Multicenter Ozone Study in Elderly Subjects (MOSES)

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and

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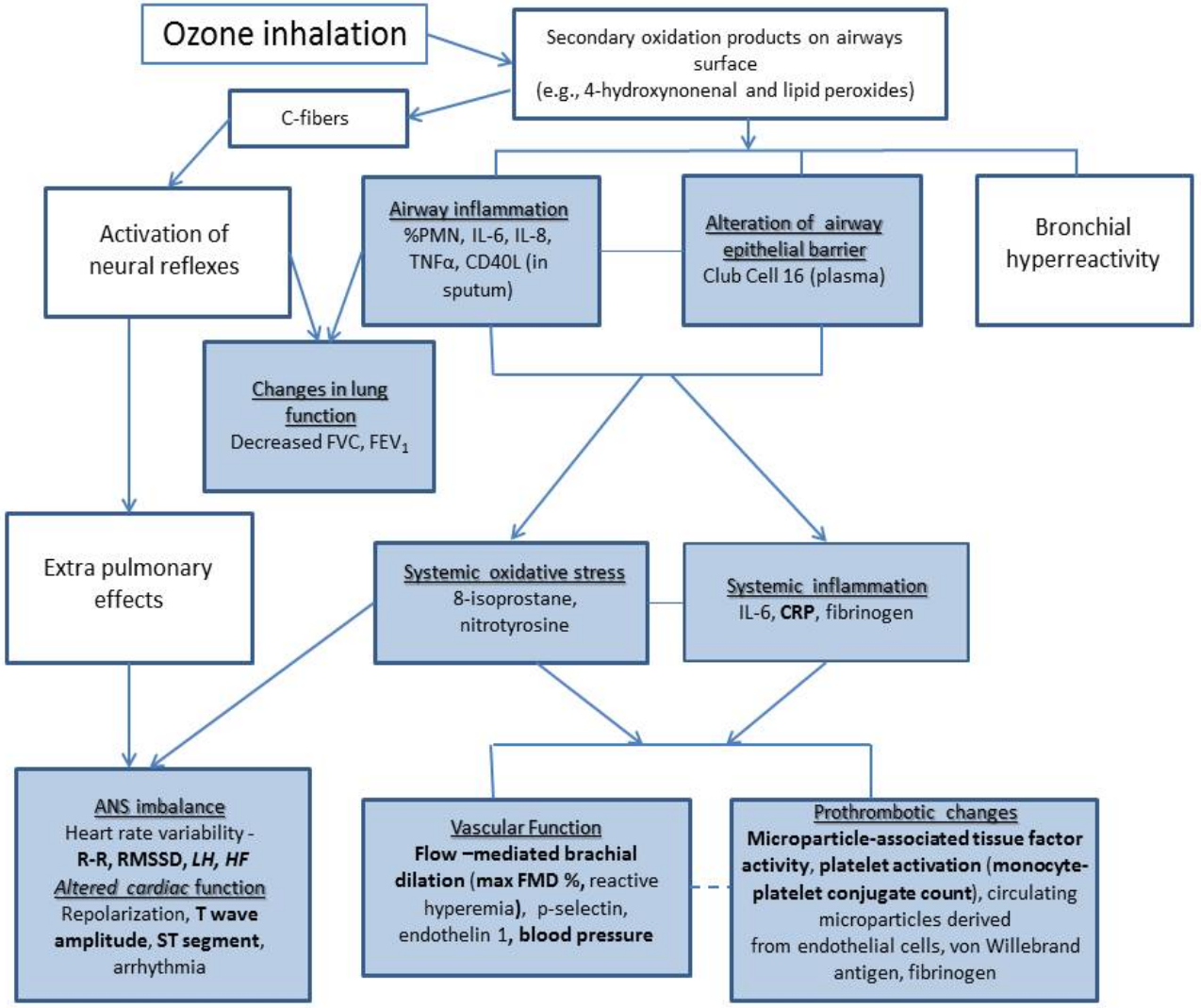
Background and Rationale

- Ozone (O_3) causes effects on lung function and airway inflammation, which are the primary basis for the current O_3 National Ambient Air Quality Standard
- To date, little attention has been paid to acute cardiovascular responses to O_3
  - Several epidemiologic studies of chronic exposure to ambient O_3 found an increased risk of mortality from cardiovascular disease
  - However, the observational evidence for impacts of acute increases in ambient O_3 levels on total cardiovascular mortality and morbidity is mixed

Hypothesized Mechanisms of Action of Ozone



MOSES – Aim 1

Aim 1. Assess whether short-term O_3 exposure:

- alters autonomic balance (heart rate variability), cardiac arrhythmia and repolarization
- alters systemic inflammation (C-reactive protein) and vascular function (blood pressure, brachial artery flow-mediated dilation)
- induces development of a pro-thrombotic vascular state (microparticle-associated Tissue Factor and monocyte-platelet conjugate count)
- induces lung function decrements (spirometry), airway inflammation (induced sputum), systemic oxidative stress (8-isoprostane), and lung injury (Club cell protein 16)

MOSES – Aim 2

The GSTM1 null genotype has been associated with susceptibility to respiratory effects of O_3, but evidence for a role of GSTM1 in increasing susceptibility to cardiac and vascular effects of ozone is lacking

Aim 2. Assess whether short-term O_3 exposure induces greater acute cardiovascular effects in subjects with the Glutathione-S-Transferase Mu 1 (GSTM1) null genotype

Study Organization

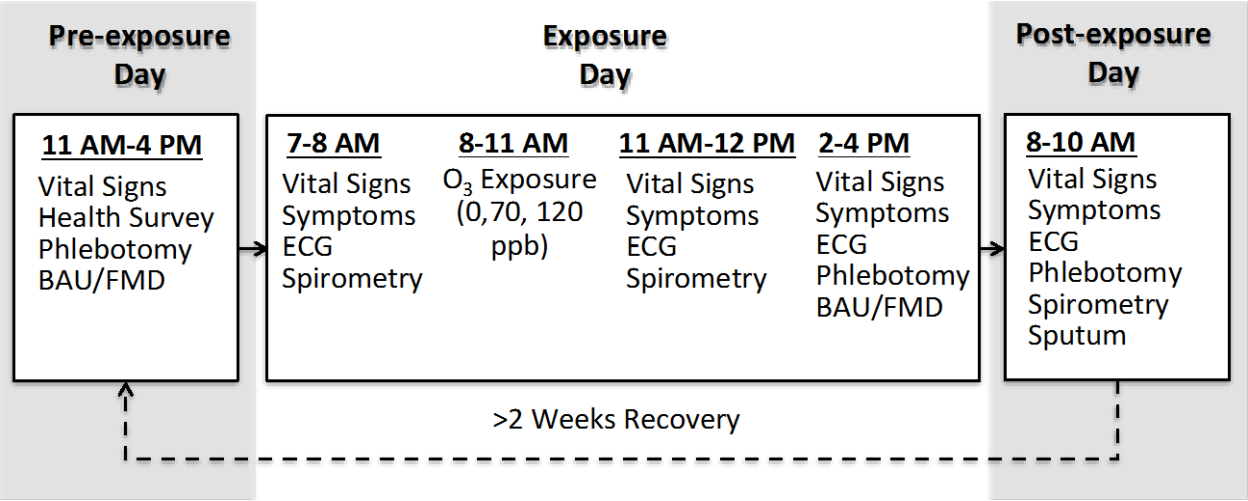
- Three clinical centers
  - University of Rochester Medical Center (URMC), M. Frampton
  - University of North Carolina (UNC), P. Bromberg
  - University of California, San Francisco (UCSF), J. Balmes
- Data Coordinating and Analysis Center
  - New England Research Institutes, P. Stark
- Seven core laboratories and commercial laboratories
  - Holter ECG (URMC, W. Zareba)
  - Brachial artery ultrasound (UCSF, P. Ganz)
  - Platelet activation (URMC, M. Frampton)
  - Sputum analyses (UNC, N. Alexis)
  - Tissue factor associated with microparticles (UNC, N. Mackman)
  - Soluble plasma markers (AssayGate)
  - Screening blood analyses (LabCorp)

Study Design

MOSES Common Protocol

87 healthy, non-smoking adults (ages 55 to 70)

- randomly exposed to clean air, 70 and 120 ppb O_3 for 3 hours while alternatively exercising and resting for 15 minutes
- majority of endpoints measured on the day before the exposure, within 4 hours after the exposure, and 22 hours after the exposure
  - with exception of Flow Mediated Dilation (which was not measured after 22 hours) and sputum markers (which were measured only 22 hours after the exposure)



Measurements during 3-day visits

MOSES Endpoints

Outcome Category	Primary Endpoints	Secondary Endpoints
Cardiac (ECG -Holter)	RMSSD (24-hr average) LF, HF (5-min average) T-wave amplitude (5-min & 24-hr average) ST in V5 (5-min & 24-hr average)	SDNN, HF, LF (24-hr average) RR, SDNN, RMSSD, LF/HF (5-min average) QTc interval (5-min averages) ST in lead II, ST in V2 (5-min & 24-hr average)
Systemic inflammation and vascular function	C-reactive protein (CRP) Systolic blood pressure (SBP) Flow Mediated Dilation (FMD)	8-isoprostane and nitrotyrosine Brachial artery diameter (BAD) Reactive hyperemia (velocity time integral, VTI) Endothelin-1 (ET-1) and P-selectin
Pro-thrombotic vascular state	Microparticle-associated Tissue Factor (MP-TF) activity Monocyte-platelet conjugate count	Von Willebrand factor (vWF) Fibrinogen Tissue Factor (TF) Activated platelet (CD62P+) count Platelet-derived microparticles (CD42b+) count Activated platelet-derived microparticles (CD42b+/62P+) count Tissue Factor expressing microparticles (CD142+) count 40 Ligand microparticle (CD40L+ (CD154+)) count

MOSES Endpoints (cont)

Outcome Category	Primary Endpoints	Secondary Endpoints
Airway inflammation and lung injury	NA	Sputum IL-6, IL-8, TNF-α, CD40L, and total protein PMNs as % of total non-epithelial cells and count#/mg sputum Serum Club cell 16 (CC16)
Pulmonary function	NA	FEV_1, FVC, FEV_1/FVC, FEF_{25-75}.
Symptoms	NA	Headache, phlegm/sputum production, eye irritation, cough, wheezing/whistling in chest, fast heart beat or pounding heart, irregular heartbeat, skipped beats

Other Data Collected

- Personal exposure to O_3 and NO_2 during the ~72 hours preceding the pre-exposure visit using Ogawa personal exposure samplers (PES)

- Temperature, relative humidity, CO, O_3, $PM_{2.5}$, NO_2, and SO_2 measurements from a central air quality monitoring station near each clinical center

Statistical Analyses

Mixed effect linear models were used to evaluate the impact of exposure to O_3 on the pre-specified primary and secondary continuous outcomes

- Site and time (when multiple measurements were taken) were controlled for in the models
- Separate interaction models were constructed for each outcome-O_3 concentration association by subject characteristics:
  - Sex
  - Age
  - GSTM1 status (wild type or null)
- To adjust for the multiple comparisons, α=0.01 was used as the threshold for statistical significance

Main Results

Characteristics of MOSES Subjects by Center

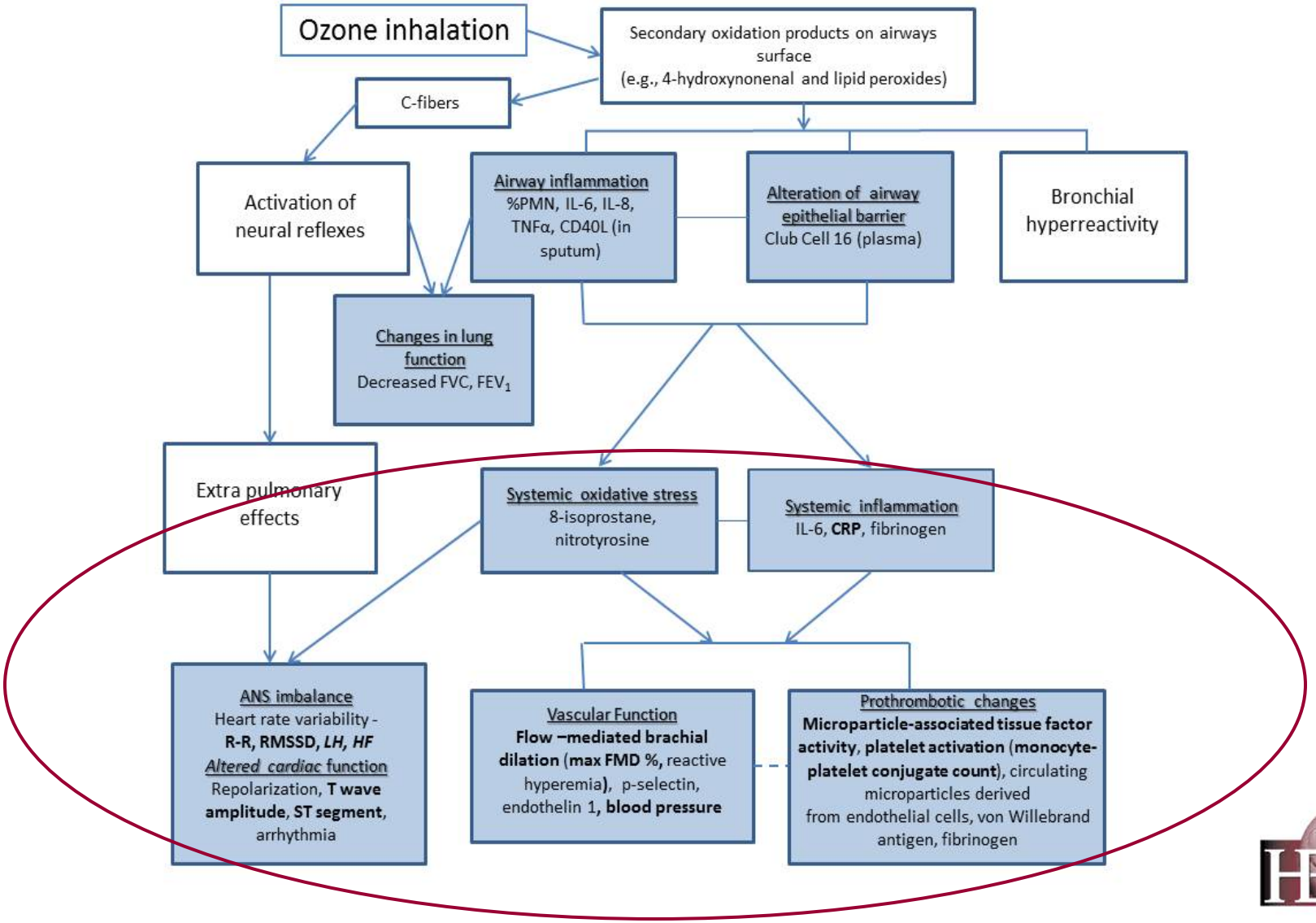
	URMC (N=32)	UNC (N=29)	UCSF (N=26)	Overall (N=87)	P-value[a]
Gender					0.236
Male	12 (38%)	9 (31%)	14 (54%)	35 (40%)	
Female	20 (63%)	20 (69%)	12 (46%)	52 (60%)	
Race					0.038
American Indian	1 (3%)	0 (0%)	0 (0%)	1 (1%)	
Asian	0 (0%)	0 (0%)	2 (8%)	2 (2%)	
Black	1 (3%)	4 (14%)	0 (0%)	5 (6%)	
White	28 (90%)	25 (86%)	23 (88%)	76 (88%)	
Hawaiian	0 (0%)	0 (0%)	1 (4%)	1 (1%)	
Unknown	1 (3%)	0 (0%)	0 (0%)	1 (1%)	
GSTM1					0.632
Wild type	15 (47%)	13 (45%)	9 (35%)	37 (43%)	
Null	17 (53%)	16 (55%)	17 (65%)	50 (57%)	
Age (yrs)	59.1 ±3.8	60.4 ±5.1	60.3 ±4.7	59.9 ±4.5	0.444
BMI (kg/m^2)	25.0 ±2.4	24.8 ±3.7	24.8 ±3.6	24.9 ±3.2	0.948
Systolic BP (mmHg)	122.4 ±11.4	120.4 ±9.7	122.2 ±12.8	121.7 ±11.2	0.750
Diastolic BP (mmHg)	69.0 ±7.5	76.1 ±7.8	73.7 ±10.7	72.8 ±9.1	0.007
Heart rate (beats/min)	65.8 ±11.4	63.9 ±9.9	65.3 ±10.1	65.0 ±10.4	0.772
Cholesterol (mg/dL)	208.3 ±34.7	215.3 ±30.7	215.8 ±47.5	212.9 ±37.6	0.696
LDL Calc (mg/dL)	118.4 ±30.0	119.6 ±29.2	123.7 ±41.8	120.4 ±33.4	0.832
% predicted FEV$_1$	104.0 ±12.8	102.4 ±13.9	102.6 ±12.9	103.0 ±13.1	0.867
FEV$_1$ (L)	3.06±0.65	2.89 ±0.59	3.24 ±0.73	3.06 ±0.66	0.144
FVC (L)	3.96 ±0.89	3.76 ±0.79	4.24 ±0.97	3.98 ±0.89	0.131

[a] P-values for categorical variables were calculated using Fisher's Exact tests.; P-value for continuous variables were calculated using ANOVA

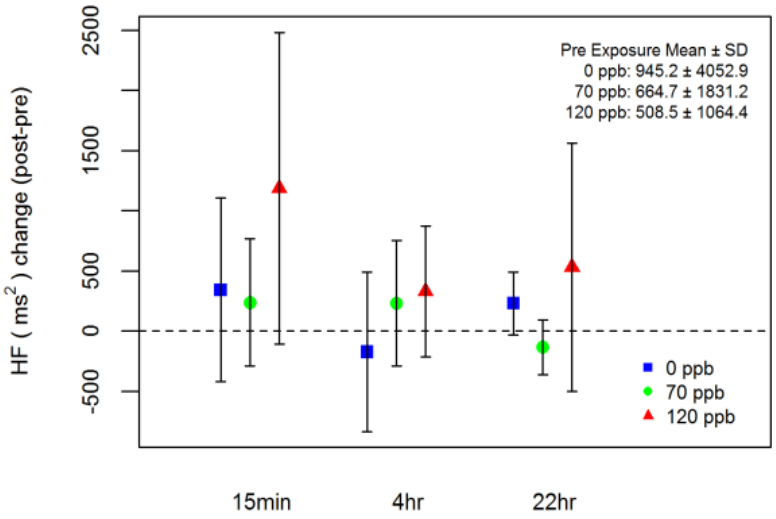
Average Chamber O_3 Concentrations Were Very Close to Target Values

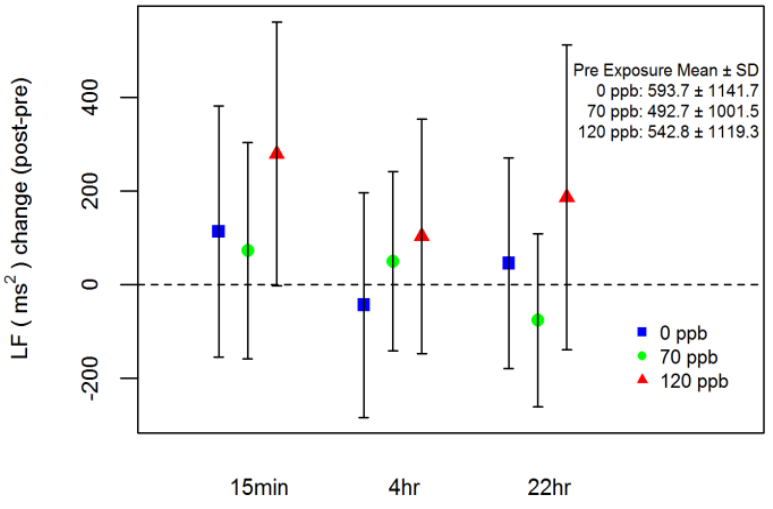
Exposure conditions	**Ozone target concentrations (mean ± SD)**			
	0 ppb	**70 ppb**	**120 ppb**	**All exposures**
All three sites	**(N=87)**	**(N=87)**	**(N=87)**	**(N=261)**
Ozone Concentration (ppb)	2.1 ± 2.4	69.9 ± 1.2	119.6 ± 1.3	
Relative Humidity (%)	41.6 ± 3.2	41.3 ± 2.9	41.4 ± 2.9	41.4 ± 3.0
Temperature (°C)	22.2 ± 0.7	22.3 ± 0.6	22.3 ± 0.7	22.3 ± 0.7
URMC	**(N=32)**	**(N=32)**	**(N=32)**	**(N=96)**
Ozone Concentration (ppb)	4.9 ± 1.6	71.0 ± 0.5	120.4 ± 0.7	
Relative Humidity (%)	44.8 ± 3.0	43.7 ± 3.4	44.2 ± 3.2	44.2 ± 3.2
Temperature (°C)	22.0 ± 1.1	22.4 ± 0.9	22.4 ± 0.9	22.3 ± 1.0
Particle count (#/cm^3)	71 ± 50	112.5 ± 142	81 ± 74	88 ± 97
UNC	**(N=29)**	**(N=29)**	**(N=29)**	**(N=87)**
Ozone Concentration (ppb)	0.4 ± 0.5	70.0 ± 0.0	120.0 ± 0.0	
Relative Humidity (%)	40.0 ± 0.0	40.0 ± 0.0	40.0 ± 0.0	40.0 ± 0.0
Temperature (°C)	22.0 ± 0.0	22.0 ± 0.0	22.0 ± 0.0	22.0 ± 0.0
Particle Count (#cm^3)	707 ± 218	795 ± 183	830 ± 193	778 ± 202
UCSF	**(N=26)**	**(N=26)**	**(N=26)**	**(N=78)**
Ozone Concentration (ppb)	0.7 ± 0.7	68.4 ± 0.9	118.1 ± 1.5	
Relative Humidity (%)	39.3 ± 1.4	39.8 ± 2.0	39.4 ± 1.0	39.5 ± 1.5
Temperature (°C)	22.5 ± 0.3	22.4 ± 0.3	22.6 ± 0.7	22.5 ± 0.5
Particle Count (#cm^3)	107.0 ± 55	191 ± 218	249 ± 151	190 ± 168

Results – Aim 1

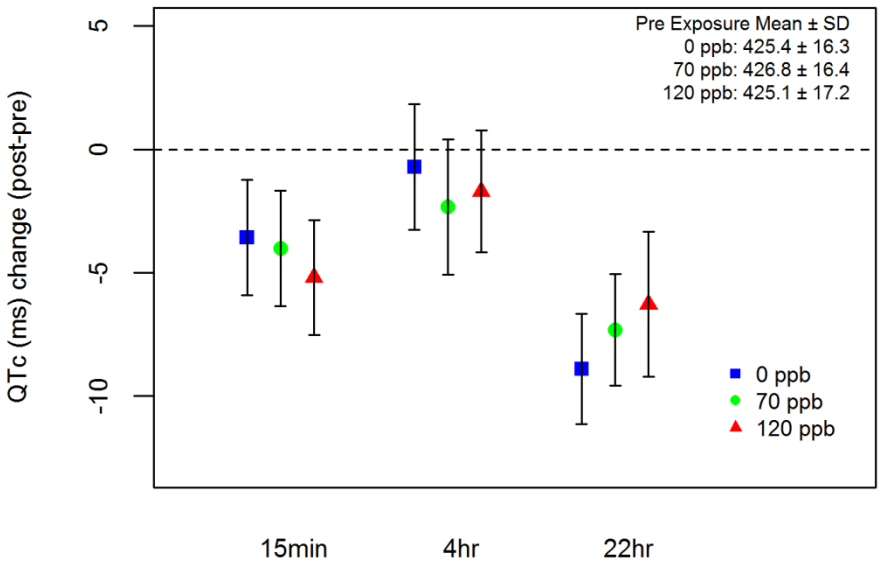


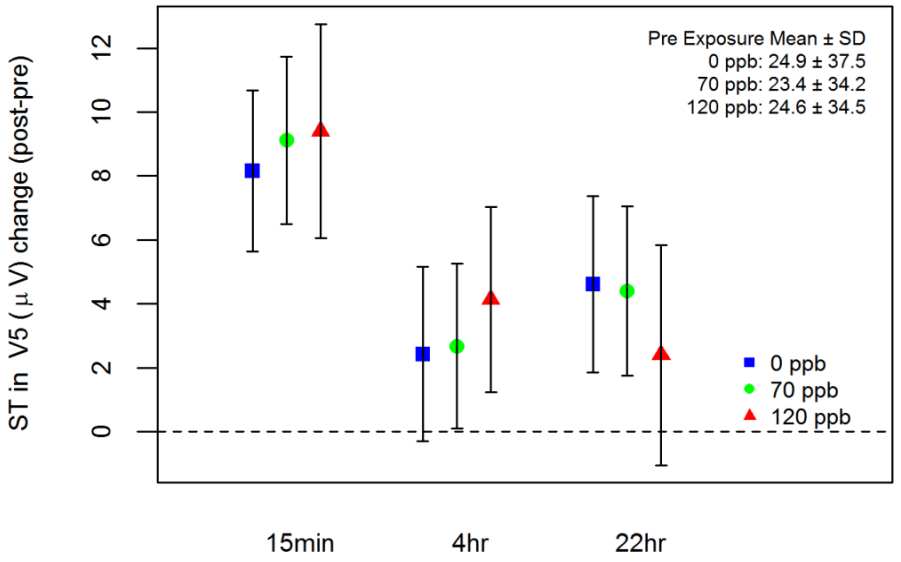
Ozone Caused no Changes in Heart Rate Variability (5-minute averages)





Ozone Caused no Other Electrocardiographic Changes (5-minute averages)





Ozone Caused no Changes in Markers of Systemic Inflammation and Oxidative Stress

Differences in CRP, IL-6, 8-isoprostane, and P-selectin associated with each ozone exposure level, compared to the 0 ppb ozone exposure

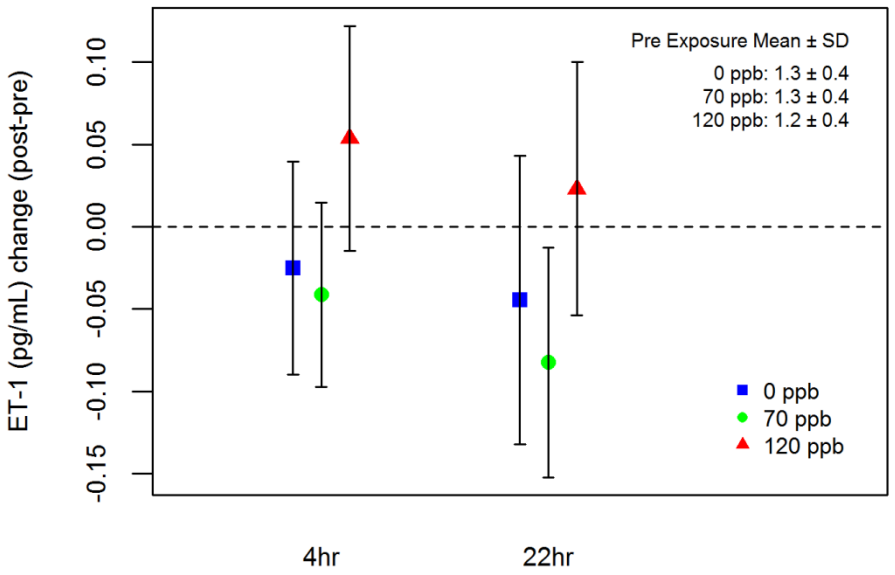
Outcome	Ozone (ppb)	Difference in estimates	95% CI	Type III SS p-value
CRP (mg/L)	120	-0.15	-0.54, 0.23	0.655
	70	-0.16	-0.54, 0.23	
	0	---	---	
IL-6 (pg/mL)	120	-0.22	-0.73, 0.29	0.567
	70	-0.25	-0.75, 0.26	
	0	---	---	
8-isoprostane (pg/mL)	120	-0.88	-5.87, 4.10	0.749
	70	-1.91	-6.85, 3.04	
	0	---	---	
P-selectin (ng/mL)	120	-14.06	-42.37, 14.26	0.235
	70	-24.28	-52.41, 3.85	
	0	---	---	

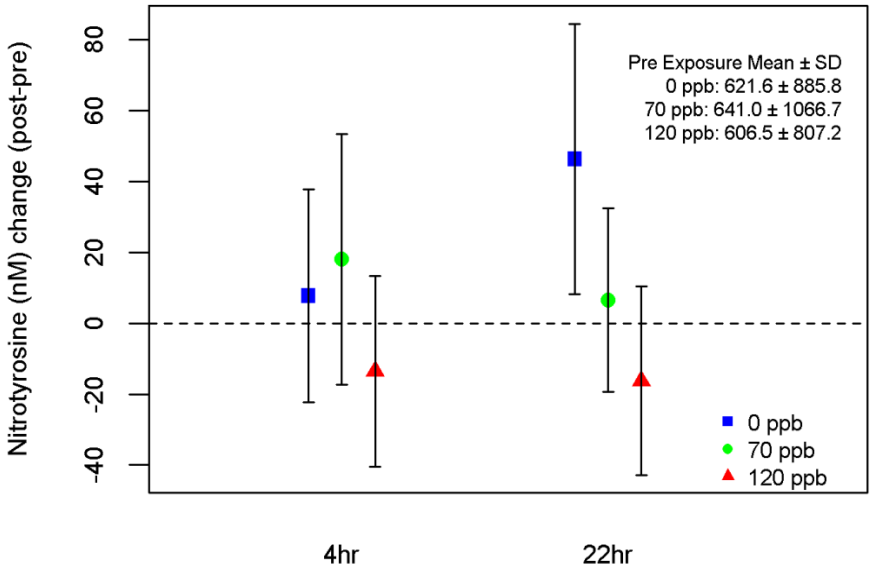
Ozone Caused no Changes in Markers of Vascular Function

Differences in systolic and diastolic blood pressure, % flow mediated dilation, and brachial artery diameter associated with each ozone exposure level, compared to the 0 ppb ozone exposure

Outcome	Ozone (ppb)	Difference in estimates	95% CI	Type III SS p-value
SBP (mmHg)	120	-1.3	-3.7, 1.2	0.950
	70	-0.6	-3.1, 1.8	
	0	---	---	
DBP (mmHg)	120	-0.1	-1.2, 1.0	0.816
	70	-0.1	-1.2, 1.0	
	0	---	---	
MaxFMD (%)	120	-0.1	-1.1, 0.9	0.637
	70	-0.6	-1.6 0.4	
	0	---	---	
VTI (cm)	120	3.9	-1.4, 9.1	0.342
	70	1.3	-3.9, 6.4	
	0	---	---	
BAD (mm)	120	0.02	-0.01, 0.05	0.523
	70	0.01	-0.02, 0.04	
	0	---	---	

Ozone Increased Plasma Endothelin-1 (ET-1) and Decreased Plasma Nitrotyrosine after 120 ppb, but not after 70 ppb



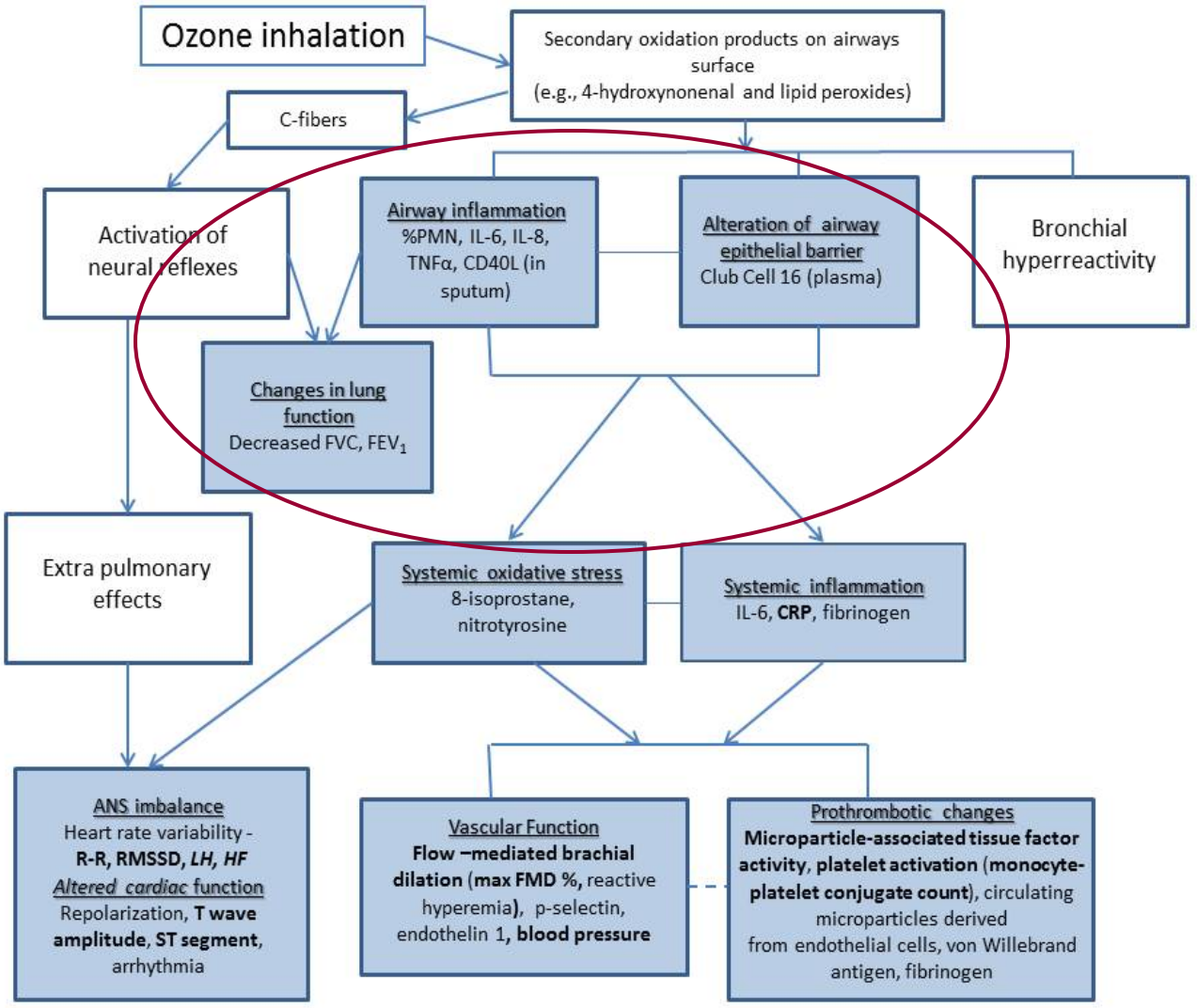


Ozone Caused no Changes in Markers of Prothrombotic Status

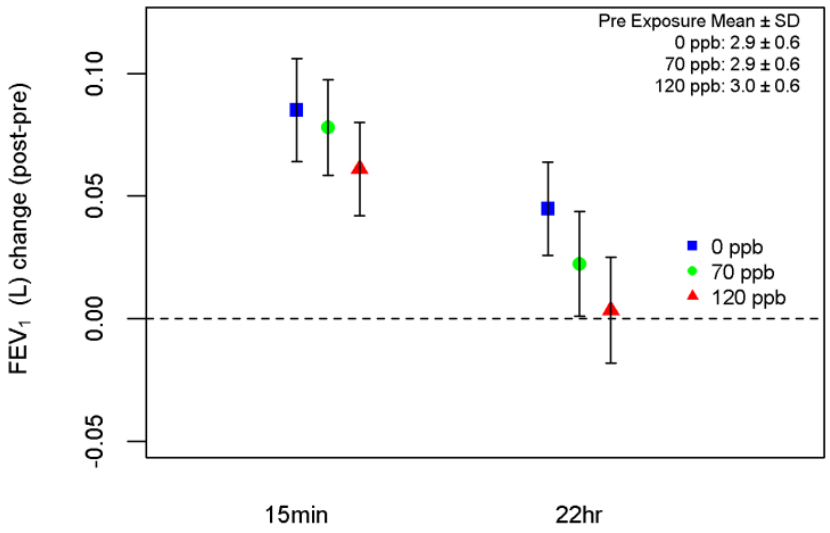
Differences in prothrombotic vascular outcomes associated with each ozone exposure level, compared to the 0 ppb ozone exposure

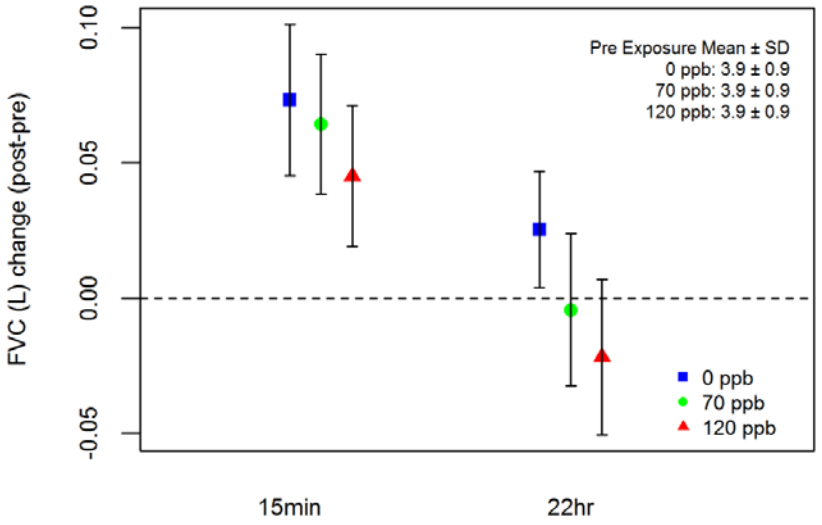
Outcome	Ozone (ppb)	Differences in estimates	95% CI	Type III SS p-value
Monocyte-platelet conjugates (count)	120	-0.2	-6.8, 6.4	0.873
	70	-1.6	-8.3, 5.0	
	0	---	---	
Activated platelets (count)	120	-1437.3	-5686.6, 2812.0	0.781
	70	-314.3	-4591.6, 3962.1	
	0	---	---	
MP-TFA (pg/mL)	120	0.009	-0.030, 0.048	0.772
	70	-0.005	-0.044, 0.034	
	0	---	---	
vWF (ng/mL)	120	-1527.6	-6719.4, 3664.2	0.765
	70	246.3	-4913.4, 5406.0	
	0	---	---	
Fibrinogen (ug/mL)	120	317.3	-67.8, 702.4	0.048
	70	-157.3	-539.9, 225.4	
	0	---	---	

Results – Aim 1

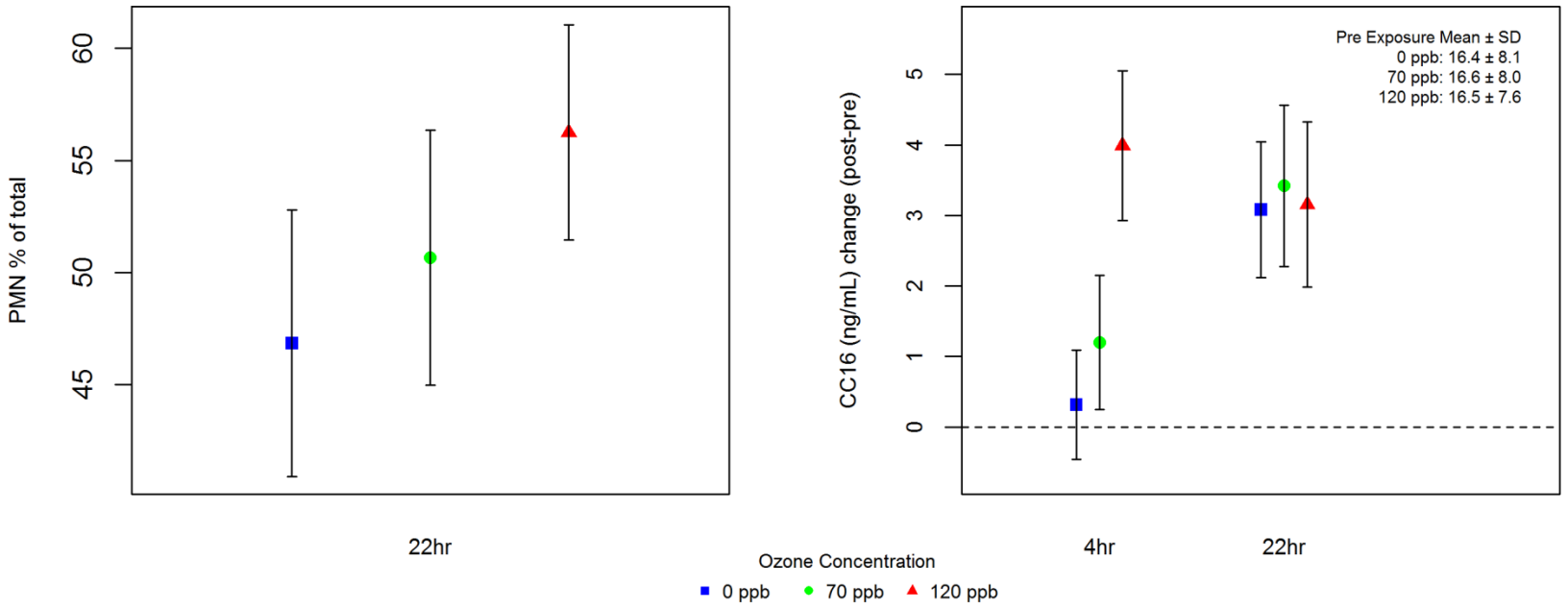


Ozone Attenuated the Increase in FEV_1 and FVC with exercise



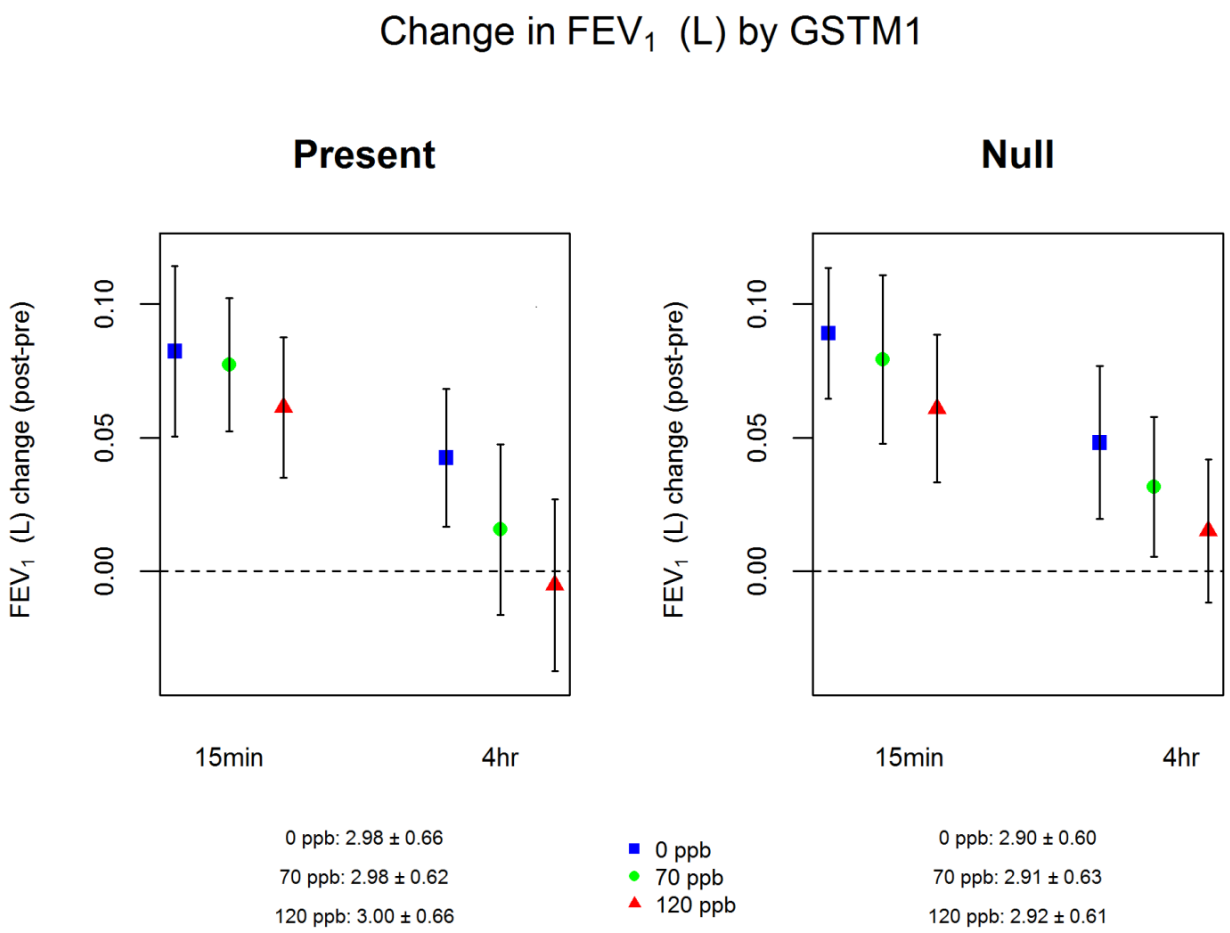


Ozone Increased Sputum Neutrophil % and Plasma Club Cell Protein 16 (CC16)



Aim 2 -No Significant Interactions Between GSTM1 Status and Effects of O_3

Example for FEV_1



Conclusions

General Considerations

- This is the first multicenter controlled O_3 exposure study, and the first to focus on cardiovascular outcomes in older subjects
- Older subjects were chosen because they may have increased susceptibility to the cardiovascular effects of air pollution
- The study was deliberately designed to assess the acute cardiovascular effects of exposure to ambient levels of O_3 (70 and 120 ppb)

Results and Implications

- We found subtle, but consistent, evidence for effects on lung function despite the relatively low inhaled dose of O_3 and the older ages of our subjects
- We also found evidence for airway inflammation and airway injury after 120 ppb exposure
- These relatively small effects are not clinically relevant for healthy people, but are of potential concern for those with underlying respiratory or cardiovascular disease

Results and Implications (cont)

- We found no convincing evidence for effects of low-level O_3 exposure on cardiovascular function or systemic inflammation
- Therefore, our study does not provide toxicological support or mechanistic plausibility for the recent epidemiological findings of ambient O_3-associated increases in cardiovascular mortality and morbidity

Acute Cardiovascular, Systemic Inflammatory and Lung Function Effects in Controlled O_3 Exposure Studies

Study	Age, yrs (# of subjects)	O_3, ppb (Exp duration)	CRP	HF	SBP	vWF	FEV_1 After exercise & clean air exposure	FEV_1 After exercise & O_3 exposure
Devlin et al 2012	28.8 median (23)	300 ppb (2 hrs)	↑	↓	NM	=	=	↓
Arjomandi et al 2015	31.8 mean (26)	100 ppb 200 ppb (4 hrs)	Dose response ↑	Dose response ↓	NM	=	=	Dose response ↓
Frampton et al 2015	26.3 mean (24)	100 ppb 200 ppb (3 hrs)	NM	NM	200 ppb ↓	NM	=	Dose response ↓
MOSES	59.9 mean (87)	70 ppb 120 ppb (3 hrs)	=	=	=	=	↑	Dose response ↓

Additional Analyses

- Effect of ozone exposure on respiratory symptoms
- Cardiovascular effects in O_3 "responders" vs. "non-responders" based on changes in FEV_1 and sputum %PMN (recommended by the HEI Review Committee)
- Effects of the levels of personal exposure to O_3 and NO_2 during the preceding 72 hrs on pre-exposure baseline values and on post-exposure changes
- Similar study of the effects of ambient pollutant concentrations during the preceding 1, 24, 48, 72 and 96 hrs

Acknowledgments

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