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Effects of short-term increases in personal and ambient pollutant concentrations on pulmonary and cardiovascular function: A panel study analysis of the Multicenter Ozone Study in oldEr subjects (MOSES 2)

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ABSTRACT

Background: The cardiovascular effects of ozone exposure are unclear. Using measurements from the 87 participants in the Multicenter Ozone Study of oldEr Subjects (MOSES), we examined whether personal and ambient pollutant exposures before the controlled exposure sessions would be associated with adverse changes in pulmonary and cardiovascular function.

Methods: We used mixed effects linear regression to evaluate associations between increased personal exposures and ambient pollutant concentrations in the 96 h before the pre-exposure visit, and 1) biomarkers measured at pre-exposure, and 2) changes in biomarkers from pre-to post-exposure.

Results: Decreases in pre-exposure forced expiratory volume in 1 s (FEV_1) were associated with interquartile-range increases in concentrations of particulate matter ≤2.5 μm ($PM_{2.5}$) 1 h before the pre-exposure visit (−0.022 L; 95% CI -0.037 to −0.006; p = 0.007), carbon monoxide (CO) in the prior 3 h (−0.046 L; 95% CI -0.076 to −0.016; p = 0.003), and nitrogen dioxide (NO_2) in the prior 72 h (−0.030 L; 95% CI -0.052 to −0.008; p = 0.007). From pre-to post-exposure, increases in FEV_1 were marginally significantly associated with increases in personal ozone exposure (0.010 L; 95% CI 0.004 to 0.026; p = 0.010), and ambient $PM_{2.5}$ and CO at all lag times. Ambient ozone concentrations in the prior 96 h were associated with both decreased pre-exposure high frequency (HF) heart rate variability (HRV) and increases in HF HRV from pre-to post-exposure.

Conclusions: We observed associations between increased ambient $PM_{2.5}$, NO_2, and CO levels and reduced pulmonary function, and increased ambient ozone concentrations and reduced HRV. Pulmonary function and HRV increased across the exposure sessions in association with these same pollutant increases, suggesting a "recovery" during the exposure sessions. These findings support an association between short term increases in ambient $PM_{2.5}$, NO_2, and CO and decreased pulmonary function, and increased ambient ozone and decreased HRV.

1. Introduction

Air pollution exposure increases the risk of both cardiovascular (CV) and respiratory events (Abed Al Ahad et al., 2020; Schraufnagel et al., 2019), and particulate matter (PM) exposure has been the most strongly linked with increased CV morbidity and mortality. However, questions remain as to the pollutants most responsible for adverse health effects, effects at concentrations below current air quality standards, the populations most at risk, exposure-response time frames, and the mechanisms involved. For example, some observational studies link ozone exposure with increased mortality from cardiovascular causes, and yet human controlled exposure studies have not consistently shown acute cardiovascular effects, even at concentrations considerably higher than ambient (U.S. EPA, 2020).

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In addition, there is limited evidence that reductions in exposure, especially in communities with already low ambient levels of air pollutants, improve health (Burns et al., 2020). This is an important consideration in supporting causality in observational epidemiological studies of air pollution health effects, and in setting air quality standards.

We recently published findings from the Multicenter Ozone Study of oldEr Subjects (MOSES), designed to evaluate whether 3-h controlled exposures, with intermittent exercise, of older (55–70 years of age), healthy individuals to near-ambient levels of ozone (120 ppb and 70 ppb), induced acute changes in cardiovascular and pulmonary biomarkers compared to filtered air with 0 ppb ozone (Arjomandi et al., 2018; Balmes et al., 2019; Rich et al., 2018). The study was conducted at three sites, using a common protocol and core laboratories for many of the measurements. It consisted of three randomized exposure sessions for each subject, with each session involving repeated measurements of biomarkers of respiratory and cardiovascular function, beginning the day before the experimental exposures, and ending the day after exposures. Subjects were housed in relative protection from ambient pollutant exposures for most of the approximately 46 h of each study session.

In general, we found an absence of acute cardiovascular responses to the controlled ozone exposures (Rich et al., 2018). These included measures of autonomic function, repolarization, ST segment change, arrhythmia, systemic inflammation, vascular function, oxidative stress, and prothrombotic vascular status. We did find evidence for relative reductions in pulmonary function and increases in airway injury and inflammation in response to the experimental ozone exposures. The forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) increased after the 3-h exposure to ozone-free filtered air. These increases were attenuated in a dose–response manner by exposure to 70 and 120 ppb ozone. We also observed a significant ozone-induced increase in the percentage of sputum polymorphonuclear leukocytes 22 h after exposure at 120 ppb compared to 0 ppb exposure ($p = 0.003$). Plasma club-cell 16 (CC16), a marker of airway epithelial injury, also increased significantly after exposure to 120 ppb ozone ($p < 0.001$). A complete description of MOSES including its protocol and findings are provided in the MOSES 1 Final Report (Frampton et al., 2017) and elsewhere (Arjomandi et al., 2018; Balmes et al., 2019; Rich et al., 2018).

In the current study, we used the MOSES biomarker data, analyzed independent of the controlled ozone exposures, to test the following two hypotheses: 1) increases in ambient and personal ozone (and other pollutant) exposures in the 96 h before the controlled exposure sessions would be associated with adverse changes in cardiovascular and pulmonary function assessed prior to the controlled exposures; and 2) these increases in prior pollutant exposures would be associated with further adverse changes when assessed after the exposure sessions, independent of the controlled ozone exposures. The biomarkers assessed changes in pulmonary function, airway inflammation, lung injury, heart rate variability, cardiac repolarization, cardiac ST-segments, blood pressure, flow mediated dilation, oxidative stress, systemic inflammation, and markers of thrombosis.

2. Methods

2.1. Study population

MOSES was conducted in three centers: the University of Rochester Medical Center (URMC; n = 32), the University of North Carolina (UNC; n = 29), and the University of California, San Francisco (UCSF; n = 26). The study was approved by institutional review boards at each center, and by the U.S. EPA Human Subjects Research Review Official. Written informed consent was obtained from all subjects. Details on subject inclusion and exclusion criteria and the study protocol were published previously (Frampton et al., 2017). Participants were healthy lifetime nonsmokers 55–70 years of age with normal spirometry and screening electrocardiogram (ECG), able to complete a training exercise session at the target minute ventilation ($\dot{V}_E$) of 15–17 L/min/m^2 body surface area (BSA) without the heart rate exceeding 80% of predicted maximum, and without arrhythmias or ST depression on cardiac monitoring during exercise.

2.2. MOSES protocol

The MOSES study was a randomized, crossover study of exposure to 0, 70, and 120 ppb ozone in filtered air for 3 h, with intermittent exercise, in an environmental chamber. All subjects and study personnel, except the technician controlling the exposure, were blinded to the nature of the exposure. The day before exposure, vital signs were measured, brachial artery ultrasound (BAU) was performed, and a 30 mL venous blood sample was collected. A box dinner was provided, after which each subject spent the night in a nearby, non-smoking hotel room. The next morning after a light breakfast, blood pressure (BP) and other vital signs were measured, Holter monitor leads were attached and resting ECG was recorded, and spirometry was performed. The Holter recording then continued for 24 h. Exposure (alternating 15 min of exercise and rest at the workload determined at the training session) started between 8:00 and 8:45am. $\dot{V}_E$ was measured intermittently during exercise with workload adjusted as needed to maintain $\dot{V}_E$ in the targeted range. Resting, sitting BP was measured 5 min before the third and fifth exercise periods.

Approximately 15 min after exposure ended, vital signs, ECG, and spirometry were recorded, and a low-fat lunch was provided. Three hours after the end of the exposure, ECG recordings were made, vital signs were measured, venous blood was collected, and BAU was performed. Subjects returned home at 4:00–4:30 p.m. wearing the Holter monitor. They returned at ~8:00 a.m. the next morning, for the following procedures: vital signs, ECG recordings (removing Holter leads when done), venous blood sampling, spirometry, and sputum induction.

2.3. Outcomes

The primary and secondary outcomes for each outcome group (i.e., pulmonary function, lung injury, airway inflammation, heart rate variability (HRV), repolarization, ST segment change, vascular function, systemic inflammation, systemic oxidative stress, and prothrombotic vascular state) are shown in Table 1. The laboratory analysis methods of all study outcomes/biomarkers have been described previously (Arjomandi et al., 2018; Balmes et al., 2019; Frampton et al., 2017; Rich et al., 2018).

2.4. Personal ozone and NO_2 exposure sampler

Personal exposures to ozone and NO_2, during the ~72 h preceding the pre-exposure visit, were measured as described previously (Rich et al., 2020), using passive personal exposure samplers (PES) from Ogawa & Company (Pompano Beach, FL). At the end of the training session and each of the first two post-exposure visits, the subject was given a PES with written instructions to store in the refrigerator and start wearing it at noon of the third day before the pre-exposure visit. Subjects were also reminded by telephone 3–4 days prior to each exposure visit. At arrival for each exposure session, the PES was collected and disassembled, and the filters were shipped in batches for analysis, with appropriate blanks. The detection limits were 0.77 ppb and 0.9 ppb for NO_2 and ozone, respectively.

2.5. Ambient air pollutant and weather measurements

Hourly averages of temperature, relative humidity, ozone, $PM_{2.5}$,

Table 1
Primary and secondary outcomes for each outcome group.

Outcome Group	Primary Endpoints	Secondary Endpoints
Pulmonary Function	Forced Expiratory Volume in 1 Second (FEV_1) Forced Vital Capacity (FVC)	–
Lung Injury	Club Cell protein (CC16)	–
Airway Inflammation	Sputum polymorphonuclear leukocytes as % of total non-epithelial cells (PMN%)	–
Heart Rate Variability	High frequency power: 0.15–0.40 Hz (HF) - 5 min average	Standard deviation of the NN intervals (SDNN) - 5 min average
	Low frequency power: 0.024–0.15 Hz (LF) – 5 min average	RMSSD - 5 min average
	Root mean square of successive differences in the NN intervals (RMSSD) – 24 h average	LF/HF Ratio – 5 min average
Repolarization	T-wave amplitude - 5 min average T-wave amplitude – 24 h average	QTc interval - 5 min average
ST-Segment Change	ST in lead V5 – 5 min average ST in lead V5 – 24 h average	ST in lead II - 5 min average ST in lead II – 24 h average ST in lead V2 – 5 min average ST in lead V2 – 24 h average
Vascular Function	Systolic Blood pressure (SBP) Flow-mediated dilation (FMD)	Diastolic Blood Pressure (DBP) von Willebrand Factor (vWF) Endothelin-1 (ET-1) Reactive hyperemic velocity-time integral (VTI) Brachial Artery Diameter (BAD)
Systemic Inflammation	C-reactive protein (CRP)	Fibrinogen P-selectin Interleukin-6 (IL-6)
Systemic Oxidative Stress	Nitrotyrosine	–
Prothrombotic Vascular State	Monocyte platelet conjugate count	–

NO_2, SO_2, and CO levels were obtained from monitoring stations near each of the three sites (Rich et al., 2020). Each monitoring site adhered to protocols and quality assurance procedures required for sites operated by state and local air monitoring agencies. We calculated mean concentrations at lag hours 0, 0–2, 0–11, 0–23, 0–47, 0–71, and 0–95, for use in the statistical analyses described below.

2.6. Statistical analysis

For each outcome separately, we examined whether or not to use its logarithmic transformation in our statistical analysis models. The decision was based on plots of residuals versus predicted values from the models described below, separately for each untransformed and log-transformed biomarker, and plots of the outcome at each post-exposure time versus the same outcome at the pre-exposure visit, separately for both the untransformed and log-transformed scales. These plots allowed us to assess whether the residual variance was more constant with or without the log transformation. For variables that were log-transformed, the "pre-to post-exposure change in biomarker" was defined as the difference in the log-transformed pre- and post-exposure values.

First, we used a panel study design with three (pre-exposure) biomarker measurements per subject (one prior to each controlled ozone exposure). Using a model similar to our previous MOSES analyses, we regressed the pre-exposure biomarker concentration for each subject visit as a linear function of the PES ozone concentration in the 72 h before the pre-exposure visit, temperature and relative humidity each averaged over the same 72 h using natural splines, day of the week, season, study site, and study time (1st, 2nd, or 3rd exposure). Study subject was treated as a random effect to account for correlation between multiple measurements of the biomarker from the same subject. From this model, we estimated the change in a pre-exposure biomarker associated with the PES ozone concentration. We then refit this model replacing the 72-h personal ozone exposure with the 72-h PES NO_2 concentration, and then with averages of ambient ozone, $PM_{2.5}$, NO_2, CO, and SO_2 concentrations, temperature, and relative humidity in the 1, 3, 12, 24, 48, 72, and 96 h before the pre-exposure visit.

Second, again using a panel study design (three exposures per subject) and the same linear mixed effects models as in our previous MOSES analyses, we regressed the pre-to post-exposure biomarker change for each subject exposure visit as a linear function of two indicator variables for controlled ozone concentration (120 ppb, 70 ppb, 0 ppb), PES ozone concentration in the 72 h before the pre-exposure visit, temperature and relative humidity averaged over the same 72 h using natural splines, day of the week, season, study site, and study time. Again, study subject was treated as a random effect to account for correlation between multiple measurements of the biomarker change from the same subject. From this model, we separately estimated the pre-to post-exposure change in a biomarker associated with the PES ozone concentration, independent of the controlled MOSES ozone exposures. We then refit this model replacing the 72-h personal ozone exposure with the 72-h PES NO_2 concentration, and then with averages of ambient ozone, $PM_{2.5}$, NO_2, CO, and SO_2 concentrations, temperature, and relative humidity in the 1, 3, 12, 24, 48, and 96 h before the pre-exposure visit. We did not include the controlled ozone exposures in this model because the randomized design precluded any correlation between these controlled exposures and the prior ambient air pollutant concentrations, thus avoiding bias or confounding in this regard.

We focused initially on the primary outcomes in each outcome group. If increased PES or ambient pollutant concentrations were associated with significant changes in a primary outcome, findings for related secondary outcomes were assessed in the same manner. As in our previous MOSES analyses, $p < 0.01$ defined statistical significance, and $0.01 \leq p < 0.05$ marginal significance, in order to adjust for multiple comparisons.

3. Results

Characteristics of MOSES subjects have been described previously (Arjomandi et al., 2018; Balmes et al., 2019; Frampton et al., 2017; Rich et al., 2018). Briefly, MOSES subjects were predominantly female (60%) and white (88%), with a mean (±SD) age of 59.9 ± 4.5 years, and were generally similar across sites. However, at UNC, subjects were either African American (14%) or white (86%), while at UCSF, subjects were Asian (8%), white (88%), or Hawaiian (4%), and at URMC they were African American (3%), white (87%), or of unknown race (7%). Diastolic blood pressure was higher at UNC (mean ± SD, 76.1 ± 7.8) than UCSF (73.7 ± 10.7) and URMC (69.0 ± 7.5). There were no site differences in systolic blood pressure. Distributions of ambient and PES pollutant concentrations are shown in Supplement Tables 1 and 2, and their correlations are shown in Supplement Table 3.

3.1. Pulmonary function

3.1.1. Hypothesis 1

Pre-exposure FEV_1 decreased significantly in association with interquartile-range (IQR) increases in concentrations of $PM_{2.5}$ in the 1 h before the pre-exposure visit (−0.022 L; 95% CI -0.037 to −0.006; $p = 0.007$), CO in the 3 h before the pre-exposure visit (−0.046 L; 95% CI -0.076 to −0.016; $p = 0.003$), and NO_2 in the 72 h before the pre-exposure visit (−0.030 L; 95% CI -0.052 to −0.008; $p = 0.007$)

Table 2
Change in pre-exposure FEV_1 associated with each interquartile range (IQR) increase in PES and ambient air pollutant concentration, by lag hours.

Pollutant	Lag Hours	IQR	N	Change in FEV_1 (L)	95% Confidence Interval	p-value
PES O_3 (ppb)	0–71	4.1	228	−0.001	−0.022, 0.020	0.920
PES NO_2 (ppb)	0–71	9.3	228	−0.002	−0.018, 0.014	0.798
Ambient O_3 (ppb)	0	15.2	210	0.012	−0.016, 0.039	0.410
	0–2	15.2	199	0.011	−0.017, 0.039	0.433
	0–11	16.1	215	0.003	−0.024, 0.029	0.841
	0–23	13.7	217	0.003	−0.022, 0.029	0.793
	0–47	11.3	218	0.010	−0.015, 0.035	0.430
	0–71	11.0	217	0.021	−0.006, 0.048	0.121
	0–95	10.3	218	0.026	−0.002, 0.055	0.069
Ambient $PM_{2.5}$ ($\mu g/m^3$)	**0**	**5.9**	**207**	**−0.022**	**−0.037, -0.006**	**0.007**
	0–2	5.8	201	−0.022	−0.039, −0.005	0.010
	0–11	5.4	209	−0.020	−0.037, −0.003	0.020
	0–23	4.9	211	−0.021	−0.041, −0.002	0.031
	0–47	4.6	211	−0.023	−0.043, −0.002	0.030
	0–71	4.7	212	−0.022	−0.045, 0.000	0.053
	0–95	4.3	211	−0.017	−0.039, 0.005	0.119
Ambient CO (ppm)	**0**	**0.126**	**210**	**−0.040**	**−0.068, -0.012**	**0.006**
	0–2	**0.143**	**206**	**−0.046**	**−0.076, -0.016**	**0.003**
	0–11	0.144	212	−0.019	−0.041, 0.003	0.086
	0–23	0.129	213	−0.027	−0.052, −0.002	0.035
	0–47	0.109	213	−0.029	−0.053, −0.005	0.017
	0–71	0.106	213	−0.027	−0.052, −0.002	0.031
	0–95	**0.108**	**212**	**−0.029**	**−0.056, -0.002**	**0.034**
Ambient NO_2 (ppb)	0	4.6	201	−0.009	−0.022, 0.004	0.189
	0–2	5.5	197	−0.016	−0.035, 0.003	0.094
	0–11	7.9	204	−0.003	−0.024, 0.017	0.746
	0–23	6.1	206	−0.010	−0.032, 0.011	0.332
	0–47	5.1	206	−0.018	−0.038, 0.002	0.078
	0–71	**5.2**	**205**	**−0.030**	**−0.052, -0.008**	**0.007**
	0–95	4.2	204	−0.024	−0.043, −0.005	0.014
Ambient SO_2 (ppb)	0	0.8	148	0.001	−0.013, 0.016	0.870
	0–2	0.9	147	−0.004	−0.016, 0.008	0.534
	0–11	0.8	149	0.003	−0.014, 0.019	0.737
	0–23	0.9	151	−0.003	−0.022, 0.017	0.775
	0–47	0.9	152	−0.004	−0.026, 0.019	0.746
	0–71	0.9	152	−0.006	−0.038, 0.025	0.679
	0–95	0.9	151	−0.001	−0.035, 0.032	0.936

(Table 2, Fig. 1A). With CO, FEV_1 changes associated with increased concentrations were largest with the three earliest lag times, and with $PM_{2.5}$, FEV_1 changes associated with increased concentrations were similar for all lag times. Decreased FEV_1 was not associated with concentrations of ambient ozone or SO_2, or PES ozone or NO_2. FVC showed similar associations, with patterns of decreased pre-exposure FVC associated with increased $PM_{2.5}$, CO, and NO_2 at most lag times (Supplement Table 4; Supplement Figure 1A). However, only NO_2 was significant, with each 5.2 ppb increase in NO_2 concentration in the 72 h before the pre-exposure visit associated with a decrease in FVC of 0.033 L (95% CI -0.057 to −0.008; p = 0.009).

3.1.2. Hypothesis 2

FEV_1 increased from pre-to post-exposure in association with each 4.1 ppb increase in PES ozone concentration (0.010 L; 95% CI 0.004 to 0.026; p = 0.010) (Table 3, Fig. 1B), as well as with IQR increases in ambient $PM_{2.5}$ and CO concentrations at all lag times. For $PM_{2.5}$ the largest FEV_1 increase was associated with each 4.7 $\mu g/m^3$ increase in $PM_{2.5}$ concentration in the 72 h before the pre-exposure visit (0.018 L; 95% CI 0.005 to 0.031; p = 0.007). Again, patterns of associations were similar for FVC and $PM_{2.5}$, with the largest FVC increase associated with each 4.9 $\mu g/m^3$ increase in $PM_{2.5}$ concentration in the 24 h before the pre-exposure visit (0.015 L; 95% CI 0.001 to 0.029; p = 0.039), with similar sized FVC increases observed for almost all lags (Supplement Table 5; Supplement Figure 1B). There were no associations between changes in FVC across the exposure session and any other pollutant.

These findings indicate that increases in ambient concentrations of $PM_{2.5}$, CO, and NO_2 in the 96 h before the pre-exposure visit were significantly associated with decrements in pre-exposure lung function, with subsequent increases in lung function over the hours of the exposure sessions.

3.2. Airway inflammation and injury

Airway inflammation was measured as the proportion of inflammatory cells (PMN%) in induced sputum, and the primary marker of lung injury was CC16.

3.2.1. Hypothesis 1

There were no clear patterns or statistically significant associations of sputum PMN% (Supplement Figure 2A), or pre-exposure CC16 (Supplement Table 6) with any pollutant.

3.2.2. Hypothesis 2

Increases in $PM_{2.5}$ and CO at multiple lag times were associated with decreases in CC16 across the exposure sessions, with the largest CC16 decreases associated with each 5.4 $\mu g/m^3$ increase in $PM_{2.5}$ concentration in the 12 h before the pre-exposure visit (−0.72 ng/mL; 95% CI -1.24 to −0.21; p = 0.006) and each 0.126 ppm increase in ambient CO concentration in the 1 h before the pre-exposure visit (−0.90 ng/mL; 95% CI -1.76 to −0.04; p = 0.041) (Supplement Table 7; Supplement Figure 2B). Decreased CC16 across the exposure sessions was significantly associated with increased $PM_{2.5}$ concentration, but the absence of a $PM_{2.5}$-associated increase in CC16 at the pre-exposure measurement makes the $PM_{2.5}$ findings inconclusive for this outcome.

3.3. Heart rate variability

3.3.1. Hypothesis 1

IQR increases in ambient ozone concentrations in the 1, 3, 12, 24, 48, 72, and 96 h before the pre-exposure visit were all associated with decreases in pre-exposure HF HRV (Fig. 2A, Supplement Table 8). The largest HF decrease was associated with increased ozone concentrations averaged over the 96 h before the pre-exposure visit (−0.460 ln[ms^2]; 95% CI, −0.743, to −0.177 for each 10.35 ppb increase in ozone; p = 0.002). IQR increases in ambient CO, $PM_{2.5}$, and NO_2 concentrations over these same lags were associated with non-significant increases in HF. IQR increases in PES ozone, PES NO_2, and ambient SO_2 concentrations (at the same lags) were not associated with changes in HF. LF HRV showed similar patterns of association with ambient and personal pollutant concentrations; details are provided in the Supplement page 2, Supplement Table 9, and Supplement Figure 3A.

Because the hypothesized relationships between the primary markers of HRV (HF and LF) and ambient ozone concentrations were confirmed, we analyzed the secondary markers of HRV: RMSSD, SDNN, and LF/HF (Supplement Tables 10, 11, 12 respectively). IQR increases in ambient ozone concentrations were significantly associated with decreases in both RMSSD and SDNN (Fig. 3A and B), in a pattern similar to that seen for HF and LF. The pattern of RMSSD and SDNN associations with increased ambient $PM_{2.5}$, CO, and NO_2 was similar to that of HF and LF, although not statistically significant. There were no such

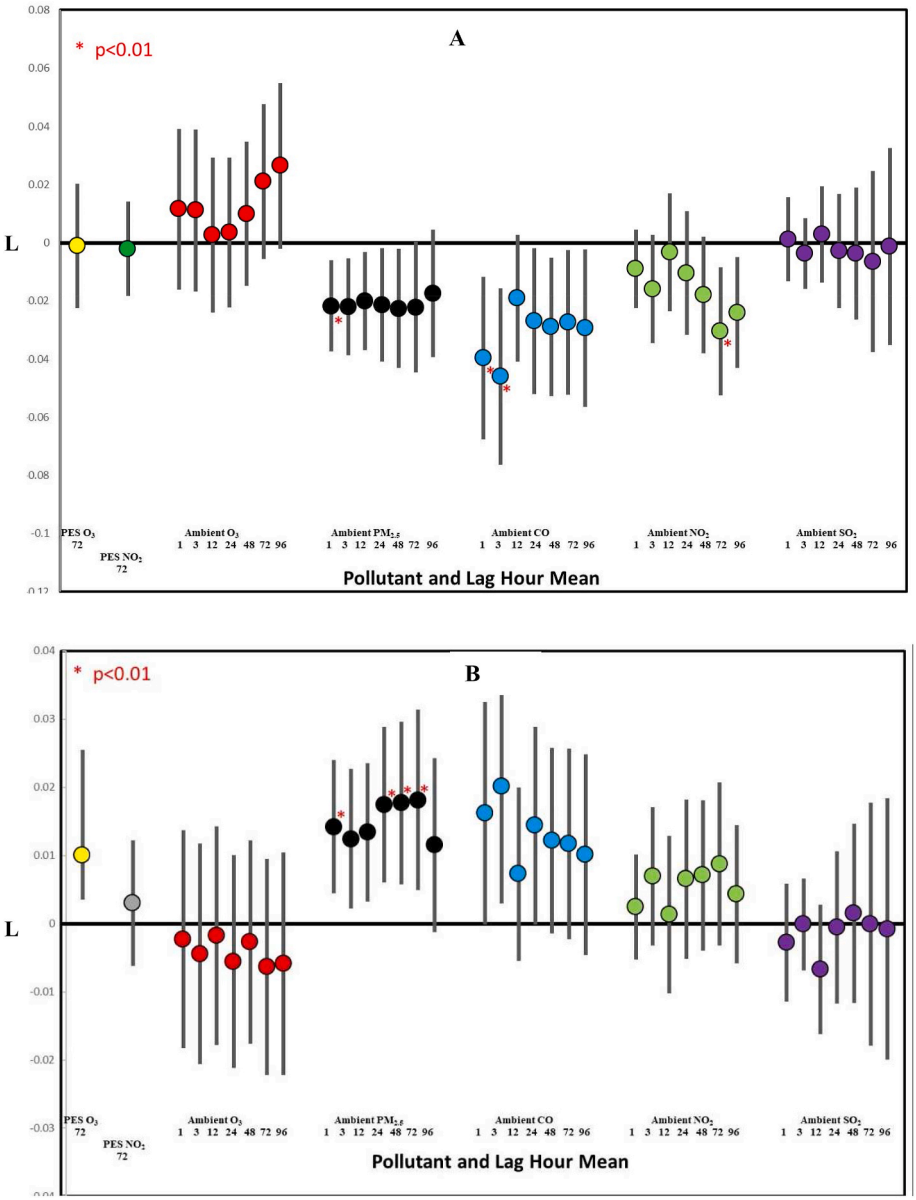

Fig. 1. A. Change in pre-exposure FEV1 (L), and **B.** Difference in the pre-to post-exposure change in FEV1, associated with each interquartile range increase in pollutant concentration, by pollutant and lag hour mean. Lag hour "1" is the first lag hour (time 0–60 min, "3" is the first 3 lag hours (time 0–180 min), etc.

associations with any pollutant and LF/HF (Supplement Figure 3B). We therefore conclude that increases in ambient ozone, but not PES ozone or other pollutants, were associated with decreases in pre-exposure HRV.

3.3.2. *Hypothesis 2*

Increases in ambient ozone concentrations were marginally significantly associated with increases in HF from pre-to post-exposure (Fig. 2B, Supplement Table 13). The largest increases in HF were associated with increased ozone concentrations in the 48, 72, and 96 h before the pre-exposure visit. Increases in $PM_{2.5}$ concentrations across all lag times were associated with pre-to post-exposure decreases in LF, with the largest LF decrease associated with each 4.3 $\mu g/m^3$ increase in $PM_{2.5}$ concentration in the 96 h before the pre-exposure visit (−0.203 ln [ms^2]; 95% CI -0.350 to −0.055; p = 0.007) (Supplement Table 14; Supplement Figure 4A). However, there were no clear patterns of LF associations with the other pollutants.

Analysis of the secondary HRV markers showed that increased ambient ozone concentrations were marginally significantly associated with increased RMSSD and SDNN across the exposure sessions. The largest RMSSD change was associated with each 10.3 ppb increase in ozone concentration in the 96 h before the pre-exposure visit (0.093 ln [ms]; 95%

CI 0.010 to 0.176; p = 0.027) (Supplement Table 15; Supplement Figure 4B). However, increased $PM_{2.5}$ concentrations were marginally significantly associated with decreased RMSSD, with the largest RMSSD change associated with each 4.3 $\mu g/m^3$ increase in $PM_{2.5}$ concentration in the same 96 h (−0.071 ln[ms]; 95% CI -0.132 to −0.009; p = 0.026). Increased PES NO_2 was marginally significantly associated with increased LF/HF, but increased ambient ozone in the 3 h before the pre-exposure visit was marginally significantly associated with decreased LF/HF (Supplement Table 17; Supplement Figure 5B). The patterns and direction of change for RMSSD and SDNN were consistent with the findings for HF. The marginally significant decrease in LF/HF associated with ozone was consistent with the observed larger increase in HF than in LF.

Table 3
Pre-to post-exposure change in FEV_1 associated with each interquartile range (IQR) increase in PES and ambient air pollutant concentration, by lag hours.

Pollutant	Lag Hours	IQR	N	Change in FEV_1 (L)	95% Confidence Interval	p-value
PES O_3 (ppb)	0–71	4.1	455	0.010	0.004, 0.026	0.010
PES NO_2 (ppb)	0–71	9.3	455	0.003	−0.006, 0.012	0.524
Ambient O_3 (ppb)	0	15.2	419	−0.002	−0.018, 0.014	0.777
	0–2	15.2	397	−0.004	−0.021, 0.012	0.591
	0–11	16.1	429	−0.002	−0.018, 0.014	0.829
	0–23	13.7	433	−0.006	−0.021, 0.010	0.486
	0–47	11.3	435	−0.003	−0.018, 0.012	0.722
	0–71	11.0	433	−0.006	−0.022, 0.010	0.432
	0–95	10.3	435	−0.006	−0.022, 0.010	0.477
Ambient $PM_{2.5}$ ($\mu g/m^3$)	**0**	**5.9**	**413**	**0.014**	**0.005, 0.024**	**0.004**
	0–2	5.8	401	0.012	0.002, 0.023	0.017
	0–11	5.4	417	0.013	0.003, 0.024	0.010
	0–23	**4.9**	**421**	**0.017**	**0.006, 0.029**	**0.003**
	0–47	**4.6**	**421**	**0.018**	**0.006, 0.030**	**0.004**
	0–71	**4.7**	**423**	**0.018**	**0.005, 0.031**	**0.007**
	0–95	4.3	421	0.012	−0.001, 0.024	0.077
Ambient CO (ppm)	0	0.126	419	0.016	−0.000, 0.033	0.051
	0–2	0.143	411	0.020	0.003, 0.037	0.022
	0–11	0.144	423	0.007	−0.005, 0.020	0.261
	0–23	0.129	425	0.014	−0.000, 0.029	0.052
	0–47	0.109	425	0.012	−0.001, 0.026	0.080
	0–71	0.106	425	0.012	−0.002, 0.026	0.100
	0–95	0.108	423	0.010	−0.005, 0.025	0.179
Ambient NO_2 (ppb)	0	4.6	401	0.002	−0.005, 0.010	0.535
	0–2	5.5	393	0.007	−0.003, 0.017	0.178
	0–11	7.9	407	0.001	−0.010, 0.013	0.825
	0–23	6.1	411	0.007	−0.005, 0.018	0.273
	0–47	5.1	411	0.007	−0.004, 0.018	0.206
	0–71	5.2	409	0.009	−0.003, 0.021	0.152
	0–95	4.2	407	0.004	−0.006, 0.015	0.404
Ambient SO_2 (ppb)	0	0.8	295	−0.003	−0.012, 0.006	0.527
	0–2	0.9	293	0.000	−0.007, 0.007	0.971
	0–11	0.8	297	−0.007	−0.016, 0.003	0.164
	0–23	0.9	301	−0.001	−0.012, 0.011	0.926
	0–47	0.9	303	0.002	−0.012, 0.015	0.820
	0–71	0.9	303	0.000	−0.018, 0.018	0.992
	0–95	0.9	301	−0.001	−0.020, 0.018	0.936

3.4. Two-pollutant models

Because we found pre-to post-exposure increases in HF and LF associated with increased ozone concentrations, but decreased HF and LF associated with increased $PM_{2.5}$, CO, and NO_2 in some single pollutant models, we fit a series of two-pollutant models for the primary pre-exposure HRV outcomes, HF and LF. We then compared the change in ozone concentration when adjusting for each of the other pollutants to that from the single pollutant model. The estimated change in HF associated with increased ozone concentrations at each lag time was robust with $PM_{2.5}$ in the model (Table 4). In the two-pollutant model, HF decreased −0.472 ln(ms^2) (95% CI -0.777 to −0.167, p = 0.003) with each 10.35 ppb increase in ozone concentration averaged over the 96 h before the pre-exposure visit, compared with −0.460 ln(ms^2) (95% CI, −0.743, to −0.177 p = 0.002) for the single pollutant model with ozone alone. The ozone associations were also little changed after adjustment for CO and NO_2 concentrations.

The pre-to post-exposure changes in LF associated with increased ozone concentrations in the 1–96 h before the pre-exposure visit were reduced with adjustment for $PM_{2.5}$, CO, and NO_2 concentrations at the same time lag (Table 5). When adjusting for $PM_{2.5}$, the decrease in LF associated with each IQR increase in ambient ozone concentration in the 96 h before the pre-exposure visit was essentially removed (−0.265 ln [ms^2] for ozone alone, −0.017 ln[ms^2] for ozone adjusted for $PM_{2.5}$). The decreases in LF associated with IQR increases in ambient ozone concentration in the 48, 72, and 96 h before the pre-exposure visit were variably reduced in the two-pollutant models adjusting for CO and NO_2 concentrations.

In summary, these findings suggest that increased ambient ozone concentrations adversely affected pre-exposure HRV levels, with reductions in HF, LF, RMSSD, and SDNN. HRV increased from pre-to post-exposure in association with the same increased ozone concentrations. The ozone associations with HF were independent of $PM_{2.5}$, CO, and NO_2 in two-pollutant models.

3.5. Cardiac repolarization

3.5.1. Hypothesis 1

We found no clear patterns or significant associations between increased pollutant concentrations and the primary markers of repolarization, T-wave amplitude and the ST segment in V5, at the pre-exposure visit (Supplement Tables 18, 19; Supplement Fig. 6A and B).

3.5.2. Hypothesis 2

There were also no significant associations between increased pollutant concentrations and pre-to post-exposure change in T-wave amplitude (Supplement Table 18; Supplement Figure 7A). Increased ambient ozone concentrations across all lags were associated with pre-to post-exposure decreases in the ST segment in V5, with the largest change associated with each 10.3 ppb increase in ozone concentration in the 96 h before the pre-exposure visit (−3.0 μV; 95% CI, −5.0 to −1.0; p = 0.003) (Supplement Table 19; Supplement Figure 7B). We therefore examined associations with the pre-to post-exposure changes in secondary ST segment outcomes. Changes in the ST segment in lead II and V2 were not associated with any increased pollutant concentrations at any lag (Supplement Tables 21, 22). We conclude that increased ambient and personal pollutant concentrations were not associated with adverse changes in ECG markers of repolarization.

3.6. Vascular function

We found a few significant and marginally significant associations between pollutant concentrations and vascular endpoints for both hypotheses. For example, each 9.4 ppb increase in PES NO_2 concentration was associated with a significant reduction in pre-exposure SBP (−1.457 mm Hg, 95% CI -2.490 to −0.425, p = 0.006) (Supplement Table 23; Supplement Figure 8A). For pre-to post-exposure changes, each 9.4 ppb increase in PES NO_2 concentration was marginally significantly associated with increases in SBP across the exposure session (0.8 mm Hg, 95% CI 0.0 to 1.6, p = 0.043) (Supplement Table 24; Supplement Figure 8B), consistent with "recovery" of the decrease at pre-exposure. There were no significant pollutant associations for FMD (Supplement Tables 25, 26; Supplement Fig. 9A and B). Because of these findings, we examined the secondary markers of vascular function: diastolic BP (DBP) (Supplement Tables 27, 32; Supplement Fig. 10A and B), plasma vWF (Supplement Tables 28, 33; Supplement Fig. 11A and B), ET-1 (Supplement Tables 29, 34; Supplement Fig. 12A and B), and the secondary outcomes from BAU testing: VTI (Supplement Tables 30, 35; Supplement Fig. 13A and B) and BAD (Supplement Tables 31, 36; Supplement Figure 13C). Details of these findings are provided in the Supplement, beginning on page 5. When the findings were considered together, inconsistencies in the directions of associations and the pollutants involved precluded any definitive conclusions about effects of prior pollutant exposure on vascular function, for either hypothesis.

3.7. Systemic oxidative stress

3.7.1. Hypothesis 1

IQR increases in ambient NO_2 concentrations were associated with decreased pre-exposure nitrotyrosine levels at all lag times, with the largest increase in the 12 h before the pre-exposure visit (−0.094 ln [nM]; 95% CI -0.164 to −0.023; p = 0.010) (Supplement Table 37;

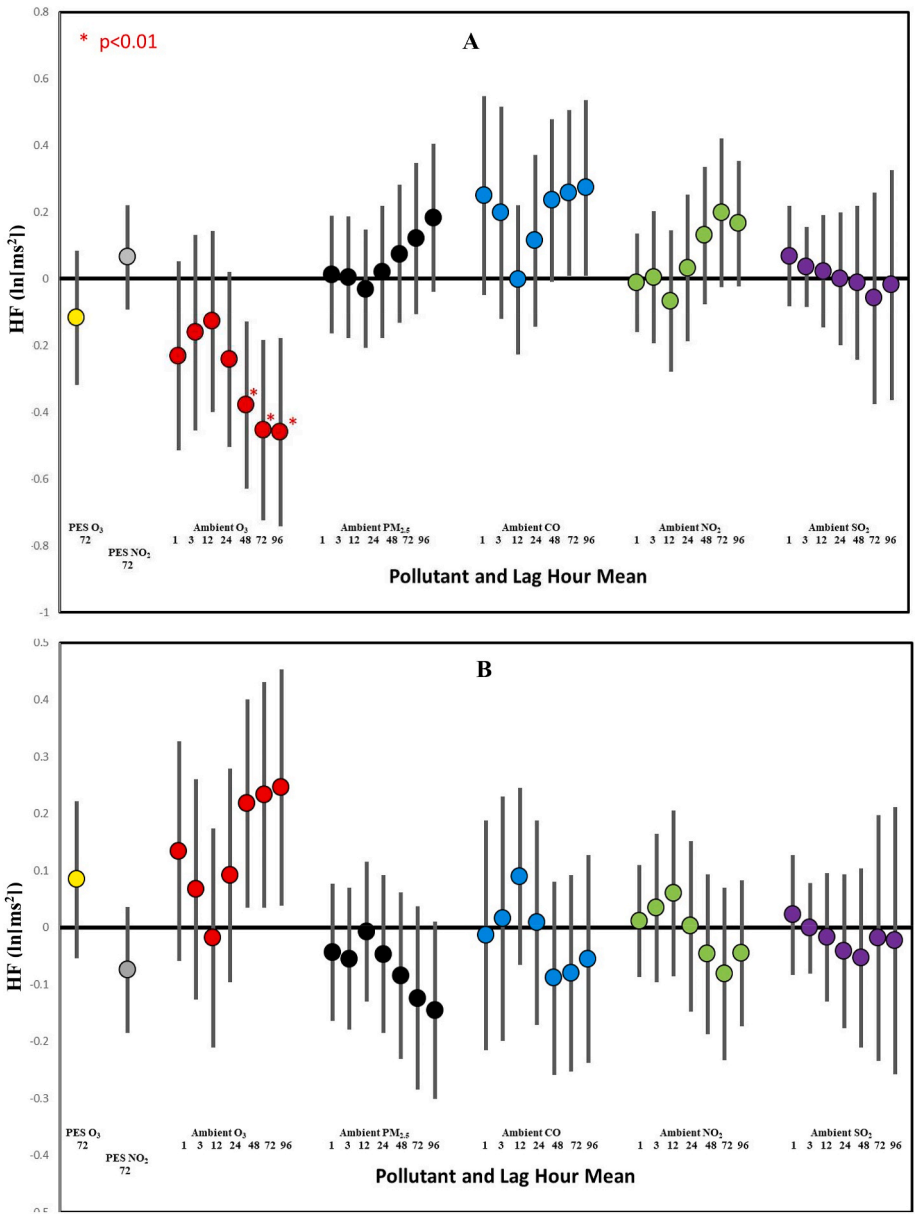

Fig. 2. A. Change in pre-exposure HF (ln[ms^2]), and **B.** Difference in the pre-to post-exposure change in HF, associated with each interquartile range increase in pollutant concentration, by pollutant and lag hour mean.

Supplement Figure 14A).

3.7.2. Hypothesis 2

Increased ambient NO_2 concentrations at all lags were associated with pre-to post-exposure increases in nitrotyrosine, with the largest increase in the 12 h before the pre-exposure visit (0.55 ln[nM], 95% CI 0.015 to 0.095, p = 0.007) (Supplement Table 38; Supplement Figure 14B). Similarly, increased ambient CO concentrations in the 1 h before the pre-exposure visit were marginally significantly associated with increased pre-to post-exposure nitrotyrosine (0.070 ln[nM], 95% CI 0.012 to 0.129, p = 0.019). However, increased ambient ozone concentrations at all lags were associated with decreases in nitrotyrosine across exposure sessions, reaching marginal significance at the 72-h lag (−0.072 ln[nM], 95% CI -0.131 to −0.013. p = 0.017). There were no patterns of association or significant nitrotyrosine associations with any other pollutant. Because the ambient NO_2-nitrotyrosine associations were opposite of the direction hypothesized, we concluded there was no clear association between increased pollutant concentrations and either pre-exposure levels or pre-to post-exposure changes in nitrotyrosine.

3.8. Systemic inflammation

3.8.1. Hypothesis 1

IQR increases in ambient concentrations of CO, $PM_{2.5}$, and NO_2 were marginally significantly associated with increased pre-exposure levels of C-reactive protein (CRP) (Supplement Table 39; Supplement Figure 15A).

3.8.2. Hypothesis 2

IQR increases in PES NO_2 and ambient NO_2 concentrations were significantly associated with decreases in CRP across the exposure sessions (Supplement Table 43; Supplement Figure 15B), and increases in ambient $PM_{2.5}$ concentrations in the 3 h before the pre-exposure visit were marginally significantly associated with decreased CRP. Increased CO concentrations showed a similar pattern of association with CRP, without statistical significance.

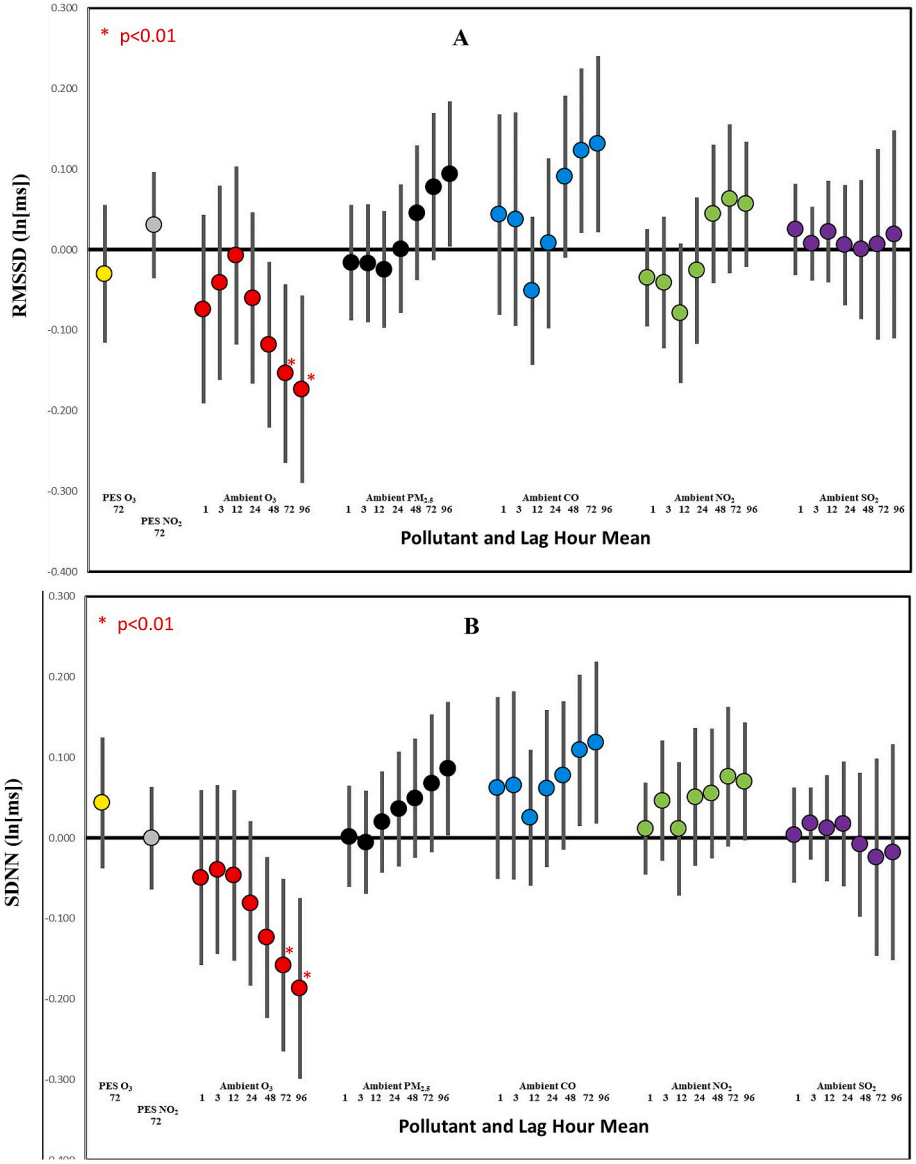

Fig. 3. A. Change in pre-exposure RMSSD (ln[ms]), and **B.** Difference in the pre-to post-exposure change in RMSSD, associated with each interquartile range increase in pollutant concentration, by pollutant and lag hour mean.

Table 4
Two pollutant models: Change in pre-exposure HF (ln[ms^2]) associated with each interquartile range (IQR) increase in ozone concentration, by outcome and lag hours.

Lag Hours	Ozone Single Pollutant			Ozone with $PM_{2.5}$			Ozone with CO			Ozone with NO_2		
	n	Change (95% CI)	p-value	n	Change (95% CI)	p-value	n	Change (95% CI)	p-value	n	Change (95% CI)	p-value
0	206	−0.231 (−0.515, 0.052)	0.109	192	−0.240 (−0.556, 0.077)	0.136	200	−0.201 (−0.504, 0.102)	0.192	189	−0.215 (−0.543, 0.113)	0.196
0-2	195	−0.162 (−0.455, 0.131)	0.277	181	−0.165 (−0.493, 0.162)	0.319	190	−0.133 (−0.452, 0.187)	0.413	178	−0.150 (−0.497, 0.198)	0.395
0-11	211	−0.128 (−0.400, 0.144)	0.354	201	−0.110 (−0.399, 0.179)	0.451	203	−0.203 (−0.548, 0.142)	0.247	196	−0.264 (−0.637, 0.110)	0.165
0-23	213	−0.242 (−0.505, 0.020)	0.070	205	−0.235 (−0.507, 0.036)	0.089	205	−0.252 (−0.563, 0.059)	0.111	199	−0.304 (−0.631, 0.024)	0.069
0–47	**214**	**−0.379 (-0.629, -0.128)**	**0.003**	**205**	**−0.389 (-0.650, -0.128)**	**0.004**	206	−0.378 (−0.667, −0.089)	0.011	199	−0.381 (−0.690, −0.071)	0.016
0–71	**213**	**−0.454 (-0.725, -0.184)**	**0.001**	**205**	**−0.460 (-0.741, -0.180)**	**0.002**	**206**	**−0.444 (-0.755, -0.133)**	**0.006**	199	−0.422 (−0.752, −0.092)	0.013
0–95	**214**	**−0.460 (-0.743, -0.177)**	**0.002**	**204**	**−0.472 (-0.777, -0.167)**	**0.003**	**206**	**−0.451 (-0.784, -0.119)**	**0.008**	199	−0.450 (−0.810, −0.090)	0.015

Table 5
Two pollutant models: Change in pre-exposure LF (ln[ms^2]) associated with each interquartile range (IQR) increase in ozone concentration, by outcome and lag hours.

Lag Hours	Ozone Single Pollutant			Ozone with $PM_{2.5}$			Ozone with CO			Ozone with NO_2		
	n	Change (95% CI)	p-value	n	Change (95% CI)	p-value	n	Change (95% CI)	p-value	n	Change (95% CI)	p-value
0	206	−0.068 (−0.325, 0.189)	0.603	192	−0.043 (−0.327, 0.241)	0.363	200	0.050 (−0.236, 0.337)	0.900	189	−0.010 (−0.404, 0.205)	0.317
0-2	195	−0.005 (−0.268, 0.258)	0.968	181	0.006 (−0.252, 0.264)	0.767	190	−0.010 (−0.324, 0.304)	0.728	178	−0.143 (−0.484, 0.198)	0.518
0-11	211	−0.058 (−0.307, 0.191)	0.646	201	−0.118 (−0.369, 0.133)	0.961	203	−0.071 (−0.355, 0.214)	0.951	196	−0.158 (−0.457, 0.141)	0.409
0-23	213	−0.138 (−0.380, 0.104)	0.261	205	−0.191 (−0.434, 0.051)	0.352	205	−0.152 (−0.420, 0.117)	0.624	199	−0.196 (−0.479, 0.088)	0.297
0-47	214	−0.224 (−0.457, 0.009)	0.060	205	−0.228 (−0.490, 0.033)	0.121	206	−0.168 (−0.456, 0.120)	0.265	199	−0.211 (−0.514, 0.091)	0.175
0-71	213	−0.277 (−0.529, −0.025)	0.031	205	−0.195 (−0.475, 0.086)	0.086	206	−0.125 (−0.433, 0.183)	0.250	199	−0.218 (−0.549, 0.113)	0.169
0-95	214	−0.265 (−0.529, −0.001)	0.049	204	−0.017 (−0.290, 0.255)	0.172	206	−0.148 (−0.439, 0.144)	0.424	199	−0.240 (−0.556, 0.077)	0.195

These findings suggest the possibility of a pro-inflammatory pollutant effect with $PM_{2.5}$, CO, and NO_2, with "recovery" while the subjects were indoors (either in the hotel the night before or in the clinical research facility during the exposure session) breathing cleaner air. We therefore examined changes in secondary outcomes related to systemic inflammation: fibrinogen (Supplement Tables 40, 44; Supplement Fig. 16A, B), P-selectin (Supplement Tables 41, 45; Supplement Fig. 17A and B), and IL-6 (Supplement Tables 42, 46;Supplement Fig. 18A and B). Detailed results are provided in the Supplement page 7. The results for the secondary inflammatory markers were not consistently supportive of a pro-inflammatory effect of these pollutants, with some pollutant concentrations associated with decreases and others with increases in the secondary markers of inflammation. These findings may reflect, in part, random or spurious findings, or there may be more complicated processes involving multiple pathways that cannot be deciphered with these data.

3.9. Prothrombotic vascular state

3.9.1. Hypothesis 1

IQR increases in ambient $PM_{2.5}$ concentrations across all lag times were associated with decreased pre-exposure MP-TFA, with the largest decrease in the 48 h before the pre-exposure visit (−0.034 pg/mL; 95% CI -0.063 to −0.005; p = 0.024) (Supplement Table 47; Supplement Figure 19A). Increases in PES ozone concentrations were marginally significantly associated with decreases in monocyte platelet conjugate count (−0.107; 95% CI -0.213 to −0.002; p = 0.046) (Supplement Table 48; Supplement Figure 20A). However, there were no clear patterns of association with any other pollutant.

3.9.2. Hypothesis 2

There were a few marginally significant associations between pollutant concentrations and the pre-to post-exposure change in the primary prothrombotic outcomes; details are provided in the Supplement page 8, Supplement Tables 49, 50, and Supplement Figures 19B, 20B. However, the aggregate findings do not consistently support an association between increases in ambient and personal pollutant concentrations in the 96 h before the pre-exposure visit and increases in prothrombotic biomarkers.

4. Discussion

Previously, in the Multicenter Ozone Study of oldEr Subjects (MOSES) (Arjomandi et al., 2018; Balmes et al., 2019; Frampton et al., 2017; Rich et al., 2018), we found subtle adverse effects of ozone exposure (120, 70, and 0 ppb for 3 h with intermittent exercise) on pulmonary function, airway inflammation, and airway epithelial injury, with no convincing effects on autonomic function, cardiac repolarization, ST segment change, arrhythmia, prothrombotic vascular status, systemic inflammation, vascular function, or oxidative stress. Using these MOSES data and a repeated-measures panel study design, we tested two hypotheses: that increases in ambient and personal pollutant exposures in the 96 h before the pre-exposure visit, independent of the experimental ozone exposures, would adversely affect pulmonary and cardiovascular function 1) when measured prior to each experimental exposure, and 2) when measured across each exposure session.

4.1. Hypothesis 1

We found that increases in ambient ozone concentrations in the preceding 72 and 96 h, but not at shorter time lags, were associated with reduced HF, LF, RMSSD, and SDNN, confirming hypothesis 1 for HRV. The decreases in HF associated with increased ambient ozone concentrations were robust with adjustments for other pollutants.

This contrasts with the absence of effects on HRV of the experimental ozone exposures in MOSES, even though those concentrations were higher than ambient. However, effects were not assessed beyond 24 h after the experimental exposure, and we cannot exclude the possibility of delayed effects. The timing of this association is also inconsistent with our previous work examining $PM_{2.5}$ and ultrafine particle effects on HRV responses (Rich et al., 2016). It is possible that any ambient ozone effects on HRV were indirect, and involved intermediary processes such as systemic inflammation, which may take a few days to develop.

There may be an alternative explanation for the inconsistent HRV effects seen with chamber ozone exposures and prior ambient ozone concentrations. The MOSES study tested the effects of ozone (and only ozone) under controlled laboratory conditions in the absence of other pollutants, and found no convincing effects on HRV. However, we did find associations between ambient ozone concentrations and reductions in markers of HRV, at ozone concentrations lower than those used in the chamber exposures. Other oxidant pollutants in ambient air, such as the peroxyacyl nitrates (Fischer et al., 2014), nitric acid, hydrogen peroxide, and secondary aldehydes that, like ozone, are known to be products of atmospheric photochemistry (Altshuller, 1983), could exert synergistic, potentiating, or attenuating cardiovascular effects in combination with ozone. Thus, although the ozone exposure in the chamber was just ozone, the measured ambient ozone concentration may reflect other un-measured pollutants or oxidants co-existing with ozone in the air pollution milieu.

We observed a similar confirmation of hypothesis 1 for analyses of pulmonary function. Increases in $PM_{2.5}$, CO, and ambient NO_2 concentrations, but not ambient ozone, were associated with decreases in pre-exposure FEV_1. These associations may reflect an effect of traffic exposure; CO and NO2 are considered markers of traffic-related pollutant

mixtures. We found no convincing pollutant effects on markers of the other outcome categories: cardiac repolarization, vascular function, oxidative stress, prothrombotic state, systemic inflammation, lung injury, and airway inflammation.

4.2. Hypothesis 2

Contrary to hypothesis 2, pre-exposure markers of HRV and pulmonary function that decreased in association with increases in prior pollutant concentrations appeared to "recover" during the exposure sessions. It is important to recall that in MOSES, FEV_1 and FVC actually increased across the exposure sessions, and the controlled ozone exposure attenuated those increases in a concentration-response fashion (Arjomandi et al., 2018; Frampton et al., 2017).

We speculate that the "recovery" of pollutant effects on HRV (following increases in ambient ozone) and pulmonary function (following increases in ambient $PM_{2.5}$, NO_2, and CO) were related to reduced pollutant exposures during the time spent in controlled indoor environments as part of the exposure sessions. For example, subjects spent the night prior to exposure in a local hotel, and then 8–9 h in the research laboratory, before returning home. This included 3 h in the exposure chamber, breathing air that was both HEPA and Purafil-filtered (with ozone added per study protocol). During the time in the laboratory, the subjects remained indoors without open windows or exposure to traffic, and without indoor pollutant sources that occur in the home such as cooking, candles, or fireplaces. Previous studies have shown improvement in markers of cardiovascular function with relatively short-term indoor air filtration (Bräuner et al., 2008; Chen et al., 2015). It is important to note that pre-exposure measurements of HRV and spirometry took place on the morning of the day of exposure, whereas the baseline measures of FMD and other biomarkers took place the day before exposure (i.e., pre-exposure visit). It is therefore possible that pre-exposure HRV and lung function had already "recovered" to some degree from prior ambient exposure effects.

This recovery from the observed reductions in HRV and lung function, in association with exposure to cleaner indoor air, provides support for the causality of the observed association between the ambient pollutant increases and the reductions in biomarkers. In other words, by itself, the finding of significant associations between prior increases in ozone concentrations and reductions in HRV does not establish causality. However the subsequent increases in HRV while the subjects were breathing cleaner air during the exposure sessions, in association with those same prior increases in ambient ozone, strengthens the causal inference by providing evidence that exposure to cleaner air leads to reversal of the adverse effects. It is important to note however, that the reversal of the decrements in lung function and HRV seen in association with prior increased pollutant concentrations, could also represent homeostatic recovery.

Panel studies have examined acute heart rate variability responses to ambient ozone concentrations with mixed findings, and have been recently reviewed (Buteau and Goldberg, 2016; U.S. EPA, 2020). Our findings are consistent with those studies reporting significant decreases in HRV associated with increased ozone concentrations (Holguin et al., 2003; Jia et al., 2011; Shields et al., 2013). For example, although the time lags differed from our findings, Jia et al. (2011) studied non-smoking, healthy Beijing residents 52–73 years of age, and reported a −4.87% (95% CI = −8.62%, −0.97%) reduction in 5-min average HF associated with each 10 ppb increase in ambient ozone concentration in the previous 5 min. Other studies have reported no significant association between increased ambient ozone and HRV (Mirowsky et al., 2017; Park et al., 2005; Suh and Zanobetti, 2010; Wheeler et al., 2006; Wu et al., 2010). However, some reported similarly sized, non-statistically significant HF reductions associated with increased ozone concentrations. For example, Mirowsky et al. (2017) reported a non-significant −21.9% reduction in HF associated with each IQR increase (actual IQR not provided) in mean ozone concentration in the previous 5 days, among 13 adult patients 40–75 years of age with coronary artery disease. A remarkable finding in our study is that increases in ambient ozone concentrations adversely affected HRV in these older healthy subjects, whereas controlled exposures in these same subjects, to considerably higher concentrations, did not affect HRV. The reasons for this, as discussed above, are speculative.

Panel studies also support acute pulmonary function responses to ambient pollutants. Studies in healthy children, and in both children and adults with asthma, have shown significant decrements in lung function associated with ambient ozone exposure, especially with physical activity outdoors, such as with children attending summer camp and outdoor workers. These studies are generally consistent with controlled exposure studies of ozone in adults. However, studies of ozone and other ambient pollutants have been less consistent with regard to short-term pulmonary function effects in older healthy adults (U.S. EPA, 2019; U.S. EPA, 2020). Traffic exposure may adversely affect lung function, including in older adults. For example, Lepeule et al. (2014) used data from the VA Normative Aging Study and found lung function effects of the traffic-related pollutants black carbon, CO, and NO_2, but not ozone, in older subjects. Sinharay et al. (2018) found adverse effects on lung function in older adults who walked on a London street busy with diesel vehicles, compared with walking in Hyde Park. We found adverse lung function changes in association with the traffic pollutants CO, NO_2, and $PM_{2.5}$, but not ozone.

This study had several strengths. Although other groups have applied a repeated measures panel study approach using pre-exposure data from controlled pollutant exposure studies (Gandhi et al., 2014; Thompson et al., 2010), to our knowledge this is the first such study to additionally examine effects while spending time in a relatively clean indoor environment, breathing filtered air away from traffic and home exposures for a number of hours during the experimental exposures. The study is further strengthened by the participation of three study sites, the relatively large number of subjects, the careful clinical characterization of the healthy older subjects, the examination of co-pollutant effects, and the opportunity to compare responses to ambient and PES ozone with chamber ozone exposures.

There are also several limitations. First, although we have controlled for several potential confounders in these analyses, including temperature, relative humidity, site, and time, residual confounding is still possible. Second, all MOSES subjects at a given study site were assigned the same ambient pollutant concentrations for a specific hour/day, regardless of proximity to the monitoring site, which likely resulted in exposure misclassification. However, this exposure misclassification/error is likely a combination of Berkson and classical errors, resulting in a bias toward the null and underestimates of effect (Bateson et al., 2007; Zeger et al., 2000).

Third, the PES samples, intended to provide estimates of each subject's personal NO_2 and ozone exposure during the 72 h before each pre-exposure visit, were subject to misclassification. We did not have information on each study subject's compliance with the PES monitoring protocol (i.e., did they wear the PES for all hours during the 72 h before the pre-exposure visit?). In our analyses, this error was likely non-differential, resulting in a bias towards the null, and underestimates of effect.

Last, for our pre-to post-exposure biomarker change analyses, we used the same analytic approach as MOSES, a mixed effects linear regression with the pre-to post-exposure biomarker changes as the outcome in all models. However, one potential disadvantage of using change as the primary outcome measure is the possibility of random baseline differences with regression to the mean for subsequent measurements. This can result in a statistically significant treatment effect that is in fact spurious. The apparent difference in outcome (change from baseline) may be caused by a chance difference between the baseline measurements, before the air and ozone exposures, with post-exposure values closer to the mean, resulting in change in opposite directions for the experimental and control exposures. We addressed this in MOSES

by examining baseline data as well as the change data, and by providing the mean baseline data for each of the three exposure conditions in all graphic representations of changes. Differences at baseline were considered in our interpretation, as well as hypothesized direction of change and concentration-response relationships. In this study, we also used coherence of related outcomes in determining whether statistically significant effects could be spurious.

In summary, when using data from MOSES in a longitudinal panel study approach, decreases in pre-exposure HRV were associated with short-term increases in ambient ozone concentrations, while decrements in pre-exposure pulmonary function were associated with increases in CO, NO_2, and $PM_{2.5}$, but not ozone. Other outcomes were not significantly affected. There appeared to be an increase or "recovery" in these HRV and pulmonary function responses during the exposure sessions, possibly related to removal of the subjects from exposure to ambient ozone and traffic. The lack of concurrence of the observed associations with PES and with ambient ozone and NO_2 concentrations are, in part, explained by scavenging of ozone indoors, potential indoor sources of NO_2, and possible other outdoor oxidants/pollutants that correlate with ambient but not PES ozone.

Future controlled exposure studies should consider the impact of ambient pollutants on pre- and post-exposure biomarker levels, and whether those prior exposures modify any health response to the controlled pollutant exposure, especially if the exposure is to a relatively low, near-ambient concentration. We conclude that the repeated biomarker measurements in controlled air pollution exposure studies can be leveraged to better understand the acute health effects of ambient air pollution. Such assessments can also serve to determine potential interactions between the results of the experimental exposures and the prior ambient exposures.

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Human subjects

The study was approved by institutional review boards at each center (University of Rochester Medical Center, University of North Carolina, and the University of California, San Francisco), and by the U.S. EPA Human Subjects Research Review Official. Written informed consent was obtained from all subjects.

Declaration of competing interest

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.

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Appendix A. Supplementary data

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