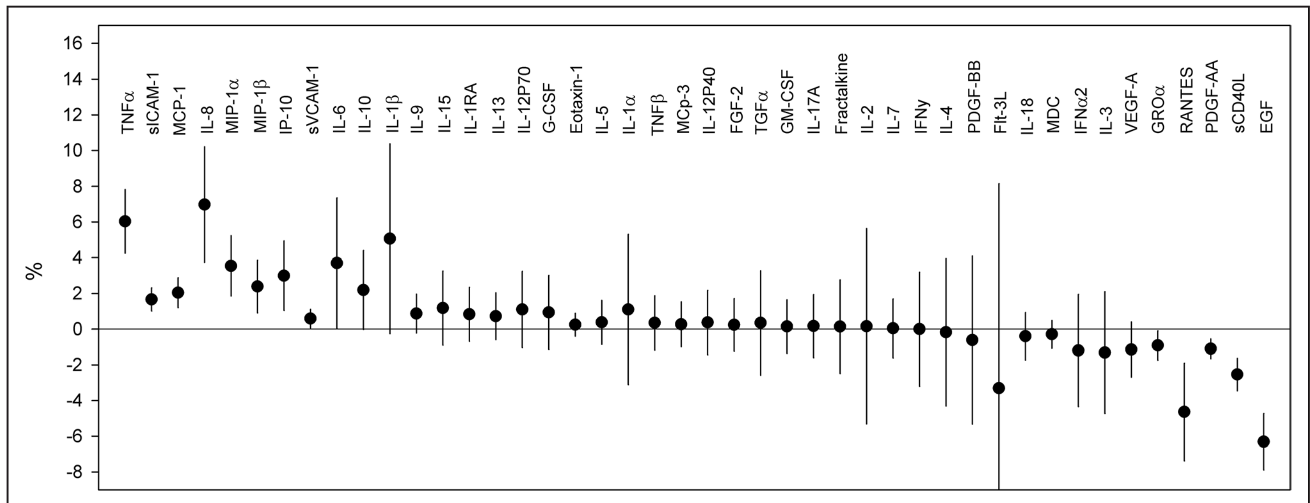

Figure 3. Associations between elevated fine particulate matter < 2.5 µm in aerodynamic diameter ($PM_{2.5}$) exposures biomarkers of inflammation. The biomarkers include all 42 measured cytokines and 2 adhesion molecules. The results are presented as percent change (and 95% confidence intervals) in each analyte per 10 µg/m^3 increase in $PM_{2.5}$ relative to the mean. Estimates are derived from the subject-mean–adjusted regressions and are ordered from left to right based on t values, resulting in the most statistically significant positive associations being on the left and the most statistically significant negative associations being on the right. EGF indicates epidermal growth factor; FGF, fibroblast growth factor; Flt-3L, FMS-like tyrosine kinase 3 ligand; G-CSF, granulocyte colony-stimulating factor; GROα, growth-regulated protein α; ICAM-1, intercellular adhesion molecule 1; IFNα, interferon alpha; IL-8, interleukin 8; IP-10, interferon γ-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MDC, macrophage-derived chemokine; MIP-1α, macrophage inflammatory protein 1α; PDGF, platelet-derived growth factor; sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cellular adhesion molecule 1; RANTES, regulated on activation, normal T-cell–expressed and secreted; and TNF-α, tumor necrosis factor α.

with $PM_{2.5}$ concentrations are presented in Figure 2C and 2D. Highly significant positive associations are observed, but this illustration also demonstrates that most of the variability remains unexplained by $PM_{2.5}$.

Sensitivity Analysis

Overall, the results were not highly sensitive to alternative modeling choices. Similar results were obtained for the fixed-effects and subject-mean–adjusted regression models, as well as models that treated all out-of-range cytokine/growth factor observations as missing observations (Online Tables I, II, and III). Also, similar results were obtained from models excluding draws from participants who reported illness, models controlling for concurrent and previous days' time exercising, models controlling for menstruation, models that exclude days with $PM_{2.5}$ concentrations >100 μg/m^3, and models that used $PM_{2.5}$ concentrations 12 and 48 hours before blood draws (rather than 24 hours). A formal comparison of effect estimates for these regression models for selected variables is presented in Online Figure II. Furthermore, for the final 2 study periods, models that included sex and $PM_{2.5}$ and fish oil (versus placebo) and $PM_{2.5,}$ interaction terms were estimated to test for effect modification. Overall, there was no consistent evidence of effect modification by either sex or use of fish oil supplements.

Discussion

The major findings of this study, as stylistically illustrated in Figure 4, are that episodic exposure to $PM_{2.5}$ was associated with an increase in circulating microparticles indicative of endothelial apoptosis, and an inflammatory, antiangiogenic blood profile associated with selective increases in T, rather than B, lymphocytes. Taken together, these findings reveal a characteristic signature of systemic injury inflicted by $PM_{2.5}$ exposure and could provide new insights into potential mechanisms by which inhalation of $PM_{2.5}$ increases CVD risk and severity, leading to premature cardiovascular mortality. Because plasmatic changes were observed in a young, healthy population with low CVD risk burden, these findings suggest that even in the absence of pre-existing disease, and unconfounded by significant disease progression, inhalation of $PM_{2.5}$ induces endothelial injury and inflammation. Therefore, these changes are likely to be early signs of systemic injury, which if sustained, could contribute to the development or exacerbation of atherosclerotic disease and the precipitation of acute cardiovascular events in susceptible individuals.

Although previous epidemiological evidence suggests that exposure to $PM_{2.5}$ contributes to the development of CVD,[1–6,9,12,14–16] the mechanistic basis of this injury is not fully understood. One potential pathway is the release of proinflammatory mediators by alveolar macrophages or epithelial cells on exposure to airborne particles. Indeed, exposure to concentrated PM results in the release of proinflammatory cytokines, such as IL-6 or TNF-α from alveolar[32] or peritoneal[33] macrophages. Similarly, human airway cells[34] or bronchial epithelial cells[35] release TNF-α, IL-6, or IL-8 on incubation of ambient air particles. Our observation showing that circulating levels of TNF-α, IL-8, and IL-6 are increased on $PM_{2.5}$ inhalation is consistent with the possibility that the release of cytokines in the lung could trigger and sustain a state of mild systemic inflammation.

Systemic inflammation due to $PM_{2.5}$ inhalation could by itself contribute to both an increase in the thrombotic propensity of the blood and proatherogenic changes in the vessel wall. Our observation of an association between increased platelet-monocyte aggregate levels in the peripheral blood and $PM_{2.5}$ exposure (Table 2) suggests that $PM_{2.5}$ exposure does indeed establish an early procoagulation state, even in healthy adults, which might be an important contributor to CVD risk associated with $PM_{2.5}$ exposure. Although in humans it is difficult to directly measure proatherogenic changes in the vessel wall and atherosclerotic disease progression in young healthy humans, we did find clear signs of endothelial injury, reflected by an increase in the circulating levels of endothelial microparticles, derived mostly from the venous endothelium. Notably, this location is just upstream of the coronary circulation putting these particles potentially at higher levels near susceptible coronary artery plaques in high-risk individuals. Thus, the increased levels of endothelial microparticles in disease-free individuals are likely suggestive of injury that precedes the development of frank disease. Nonetheless, the mechanisms by which $PM_{2.5}$ triggers the release of microparticles from the lung remain unclear. Exposure to inflammatory cytokines such as TNF-α could stimulate the release of microparticles by activating the endothelium,[36] and increased levels of activated ($CD62^+$) endothelial microparticles are associated with the states of high inflammation that accompany acute cardiovascular events.[37] However, we found no association between $CD62^+$ microparticles and $PM_{2.5}$ exposure, suggesting that most endothelial microparticles released on exposure to $PM_{2.5}$ are derived from apoptosis, not cytokine-induced activation, of the endothelium. A similar increase in the plasma levels of apoptotic, not activated, endothelial ACE^+ microparticles has been observed also in smokers with normal spirometry,[38] suggesting that pathological changes associated with smoking and $PM_{2.5}$ exposure share overlapping mechanisms that involve early lung destruction, followed by subsequent cardiovascular injury.

Although elevated levels of specific cytokines such as TNF-α cannot be linked to the generation of activated endothelial microparticles, the collective pattern of changes in plasma cytokines is indicative of an antiangiogenic state. All the cytokines and growth factors that were suppressed with $PM_{2.5}$ exposure—EGF, CD40L, platelet-derived growth factor-AA, GROα, RANTES, and vascular endothelial growth factor (VEGF)—have a key property in common. They are all potent angiogenic factors. The growth factor VEGF is required for in vivo angiogenesis and the growth of macrovascular endothelial cells,[39] whereas EGF increases the growth and proliferation of microvascular endothelial cells, particularly in the presence of platelet-derived growth factor.[40,41] EGF is also a strong trophic factor that prevents endothelial cells against TNF-α–induced apoptosis,[42] and the blockage of EGFR signaling in vivo induces endothelial apoptosis.[43] RANTES is also a proangiogenic factor. It promotes endothelial cell migration, spreading and neovessel formation, and RANTES-mediated angiogenesis depends at least partly on VEGF.[44,45] The chemokine, GROα is essential for thrombin-induced angiogenesis and it increases VEGF production by endothelial cells.[46] Downregulation or inhibition of GROα markedly decreases VEGF expression

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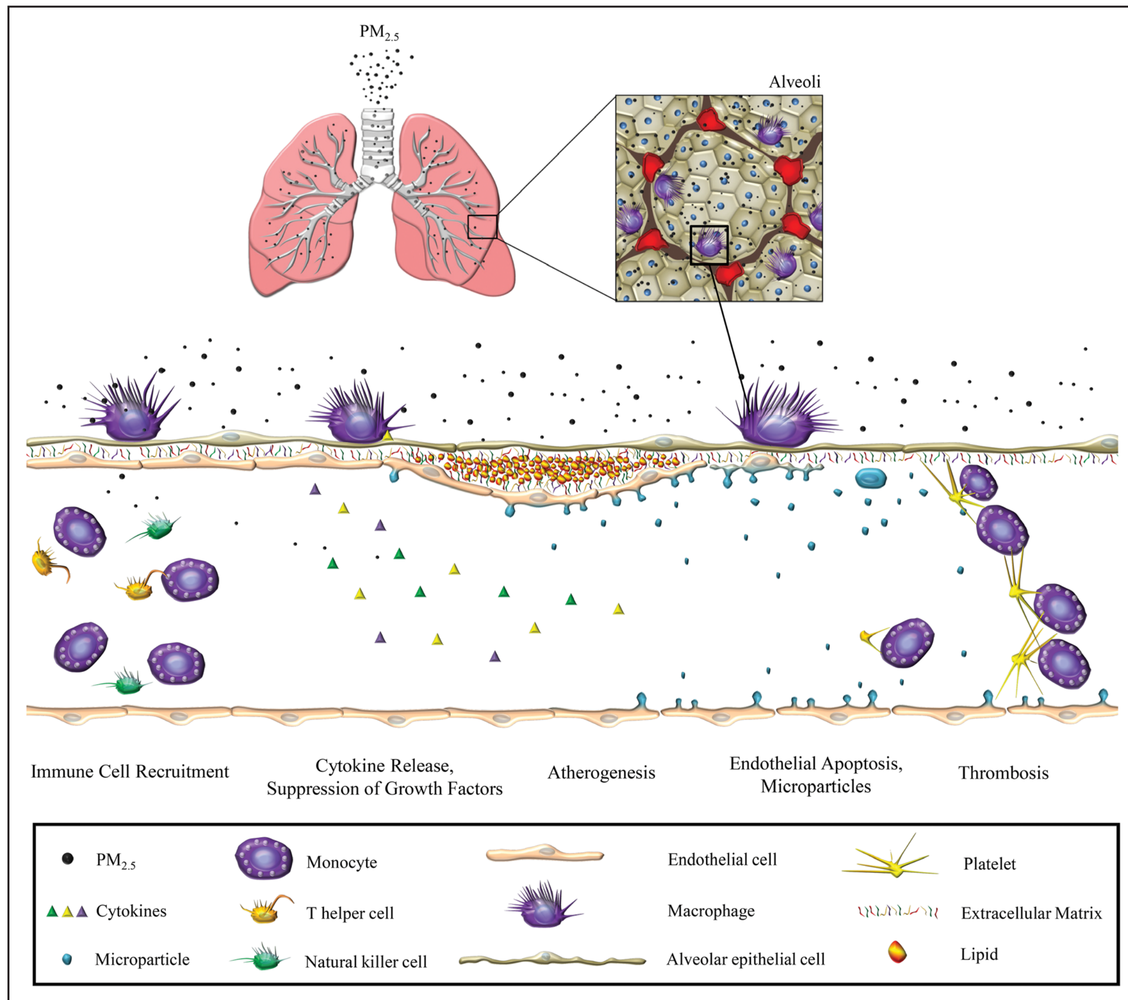

Figure 4. Schematic representation of the proposed mechanism by which fine particulate matter <2.5 µm in aerodynamic diameter ($PM_{2.5}$) induces vascular injury. Inhalation and deposition of $PM_{2.5}$ in the lungs triggers inflammatory responses that lead to the release of inflammatory cytokines (tumor necrosis factor α [TNF-α], monocyte chemoattractant protein 1, and interleukin 8), the recruitment of immune cells ($CD14^+$ monocytes, $CD16^+$ natural killer cells, and $CD4^+$ and $CD8^+$ T cells), suppression of growth/angiogenic factors (epidermal growth factor, CD40L, platelet-derived growth factor-AA, regulated on activation, normal T cell expressed and secreted, and growth-regulated protein α), and an increase in anti-angiogenic factors (TNF-α and interferon γ-induced protein 10), resulting in endothelial cell apoptosis and the generation of endothelial microparticles in blood. These events are associated with an increase in circulating levels of soluble adhesion molecules (sICAM-1 [soluble intercellular adhesion molecule 1] and sVCAM-1 [soluble vascular cellular adhesion molecule 1]) and platelet–monocyte aggregates. Collectively, these responses contribute to the pathogenic sequelae of atherogenesis and may increase thrombotic potential increasing risk of acute cardiovascular events.

and the angiogenic potential of endothelial cells.[47] Likewise, the interaction of sCD40L with CD40 on endothelial cells has been shown to increase the expression of VEGF and stimulate angiogenesis.[48,49] Overall, the association of $PM_{2.5}$ with lower circulating levels of sCD40L, EGF, platelet-derived growth factor, GROα, RANTES, and VEGF suggests that the loss of trophic, angiogenic factors could account for the increase in apoptosis of endothelial cells on $PM_{2.5}$ exposure.

The antiangiogenic state established by the loss of angiogenic growth factors seems to be further exacerbated and reinforced by a corresponding increase in antiangiogenic cytokines, such as TNF-α and IP-10. IP-10 is secreted by activated T cells, monocytes and endothelial cells, and elevated levels of this cytokine has been linked to inflammatory disorders, such as asthma.[50] It inhibits the development of new vasculature and causes the regression of newly formed vessels[51] and is strongly induced on the stimulation of monocytes with TNF-α.[52] Elevation in the circulating levels of TNF-α and other cytokines such as MCP-1, IL-8, and MIP-1α/MIP-1β are indicative of a proinflammatory state associated with increased chemotaxis and atherogenesis. High circulating levels of MCP-1[53] and IL-8[54] are associated with increased risk of all-cause mortality in patients with CVD. Interestingly, circulating levels of MIP-1β and TNF-α correlate significantly with plaque levels of these cytokines,[55] suggesting that $PM_{2.5}$ associated increases in the plasma levels of these cytokines may be reflective of increased vascular inflammation.

The pattern of changes in plasma cytokine levels associated with $PM_{2.5}$ exposure may also be linked with the observed increases in circulating lymphocytes. Cytokines such as MCP-1[56] and IP-10[57] exert potent chemotactic activity toward monocytes and T lymphocytes, whereas IL-9 stimulates the

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proliferation of activated T cells. Hence, an increase in these cytokines may be linked to the increased levels of monocytes and T cells. Notably, no change in the levels of B cells was observed, suggesting a lack of an adaptive immune response. Because $PM_{2.5}$ lacks proteins or other T-cell–dependent antigens, as well as non–T-cell–dependent antigens such as foreign polysaccharides or DNA, it may be insufficient to induce a humoral response. Nevertheless, selective increase in T, but not B, cell populations may be indicative of increased cytokine production and the formation of autoantigens generated by injured or dying endothelial cells.

Previous studies have shown that $PM_{2.5}$ exposure of just a few hours or days is associated with both fatal and nonfatal ischemic stroke and acute coronary syndrome events.[12–16] This evidence suggests that there are systemic responses to even short-term $PM_{2.5}$ exposure that play a role in triggering acute cardiovascular events.[1] Our observation that episodic increases in $PM_{2.5}$ are associated with an increase in endothelial microparticles, lymphocytes, and cytokines suggests a potential mechanism for the acute effects of $PM_{2.5}$. Although cause-and-effect relationships are not easily disentangled, our results are consistent with a scenario in which the inhalation of $PM_{2.5}$ triggers proinflammatory and antiangiogenic cytokine profile, which in turn induces endothelial cell apoptosis, leading to the increased levels of circulating microparticles. These microparticles carry metalloproteases,[58] which can induce endothelial cell apoptosis[28] or contribute to the disruption or erosion of unstable plaques in susceptible individuals. Notably, metalloproteases are responsible for the cleavage and generation of soluble adhesion molecules, including sICAM-1 and sVCAM-1 from the endothelium, both of which were increased in association with $PM_{2.5}$ exposure. Thus, an increase in microparticles on $PM_{2.5}$ exposure could disseminate proinflammatory mediators and spread inflammation beyond the lung to establish and sustain low-grade inflammation and aggravate the atherothrombotic process.[29]

The characteristic pattern of changes in cytokines, chemokines, endothelial microparticles, and blood lymphocytes reported here may be diagnostic of exposure to $PM_{2.5}$ and may be useful in distinguishing $PM_{2.5}$-induced injury from that induced by the inhalation of other toxins, such as tobacco smoke. Although direct comparisons are not yet available, the pattern of changes we observed on exposure to $PM_{2.5}$ seems to differ from that observed with smoking. Although like $PM_{2.5}$ exposure, smoking is also associated with endothelial apoptosis and a decrease in the plasma levels of CD40L,[59] EGF,[60] and GROα,[61] it is generally associated with the increased blood levels of VEGF,[62] which is due to the stimulatory effect of nicotine on VEGF[63] or due to hypoxia induced by CO in cigarette smoke. Also, unlike $PM_{2.5}$ exposure, smoking is not usually associated with an increase in plasma TNF-α or MCP-1 levels.[64] Hence, although further research is clearly needed, direct future comparisons between the pattern of changes with $PM_{2.5}$ and other inhaled toxic substances may help discern, and thereby more reliably attribute, cardiovascular injury due to $PM_{2.5}$ exposure.

Strengths of this study are its size and design. The number of available observations during the 3-year period, multiple observations per subject, and the ability to control for subject-specific differences using fixed-effects models or models using subject-mean–adjusted variables provided adequate power to test the primary hypotheses. This approach allows for matching and control for subject-specific differences, such as age, sex, race, genetics, health, etc. In this study, there was minimal potential for confounding by active smoking or exposure to second-hand cigarette smoke. Another primary strength of our study is that we evaluated $PM_{2.5}$ associations under real-world conditions with relevant exposures. Despite these strengths, the study also has limitations. Although pollution episodes were predictable, we could only control the timing of the blood draws. We were generally successful at getting observations during pollution episodes but, in the third-year study period, no substantive pollution episode occurred.

In conclusion, episodic $PM_{2.5}$ air pollution exposures were associated with the increased levels of endothelial microparticles and systemic increase in antiangiogenic cytokines and a suppression of angiogenic growth factors in young healthy adults. The effects are statistically significant, and the pattern of results is coherent and consistent. Although these findings need validation with additional research, they suggest that the inhalation of $PM_{2.5}$ can instigate adverse cardiovascular responses through changes in cytokine and growth factor levels, leading to endothelial injury and increased abundance of monocytes and T cells in the peripheral blood; responses that could potentially initiate and promote atherosclerotic lesions and trigger acute cardiovascular and cerebrovascular events. Nevertheless, we found that air pollution exposure explained only a small amount of the variability in endothelial microparticles and markers of inflammation, suggesting that exposure to air pollution is one of multiple factors that influences cardiovascular health.

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Disclosures

None.

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Novelty and Significance

What Is Known?

- Exposure to fine particulate air pollution ($PM_{2.5}$) is associated with increased risk of cardiovascular disease and mortality and contributes substantially to global burden of disease.
- Although $PM_{2.5}$-induced systemic inflammation and endothelial dysfunction have been implicated, it remains unclear how inhaled $PM_{2.5}$, a pulmonary insult, can induce cardiovascular injury and exacerbate cardiovascular disease.

What New Information Does This Article Contribute?

- In healthy, nonsmoking, young adults, episodic exposure to $PM_{2.5}$ was associated with elevated circulating endothelial microparticles, indicative of endothelial cell apoptosis and endothelial injury.
- $PM_{2.5}$ exposure was associated with inflammatory responses, including an increase in immune cells, a systemic increase in antiangiogenic cytokines, and a suppression of proangiogenic growth factors.
- Circulating levels of soluble adhesion molecules and platelet–monocyte aggregates were also elevated with $PM_{2.5}$ exposure.

Extensive epidemiological evidence indicates that exposure to ambient $PM_{2.5}$ contributes to cardiovascular disease and mortality, but it is unclear how exposure to $PM_{2.5}$ causes cardiovascular injury. We collected blood from panels of healthy, nonsmoking young adults who were environmentally exposed to episodes of elevated $PM_{2.5}$ levels. Exposure to this pollution was positively associated with markers of endothelial injury and systemic inflammation. The evidence suggests that inhalation and pulmonary deposition of $PM_{2.5}$ triggers inflammatory responses characterized by an increase in antiangiogenic cytokines and suppression of proangiogenic growth factors, which could result in increased endothelial cell death and the generation of endothelial microparticles. In combination with observed elevated levels of soluble adhesion molecules and platelet–monocyte aggregates, these responses could contribute to atherogenesis, and thrombosis, and thereby increase the risk of acute cardiovascular events.

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Exposure to Fine Particulate Air Pollution Is Associated With Endothelial Injury and Systemic Inflammation

C. Arden Pope III, Aruni Bhatnagar, James P. McCracken, Wesley Abplanalp, Daniel J. Conklin and Timothy O'Toole

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SUPPLEMENTAL MATERIAL

EXPANDED STATISITCAL METHODS

Associations with $PM_{2.5}$ exposures and microparticles and markers of inflammation were evaluated by estimating two similar regression models. The first model used a fixed effects regression approach that controls for subject-specific differences by estimating a fixed effects model as follows:

$$Y_{it} = \alpha_1 + \alpha_2 + \ldots + \alpha_{72} + \beta(P_t) + \varepsilon_{it}$$

where Y_{it} is the outcome variable for individual i on day t; $\alpha_1 + \alpha_2 + \ldots + \alpha_{72}$ are the fixed effects coefficients that adjust for subject-specific differences; β is the coefficient that measures the association between $PM_{2.5}$ and the outcome variable, P_t is $PM_{2.5}$ averaged over the 24 h period prior to the blood draw on day *t*; and ε_{it} is the model error. The second model uses a subject-mean adjusted approach that accounts for subject-specific differences by subtracting out subject-level means as follows:

$$\Delta Y_{it} = (Y_{it} - \bar{Y}_i)_{it} = \alpha + \beta(P_t) + \varepsilon_{it}$$

where $\bar{Y}_i$ is the mean value for individual *i*. The difference between the two models is that the fixed effects model controls for subject-level differences by estimating subject-specific fixed effects as part of the model. The subject-mean adjusted model controls for subject-level differences by first subtracting out the subject-level means, and regressing deviations from these means (ΔY_{it}) on $PM_{2.5}$.

To illustrate associations, plots of the subject-specific differences over pollution concentrations along with regression plots were generated. To explore the sensitivity of the results, models were estimated that excluded observations from any participant who reported any acute illness at time of the blood draw, models that controlled for time exercised on the day of the draw and the day before the draw, models that controlled for whether or not female subjects were menstruating, and models that excluded observations for days with $PM_{2.5}$ concentrations greater than 100 $\mu g/m^3$. Additionally, rather than using $PM_{2.5}$ concentrations 24 h prior to the blood draws, models that used $PM_{2.5}$ concentrations 12 h and 48 h, respectively, were estimated. Finally, using data for the final two winter/spring time periods, models that included interaction terms for gender and $PM_{2.5}$, and fish oil (versus placebo) and $PM_{2.5}$ were estimated to test for effect modification by gender and by fish oil supplement use.

For the 42 analyzed human cytokines and the 2 adhesion proteins, we also estimated the fixed effects and subject-means adjusted models as described above. The percent change (and 95% Cis) for each analyte per 10 $\mu g/m^3$ increase in $PM_{2.5}$ relative to the mean value of the analyte were calculated and plotted. The results are ordered based on t-values—resulting in the most statistically significant positive associations being on the left. This approach was used in order comprehensively evaluate all of the measures analytes, allow for direct comparisons and evaluation of the strength of the statistical associations with key markers of systemic inflammation, and to mitigate concerns regarding multiple testing and selective reporting.

All of the statistical analyses were conducted using SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina).

Supplemental Table I. Regression coefficients for $PM_{2.5}$ from fixed effect and subject-mean adjusted modeling approaches

	Fixed Effect		Subject-mean adjusted		
Outcome variables	**Coefficient (x 10) (Std. Error)**	**P-value**	**Coefficient (x 10) (Std. Error)**	**P-value**	R^2
Microparticles					
MP, EPC	-0.12 (0.45)	0.787	-0.09 (0.34)	0.796	0.00
MP, Platelet	-1.85 (0.73)	0.012	-1.33 (0.55)	0.017	0.02
MP, Endothelial	1.39 (0.20)	<0.001	1.00 (0.16)	<0.001	0.11
MP, Lung Endothelial	0.58 (0.09)	<0.001	0.42 (0.07)	<0.001	0.10
MP, Non-lung Endothelial	0.79 (0.13)	<0.001	0.56 (0.10)	<0.001	0.09
MP, Venous Endothelial	0.67 (0.11)	<0.001	0.48 (0.09)	<0.001	0.09
MP, Lung Venous Endothelial	0.54 (0.09)	<0.001	0.39 (0.07)	<0.001	0.08
MP, Arterial Endothelial	0.51 (0.1)	<0.001	0.37 (0.07)	<0.001	0.07
MP, Lung Arterial Endothelial	0.43 (0.09)	<0.001	0.31 (0.07)	<0.001	0.06
MP, Activated Endothelial	-0.88 (0.34)	0.010	-0.63 (0.26)	0.014	0.02
MP, Lung Activated Endothelial	0.006 (0.09)	0.940	0.005 (0.06)	0.943	0.00
MP, Venous Activated Endothelial	-0.02 (0.14)	0.871	-0.02 (0.10)	0.876	0.00
MP, Lung Venous Activated Endothelial	-0.002 (0.08)	0.979	-0.002 (0.06)	0.980	0.00
MP, Arterial Activated Endothelial	0.04 (0.11)	0.690	0.03 (0.08)	0.702	0.00
MP, Lung Arterial Activated Endothelial	0.07 (0.10)	0.501	0.05 (0.07)	0.518	0.00
Immune cells					
Monocytes	1063.78 (228.46)	<0.001	863.99 (185.95)	<0.001	0.06
Natural killer cells	812.89 (224.51)	<0.001	660.22 (182.24)	<0.001	0.03
Helper T cells	2649.31 (620.36)	<0.001	2151.75 (504.36)	<0.001	0.05
Killer T cells	1278.28 (398.93)	0.002	1038.21 (323.52)	0.001	0.03
B cells	-382.57 (376.38)	0.310	-310.72 (304.33)	0.308	0.00
Platelet-monocyte aggregates	0.25 (0.11)	0.019	0.20 (0.09)	0.020	0.01

Supplemental Table II. Summary statistics and regression coefficients (and standard errors) for $PM_{2.5}$ from subject-mean adjusted regression results for all measured cytokines and adhesion molecules with out-of-range observations imputed using the florescent intensity and the relevant minimum or maximum values of the standard curve as recommended.

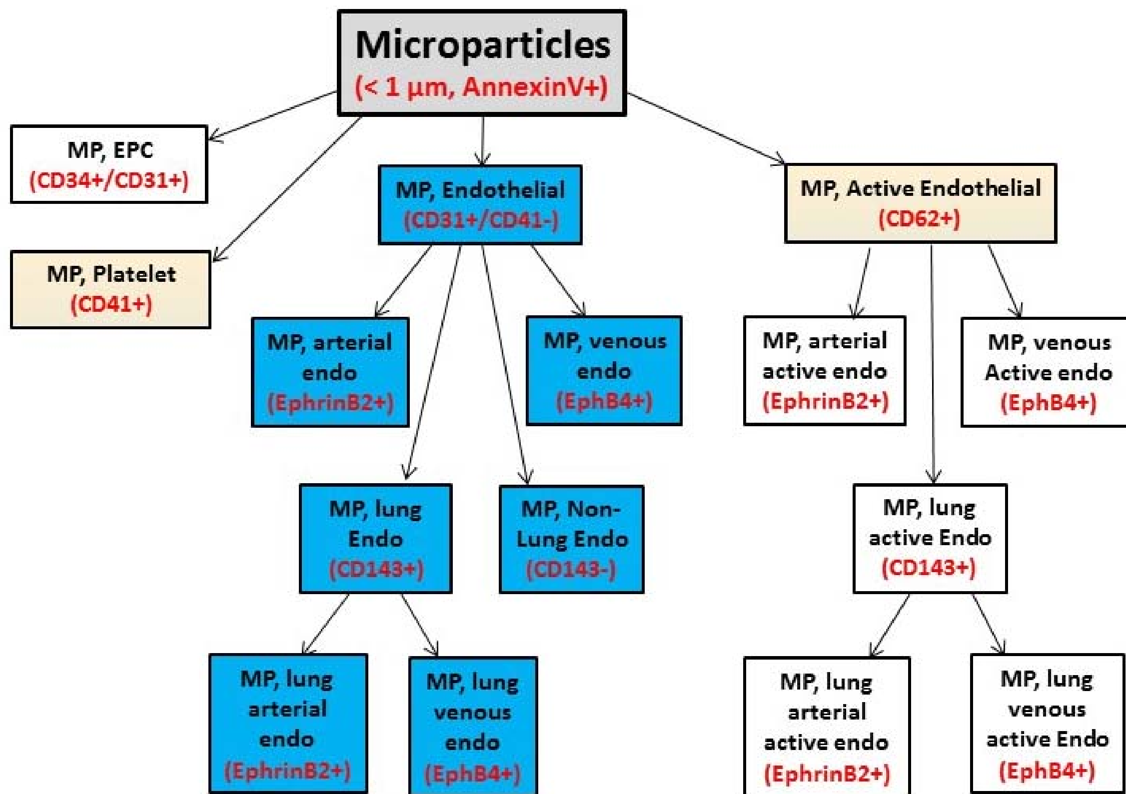
Analyte	N of Obs.	Mean	SD	Fixed Effects Estimate	P-value	Subject Mean Estimate	P-value	R square
TNFα	370	20.79	18.91	1.54 (0.23)	<0.0001	1.25 (0.19)	<0.0001	0.1063
sICAM-1	370	37723.12	11579.62	771.58 (153.2)	<0.0001	628.43 (125.19)	<0.0001	0.0641
MCP-1	370	255.7	105.67	6.41 (1.34)	<0.0001	5.22 (1.09)	<0.0001	0.0583
IL-8	370	42.42	68.69	3.63 (0.86)	<0.0001	2.96 (0.7)	<0.0001	0.0464
MIP-1α	370	24.35	30.61	1.06 (0.26)	<0.0001	0.86 (0.21)	<0.0001	0.0442
MIP-1β	370	68.95	116.06	2.02 (0.64)	0.0017	1.65 (0.52)	0.0016	0.0266
IP-10	370	135.17	92.6	4.97 (1.65)	0.0028	4.05 (1.34)	0.0027	0.0242
sVCAM-1	370	389023.08	89651.48	2809.7 (1268.56)	0.0275	2288.44 (1030.08)	0.0269	0.0132
IL-6	370	2.45	4.92	0.11 (0.06)	0.0494	0.09 (0.05)	0.0484	0.0105
IL-10	370	5.05	9.17	0.14 (0.07)	0.0532	0.11 (0.06)	0.0523	0.0102
IL-1β	370	5.7	16.12	0.35 (0.19)	0.0639	0.29 (0.15)	0.0628	0.0094
IL-9	370	2.39	4.47	0.03 (0.02)	0.1209	0.02 (0.01)	0.1193	0.0066
IL-15	370	5.38	12.96	0.08 (0.07)	0.2678	0.06 (0.06)	0.2657	0.0034
IL-1RA	370	109.75	248.43	1.12 (1.05)	0.2837	0.92 (0.85)	0.2815	0.0032
IL-13	370	33.31	67.58	0.29 (0.28)	0.2876	0.24 (0.22)	0.2855	0.0031
IL-12P70	370	16.23	38.92	0.22 (0.22)	0.3169	0.18 (0.18)	0.3147	0.0027
G-CSF	370	29.22	84.05	0.34 (0.38)	0.3806	0.27 (0.31)	0.3784	0.0021
Eotaxin-1	370	101.6	44.54	0.31 (0.41)	0.4538	0.25 (0.33)	0.4517	0.0015
IL-5	370	3.19	6.6	0.01 (0.02)	0.5475	0.01 (0.02)	0.5456	0.001
IL-1α	370	32.2	94.66	0.43 (0.85)	0.6106	0.35 (0.69)	0.609	0.0007
TNFβ	370	224.79	500.19	0.95 (2.16)	0.6592	0.78 (1.75)	0.6577	0.0005
MCp-3	368	124.65	225.41	0.41 (0.99)	0.6759	0.34 (0.8)	0.6743	0.0005
IL-12P40	370	70.62	190.82	0.32 (0.8)	0.69	0.26 (0.65)	0.6886	0.0004
FGF-2	370	124.34	137.68	0.36 (1.16)	0.7545	0.29 (0.94)	0.7534	0.0003
TGF-α	370	8.23	23.9	0.04 (0.15)	0.8154	0.03 (0.12)	0.8146	0.0001
GM-CSF	370	25.73	55.6	0.05 (0.24)	0.8507	0.04 (0.2)	0.85	<0.0001
IL-17A	370	8.46	13.86	0.02 (0.09)	0.8507	0.01 (0.08)	0.85	<0.0001
Fractalkine	370	82.53	217.07	0.14 (1.36)	0.9167	0.12 (1.11)	0.9163	<0.0001
IL-2	370	4.76	14.34	0.01 (0.16)	0.9562	0.01 (0.13)	0.956	<0.0001
IL-7	370	1.86	3.01	0 (0.02)	0.957	0 (0.02)	0.9568	<0.0001
IFNy	370	29.22	77.66	0 (0.59)	0.9994	0 (0.48)	0.9994	<0.0001
IL-4	370	2.36	10.74	-0.01 (0.06)	0.9336	0 (0.05)	0.9333	<0.0001
PDGF-BB	370	18537.05	27508.78	-140.45 (549.09)	0.7983	-114.39 (445.19)	0.7974	0.0002
Flt-3L	370	3.58	21.98	-0.15 (0.26)	0.5736	-0.12 (0.21)	0.5718	0.0009
IL-18	370	94.8	81.4	-0.47 (0.79)	0.554	-0.38 (0.64)	0.5522	0.001
MDC	370	547.14	243.61	-1.97 (2.66)	0.4593	-1.61 (2.16)	0.4572	0.0015
IFNa2	370	74.23	181.96	-1.09 (1.47)	0.4585	-0.89 (1.19)	0.4564	0.0015
IL-3	370	1.97	6.7	-0.03 (0.04)	0.4532	-0.03 (0.03)	0.4511	0.0015
VEGF-A	355	95.53	118.99	-1.34 (0.93)	0.1511	-1.09 (0.75)	0.1478	0.0059
GROα	370	2830.76	1317.08	-31.78 (14.59)	0.0302	-25.89 (11.85)	0.0296	0.0128

RANTES	370	2513.11	2784.59	-143.17 (43.13)	0.001	-116.61 (35.09)	0.001	0.0291
PDGF-AA	370	1438.19	466.94	-19.39 (4.96)	0.0001	-15.79 (4.04)	0.0001	0.0399
sCD40L	370	1521.46	713.2	-47.38 (8.63)	<0.0001	-38.59 (7.07)	<0.0001	0.075
EGF	370	197.96	137.85	-15.34 (1.93)	<0.0001	-12.5 (1.6)	<0.0001	0.1429

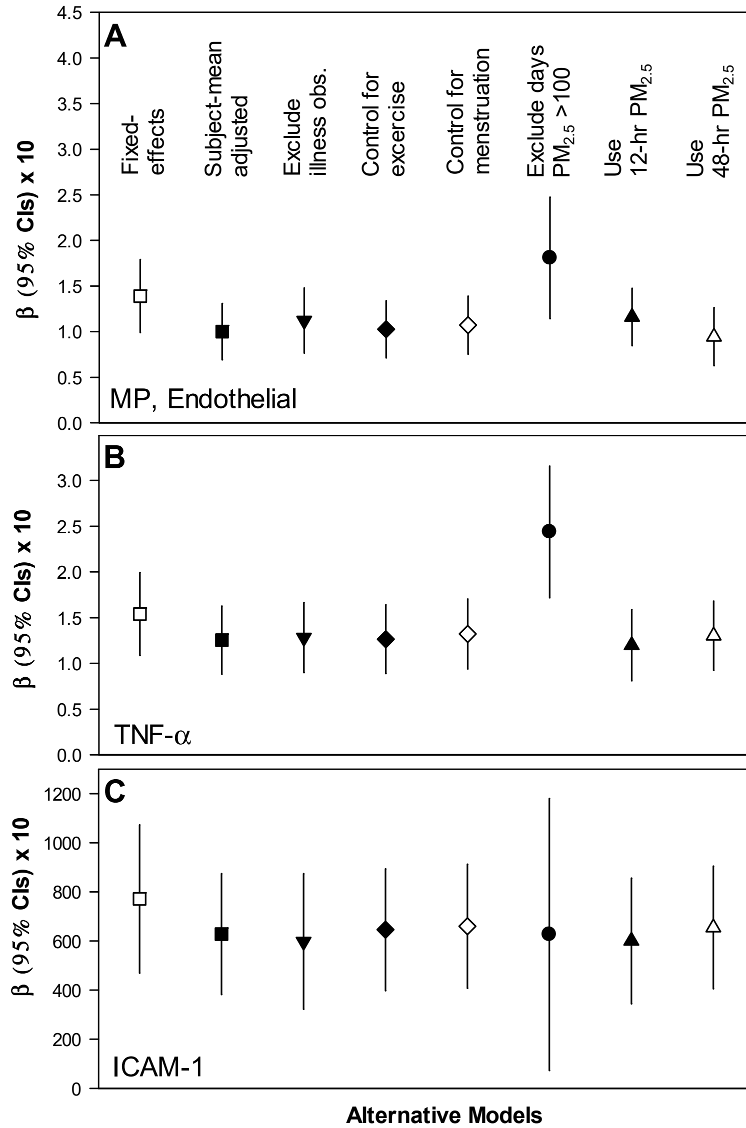
Supplemental Table III. Summary statistics and regression coefficients (and standard errors) for $PM_{2.5}$ from all of the measured cytokines and adhesion molecules with out-of-range observations treated as missing observations.

Analyte	N of Obs.	Mean	SD	Fixed Effects Estimate	P-value	Subject Mean Estimate	P-value	R^2
TNFα	366	21.01	18.89	1.55 (0.23)	<0.0001	1.26 (0.19)	<0.0001	0.1066
sICAM-1	370	37723.12	11579.62	771.58 (153.2)	<0.0001	628.43 (125.19)	<0.0001	0.0641
MCP-1	370	255.7	105.67	6.41 (1.34)	<0.0001	5.22 (1.09)	<0.0001	0.0583
MIP-1a	222	40.16	30.61	1.1 (0.29)	0.0002	0.81 (0.21)	0.0002	0.0601
IL-8	266	58.75	74.95	3.74 (1.19)	0.0019	2.64 (0.87)	0.0028	0.0334
IP-10	370	135.17	92.6	4.97 (1.65)	0.0028	4.05 (1.34)	0.0027	0.0242
MIP-1β	287	88.7	125.03	2.06 (0.72)	0.0048	1.59 (0.56)	0.0049	0.0274
sVCAM-1	370	389023.08	89651.48	2809.7 (1268.56)	0.0275	2288.44 (1030.08)	0.0269	0.0132
IL-9	370	2.39	4.47	0.03 (0.02)	0.1209	0.02 (0.01)	0.1193	0.0066
IL-10	179	9.76	11.45	0.21 (0.14)	0.13	0.16 (0.11)	0.122	0.0135
IL-6	155	4.96	6.86	0.16 (0.12)	0.1764	0.1 (0.08)	0.1982	0.0108
IL-1β	232	8.72	19.77	0.39 (0.32)	0.2343	0.26 (0.22)	0.2512	0.0057
IL-13	150	81.22	86.17	0.57 (0.53)	0.2842	0.46 (0.42)	0.2776	0.0080
IL-1RA	161	251.39	326.49	2 (2.21)	0.3679	1.58 (1.73)	0.3636	0.0052
IL-15	198	9.5	16.67	0.11 (0.14)	0.432	0.08 (0.1)	0.433	0.0031
Eotaxin-1	370	101.6	44.54	0.31 (0.41)	0.4538	0.25 (0.33)	0.4517	0.0015
IL-12P70	274	21.69	43.96	0.24 (0.32)	0.4478	0.17 (0.23)	0.454	0.0021
G-CSF	225	47.63	103.77	0.46 (0.68)	0.4929	0.33 (0.49)	0.4921	0.0021
IL-5	175	6.04	8.76	0.03 (0.05)	0.5587	0.02 (0.04)	0.5476	0.0021
MCp-3	139	328.95	259.93	0.91 (1.88)	0.6284	0.73 (1.49)	0.6236	0.0018
TNFβ	153	542.69	658.72	1.92 (4.15)	0.645	1.5 (3.24)	0.6452	0.0014
IL-1α	258	45.9	110.65	0.51 (1.17)	0.6624	0.4 (0.89)	0.6525	0.0008
IL-12P40	182	142.9	252.77	0.38 (1.62)	0.8132	0.29 (1.23)	0.8125	0.0003
GM-CSF	277	34.16	62.04	0.04 (0.31)	0.8982	0.03 (0.24)	0.8956	<0.0001
FGF-2	306	149.68	138.6	0.1 (1.15)	0.9336	0.08 (0.89)	0.9328	<0.0001
IL-7	140	3.85	4.19	0 (0.05)	0.9789	0 (0.04)	0.9782	<0.0001
Fractalkine	220	138.37	267.7	-0.02 (2.5)	0.9924	-0.02 (1.79)	0.9923	<0.0001
TGF-α	168	17.35	33.3	0 (0.34)	0.9907	0 (0.25)	0.9906	<0.0001
IL-2	202	8.19	18.74	-0.03 (0.31)	0.9282	-0.02 (0.22)	0.9262	<0.0001
IFNy	193	55.43	100.74	-0.19 (1.23)	0.8792	-0.13 (0.88)	0.8783	0.0001
IL-4	54	12.45	26.11	-0.08 (0.55)	0.8797	-0.06 (0.36)	0.874	0.0005
IL-17A	271	11.31	15.23	-0.05 (0.12)	0.6486	-0.04 (0.09)	0.6499	0.0008
PDGF-BB	332	19514.18	28883.97	-258.72 (566.73)	0.6484	-204.19 (446.09)	0.6474	0.0006
IL-18	350	100.03	80.6	-0.49 (0.84)	0.5601	-0.39 (0.67)	0.5594	0.0010
MDC	370	547.14	243.61	-1.97 (2.66)	0.4593	-1.61 (2.16)	0.4572	0.0015
IL-3	370	1.97	6.7	-0.03 (0.04)	0.4532	-0.03 (0.03)	0.4511	0.0015

IFNa2	301	91.1	197.97	-1.54 (1.89)	0.418	-1.2 (1.47)	0.4134	0.0022
Flt-3L	8	136.71	69.01	-51.81 (48.5)	0.3343	-47.57 (42.82)	0.3091	0.1706
VEGF-A	218	155.17	117.64	-1.55 (1.19)	0.1953	-1.19 (0.89)	0.1804	0.0083
GROα	370	2830.76	1317.08	-31.78 (14.59)	0.0302	-25.89 (11.85)	0.0296	0.0128
RANTES	350	2085.29	2191.45	-92.64 (36.08)	0.0108	-73.68 (28.78)	0.0109	0.0185
PDGF-AA	370	1438.19	466.94	-19.39 (4.96)	0.0001	-15.79 (4.04)	0.0001	0.0399
sCD40L	370	1521.46	713.2	-47.38 (8.63)	<0.0001	-38.59 (7.07)	<0.0001	0.0750
EGF	370	197.96	137.85	-15.34 (1.93)	<0.0001	-12.5 (1.6)	<0.0001	0.1429



Supplemental Figure I. Stylized summary of findings regarding $PM_{2.5}$ associations with circulating microparticles. The color shading indicates the following: white, not significantly associated with $PM_{2.5}$; blue, positively associated with $PM_{2.5}$; tan, negatively associated with $PM_{2.5}$.



Supplemental Figure II. Selected sensitivity analysis. Forrest plot of point estimates and 95% CIs of the coefficient of $PM_{2.5}$ (x 10) for endothelial microparticles (Panel A), TNFα (Panel B), and sICAM-1 (Panel C) using various regression models. These models include: fixed-effects and subject-mean adjusted models with all observations; subject-mean adjusted models excluding draws from participants who reported illness; models controlling for concurrent and previous days' time exercising; models controlling for menstruation; models that exclude days with $PM_{2.5}$ concentrations greater than 100 $\mu g/m^3$; and models that used $PM_{2.5}$ concentrations 12 h and 48 h prior to blood draws (rather than 24 h).