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Exposure to airborne fine particulate matter is associated with impaired endothelial function and biomarkers of oxidative stress and inflammation

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Abstract

Epidemiological evidence suggests that exposure to air pollution is a leading risk factor for cardiovascular disease (CVD). However, there is little direct evidence linking exposure to vascular dysfunction. We conducted a cross-sectional study of 100 participants, recruited from the University of Louisville Clinics. Endothelial function was assessed by calculating the reactive hyperemia index (RHI). Oxidative stress was indexed by measuring urinary levels of isoprostanes (n=91). Inflammatory biomarkers were measured in the plasma (n=80). Daily average PM_{2.5} levels were obtained from regional monitoring stations. Adjusted associations between PM_{2.5} levels and measured outcomes were tested using generalized linear models. The average age of participants was 48 years (44% male, 62% white); 52% had a diagnosis of hypertension, and 44% had type-2 diabetes. A 12.4% decrease in RHI was associated with 10 μ g/m³ increase in PM_{2.5} (95% CI: -21.0, -2.7). The F-2 isoprostane metabolite showed a positive association of 28.4% (95% CI: 2.7, 60.3) increase per 10 μ g/m³ PM_{2.5}. Positive associations were observed with angiotensin 1

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The study was approved by the Institutional Review Board (IRB number 10.0350) at the University of Louisville, and all participants gave written informed consent.

(17.4%; 95% CI: 2.8, 33.8), vascular endothelial growth factor (10.4%; 95% CI: 0.6, 21.0), placental growth factor (31.7%; 95% CI: 12.2, 54.5), intracellular adhesion molecule-1 (24.6%; 95% CI: 1.6, 52.8), and matrix metalloproteinase-9 (30.3%; 95% CI: 8.0, 57.5) per $10\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$. Additionally, a $10\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ was associated with 15.9% decrease in vascular adhesion molecule-1 (95% CI: -28.3, -1.3). These findings suggest that exposure to $\text{PM}_{2.5}$ is associated with impaired vascular function, which may result from oxidative stress and inflammation, thereby leading to a pro-atherogenic state.

Keywords

air pollution; endothelial dysfunction; $\text{PM}_{2.5}$; isoprostanes; reactive hyperemia index; cytokines

INTRODUCTION

Extensive epidemiological evidence suggests that exposure to air pollution is associated with adverse health outcomes.¹ A recent report by the World Health Organization (WHO), estimated that exposure to indoor and outdoor air pollution could be linked to 6.5 million deaths (approximately 12% of total deaths) per year worldwide.² Furthermore, it has been estimated that 92% of the world's population lives in areas where levels of the fine particulate matter fraction ($\text{PM}_{2.5}$; 0.1 – 2.5 μm in diameter) exceeds the WHO-recommended annual mean concentration limit of $10\mu\text{g}/\text{m}^3$.² Thus, exposure to polluted air is a current global health threat, especially in developing countries, and is likely to remain so in the future.

Polluted air is a complex mix of gases, including volatile organic compounds, metals and particles of various sizes. As air pollution particles initially contact the lung, pulmonary diseases (e.g. asthma, cancer, COPD) are common outcomes of these exposures. However, cardiovascular disease (CVD) has a higher prevalence in populations exposed to fine particulate ($\text{PM}_{2.5}$) air pollution^{3, 4}. Indeed, previous studies have shown that acute and chronic exposures promote CVD risk factors such as hypertension^{5, 6} and insulin resistance,^{7–9} thereby contributing to atherogenesis, thrombosis and the incidence of myocardial infarction, stroke and sudden cardiac death.^{4, 10, 11} These adverse cardiovascular outcomes likely result from a damaged or dysfunctional endothelium. Prior studies addressing the impact of $\text{PM}_{2.5}$ exposure on vascular function have done so indirectly, or have done so using cohorts presenting with confounding factors. Even less is known about the impact of exposure on signaling molecules, which regulate endothelial function or angiogenesis. Nevertheless, in a young, healthy human cohort, we previously found that acute exposure to $\text{PM}_{2.5}$ reduces the levels of proangiogenic growth factors and is associated with elevated levels of circulating endothelial microparticles, indicative of vascular apoptosis.¹² We also identified a deficit of circulating endothelial progenitor cells (EPCs),¹³ a stem cell type that promotes vascular regeneration and tissue repair. Furthermore, mice exposed to concentrated ambient particles (CAPs) demonstrated impairments in both EPC mobilization and function.^{13–15} Nevertheless, these animal and human studies lack assessments of endothelial function and angiogenesis in response to $\text{PM}_{2.5}$. Thus, the immediate physiological impact of

exposure on the vasculature remains largely speculative and whether an at risk population is more susceptible to the effects of PM_{2.5} exposure remains uncertain.

To examine how PM_{2.5} exposure affects endothelial function, we calculated the reactive hyperemia index and measured angiogenic signaling molecules in a study group of individuals at risk of developing CVD. Our results support the notion that PM_{2.5} induces oxidative stress and may directly impair endothelial function. Attenuated endothelial function may form the basis of cardiovascular pathologies associated with PM_{2.5} exposure.

METHODS

Participant characteristics and specimen collection

Participants between the ages of 22 and 65 years were recruited from the Diabetes and Primary Care Clinics of the University of Louisville.¹⁶ The study period extended from June 2011 to May 2013. All participants signed informed consents and the Institutional Review Board of the University of Louisville approved the study. Participants were prescreened, and those with conditions known to effect peripheral blood cell counts and bone marrow function were excluded from the study. These conditions included malignancies, organ transplant, renal replacement therapy, type 1 diabetes, untreated thyroid disease, anemia, acute infections, HIV infection, hepatitis, and unhealed wounds. Participants on hormone replacement therapy or medications affecting bone marrow function or peripheral blood cell counts were also excluded from the study. We also excluded those unwilling or unable to provide informed consent, pregnant or lactating women, prisoners and other vulnerable populations. All menstruating females were in the luteal phase of their cycle. A total of 100 participants were enrolled in the study.

Prior to biospecimen collection, the participants fasted for a minimum of eight hours. At the clinic, each participant filled out a health and diabetes questionnaire and biometrics were recorded. Four ml of blood was obtained from the participants using citrate as an anti-coagulant and standard venipuncture procedures. Within 30 min of collection, the tubes were spun at 1000×g for 15 min, the plasma was collected and spun again at 10,000×g for 10 min. The plasma was collected and stored at −80°C until use. Standard clean catch urine specimens were obtained from participants, and frozen in multiple aliquots at −80°C until use.

Exposure assessment

Ambient levels of PM_{2.5} in Louisville were obtained by calculating the daily average of five EPA-validated monitoring stations in Jefferson County, KY that report daily PM_{2.5} levels (Figure 1). Variations in PM_{2.5} between these monitors was limited, and over the study period, the mean PM_{2.5} concentration ranged from 11.49µg/m³ to 13.99µg/m³ (Figure 1). In general, PM_{2.5} measurements usually varied in accordance with each other and to approximately the same extent over time. However, we did observe higher values adjacent to a coal-fired power plant and lower values at a rural location. We observed that temporal variation was much higher than spatial variation between the monitors.

To examine associations with measured parameters from our study participants, PM_{2.5} levels were obtained for the day the study participants visited the clinic, and for six days prior to their visit. From this we calculated 1-day, 3-day, and 7-day average PM_{2.5} levels for each participant.

Reactive hyperemia index

To assess endothelial function, we calculated a reactive hyperemia index (RHI) value on the study participants, where a lower value indicates diminished function. This was accomplished using EndoPAT as stipulated by the manufacturer (Itamar Medical). After baseline recordings of the pulse wave amplitude from a fingertip on each arm, supra systolic pressure was applied to the non-dominant upper arm by a blood pressure cuff. The cuff was deflated after 5 min and reactive hyperemia recorded by the change in finger arterial pulse volume amplitude (PVA). The ratio between hyperemic and baseline PVA was normalized to the same ratio in the contralateral arm and reported as the reactive hyperemia index (RHI).¹⁷

Biochemical measurements

To examine the effects of PM_{2.5} on angiogenic signaling and inflammation, we examined the plasma levels of pro-angiogenic proteins (angiogenin, angiopoietin-1,2), growth factors, (vascular endothelial growth factors A,D,C, placental growth factor, stem cell factor, epidermal growth factor, platelet-derived growth factor, basic fibroblast growth factor, hepatocyte growth factor, erythropoietin), adhesion molecules (E-selectin, vascular cell adhesion molecule-1, intracellular adhesion molecule-1) and proteases (matrix metalloproteases 2, 9). The biomarkers were measured using laser microbead (Luminex) technology and specific kits from ThermoFisher. To examine systemic oxidative stress, we measured urinary levels of isoprostanes, which are formed from peroxidation of fatty acids and are indicative of oxidative stress in smokers.¹⁸ Urinary F2-isoprostane (Isop) and metabolite (Isom) were measured using GC-MS by the Vanderbilt University Eicosanoid Core Laboratory.¹⁹

Statistical analysis

Participant characteristics are presented as frequency (%) for categorical variables, and mean ($\pm$ S.D.) for continuous variables. As outcome variables were positive, and heavily right-skewed, generalized linear models with the gamma distribution and log link were used to test for associations with PM_{2.5} levels. We performed both unadjusted and adjusted models for all outcomes. To control for potential meteorological impacts, all models were adjusted for average 24 h temperature on the day of the study visit. Additionally, because certain covariates may have unequal influence on the outcome variables, we tested for significant differences in covariates between high and low outcome groups using the median cutoff. Any variables significant at the $p < 0.1$ level, as well as potential confounders and effect modifiers, were added to the final model, with final models chosen using the lowest Akaike Information Criterion value. Final models for RHI are adjusted for ambient temperature, sex, diabetes, hypertension, and waist size. Final models for isoprostanes are adjusted for ambient temperature, sex, diabetes, smoking status, systolic blood pressure, waist to hip ratio, hyperlipidemia, hsCRP, and aspirin use. Final models for cytokines are adjusted for ambient temperature, diabetes, smoking status, systolic blood pressure, waist to hip ratio,

hsCRP, and ACE inhibitor use. We also included interaction terms to test for effect modification by sex and diabetes status, but interactions were not significant and were dropped from final models. As a sensitivity analysis, we performed augmented backward elimination for confounder identification (Supplemental Table 1).²⁰ We also performed separate models using 3-day and 7-day PM_{2.5} levels (Supplemental Table 2), as well as adjustments for humidity and season (Supplemental Table 3). Seasonal variations were adjusted for by including sine and cosine terms of calendar dates for the day of blood draw. Predicted mean results were obtained for RHI, and plotted against PM_{2.5} levels, along with 95% confidence limits, and 95% prediction limits. All effect estimates were back transformed from the log scale to represent a percent change of the outcome, along with corresponding 95% confidence intervals per 10µg/m³ PM_{2.5}. Statistical significance was accepted at p-value <0.05. All analyses were performed using SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina) and GraphPad Prism, version 7 (GraphPad Software, La Jolla, California).

RESULTS

Participant characteristics

Participants in this study were of middle age (48.1 ± 10.6 yr), mostly female (56%), and overweight (avg. BMI=33.3) (Table 1). The racial makeup was representative of the metropolitan Louisville area as a whole (62% Caucasian, 28% Black). Our study group presented with a range of CVD risk levels where 21–52% of the participants presented with a CVD risk factor while 5–36% were on some medication to improve cardiovascular function. Total cholesterol levels were borderline high (190.3mg/dL), while HDL, LDL, and triglyceride levels were within normal ranges.

Particulate matter air pollution and endothelial function

Using a generalized linear model with gamma distribution we determined associations between RHI and average PM_{2.5} levels on the day of blood draw. We found a statistically significant negative association between PM_{2.5} levels and RHI (Figure 2). Specifically, there was a 12.4% decrease in RHI for every 10µg/m³ increase in PM_{2.5} (Table 2). These negative associations were found for both the unadjusted RHI (Figure 2A) and for the adjusted model (Figure 2B). No association was observed when the analysis was done using 3- and 7-day PM_{2.5} exposure levels (Supplemental Table 2), but the associations were maintained when adjusting for humidity and season (Supplemental Table 3).

Particulate matter air pollution, pro-angiogenic molecules and oxidative stress

Circulating levels of PlGF, angiopoietin1, VEGF, and ICAM-1 showed a positive association with PM_{2.5} levels after adjustment for temperature, diabetes, smoking, systolic blood pressure, waist to hip ratio, hsCRP and ACE inhibitor use (Table 2; Figure 3). In addition, MMP-9, which supports new vessel growth, showed a positive association with PM_{2.5}. In contrast, VCAM-1 demonstrated a negative association with air pollution levels (Table 2; Figure 3). Finally, when adjusted for temperature, sex, diabetes, smoking, systolic blood pressure, waist to hip ratio, hyperlipidemia, hsCRP and aspirin use, we found a positive association of PM_{2.5} with the F2 isoprostane metabolite (IsoM; Table 2) but not with F2-

isoprostane (IsoP; Table 2). Fewer associations were observed when using 3- and 7-day PM_{2.5} exposure levels (Supplemental Table 2), but associations were maintained when adjusting for humidity and season (Supplemental Table 3).

DISCUSSION

While the associations between PM_{2.5} exposure and cardiovascular morbidity and mortality suggest that PM_{2.5} might directly affect endothelial integrity or function, there is little evidence to support this assertion. Although the direct application of PM_{2.5} particles to endothelial cells in culture induced oxidative stress and subsequent responses such as autophagy,^{21, 22} these outcomes were identified in a non-physiological model of exposure. Even in animal models, only the *ex vivo* constriction and relaxation of isolated endothelial vessels has been studied.²³ In still other mouse or human studies, endothelial dysfunction has been assessed indirectly by measuring the expression levels of endothelin-1 and eNOS.^{24, 25} While some human-based studies do make direct assessments of endothelial function and identify associations with PM_{2.5} levels, these findings were limited to specific cohort populations. For instance, PM_{2.5} levels have been found to be negatively associated with endothelial dysfunction as identified by flow mediated dilation or a reactive hyperemia index, but these studies only used diabetics^{26, 27} or an elderly population,²⁸ respectively. When using a more diverse cohort, another study identified negative associations with measured endothelial function but only in diabetics and not in non-diabetics.²⁹ Still other, related studies found negative associations but only when analyzing one-year average PM_{2.5} exposure trends³⁰ or found no association at all between measured exposure or intake and RHI.³¹ Thus, the present study has several advantages over previous human or animal studies in so far as it provides strong support to the idea that PM_{2.5} inhalation induces endothelial dysfunction. First, rather than quantifying biomarkers reflective of endothelial function, we assess function by calculating a reactive hyperemia index. Second, since our study group has a mix of CVD risk, our results indicate that the effects of PM_{2.5} on endothelial function is independent of CVD risk status. In fact, we continued to observe the association after adjustment for diabetes. Finally, we observed these associations when using measures of average daily PM_{2.5} levels. Thus, endothelial dysfunction resulting from acute PM_{2.5} inhalation may be pervasive in the general population.

We found that several growth factors (PIGF, angiopoietin-1, VEGF, PDGF-BB, erythropoietin) and signaling molecules (MMP9) that support angiogenesis showed a significant, or near significant, positive association with PM_{2.5} exposure. Our observed positive associations with VEGF and PDGF levels are consistent with the results from a recent meta-analysis of VEGF expression³² and a cell culture study of particle-induced PDGF expression,³³ respectively. Similarly, positive associations with MMP-9 have also been observed in a cell culture model of exposure,³⁴ in a human exposure study,³⁵ and in endothelial³⁶ and pulmonary³⁷ tissues in murine models of exposure. Thus, persistent vascular injury resulting from exposure might stimulate the expression of several pro-angiogenic agonists to maintain vascular function. However, this assertion seems to be inconsistent with our measured assessment of vascular function, which identified impairments (decreased RHI) with increasing PM_{2.5}. This apparent discrepancy may be due to resistance in downstream signaling that results in greater production of cytokines and

growth factors. This is consistent with our previous findings in mice which showed that PM exposure induces resistance to VEGF signaling and limits EPC mobilization.¹⁵ Thus even in the presence of increased levels of VEGF, such as in the present study, reparative cells may have limited responsiveness to this agonist and angiogenesis or endothelial function would remain impaired. While a complete understanding of the mechanisms whereby PM_{2.5} impacts signaling downstream of the VEGF receptor is lacking, similar reductions in the efficacy of growth factor signaling might also account for the discrepancies between increased PlGF, angiopoietin1, PDGF-BB, and erythropoietin and impaired vascular function.

It is interesting to point out that the effect of PM_{2.5} on some growth factors differed between the current study and previous work from our group.¹² Thus, while VEGF, PDGF, and EGF demonstrated an increase with PM_{2.5} in the current study (using participants residing in metropolitan Louisville, KY), we observed that these same growth factors decreased with PM_{2.5} in the previous study (using a Provo, Utah cohort). Although the basis for these differences is uncertain, they may be reflective of the different participants used in these studies as somewhat older participants of variable CVD risk were studied here, while healthy individuals participated in the previous study. Thus, while PM_{2.5} exposures may decrease VEGF (and other pro-angiogenic molecules) acutely in a healthy population, this outcome may be reversed in a more at-risk population presenting with other confounders and co-morbidities. Alternatively, the observed differences in growth factor expression may be reflective of differences in PM_{2.5} composition between the two locales. Little is known about the impact of individual PM_{2.5} components on growth factor expression or signaling competency. Finally, the observed differences in growth factor levels between the two studies might be reflective of experimental protocol and exposure duration. Blood in the Provo, UT study was collected at times of high PM_{2.5} during a wintertime inversion episode, when PM_{2.5} levels rapidly increase over a short time period (1–2 days). Thus while an acute increase in PM_{2.5} may deplete existing stores of certain growth factors in the Utah cohort, persistent exposure to lower levels of PM_{2.5} may serve as a stimulus for growth factor expression in the Louisville study participants.

Progressive atherosclerotic disease is a major adverse outcome associated with chronic exposure to PM_{2.5},^{38, 39} and extensive studies have shown that atherogenesis is facilitated by increased cell adhesion. In the present study we found a positive association of ICAM-1 with PM_{2.5}, suggesting that exposure can increase atherogenesis by promoting cell adhesion. Indeed, increased adhesion molecule expression seems to be a common outcome of PM_{2.5} exposure.^{12, 40, 41} However, in contrast to the results obtained with ICAM-1, we observed a negative association with VCAM-1. While the responses of ICAM-1 and VCAM-1 are generally similar, the divergent outcomes in this study may result from some unique characteristic of our study population or local particle composition leading to the differential responses of these adhesion molecules. A clarification of these differing outcomes awaits further study.

The diverse pathological outcomes deriving from particulate inhalation may derive from the initiation of oxidative stress.⁴² It has been suggested that biomolecules (e.g. lipids) oxidized in the tissue of first exposure (lungs) are distributed throughout the vasculature to affect the

function of distal cells and tissues.⁴³ PM_{2.5}-induced oxidative stress could also be a strong driver of the atherosclerotic response characteristic of chronic exposures.⁴⁴ Our earlier animal studies support the idea that pathologies associated with PM_{2.5} exposure result from oxidative stress. Specifically, we found that pulmonary over-expression of extracellular superoxide dismutase (ecSOD) in mice mitigated PM_{2.5}-induced defects in EPC function and increased their availability as well.¹⁴ Similarly, ecSOD over-expression limited PM_{2.5}-induced vascular insulin resistance.⁷ Nevertheless, the results derived from the use of these anti-oxidants are only correlative and direct evidence that PM_{2.5} exposure induces oxidative stress is minimal. To address this issue, we measured urinary levels of isoprostanes. These products of oxidized lipids have found widespread use as indicators of oxidative stress and their levels are increased upon acute exposure to particles generated from multiple sources.^{45–48} In this study, we specifically identified a positive association between PM_{2.5} levels and the F2-isoprostane metabolite, but not the parent compound, F2-isoprostane. Consistent with these findings, metabolite levels have been shown to be a more robust indicator of oxidative stress.⁴⁹

Although our study provides strong support for an association between exposure to PM_{2.5} and endothelial dysfunction, it has some limitations. First, the size of the study was relatively small, consisting mostly of middle aged participants, which might limit the generalizability of our findings. Second, many participants had multiple risk factors for cardiovascular disease, necessitating adjustments for multiple confounders. Third, the study has a cross-sectional design and only single measures were obtained from each participant, precluding any causal inferences. Finally, we did not assess exposure at an individual level. Therefore, we cannot account for specific residential or occupational exposure to PM_{2.5} at levels which may deviate from that in the larger metropolitan Louisville area.

Nevertheless, we found a consistent and robust association between PM_{2.5} and impaired vascular function. Based on our measurements of multiple biomarkers, we speculate that these impairments result from oxidative stress and occur despite increases in pro-angiogenic signaling molecules. Taken together, the findings of this study provide important evidence suggesting that acute exposure to airborne particulate matter directly impacts endothelial function, thereby leading to an increase in the risk of cardiovascular morbidity and mortality.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Exposure to fine airborne particulate matter (PM_{2.5}) is associated impaired vascular function.
- Exposure to PM_{2.5} is associated with oxidative stress and changes in plasma levels of growth factors and cytokines.
- These effects may underlie the association of PM_{2.5} exposure with increased cardiovascular morbidity and mortality.

Figure 1. PM_{2.5} monitoring.

Illustrated are the approximate locations of EPA-validated monitoring sites in the Louisville metropolitan area and the measured, average PM_{2.5} concentrations over the study period.

Figure 2. $PM_{2.5}$ and RHI.

Illustrated are the regression analyses between $PM_{2.5}$ and unadjusted RHI (A) and adjusted predicted mean RHI (B) with 95% confidence limits, and 95% prediction limits. The predicted mean results (B) were generated from a generalized linear model with gamma distribution, adjusting for temperature, sex, diabetes, hypertension, and waist size.

Figure 3. Associations between plasma biomarkers and PM_{2.5}.

The biomarkers include 18 analytes supportive of vascular function and angiogenesis or indicative of inflammation. Results are presented as percent change (and 95% confidence intervals) per 10 µg/m³ increase of PM_{2.5}. Generalized linear models with gamma distribution were used to test associations between outcomes and PM_{2.5} (n=80). Models are adjusted for temperature, diabetes, current smokers, systolic blood pressure, waist to hip ratio, hsCRP, and ACE inhibitor use. Ang2 indicates angiopoietin-2; VEGF, vascular endothelial growth factor; PIGF, placental growth factor; SCF, stem cell factor; EGF, epidermal growth factor; PDGFBB, platelet derived growth factor-BB; BFGF, basic fibroblast growth factor; HGF, hepatocyte growth factor; Epo, erythropoietin; VCAM1, vascular cellular adhesion molecule 1; ICAM1, intercellular adhesion molecule 1; MMP2, matrix metalloproteinase-2; MMP9, matrix metalloproteinase-9.

Table 1.

Cohort Demographics and Cardiovascular Disease History (n=100)

Characteristics	Frequency
Sex	
Male	44
Race	
White	62
Black	28
Other	10
CVD Risk Factors	
RHI (<1.67)	23
Hypertension	52
Hyperlipidemia	39
Diabetes	44
Current smoker	21
Former smoker	12
Medications	
Metformin	36
Insulin	28
ACE	31
ARB	9
β -Blocker	21
Calcium-channel blocker	17
Diuretics	35
Statins	36
Vasodilators	5
Aspirin	31
Continuous Characteristics	Means (SD)
Age	48.1 (10.6)
BMI	33.26 (8.44)
WHR	0.95 (0.16)
Waist size	42.19 (8.72)
hsCRP	3.51 (4.38)
Systolic BP (mmHg)	128.0 (20.2)
Diastolic BP (mmHg)	80.0 (10.6)
Lipid Levels (md/dL)	
Cholesterol	190.3 (43.8)
HDL	47.5 (16.4)
LDL	102.1 (29.2)
Triglycerides	147.3 (130.5)
Temperature (°C)	11.63 (12.50)
Humidity (%)	22.51 (13.33)

PM_{2.5} (µg/m³)	11.45 (5.07)
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Abbreviations: BMI: body mass index (weight(kg)/height(m)²); WHR: waist to hip ratio; hsCRP: high sensitivity C-reactive protein; CVD: cardiovascular disease; ACE: angiotensin-converting enzyme inhibitor; ARB: angiotensin-receptor blockers.

Table 2.Associations between PM_{2.5}, and reactive hyperemia index, isoprostanes, and cytokines.

Outcome	n	Mean (SD)	Model 1 (unadjusted)			Model 2 (adjusted)		
			% Change*	95% C.I.	P-Value	% Change*	95% C.I.	P-Value
RHI	100	2.14 (0.64)	-16.2	-24.9, -6.6	0.002	-12.4	-21.0, -2.7	0.014
Isop	91	2.24 (2.26)	-7.3	-30.0, 22.6	0.594	19.4	-16.9, 71.6	0.339
Isom	91	0.93 (0.61)	21.2	-1.6, 49.2	0.071	28.4	2.7, 60.3	0.028
Angiogenin	80	121.10 (15.64)	-3.1	-9.3, 3.5	0.347	-4.7	-10.4, 1.4	0.129
Angiopoietin1	80	615.11 (190.44)	24.5	7.5, 44.2	0.004	17.4	2.8, 33.8	0.017
Ang2	80	3.33 (1.65)	14.8	-11.0, 48.1	0.286	3.1	-19.2, 31.7	0.804
VEGF	80	14.70 (3.42)	16.8	4.4, 30.5	0.007	10.4	0.6, 21.0	0.037
VEGFd	80	222.90 (15.71)	1.9	-1.6, 5.5	0.277	1.3	-2.1, 4.7	0.468
VEGFc	80	103.34 (47.71)	9.9	-16.6, 44.6	0.505	5.4	-19.5, 38.3	0.699
PIGF	80	3.35 (1.24)	39.8	16.9, 67.0	<0.001	31.7	12.2, 54.5	<0.001
SCF	80	51.17 (14.83)	-3.8	-17.1, 11.5	0.602	-6.7	-19.6, 8.3	0.366
EGF	80	2.23 (1.06)	10.4	-12.3, 39.1	0.397	9.2	-13.0, 37.0	0.449
PDGFBB	80	43.62 (20.55)	21.9	-3.1, 53.6	0.092	24.2	-0.1, 54.5	0.051
BFGF	80	122.61 (11.60)	1.2	-3.4, 6.1	0.605	-0.3	-4.6, 4.2	0.893
HGF	80	64.65 (25.29)	25.0	4.0, 50.4	0.017	10.1	-3.3, 25.2	0.147
Epo	80	1.09 (0.11)	5.1	0.1, 10.4	0.044	4.5	-0.5, 9.7	0.079
Eselectin	80	25.71 (9.75)	20.1	-0.4, 44.6	0.055	11.0	-4.3, 28.7	0.170
VCAM1	80	581.11 (201.14)	-13.2	-26.3, 2.1	0.088	-15.9	-28.3, -1.3	0.034
ICAM1	80	193.49 (83.01)	32.2	6.3, 64.5	0.012	24.6	1.6, 52.8	0.035
MMP2	80	197.75 (58.47)	4.5	-17.6, 11.0	0.560	-4.6	-17.1, 9.9	0.516
MMP9	80	18.22 (7.75)	29.0	5.9, 57.5	0.012	30.3	8.0, 57.5	0.006

* Represents % change per 10 μ g/m³ PM_{2.5}. Generalized linear models with gamma distribution were used to test associations between outcomes and PM_{2.5}. Isoprostanes and cytokines were measured in a subset of participants, n=91 and n=80, respectively. RHI was adjusted for temperature, sex, diabetes, hypertension, and waist size; isoprostanes were adjusted for temperature, sex, diabetes, current smokers, systolic blood pressure, waist to hip ratio, hyperlipidemia, hsCRP, and aspirin use; cytokines were adjusted for temperature, diabetes, current smokers, systolic blood pressure, waist to hip ratio, hsCRP, and ACE inhibitor use. RHI indicated reactive hyperemia index; Isop, F2-isoprostane; Isom, F-2 isoprostane metabolite; Ang2, angiopoietin-2; VEGF, vascular endothelial growth factor; PIGF, placental growth factor; SCF, stem cell factor; EGF, epidermal growth factor; PDGFBB, platelet derived growth factor-BB; BFGF, basic fibroblast growth factor; HGF, hepatocyte growth factor; Epo, erythropoietin; VCAM1, vascular cellular adhesion molecule 1; ICAM1, intercellular adhesion molecule 1; MMP2, matrix metalloproteinase-2; MMP9, matrix metalloproteinase-9.