

Air Pollution and Blood Pressure

Insights Into the Mechanisms and Mediators of the Effects of Air Pollution Exposure on Blood Pressure and Vascular Function in Healthy Humans

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Abstract—Fine particulate matter air pollution plus ozone impairs vascular function and raises diastolic blood pressure. We aimed to determine the mechanism and air pollutant responsible. The effects of pollution on heart rate variability, blood pressure, biomarkers, and brachial flow-mediated dilatation were determined in 2 randomized, double-blind, crossover studies. In Ann Arbor, 50 subjects were exposed to fine particles ($150 \mu\text{g}/\text{m}^3$) plus ozone (120 parts per billion) for 2 hours on 3 occasions with pretreatments of an endothelin antagonist (Bosentan, 250 mg), antioxidant (Vitamin C, 2 g), or placebo. In Toronto, 31 subjects were exposed to 4 different conditions (particles plus ozone, particles, ozone, and filtered air). In Toronto, diastolic blood pressure significantly increased (2.9 and 3.6 mm Hg) only during particle-containing exposures in association with particulate matter concentration and reductions in heart rate variability. Flow-mediated dilatation significantly decreased (2.0% and 2.9%) only 24 hours after particle-containing exposures in association with particulate matter concentration and increases in blood tumor necrosis factor α . In Ann Arbor, diastolic blood pressure significantly similarly increased during all of the exposures (2.5 to 4.0 mm Hg), a response not mitigated by pretreatments. Flow-mediated dilatation remained unaltered. Particulate matter, not ozone, was responsible for increasing diastolic blood pressure during air pollution inhalation, most plausibly by instigating acute autonomic imbalance. Only particles from urban Toronto additionally impaired endothelial function, likely via slower proinflammatory pathways. Our findings demonstrate credible mechanisms whereby fine particulate matter could trigger acute cardiovascular events and that aspects of exposure location may be an important determinant of the health consequences. (*Hypertension*. 2009;54:659-667.)

Key Words: hypertension ■ endothelium ■ sympathetic nervous system ■ inflammation ■ oxidative stress

Fine particulate matter air pollution $<2.5 \mu\text{m}$ in diameter ($\text{PM}_{2.5}$) is a major worldwide cause of cardiovascular (CV) mortality.^{1,2} Although $\text{PM}_{2.5}$ mass is related to the degree of CV risk, specific sources (eg, traffic), chemical components, and gases (eg, ozone) contribute to the health consequences.³⁻⁷ Because gas and particle pollution are typically present together, it is important to understand their individual and combined effects.^{1,2}

$\text{PM}_{2.5}$ promotes CV events via several mechanisms^{1,2}; however, vascular dysfunction likely plays an integral role. An imbalance in vasomotor tone and/or a related prohypertensive response could trigger ischemic cardiac events and

contribute to the observed risk for heart failure and stroke.⁸ Indeed, we demonstrated that a 2-hour-long exposure to concentrated ambient $\text{PM}_{2.5}$ (CAP) plus ozone raises diastolic blood pressure (BP) and triggers vasoconstriction in healthy adults.^{9,10} Diesel exhaust, a mixture of particles and gases, can also instigate endothelial dysfunction and cardiac ischemia.^{11,12} Although the degrees of vasoconstriction and BP elevation were both associated with the concentration of organic carbon within $\text{PM}_{2.5}$ in our original studies,¹³ some effect of ozone could not be discounted. Ozone (O_3) could have directly impaired arterial function or interacted with the particles enhancing their toxicity.^{3,14} Because both are targets

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for regulations, it is important to elucidate the responsible pollutant(s).¹⁵

In addition, the mechanism underlying the CV responses remained a matter of speculation.^{9,10} Three pathways appeared most plausible at the time of this study design: (1) pollution-induced systemic oxidative stress/inflammation; (2) elevated endothelin (ET) levels or activity; and (3) altered autonomic nervous system (ANS) balance.^{2,16} The inhalation of PM_{2.5} has been shown to cause a systemic inflammatory response (eg, elevation in proinflammatory cytokines, eg, interleukin 6 and tumor necrosis factor α [TNF- α]). Previous studies suggest that particle-induced oxidative stress, within the lungs and/or systemically, likely plays a key role in initiating this response.^{1,2} Additional experiments show that air pollution exposure is associated with rapid changes in ANS balance, favoring sympathetic nervous system activation and parasympathetic withdrawal. Several other lines of evidence also suggest that PM inhalation can rapidly trigger an increase in circulating levels and/or bioactivity of ET within the vasculature.^{1,2} Each of these responses may be potentially responsible for causing systemic vascular dysfunction and increasing BP by favoring provasoconstrictive cellular signaling pathways after acute air pollution exposure.² Therefore, we explicitly designed this experiment to test the viability of each of these putative pathways.

This study aimed to determine the responsible air pollutant(s) (ozone, PM_{2.5}, or their combination) and the most credible mechanism underlying our previous findings.^{9,10} To meet these goals, we designed a controlled air pollution exposure study purposely involving well-coordinated experimental limbs performed concomitantly at 2 different sites. This design contributed to the increased sample size compared with previous studies^{9,11} and facilitated the investigation of the 2 different study aims along with our ability to discern whether responses differ between locations where PM_{2.5} can be dissimilar in composition and sources.

Methods

The study was approved by the human research ethics committees of St Michael's Hospital and the University of Toronto, as well as the institutional review board of the University of Michigan. Subjects at both locations were healthy 18- to 50-year-old nonsmokers without any CV disease or risk factor and not taking medications. We excluded subjects with a screening fasting total cholesterol >240 mg/dL or glucose >126 mg/dL.

Study Design

The overall study design is illustrated in the online Data Supplement (please see <http://hyper.ahajournals.org>; Figure S1). The 2 specific site locations were selected to provide exposures to fine particles that differ in source and likely also chemical composition. In Ann Arbor, exposures and CV testing were performed in the AirCare1 mobile facility adapted for humans and stationed at the University of Michigan North Campus.¹⁷ Subjects were exposed to CAP plus ozone for 2 hours on 3 separate occasions 2 to 4 weeks apart. Two hours before each exposure (1 hour before pre-exposure testing), subjects were given 1 randomized, double-blind oral pretreatment of Bosentan (250 mg), vitamin C (2000 mg), or placebo. Neither the subject nor the investigative personnel was aware of the pretreatment type during the study. In Toronto, exposures and CV testing were performed at the Gage Occupational and Environmental Health Unit in downtown Toronto.⁹ Subjects were exposed in a randomized, blinded fashion to 4 conditions for 2 hours (CAP plus ozone, CAP,

ozone, or filtered air) without pretreatments ≥ 2 weeks apart. Subjects were not aware of the order and could not discern the exposure type. All of the study personnel who performed the CV outcome measurements were blinded to the exposure types during the study. Only the investigator responsible for generating the exposure was aware of its composition during the study period.

Subjects arrived fasting (>8 hours) to the facilities between 8:00 and 9:00 AM. Pre-exposure testing was performed first (1-hour duration). Afterward, subjects underwent the 2-hour-long exposure, followed immediately by repeat testing on completion. Subjects returned fasting the following morning for repeat testing between 8:00 and 9:00 AM. In Ann Arbor, subjects wore a 24-hour ambulatory BP monitor (SpaceLabs 90207 ABP Monitor) the day before and after all of the exposures.

Exposures

Ambient PM_{2.5} was concentrated to a target level of 150 $\mu\text{g}/\text{m}^3$. In Toronto, CAP exposures were produced with a 2-stage Harvard virtual impactor system.⁹ During filtered air exposures, a high-efficiency particulate arresting filter was inserted downstream of the concentrator. In Ann Arbor, CAP exposures were produced with a 3-stage Harvard virtual impactor system.¹⁷ Ozone (120 parts per billion) was produced by an arc generator and added to the CAP airflow. Ozone was monitored continuously by using a photometric analyzer. Both human chambers were modified air-tight body plethysmographs with exposure air flows (15 to 20 L/min) entering the chamber via ducting ending in a face mask facilitating nasal inhalation. PM_{2.5} levels were monitored during exposures by a tapered element oscillating microbalance.

A PM_{2.5} filter sample was collected immediately upstream of the chambers on a 47-mm Gelman Teflon filter with a 2- μm pore size at an air flow of 8 L/min. The sample was analyzed gravimetrically for total mass on conditioned filters using a climate-controlled clean room.^{9,17}

CV Outcomes

Technicians at both sites performed all of the protocols using identical methodologies after combined training.⁹ Subjects rested supine for >10 minutes before all of the testing periods in a temperature-controlled room. In Ann Arbor, the order of testing was as follows: average of 3 supine BP levels (Omron 780), brachial artery diameter (BAD), flow-mediated dilatation (FMD), arterial compliance, nitroglycerin-mediated dilatation (NMD), and blood draws for biomarkers. In Toronto, the order of testing was as follows: average of 3 supine BP levels (Oscar-1 or -2; SunTech); supine 10-minute ECG using a Holter monitor, which subsequently recorded continuously throughout the study; BAD; FMD; NMD; and blood draws for biomarkers.

BAD, FMD, and NMD were measured using a Terason 2000 ultrasound with a 7.5- to 10.0-mHz linear array transducer with ECG-gated image acquisition and storage of digital loops (Teratech, Inc). Upper arm cuff inflation above the site of ultrasound image acquisition for 4 minutes was used to generate reactive hyperemia, and images were obtained continuously from 50 to 120 seconds after cuff deflation. Peak FMD within this period was used as the study outcome for "endothelial function." Image analyses were performed using semiautomated software (Medical Imaging Applications, Inc). Arterial compliance was measured by radial artery tonometry using the CVProfilor, as described previously (Hypertension Diagnostics, Inc).¹⁸

BP and heart rate were measured during the exposures while a subject was seated within the chamber. At both sites, subjects wore the automated BP monitors on their left upper arm (Ann Arbor: Omron 780; Toronto: Oscar-1 or -2). Readings were measured at the start, at 30-minute intervals, and immediately after exposure completion while in the chamber. At both sites, subjects showed the readings obtained by the automated devices to the investigators but were blinded to the results. The average of the second and third BP and heart rate readings was used in Toronto (7 subjects had only a single reading, which was used). In Ann Arbor, we only performed

Table 1. Subject Characteristics

Parameter	Toronto Cohort (n=31)	Ann Arbor Cohort (n=50)
Demographics		
Age, y	27±8	27±8
Sex, female/male	15/16	31/19*
Body mass index, kg · m ⁻²	24±4	24±3
Blood laboratory tests		
Total cholesterol, mg · dL ⁻¹	169±28	166±35
LDL-C, mg · dL ⁻¹	107±26	90±31*
HDL-C, mg · dL ⁻¹	49±12	59±14*
Triglycerides, mg · dL ⁻¹	69±32	88±58*

All of the values are mean±SD. LDL-C indicates low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

**P*<0.05 for differences between sites.

a single BP reading at each time point during the exposure, and, therefore, only this single result was used in the analyses.

Holter monitoring was performed in Toronto with a SEER MC ambulatory digital recorder (GE Medical Systems). The 2-channel ECG data were recorded on an 8-Mb flash card and downloaded onto a MARS 8000 workstation (GE Medical Systems) to determine time and frequency domain heart rate variability (HRV).¹⁹ HRV measures were carried out by comparing a 10-minute epoch at the start of all of the exposures while seated within the chamber to one just before the end of the exposures.

Screening laboratory tests were analyzed for fasting lipoproteins and glucose. In Toronto, venous blood was collected before, after, and 24 hours after exposures for ET-1, cytokines, complete blood cell count, and high-sensitivity C-reactive protein. Blood samples were centrifuged and the resulting plasma (ET-1 and cytokines) and serum (C-reactive protein) stored at -70°C. Whole blood was used to make smears for complete blood cell count with an automated analyzer (Coulter, model LH755, Beckman Coulter Canada). A panel of 10 cytokines (see the online Data Supplement) that included TNF- α was analyzed using LiquiChip cytokine kits (Qiagen) and a Luminex analyzer (Luminex). C-reactive protein and lipids were analyzed using a Beckman Synchron LX analyzer (Beckman Coulter Canada). ET-1 was analyzed using a QuantiGlo ET-1 chemiluminescent immunoassay (R&D Systems) and Fluostar Optima microplate luminometer (BMG Labtechnologies).

Statistical Methods

The study was designed and powered to assess the differential effect of the 4 exposure types in Toronto, as well as the effect of the exposures across the 3 different pretreatments in Ann Arbor, on the outcomes of intrachamber diastolic BP slope and change in BAD and FMD. Other outcomes are secondary analyses. Data were analyzed using SAS version 9.1 (SAS Institute). To examine the exposure-induced change in outcomes, the postexposure minus pre-exposure difference (Δ) was calculated for each variable at each time point. Differences between the postexposure results versus the corresponding pre-exposure values for each outcome were tested by paired *t* tests. Differences in Δ responses in the outcomes at all of the time points were compared with the corresponding Δ responses across the 4 different exposure types (Toronto) and the 3 pretreatment limbs (Ann Arbor) by linear mixed-model analyses. For BP measured during exposure, linear regression was used to determine the slope (in millimeters of mercury per 30 minutes) of the line fitted over the 5 BP time point measures for each subject's exposure during the 2-hour period. The slope (β) or postexposure Δ was then used as a dependent variable in mixed models. Each model included a random subject effect with the fixed effects being CAP and/or ozone along with the CAP and ozone interaction. The effects of HRV and PM_{2.5} on diastolic BP change during exposure were examined using a mixed model that included the fixed effects: HRV exposure end

Table 2. PM_{2.5} Mass Concentration and Ozone Levels During Exposures in Toronto

Exposure Measure	Filtered Air	Ozone	CAP	CAP+Ozone
PM _{2.5} , $\mu\text{g}/\text{m}^3$ *	1.3±8.0	2.8±11.7	148.5±54.4	132.4±38.7
Ozone, ppb†	10.9±7.2	111.3±6.5	9.7±6.1	109.0±5.6

Values are mean±SD (n=31). PM_{2.5} indicates gravimetric measure (post-exposure–pre-exposure conditioned filter Δ weight) over 2-h exposure; ozone, Dasibi UV photometric ozone analyzer, mean of 15-s readings over 2-h exposure; ppb, parts per billion.

**P*<0.0001: FA vs CAP; O₃ vs CAP; FA vs CAP+O₃; and O₃ vs CAP+O₃ by ANOVA posthoc test (SAS Contrast statement).

†*P*<0.0001: FA vs O₃; CAP vs O₃; FA vs CAP+O₃; and CAP vs CAP+O₃ by ANOVA posthoc test (SAS Contrast statement).

minus start HRV (Δ), continuous CAP (integrated gravimetric exposure PM_{2.5} mass), ozone as dichotomous, and the CAP and ozone interaction. The effects of PM_{2.5} on FMD change during exposures were examined using a mixed model including the fixed effects: continuous CAP mass, ozone as dichotomous, and the CAP and ozone interaction. The Pearson correlation was used to test for bivariate associations between variables. Statistical significance is reported as *P*<0.05. Additional information regarding study power estimations and recruitment methods is provided in the online Data Supplement (please see the Supplemental Methods section).

Results

Subject characteristics are shown in Table 1. The PM_{2.5} mass and ozone concentrations were not different among the exposure limbs in Ann Arbor but significantly differed per design in Toronto (Tables 2 and 3).

Toronto Outcomes

FMD and NMD were not impaired immediately after exposures (Table 4). However, FMD significantly decreased 24 hours after CAP exposure compared with the baseline level (Table 4). Moreover, the change in FMD that occurred 24 hours after both exposures containing CAP (CAP and CAP plus ozone) was significantly different than the comparative change occurring after exposures without CAP (ozone and filtered air; Table 4). We defined this mixed-model analysis as a specific effect of "CAP-containing exposures" on outcomes (for other end points as well). The degree of decrease in FMD 24 hours after exposures was significantly associated only with the 2-hour integrated gravimetric PM_{2.5} mass concentration (pooled results for all 4 of the exposure conditions: β = -2.3% per 100 $\mu\text{g}/\text{m}^3$; *P* = 0.010; n = 117 by mixed-model analysis accounting for ozone). Higher concen-

Table 3. PM_{2.5} Mass Concentration and Ozone Levels During Exposures in Ann Arbor

Exposure Measure	Vitamin C	Bosentan	Placebo
PM _{2.5} , $\mu\text{g}/\text{m}^3$ *	133.2±48.7	142.6±51.6	126.9±55.0
Ozone, ppb†	122.5±5.5	122.3±2.3	122.0±2.8

Values are mean±SD. Posthoc tests: vitamin C vs placebo, *P* = 0.55; Bosentan vs placebo, *P* = 0.15; and vitamin C vs Bosentan, *P* = 0.36. PM_{2.5} indicates gravimetric measure (postexposure–pre-exposure conditioned filter Δ weight) over 2-h exposure; ppb, parts per billion.

*ANOVA (no group differences).

†ANOVA (no group differences).

Table 4. Vascular, Hemodynamic, and Blood Biomarker Results in Toronto (n=31)

Outcome	Filtered Air			Ozone Exposure			CAP Exposure			CAP+Ozone Exposure		
	Before	After	24 h After	Before	After	24 h After	Before	After	24 h After	Before	After	24 h After
Endothelial function												
BAD, mm	3.9±0.8	3.8±0.7†	3.8±0.8	3.8±0.7	3.8±0.8	3.7±0.7	3.9±0.8	3.8±0.8	3.9±0.8	3.8±0.7	3.7±0.7	3.7±0.7
FMD, %	7.2±4.8	8.6±5.8	9.9±7.1	11.5±6.5	9.6±7.8	10.5±6.9	8.7±5.7	8.5±7.8	5.8±5.3‡	10.7±6.5	9.7±7.0	8.8±4.2§
NMD, %	22±7	21±8	22±9	23±10	24±9	25±11	23±8	22±8	23±10	19±8	23±10‡	22±9
Blood laboratory tests												
Neutrophil, # · L ⁻⁹	3.2±1.0	3.3±0.9	3.1±1.2	3.2±1.2	3.4±1.1	3.3±1.1	3.0±1.1	3.4±1.1†	3.2±1.4	3.1±1.2	3.6±1.3*	3.2±1.2
WBC, # · L ⁻⁹	5.4±1.2	5.7±1.1	5.4±1.3	5.4±1.4	5.9±1.3†	5.5±1.3	5.2±1.4	5.9±1.4*	5.5±1.7	5.3±1.4	5.9±1.6*	5.4±1.3
log ₁₀ TNF-α, pg/mL	0.7±0.5	0.6±0.5	0.6±0.6	0.6±0.5	0.6±0.5	0.6±0.6	0.5±0.5	0.5±0.5	0.4±0.5	0.5±0.6	0.5±0.6	0.6±0.6
ET-1, pg/ml¶	0.8±0.8	1.2±1.1‡	0.9±1.0							0.9±1.0	1.1±1.1	0.7±0.9

WBC indicates white blood cell count. Values are mean±SD.

*P<0.001 vs pre-exposure value for same visit (paired t test).

†P<0.01 vs pre-exposure value for same visit (paired t test).

‡P<0.05 vs pre-exposure value for same visit (paired t test).

There were no differences in outcome Δ responses to exposure when compared across exposure types except for the following:

§P=0.016 for the comparison of the (24-h post-exposure–pre-exposure) Δ values between [(FAΔ+O₃Δ) vs (CAPΔ+(CAP+O₃)Δ)] in a linear mixed-model analysis. This is defined as an effect of CAP-containing exposures.

||P<0.05 for the comparison of the (post-exposure–pre-exposure) Δ values between [(FAΔ+O₃Δ) vs (CAPΔ+(CAP+O₃)Δ)] in a linear mixed-model analysis (effect of CAP-containing exposures).

¶n=24 subjects and 144 observations.

trations of CAP were, thus, associated with greater reductions in FMD. The changes in BAD (Table 4), BP, heart rate, and HRV measures (Tables S1 and S2) did not differ across the 4 different exposure conditions when measured at any time point outside the chamber.

WBC count and neutrophils increased immediately after CAP-containing exposures (Table 4). There was no significant differential change across exposure types in any other blood biomarker, including all of the cytokines and C-reactive protein (Table S3) or ET-1 (Table 4), except for a marginal effect on interleukin-4, likely attributed to chance. Interestingly, the log-normalized immediate postexposure change in TNF-α was significantly inversely correlated (r=−0.26; P=0.023) with the 24-hour postexposure reduction in FMD (all 4 of the exposures pooled: n=77 observations). This means that greater postexposure elevations in TNF-α were associated with larger reductions in FMD 24 hours later. There was no other significant association among the changes in all of the other blood biomarkers with BP or FMD.

Diastolic BP significantly linearly increased during CAP-containing exposures (Figure 1), resulting in a significant 2.9- and 3.6-mm Hg elevation after 2 hours for the CAP and CAP plus ozone exposures, respectively. Neither filtered air (FA) nor ozone caused a significant increase in diastolic BP (slope statistically not different from 0). The diastolic BP slope for the 2-hour period during all of the exposures pooled was associated in a linear mixed model analysis with reductions in several HRV components (Table 5) and with the 2-hour integrated PM_{2.5} gravimetric PM_{2.5} mass concentration (β=1.6 mm Hg per 100 μg/m³ of PM_{2.5}; P=0.01), even when accounting for the HRV changes. This means that greater increases in diastolic BP during exposure were associated with larger reductions in most HRV metrics (eg, SD of normal-to-normal intervals) and also with larger elevations in the PM mass level. Although systolic BP increased after CAP-containing exposures (2.3 to 3.5 mm Hg; P≤0.05) and

not after filtered air (1.5 mm Hg; P>0.2), this increase was not quite significant in the mixed model versus filtered air and ozone (P=0.23). Ozone exposure was not associated with changes in HRV or BP.

Ann Arbor Location Outcomes

BAD, arterial compliance, FMD, and NMD were not changed after exposures (Table 6). There was no significant differential change in any outcome in response to exposures at any time point (immediately and 24 hours after exposures) when compared across the 3 different pretreatment limbs (mixed-model analysis).

Throughout the exposures, diastolic BP linearly increased (between 2.5 and 4.0 mm Hg after 2 hours) to a statistically

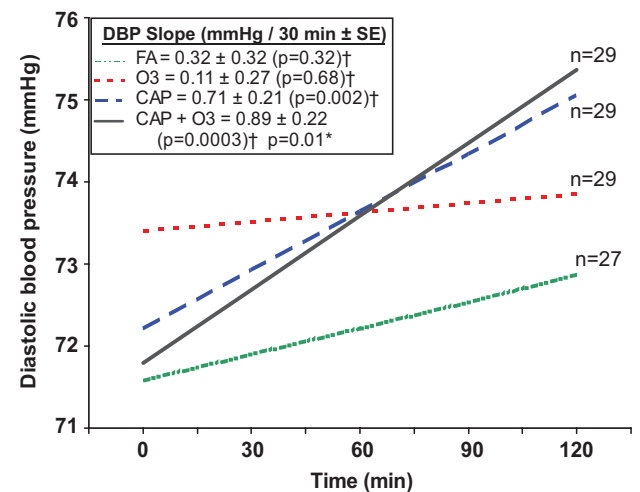


Figure 1. The diastolic BP changes occurring during exposures in Toronto. †Versus slope=0 (ie, no change from baseline diastolic BP). FA indicates filtered air; O₃, ozone; SE, standard error. *For “effect of CAP exposure”=slopes of [CAP+(CAP+O₃)] vs [O₃+FA].

Table 5. Associations Between HRV Measures and 2-h Change in Diastolic BP (per 1 mm Hg) During Exposures in Toronto

HRV Exposure End–Start Δ	HRV Measurement		
	β Estimate	95% CI	P
Frequency domain			
log ₁₀ LF, ms ²	−4.5	−8.3 to −0.7	0.0218
log ₁₀ HF, ms ²	−5.2	−8.4 to −1.9	0.0022
log ₁₀ LF/HF ratio	1.9	−1.6 to 5.5	0.2782
Time domain			
SDNN, ms	−0.09	−0.14 to −0.04	0.0006
rMSSD, ms	−0.07	−0.12 to −0.02	0.0043
Heart rate, bpm	0.01	−0.22 to 0.24	0.9216

All of the models included all 4 exposures (97 observations), a random intercept for subject, and the PM_{2.5} mass concentration during exposure. The original models included ozone (dichotomous) and the interaction of ozone*PM_{2.5} concentration, but neither were significant, and thus were removed from the final models. Only 27 subjects had HRV data and were included in these analyses. PM_{2.5} mass concentration indicates integrated gravimetric measure of PM_{2.5} in CAP airstream delivered to subject over 2-h exposure; LF, low frequency; HF, high frequency; SDNN, SD of all normal RR intervals; rMSSD, root mean square successive difference in heart rate interval.

comparable degree in each pretreatment limb (Figure 2). Although there was a slight blunting of the response, the slope of the diastolic BP increase was not statistically different in the Bosentan limb compared with the other 2 pretreatments limbs. Heart rate (slope: bpm per 30 minutes) significantly increased to a statistically similar degree ($P>0.5$ by linear mixed-model analysis) during all of the exposure limbs (placebo: 1.05 ± 0.24 , $P=0.001$; Bosentan: 0.78 ± 0.27 , $P=0.003$; vitamin C: 0.99 ± 0.27 , $P=0.001$). Systolic BP did not significantly increase.

Table 6. Vascular, Hemodynamic, and Blood Biomarker Results in Ann Arbor (n=50)

Outcome	Placebo Visit			Vitamin C Visit			Bosentan Visit		
	Pre-Exposure	Post-Exposure	24 h Post-Exposure	Pre-Exposure	Post-Exposure	24 h Post-Exposure	Pre-Exposure	Post-Exposure	24 h Post-Exposure
Endothelial function									
BAD, mm	3.7±0.8	3.7±0.7	3.7±0.7	3.7±0.7	3.7±0.7	3.8±0.7	3.7±0.7	3.7±0.7	3.7±0.7
FMD, %	5.6±4.1	6.8±5.9	6.6±4.7	5.0±5.9	5.6±7.9	7.4±5.5‡	6.4±6.7	4.4±8.4	6.3±6.5
NMD, %	17±7	18±8	18±7	19±8	19±8	20±7	21±9	19±7	19±8
Hemodynamics									
Cardiac output, L · min ^{−1}	5.8±0.9	5.6±0.8	5.8±0.9	5.9±0.8	5.6±0.8‡	5.9±0.8	5.7±0.8	5.7±0.7	5.9±0.9
SVR, dynes · s · cm ^{−5}	1188±314	1155±261	1172±333	1137±170	1114±169‡	1129±335‡	1169±248	1114±169	1129±335
C1, 10 · mL · mm Hg ^{−1}	19.0±6.6	26.1±38.1	19.0±8.0	18.1±7.2	19.9±6.1	18.1±5.4	18.1±5.4	19.9±6.1	18.1±5.4
C2, 100 · mL · mm Hg ^{−1}	9.0±2.7	10.8±11.7	8.8±3.2	9.4±3.1	8.8±2.6	9.1±2.5	9.0±2.7	8.8±2.6	11.4±14.9
Ambulatory monitoring									
SBP, mm Hg	117±7	...	115±7*	116±7	...	113±8*	116±9	...	113±8*
DBP, mm Hg	69±6	...	68±5‡	68±6	...	67±6‡	69±7	...	66±6*
HR, beats · min ^{−1}	71±9	...	71±11	72±7	...	72±10	71±9	...	74±10*§

SVR indicates systemic vascular resistance; C1, large vessel compliance; C2, small vessel compliance; SBP, systolic BP; DBP, diastolic BP; HR, heart rate. Values are mean±SD. ... indicates no data.

* $P<0.001$ vs pre-exposure value for same visit (paired *t* test).

‡ $P<0.01$ vs pre-exposure value for same visit (paired *t* test).

‡‡ $P<0.05$ vs pre-exposure value for same visit (paired *t* test).

§ $P<0.05$ for bosentan (post-exposure–pre-exposure HR) vs placebo (post-exposure–pre-exposure HR; mixed-model analysis).

Discussion

This study provides several novel insights into the acute CV effects of air pollution exposure. First, PM_{2.5}, not ozone, was responsible for raising diastolic BP and only transiently during the actual period of inhalation. Second, the results implicate ANS imbalance as the most plausible mechanism underlying this prohypertensive response. Third, PM_{2.5}, not ozone, can additionally impair endothelial function 24 hours after exposure. However, certain aspects of exposure location are major determinant of this response, because it occurred only in downtown Toronto. Fourth, the endothelial dysfunction was triggered via a slower biological pathway, the most likely being systemic inflammation.

Responsible Air Pollutant and the Importance of Exposure Location

This is the first controlled human exposure study to investigate the effect of ozone on the vasculature, moreover to compare the responses to those induced by PM_{2.5}. The results provide clear evidence that it is PM_{2.5}, not ozone, that causes both the endothelial dysfunction (Toronto) and prohypertensive response (both locations) within minutes to hours of exposure.^{9–11} Ozone did not cause any adverse CV effects by itself, nor did it augment those induced by PM_{2.5}. Furthermore, higher levels of PM_{2.5} exposure were associated with larger elevations in diastolic BP and greater reductions in FMD, supporting the linear dose-response relationship between exposure and CV risk.^{1,2}

Our findings support the concept that aspects of exposure location (eg, PM_{2.5} composition or sources) critically influence the breadth of CV responses. Particle exposures caused a similar diastolic BP elevation at both sites. On the other hand, only the fine particles from downtown Toronto, which are heavily influenced by local traffic, were capable of

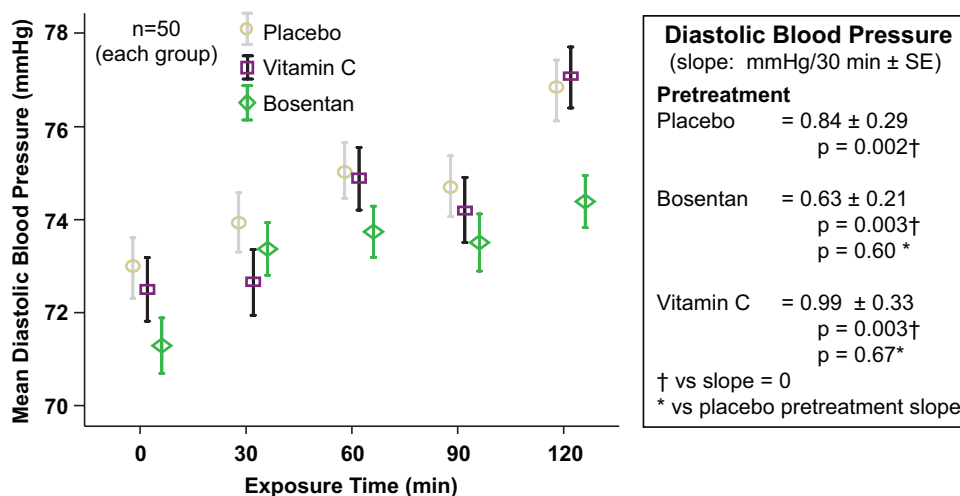


Figure 2. The diastolic BP increases occurring during exposures in Ann Arbor. FA, filtered air; O₃, ozone; DBP, diastolic BP; SE, standard error.

additionally triggering endothelial dysfunction despite the fact that mass concentrations were nearly identical between sites. Congruent with our findings, particles in Northern Scotland (low in combustion-derived compounds) were shown to have a neutral effect,²⁰ whereas diesel exhaust (high in combustion particles and gases) was capable of triggering endothelial dysfunction.¹¹

At present we can only speculate about the specific particle constituents or sources accountable for the differential responses between locations, although we can rule out a role played by gases or ozone. The exposure inlet in Toronto faces a downtown urban roadway experiencing 30 000 vehicles per day.²¹ The site is surrounded by a dense road network for several kilometers in all directions, and exposures were conducted in the morning when the impact of fresh rush-hour emissions on particle mass and composition was expected to be important.²² In contrast, Ann Arbor is a smaller urban area with much less traffic density in the vicinity of the north campus location. The region surrounding the exposure site quickly becomes rural in the prevailing wind directions, and, thus, the fine particles used in the experiments were more likely derived from long-range transport of background-aged aerosols.²³ Future analyses of the filters collected during exposures will provide more specific information regarding the particulate constituents and sources responsible. Nonetheless, the present findings provide compelling evidence that PM_{2.5} derived from different locations poses varying degrees of risk to the CV system.^{1,2}

Biological Mechanisms

Our findings implicate acute ANS imbalance as the most plausible mechanism for the prohypertensive response. It initiated and terminated rapidly and was associated with an increase in heart rate. The magnitude of diastolic BP elevation during exposures was also most strongly associated with decreases in HRV markers of parasympathetic ANS withdrawal (eg, SD of normal-to-normal intervals; Table 5).^{19,24} This hypothesis is supported by the fact that human airways are lined with receptors and nerve endings that, after stimulation by inhaled PM_{2.5}, may be capable of altering reflex

ANS pathways leading to a blunting of CV parasympathetic tone and a relative favoring of sympathetic activity.²⁴ Indeed, a recent study with dogs has corroborated that diastolic BP acutely increases during 5-hour-long CAP inhalation. This response was mitigated by α -adrenergic receptor antagonism, thus further supporting the central mechanistic importance of the ANS.²⁵ On the other hand, the later-onset elevation in BP (eg, occurring 1 to 5 days postexposure) observed in epidemiological studies is likely elicited by different slower mechanistic pathways (eg, inflammation-induced vascular dysfunction) unrelated to the acute ANS imbalance that increases BP within minutes-to-hours.

Although we had alternatively hypothesized that PM_{2.5} could raise BP attributed to a systemic oxidative stress-mediated impairment in basal NO-dependent bioavailability (favoring vasoconstriction),^{9–11} the BP change was unrelated to inflammatory biomarkers (Toronto) and not mitigated by a pretreatment dosage of vitamin C (Ann Arbor) previously shown capable of acutely obviating tobacco smoke-induced oxidative stress and endothelial dysfunction.²⁶ Moreover, the time courses of the endothelial dysfunction and BP elevation were discordant in Toronto. Diastolic BP also increased in Ann Arbor without any change in FMD or arterial compliance. Therefore, it is not likely that the later-onset endothelial dysfunction was mechanistically responsible for the earlier BP increase or that oxidative stress was centrally involved. It has also been speculated that increased ET-1 bioactivity could be responsible.²⁷ However, the nonsignificant changes in plasma ET-1 (Toronto) were unrelated to the BP elevation. Moreover, the relatively large pretreatment dose of Bosentan (Ann Arbor) effectively lowered pre-exposure diastolic BP, suggesting that it achieved physiologically relevant ET-A and -B receptor blockade that should have significantly attenuated any hemodynamic actions of CAP exposure if they had occurred via this pathway. Finally, ET-mediated vasoconstriction is typically of slower onset and of more prolonged duration than the corresponding BP changes observed.²⁸

The mechanisms responsible for the endothelial dysfunction after the CAP-containing exposures in Toronto are less

clear. However, our findings could most plausibly be interpreted to mean that the dysfunction was triggered by a systemic proinflammatory cascade. It had the expected slower onset of occurrence than responses induced by the ANS. Moreover, the decrease in FMD was associated with an increase in postexposure TNF- α levels. Epidemiological and animal studies support this hypothesis in that PM_{2.5} exposure can cause an increase in a variety of inflammatory cytokines/mediators within the circulation and vasculature.^{29,30} Although C-reactive protein and other measured blood cytokines remained largely unchanged by PM_{2.5}, white blood cell count and neutrophils (also blunt markers of inflammation) increased after the CAP-containing exposures that provoked endothelial dysfunction. It is possible that unmeasured proinflammatory factors derived from circulating leukocytes (eg, myeloperoxidase, as demonstrated elsewhere),^{31,32} the increase in TNF- α itself, or other facets of inflammation that we did not measure (or accurately quantify by the cytokine tests) were responsible for impairing FMD.³³ Nevertheless, our results corroborate that ambient PM_{2.5} exposure, at least in some urban locations, is capable of triggering vascular endothelial dysfunction within a day.^{11,32}

Clinical Implications

It is important to highlight in the context of short-term and rather low-dose exposures performed on healthy humans that the adverse, yet modest, CV responses observed in this study are actually at the very least on par with past reports.^{1,2,9,11,32} Nevertheless, the observed small degree of diastolic BP elevation and endothelial dysfunction pose little risk to healthy people. However, both are plausible instigators of ischemic events in susceptible individuals by triggering pre-existing atherosclerotic plaque instability and/or by impairing myocardial perfusion.⁸ The BP increase could also help explain the associations between PM_{2.5} with strokes and heart failure exacerbations.^{1,2} Moreover, these responses could very conceivably occur in an exaggerated manner in patients with CV risk factors or disease who have a pre-existing ANS imbalance or endothelial dysfunction that renders counterbalancing mechanisms less effectual.¹² For example, subjects with hypertension have been shown to have a greater increase in BP in response to ambient PM_{2.5} exposures than normotensives.³⁴ Diabetics are at greater risk for air pollution-mediated endothelial dysfunction than healthy patients.³⁵ These findings agree with observations that PM_{2.5} poses much greater acute risks to vulnerable individuals with pre-existing heart disease.^{1,2} Together with our present results, the evidence supports that even shorter-term PM_{2.5} averages (eg, hourly levels) than are currently regulated may need to be minimized to optimally protect vulnerable individuals from an acute CV event.^{15,36}

Strengths and Limitations

This study was the largest human-controlled air pollution exposure protocol yet completed (274 total exposures).^{9,11} Our well-coordinated design and uniform methods between 2 sites provided us the unique ability to compare CV responses with air pollution from different locations.

It is unclear why we did not replicate previous findings of brachial artery vasoconstriction after CAP plus ozone exposures.⁹ We either failed to identify its occurrence, or this vascular territory behaved in a discordant manner between experiments. It is also uncertain why exposures elicited a greater effect on diastolic rather than systolic BP. Additional studies are required, but perhaps the underlying hemodynamic changes responsible reflected a predominant vasoconstriction without changes in cardiac output or arterial compliance (as suggested by the results in Table 6).

We also limited blood biomarker interpretation to the Toronto results because this was where we could account for any filtered air (placebo) effect on these secondary outcomes. The Ann Arbor site did not have a filtered air control; therefore, the blood biomarkers were not analyzed after it was apparent from the results in the Toronto study that controlling for filtered air responses was essential for proper data interpretation. It is unclear why we did not observe the expected decreases in HRV after CAP-containing exposures,^{19,24} despite the fact that reductions in HRV during all of the exposures pooled together were associated with the diastolic BP elevation. Perhaps the sample size was inadequate for discerning dichotomous HRV differences because the study was not powered for this outcome, or the young subjects were less susceptible than studies showing such changes in elderly patients. Future studies that investigate the effect of exposures on baroreflex sensitivity and/or direct microneurography measurement of sympathetic activity are warranted to help further corroborate our findings. A lack of adequate sample size and the very healthy nature of our subjects may also explain why there were no observed increases in cytokines after CAP exposures, although the increase in TNF- α correlated with a reduction in FMD in the pooled analysis. Although the PM_{2.5} concentration was higher than that typically observed over 24 hours, levels >150 $\mu\text{g}/\text{m}^3$ can occur for 1- to 2-hour periods in many North American locations and are commonly encountered over even longer durations throughout developing nations.³⁷

It is a possible limitation that gaseous pollutants (eg, CO, nitrogen oxides, and sulfur oxides) were not measured during all of the exposures. However, these gases, along with any volatile vapor phase pollutants, are at (or below) ambient levels in the concentrator system.^{9,10,13} As such, per our study design, the concentrations of these pollutants were expected to be at similar and ambient levels during all of the exposures at both sites. They were, thus, not likely to differ between exposures to any meaningful degree, nor are they, therefore, likely to explain any of the responses. Although it is not impossible that these other pollutants (eg, gases or vapor phase compounds) could have played a small role, the sum of our findings (particularly the independent statistical associations of BP and FMD with PM_{2.5} mass) along the concentrator systems used makes it much more likely that the fine particles were principally responsible for the observed results.

There were small differences in triglycerides, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and the female:male ratio of subjects between sites (Table 1). However, the values were all well within the normal range, and all of the subjects were healthy without overt CV disease,

risk factors, and/or any parameter known to alter the risk of air pollution exposure.^{1,2} At both sites, these parameters were not associated with the FMD responses to exposures. In addition, even overt hyperlipidemia has not been shown to be a risk-effect modifier to air pollution exposure.^{1,2} Therefore, we do not believe that these small differences account for the observed FMD response differences between sites.

Conclusions

Our findings provide plausible mechanisms whereby even brief contact with high levels of real-world ambient PM_{2.5} can promote acute CV events. They also illustrate that aspects of exposure location and, hence, particle source and/or composition can play a critical role in determining the breadth of the adverse reactions. We believe it important to examine whether shorter (eg, hourly) time frames of ambient PM_{2.5} reductions beyond existing 24-hour-long hour standards can have a positive impact on the health of vulnerable populations.³⁴

Perspectives

PM_{2.5} air pollution is a leading cause of worldwide mortality. Exposure to ozone is also associated with an increased risk of premature death. We demonstrated that PM_{2.5} exposure, not ozone, causes a significant increase in diastolic BP and impairs endothelial function, but only from particles derived from an urban environment. The results implicate changes in ANS activity as the cause for the acute increase in BP, whereas the slower impairment in vascular function was likely attributable to systemic inflammatory responses. These are important new insights into the mechanisms whereby air pollution imparts biological harm. The findings confirm that even transient contact with relevant concentrations of PM_{2.5} can rapidly instigate physiological responses potentially capable of triggering acute CV events in susceptible individuals. In addition to bolstering the veracity of the epidemiological associations linking air pollution with excess CV-related mortality, the findings suggest that the characteristics of the particles are likely to be important determinants of the health consequences after exposure. Overall, these study results further strengthen the justification for environmental regulations on ambient PM_{2.5}.

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Disclosures

None.

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