

*Observational Study***Air pollution, physical activity, and markers of acute airway oxidative stress and inflammation in adolescents**Emilia Pasalic, MPH, ¹ Matthew J. Hayat, PhD, ² and Roby Greenwald, PhD³¹Master of Public Health Program, Georgia State University School of Public Health, Atlanta, GA; ²Graduate Division of Epidemiology and Biostatistics, Georgia State University School of Public Health, Atlanta, GA; and ³Graduate Division of Environmental Health, Georgia State University School of Public Health, Atlanta, GA**Corresponding Author:** Emilia Pasalic • c/o Matthew J Hayat, PhD, School of Public Health, Georgia State University, PO Box 3984, Atlanta, GA 30302-3894 • 404-809-6633 • ekaiser2@student.gsu.edu**ABSTRACT****Background:** The airway inflammatory response is likely the mechanism for adverse health effects related to exposure to air pollution. Increased ventilation rates during physical activity in the presence of air pollution increases the inhaled dose of pollutants. However, physical activity may moderate the relationship between air pollution and the inflammatory response. The present study aimed to characterize, among healthy adolescents, the relationship between dose of inhaled air pollution, physical activity, and markers of lung function, oxidative stress, and airway inflammation.**Methods:** With a non-probability sample of adolescents, this observational study estimated the association between air pollution dose and outcome measures by use of general linear mixed models with an unstructured covariance structure and a random intercept for subjects to account for repeated measures within subjects.**Results:** A one interquartile range (IQR) (i.e., 345.64 µg) increase in ozone (O₃) inhaled dose was associated with a 29.16% average decrease in the percentage of total oxidized compounds (%Oxidized). A one IQR (i.e., 2.368E+10 particle) increase in total particle number count in the inhaled dose (PNT) was associated with an average decrease in forced expiratory flow (FEF₂₅₋₇₅) of 0.168 L/second. Increasing activity levels attenuated the relationship between PNT inhaled dose and exhaled nitric oxide (eNO). The relationship between O₃ inhaled dose and percent oxidized exhaled breath condensate cystine (%CYSS) was attenuated by activity level, with increasing activity levels corresponding to smaller changes from baseline for a constant O₃ inhaled dose.**Conclusions:** The moderating effects of activity level suggest that peaks of high concentration doses of air pollution may overwhelm the endogenous redox balance of cells, resulting in increased airway inflammation. Further research that examines the relationships between dose peaks over time and inflammation could help to determine whether a high concentration dose over a short period of time has a different effect than a lower concentration dose over a longer period of time.**Key words:** air pollution, physical activity, adolescents, inhaled dose**Statement of Student-Mentor Relationship:** This report is the result of master's thesis research completed by lead author Emilia Pasalic, under the mentorship of Dr. Matt Hayat and Dr. Roby Greenwald, at the Georgia State University School of Public Health.<https://doi.org/10.21633/jgpha.6.2s19>**INTRODUCTION**

The benefits of physical activity, which are well documented, include reduced mortality and morbidity as well as increased mental and physical wellbeing (Hupin et al., 2015; Kaplan et al., 1996; Moore et al., 2012). Among healthy

subjects, physical activity reduces airway inflammatory response and increases lung function (Burnett et al., 2016; Evjenth et al., 2015; Rahman et al., 2006; Stang et al., 2015). However, increased ventilation and inspiratory flow rates due to physical activity in the presence of air pollution increases the inhaled dose of air

pollutants (Cole-Hunter et al., 2012; Greenwald et al., 2016; Kawahara et al., 2012; Nyhan et al., 2014; O'Donoghue et al., 2007; Ramos et al., 2015). The airway inflammatory response is believed to be a central mechanism in the development of adverse health effects related to exposure to air pollution (Kelly and Fussell, 2011; Øvrevik et al., 2015; Salvi and Holgate, 1999). Reactive oxygen species and oxidative stress are involved in the airway inflammatory response during exposure to airborne particles (Øvrevik et al., 2015). Airborne particles are believed to trigger oxidative stress, resulting in systemic and pulmonary inflammatory responses (Kelly and Fussell, 2015; Salvi et al., 1999, 2000).

Because both physical activity and air pollution can independently affect lung function and markers of oxidative stress and airway inflammation, understanding the interplay between these two factors is necessary to interpret the effects of air pollution on lung function and oxidative stress in the presence of physical activity (Kubesch et al., 2015). In exploring the relationship between air pollution and lung function or oxidative stress in the airways, relatively few studies have investigated interactions between physical activity and air pollution, or have adjusted for the effects of physical activity. Among those that have done so, the results are conflicting. One study of adult hikers found that, adjusting for smoking status, asthma, hours hiked, and other covariates, for every 50 ppb increase in mean O_3 , there was a 2.6% decrease in forced expiratory volume in one second (FEV_1) and a 2.2% decrease in forced vital capacity (FVC) (Korrick et al., 1998). Exposure to high levels of fine particulate matter during exercise was associated with a decrease in FEV_1 and FEF_{25-75} and a non-significant decrease in exhaled nitric oxide (eNO); however, lung function did not change after exposure to low levels of fine particulate matter during exercise (Rundell et al., 2008). Another study showed that, although exposure to high concentrations of fine and ultrafine particulate matter during exercise was associated (non-significantly) with an immediate increase in FEV_1 and FVC; at 6 hours after exposure, participants showed a non-significant decrease in these same measures (Strak et al., 2010). Kubesch et al. employed a crossover design to disentangle the effects of physical activity and traffic-related air pollution (TRAP) on respiratory and inflammatory response. Their study examined each participant in four

conditions: either moderate exercise or rest in either low TRAP or high TRAP environments. The researchers concluded that air pollution and physical activity have independent effects. Exercise was associated with increases in FEV_1 , FVC, FEF_{25-75} , and eNO and systemic inflammation markers, independent of TRAP levels, and increases in coarse particulate matter were associated with increases in eNO (Kubesch et al., 2015).

One explanation for contradictory results among studies that examine physical activity, air pollution, and respiratory response is that many relied on measures of air pollution exposure. Yet, mechanisms between air pollution and pulmonary response may be more sensitive to the inhaled dose of air pollution than to ambient air pollution exposure alone. The inhaled air pollution dose varies based on ambient air pollution levels, individual physical characteristics, and breathing rate at the time of exposure (Cole-Hunter et al., 2012; Greenwald et al., 2016; Kawahara et al., 2012; Nyhan et al., 2014; O'Donoghue et al., 2007; Ramos et al., 2015). The relationship between physical activity, air pollution, and respiratory response is further complicated in that physical activity elevates the ventilation rate, increasing the inhaled dose of air pollutants as well as particle deposition in human lungs (McNabola et al., 2008; Oravissjärvi et al., 2011). Evaluation of the inhaled dose of air pollution rather than simply the exposure allows researchers to isolate and investigate possible interactions between physical activity and air pollution, and can provide better insight into the effects of each of these factors on respiratory response.

Only a few studies have examined the human respiratory response to inhaled doses of air pollution. For asthmatic children, Buonanno et al. estimated the dose-response relationship between daily alveolar deposited surface area dose of airborne particles and measures of spirometry and eNO, finding that a daily dose increase of 100 mm^2 was associated with a 4.1 ppb increase in eNO and a 0.8% decrease in FEF_{25-75} (Buonanno et al., 2013). A limitation of this study was that the inhalation rate used in the dose calculation was determined by use of U.S. EPA inhalation rate estimates for different daily activities, which were self-reported by the participants over several days. In a randomized controlled cross-over trial, Behndig et al. exposed, in randomized order several weeks apart, each exercising group to either diluted

diesel exhaust at a steady concentration of 100 $\mu\text{g}/\text{m}^3$ or to filtered air. After diesel exposure, there was an increase in glutathione (GSH) and an increase in airway inflammation in the bronchial airway and nasal lavage samples, but not in the alveolar lavage (Behndig et al., 2006). Although the researchers did not specifically measure the inhaled dose of air pollutants, they fixed the concentration of diesel exposure and the duration and intensity of exercise; differences in individual ventilation rates and physical characteristics that would affect dose were likely controlled by the randomized crossover design. While Rundell et al. and Kubesch et al. did not calculate an inhaled dose of air pollutants, both compared respiratory response after exercise during exposure to low and high TRAP environments, demonstrating dose-response relationships between air pollution and respiratory response (Kubesch et al., 2015; Rundell et al., 2008).

The present study aimed to characterize, among adolescents, the relationship between dose of inhaled air pollution, physical activity, and respiratory response. Respiratory response measures included eNO, percent oxidized exhaled breath condensate glutathione (%GSSG), percent oxidized exhaled breath condensate cystine (%CYSS), percent of total oxidized compounds (%Oxidized), and changes in pulmonary function, namely, forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and forced expiratory flow during the middle half of FVC maneuver (FEF_{25-75}). Air pollution measures included the inhaled dose of fine particulate matter ($\text{PM}_{2.5}$), ozone (O_3), and black carbon (BC), as well as the total particle number count in the inhaled dose (PNT). We hypothesized that interactions exist between physical activity and air pollution and that, when controlling for physical activity, increased inhaled doses of air pollutants would be associated with a decrease in measures of lung function; an increase in eNO; and, as GSH and CYS are oxidized during the course of exposure, increases in %GSSG, %CYSS, and %Oxidized.

METHODS

Institutional Review Board Approval

Approval for this study was provided by the Emory University Institutional Review Board and the Georgia State University Institutional Review Board.

Participants

A convenience sample of 126 students was recruited from two high schools. All participants were healthy and engaged in one or more extracurricular sports, including marching band, track and field, football, soccer, basketball, and cheerleading. Participants over the age of 18 provided written consent. Participants under the age of 18 provided written assent as well as written parental consent.

Setting

Data collection for this observational study was conducted at two high schools in Atlanta, GA. One high school was set in a wooded, suburban area; the other was set in an urban area close to major roadways. Recruitment took place between October 2012 and July 2014, and data were collected from December 2012 to July 2014.

Data Collection

Prior to beginning sports practice and for the duration of the practice session, participants were fitted with a chest strap that recorded continuous measurements of heart rate (HR), breathing rate (F_B), and motion. Spirometry was conducted prior to and after practice. Spirometry measures were FVC, FEV_1 , and FEF_{25-75} . Baseline and post-exposure measurements of eNO, GSH, GSSG, CYS, CYSS, and mixed disulfides (MD) were also taken. Throughout the practice session, ambient levels of $\text{PM}_{2.5}$, O_3 , BC, and particle number concentration (PNC) were monitored on site. The cumulative inhaled dose of each air pollutant was calculated by multiplying ambient levels of the air pollutant at each minute of participation by the participant's minute ventilation ($\dot{V}_E$) normalized to FVC, and summing the estimated dose for each minute. The method used for estimation of air pollution dose is described in more detail below.

Ambient air pollution levels, including $\text{PM}_{2.5}$, O_3 , BC, and PNC, were measured on site. All air pollution measures were converted to concentration/L taken in one-minute intervals. Ambient PNC was measured with a Hand-held Condensation Particle Counter Model 3007 (TSI Inc., Shoreview, MN). PNC was converted to the number of particles/L. Ambient $\text{PM}_{2.5}$ was measured with a Portable Laser Aerosolspectrometer and Dust Monitor, model 1.109 (Grimm Aerosol, Ainring, Germany). $\text{PM}_{2.5}$ was measured in $\mu\text{g}/\text{m}^3$ and converted to $\mu\text{g}/\text{L}$. Ambient O_3 was measured with a Model 49i Ozone Analyzer (Thermo scientific,

Waltham, MA). O₃ was measured in parts per billion and converted to µg/L. In the event that on-site ambient pollution measurements failed, one-minute ambient levels of PM_{2.5} and O₃ were collected from the Ambient Air Monitoring Network site closest to each school that engaged in continuous sampling of PM_{2.5} and O₃. These two monitoring stations, operated by the Georgia Environmental Protection Division, were located approximately 2 and 10 miles from the respective schools. Ambient BC was measured with a microAeth Model AE51 Aethalometer (AethLabs, San Francisco, CA). BC was measured in ng/m³ and converted to ng/L.

Continuous measurements of HR (beats per minute), F_B (breaths per minute), and activity level ("the vector addition of three dimensional acceleration expressed as a fraction of standard

gravity" (Greenwald et al., 2016)) were taken in one-second intervals by use of a chest strap with a physiological monitoring module, BioHarness™ 3 (Zephyr Technology Corporation, Annapolis, MD). These data were collected in real time by laptops on site. For use as a predictor, a cumulative activity level was estimated by averaging one-second intervals of activity level over the course of one minute, and summing the activity level for all minutes.

Minute ventilation in liters ($\dot{V}_E$) was estimated with a method developed by Greenwald et al. (Greenwald et al., 2016). The present study employed Greenwald's two-predictor model with HR and BR averaged over 30-second intervals to estimate a 30-second interval of $\dot{V}_E$ normalized to the participant's highest overall measurement of FVC:

$$\frac{\dot{V}_E}{FVC} = -4.2469 + (0.0595HR) + (0.2255BR)$$

30-second intervals of $\dot{V}_E$ normalized to FVC were then multiplied by the participant's highest overall measurement of FVC to produce a unique estimate of $\dot{V}_E$ for that 30-second interval. The 30-second intervals of $\dot{V}_E$ were averaged over one minute and multiplied by the ambient level of air pollution concentration per liter measured at that minute. An inhaled dose of air pollution was estimated for each minute a participant was engaged in sports practice. Minute pollution doses over the entire period were then totaled for each participant to produce a measure of the cumulative total air pollution dose (rather than the concentration) for each pollutant to test as predictors of respiratory response.

Participants provided non-invasive samples of breath condensate, which were tested for MD, GSH, GSSG, CYS, and CYSS by use of high performance liquid chromatography (HPLC) for exhaled breath condensate as described by Yeh et al., and originally developed for plasma samples by Jones et al. (Jones et al., 1998; Yeh et al., 2008). The percentage of oxidized glutathione was calculated as %GSSG = [GSSG / (GSSH + GSH)] x 100. Similarly, the percentage of cystine was calculated as %CYSS = [CYSS / (CYSS + CYS)] x 100. The percentage of total oxidized compounds was calculated as %Oxidized = [(GSSG + CYSS + MD) / (GSSG + CYSS + MD + GSH + CYS)] x 100.

Prior to performance of spirometry maneuvers, trained study staff measured eNO with a hand-held instrument, the NIOX MINO (Aerocrine, Morrisville, NC). Study staff were trained in spirometry test procedures according to guidelines from the American Thoracic Society. Staff guided participants as they performed 3 FVC maneuvers before and after each sports practice session using the EasyOne Plus handheld spirometer (ndd Medical Technologies Inc., Andover, MA). For each maneuver, study staff recorded FVC, FEV₁, and FEF₂₅₋₇₅.

Statistical Analyses

SAS 9.4 (SAS Institute Inc., Cary, NC) was used for all data analyses. The α level was set a priori to 0.05. Normality of outcome variables was checked visually. In the event that outcome variables did not approximate a normal distribution, natural log transformations were taken to approximate normality. Multicollinearity between predictors was tested and ruled out, first by examining bivariate correlations using Pearson's correlation coefficient and scatter plots, and second by regressing each predictor on all the others and examining tolerance and variance inflation factors as well as condition indices. Observations with missing data were assumed to be missing completely at random and were excluded from the analysis. For the outcome eNO, all values below five were outside the detectable range of the instrument. A sensitivity analysis was performed to assess the sensitivity of the multi-

pollutant model to different imputed values: 0.00001, 2.5, and 5, and “missing.” The three numerical values were selected to represent the range of possible values for these observations. For each model in the sensitivity analysis, a natural log transformation of eNO was taken after the single imputation at the specified level. For the final analysis, the nine values of eNO below the detectable limit were imputed with the value 2.5.

Data were analyzed with a general linear mixed model with an unstructured covariance matrix. To select the covariance structure, multi-pollutant models for two outcomes (log of eNO and log of %GSSG) were run with unstructured, compound symmetry and variance component covariance matrices. Covariance structures were compared by use of the Akaike Information

Criterion (AIC). The final models included a random intercept for subject to account for repeated measurements taken on each individual. Random slopes for the effect of time and time*occurrence to account for repeated measurements taken on each individual as well as the repeated participation of subjects during multiple practice sessions were tested and left out of the model due to estimability problems. Separate models were constructed for each outcome. To evaluate the change between pre and post measurements, all models included fixed effects for each air pollutant dose*time and activity*time. All models were controlled for body mass index (BMI), sex, and age. The basic multi-pollutant model for each outcome contained terms for PM_{2.5}, O₃, and PNT, but not BC. The basic multi-pollutant model was as follows:

$$Y_i = \beta_0 + \beta_1(PM_{2.5}dose)_i + \beta_2(PNTdose)_i + \beta_3(O_3dose)_i + \beta_4(activity)_i + \beta_5(time)_i + \beta_6(PM_{2.5}dose*time)_i + \beta_7(PNTdose*time)_i + \beta_8(O_3dose*time)_i + \beta_9(activity * time) + \beta_{10}(sex)_i + \beta_{11}(age)_i + \beta_{12}(BMI)_i + \gamma_i + \varepsilon$$

For each multi-pollutant model, interaction terms between activity level, time, and each type of air pollution were tested individually in this multivariable model and retained in the model only if the interaction term was significant.

In addition, single pollutant models were constructed for each outcome and compared to multi-pollutant models. Single pollutant models, as follows, were constructed separately for each pollutant, including BC:

$$Y_i = \beta_0 + \beta_1(dose)_i + \beta_2(activity)_i + \beta_3(time)_i + \beta_4(dose*time)_i + \beta_5(activity*time)_i + \beta_6(sex)_i + \beta_7(age)_i + \beta_8(BMI)_i + \gamma_i + \varepsilon_i$$

Because a single unit change in air pollution dose is relatively small, and the interpretation of a change this small holds little practical value, final results were presented as the change from baseline in outcome measurement per

interquartile range (IQR) increase in inhaled dose or activity level (Δ). For natural log transformed outcomes, estimates were presented as a percent change and were calculated as

$$\Delta = [exp^{\beta_{time} + \beta_{dose*time} \times IQR} - 1] \times 100\%$$

where β_{time} was the coefficient estimate for time of outcome measurement (pre or post, coded as 0,1) in the mixed model, $\beta_{dose*time}$ was the coefficient estimate for the dose by time

interaction, and the IQR was that of the predictor in question. For non-transformed outcomes, estimates were presented as an absolute change and calculated as

$$\Delta = \beta_{time} + \beta_{dose * time} \times IQR$$

RESULTS

Participant characteristics are presented in Table 1.

Table 1. Participant characteristics

Characteristics		n (%)	Missing n (%)
Sex			
	Female	41 (32.54)	0

Characteristics		n (%)	Missing n (%)
	Male	85 (67.46)	0
Race			
	Black	122 (96.83)	0
	Hispanic	4 (3.17)	0
School			
	Rural	68 (53.97)	0
	Urban	58 (46.03)	0
Age; Mean(SD);		16.38 (1.34)	3 (0.023%)
BMI Median (IQR)		23.53 (20.93, 25.90)	1 (0.008%)
Abbreviations: BMI, body mass index; IQR, interquartile range; n, number; SD, standard deviation			

A total of 126 participants were recruited to and included in the study. The average age of participants was 16 years and 4.5 months (16.38 $\pm$ 1.34). For males, the average age was 16.49 ($\pm$ 1.37), and, for females, was 16.16 ($\pm$ 1.28). A total of 85 (67.46%) participants were male, and 41 (32.54%) were female. 122 (96.83%) participants were Black; the remaining 4 (3.17%) were Hispanic. The median BMI among all

participants was 23.53 (IQR 20.93-25.90). Among females, the median BMI was 22.33 (IQR: 20.27-24.56); among males, it was 23.54 (IQR: 21.57-26.21). All participants were non-smokers. No participants had a current physician's diagnosis of asthma. A summary of participant air pollution doses and activity levels is presented in Table 2.

Table 2. Air pollution dose and activity level characteristics

Predictor (unit)	Median (IQR)	Missing n(%)
PM _{2.5} Dose (μ g)	34.33 (19.74-50.72)	29 (11.74%)
PNT Dose (1E+7 particles)	1788.04 (1015.74-3384.07)	42 (17%)
O ₃ Dose (μ g)	249.8 (56.05-401.7)	44 (17.81%)
BC Dose (ng)	1340.8 (883.35-2562.9)	64 (25.91%)
Activity Total	28.474 (20.17-35.16)	29 (11.74%)

A summary of outcome characteristics at baseline and follow-up is presented in Table 3.

Table 3. Outcome characteristics

Outcome	Baseline	Missing n(%)	Follow-up	Missing n (%)
eNO ; Median (IQR)	18 (12-33)	1 (0.4%)	18 (11-32)	21 (8.5%)
Log of eNO; Mean (SD)	2.98 (0.83)	1 (0.4%)	2.94 (0.83)	21 (8.5%)
GSSG; Median (IQR)	0.41 (0.13-1.3)	115 (46.6%)	0.66 (0.17-2.28)	129 (52.2%)
%GSSG; Median (IQR)	1.94 (0.93-3.59)	117 (47.4%)	2.34 (1.1-5.16)	129 (52.2%)
Log of %GSSG; Mean (SD)	0.52 (1.11)	117 (47.4%)	0.70 (1.28)	129 (52.2%)
CYSS; Median (IQR)	0.97 (0.62-1.57)	115 (46.6%)	1.15 (0.71-1.79)	129 (52.2%)
%CYSS; Median (IQR)	74.26 (42.09-82.71)	115 (46.6%)	59.87 (27.09-82.97)	129 (52.2%)
Log of %CYSS; Median (IQR)	4.31 (3.74-4.42)	115 (46.6%)	4.09 (3.3-4.42)	129 (52.2%)

Outcome	Baseline	Missing n(%)	Follow-up	Missing n (%)
Log of %Oxidized; Mean (SD)	2.2 (0.68)	115 (46.6%)	2.19 (0.62)	129 (52.2%)
FEF ₂₅₋₇₅ ; Mean (SD)	3.77 (1.13)	41 (17%)	3.58 (1.09)	50 (20.2%)
FEV ₁ ; Mean (SD)	3.27 (0.66)	44 (17.8%)	3.21 (0.63)	55 (22.3%)
FVC; Mean (SD)	3.75 (0.75)	41 (17%)	3.72 (0.73)	49 (19.8%)

Missing Data

Missing data are reported in Tables 1, 2, and 3. For missing values of air pollution dose measurements, covariate values were not measurable as a result of instrument error. Missing values of %GSSG, %CYSS, and %Oxidized were a result either of contamination of the sample or because of a failure to collect the minimum amount of exhaled breath condensate necessary for analysis. Missing values of spirometry measures were a result of measurement error. The numbers of observations analyzed in each model are presented in Tables 5 (Appendix) and 6.

Multicollinearity Testing

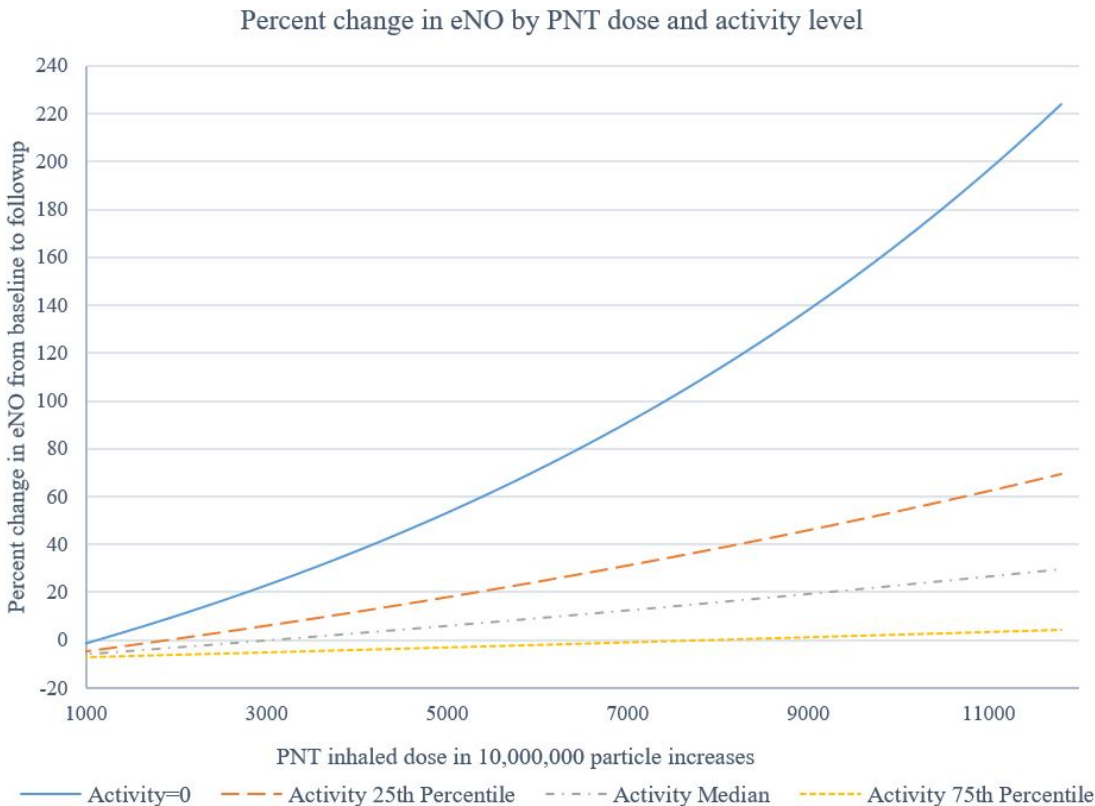
The highest bivariate correlation between any two predictors, PM_{2.5} and O₃, was $r=0.67$. The lowest tolerance level was 0.35, with a variance inflation factor of 2.85. No condition indices were higher than 5 when adjusting out the intercept using the “collinoit” option in SAS.

Multi-pollutant General Linear Mixed Models

The results of all multi-pollutant models are presented in Table 4 in the Appendix.

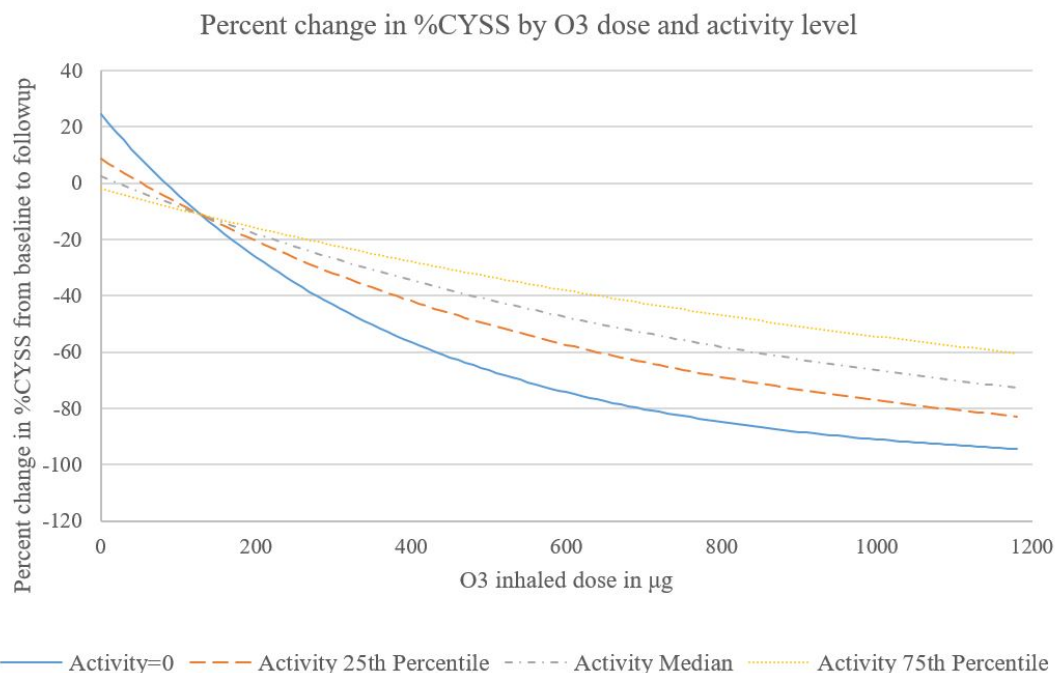
Significant associations were seen between O₃ and %Oxidized, and PNT and FEF₂₅₋₇₅. A one IQR (i.e., 345.64 µg) increase in O₃ inhaled dose was associated with a 29.16% average decrease from baseline in the percentage of total oxidized compounds. A one IQR (i.e., 23,683,300,000 particle) increase in PNT inhaled dose was significantly associated with an average decrease in FEF₂₅₋₇₅ of 0.168 L/second from baseline. A statistically significant association was also seen between PNT and eNO; however, this association was attenuated by activity level. At a total activity level of zero, a one IQR (i.e., 23,683,300,000 particle) increase in PNT inhaled dose was associated with an average increase in eNO of 14.77% above baseline; at the 25th quartile activity level of 20.17, a one IQR increase in PNT was associated with a smaller, 2.59%, increase in eNO. As activity levels rose, the relationship between PNT and eNO became negative. At the median activity level of 28.474, a one IQR increase in PNT was associated with a 2.05% decrease in eNO, and, at the 75th quartile of activity level, 35.15, PNT was associated with a decrease of 5.62% in eNO. A graphical depiction of this relationship is in Figure 1.

Figure 1. The relationship between PNT and eNO is moderated by activity level



Similarly, the relationship between O_3 and %CYSS was attenuated by activity level, with increasing activity levels corresponding to smaller changes from baseline for a constant level of O_3 . When the activity level was zero, an IQR change of 345.64 μg O_3 was associated

with a 49.81% decrease in %CYSS. However, at the 25th quartile of activity level, the decrease was 36.71%, and, at the 75th percentile of activity level, a 24.81% decrease from baseline was seen for %CYSS. See Figure 2 for a depiction of this relationship.

Figure 2. The relationship between O3 inhaled dose and %CYSS is moderated by activity level

Single Pollutant General Linear Mixed Models

The results of all single pollutant models are presented in Table 5 in the Appendix.

In single pollutant models, significant relationships were observed between different types of air pollution doses and %CYSS, %Oxidized, FEF₂₅₋₇₅, and FEV₁. A one IQR increase in PM_{2.5} inhaled dose (i.e., 30.97 µg) was associated with a 6.9% decrease in %CYSS and a 9.68% increase in %Oxidized. However, at inhaled dose levels of PM_{2.5} greater than 41 µg, the relationship between PM_{2.5} and %Oxidized became negative. A one IQR increase in PNT (i.e., 23,683,300,000 particles) was associated with a 0.179 L/second decrease in FEF₂₅₋₇₅. A one IQR increase in ozone inhaled dose (i.e., 345.64 µg) was associated with a 31.42% decrease in %CYSS and an 18.16% decrease in %Oxidized. A one IQR increase in BC (i.e., 1680 ng) was associated with a 23.35% decrease in %CYSS, a 12.67% decrease in %Oxidized, and a 0.028L decrease in FEV₁.

Sensitivity Analyses

During data collection, nine observations of eNO were flagged as below the detectable limit. In multi-pollutant models, significant coefficient estimates for PNT*Time using imputed values of 2.5 and 5 were not significant for imputed values of 0.00001 and missing. Significant coefficient

estimates for PNT*Activity*Time using an imputed value of 2.5 were not significant for imputed values of 0.00001, 5, and missing. Differences in effect size and standard error were noted for models using an imputed value of 0.00001.

DISCUSSION

This study has several limitations that warrant consideration and suggest that the results should be interpreted with caution. First, the non-probability sample is not representative of the general population of adolescents in the U.S., thus the results are not generalizable to all healthy adolescents. Second, the data collection process for the Study of Air Pollution and Physical Activity is ongoing, and the study has not yet reached its intended sample size. Thus, this analysis may be underpowered. Third, due to the difficulty of measuring multiple outcomes quickly among energetic adolescents in a field setting, as well as repeated failures of air quality monitoring equipment, much of the data are missing. While the missingness of the data is unlikely to be correlated with either the predictors or the outcomes, with the exception of observations where eNO is below the detectable limit, there is a possibility that excluding observations with missing data could have introduced bias. Furthermore, missing data may have been the source of estimability problems of

the models with random effects for time and time*occurrence. Not including these random effects in the final models may have underestimated the standard error and inflated the possibility of type one error. Fourth, while a single imputation of the value 2.5 is unlikely to approximate well the actual distribution of values of eNO below the detectable limit, leaving these values as missing would ignore information about the nature of their missingness and would bias the results towards the null. The value 2.5 represents a best guess, avoiding extremes within the possible range of real values. Given the sensitivity of the eNO model to different imputed values of eNO, the results of this model should be interpreted with caution. In light of these limitations and the present findings, we offer five considerations:

First, the lack of significant relationships between air pollution and %GSSG in either multi-pollutant models or single pollutant models is consistent with similar findings, which showed that, in mouse models, combined exposure to diesel exhaust particles and house dust mite allergens had significant effects on the CYS redox state but no effect on the GSH redox state, which suggests that the CYS redox state may be a better biomarker for oxidative stress induced by diesel exhaust particles and allergens (Lee et al., 2013).

Second, the presence of unmeasured factors could have affected the results. The study by Lee et al. suggests that diesel exhaust particles alone do not significantly alter the redox balance in mice, but that, in combination with allergens, diesel exhaust induces oxidative stress and may amplify the cellular inflammatory response (Lee et al., 2013). The present study did not measure or control for the presence of allergens. Thus, the possibility of a synergistic relationship between allergens and pollution exposure could introduce bias. Another unmeasured factor that may lead to variability in redox status after exposure to particulate matter is the oxidative potential of the specific mix of particles inhaled at the time of exposure. Several studies have demonstrated that, for a given mass concentration of particulate matter, the oxidative potential can vary according to the composition, particularly the presence of redox-active metals, which is affected by proximity to roadways and other sources of particulate pollution (Godri et al., 2011; Janssen et al., 2014; Kelly and Fussell, 2015).

Third, although the associations between pollutant dose and markers of oxidative stress are the opposite of what was hypothesized, the negative relationship between air pollution and percent of oxidized compounds may signal the predominance of a protective antioxidant response to oxidative stress induced by increasing O₃ dose (Kelly and Fussell, 2015). These findings are consistent with other research showing, in mice, a nonsignificant increase in CYS and a corresponding decrease in %CYSS after exposure to diesel exhaust compared to exposure to saline (Lee et al., 2013). Similarly, Behndig et al. observed an early adaptive increase in the antioxidant GSH in both the bronchial and the alveolar compartments within six hours of exposure to diesel exhaust. This increase in antioxidants was subsequently overwhelmed and followed by the development of an inflammatory response, within the bronchial lavage, but not in the alveolar lavage (Behndig et al., 2006). The authors offer the explanation that, within the alveolar compartment, deeper into the airway, the tissue particle doses are lower, and thus the adaptive antioxidant response of cells can cope with the onslaught of oxidants, demonstrating a dose threshold for respiratory response to diesel exhaust (Behndig et al., 2006).

Fourth, with a few exceptions, single pollutant and multi-pollutant models reflected similar significant relationships between air pollutant inhaled doses and outcomes, though varying slightly in effect size. PM_{2.5} showed significant relationships with markers of oxidative stress in single pollutant models, but not in multi-pollutant models, perhaps reflecting that, in the single pollutant model, the relationship between PM_{2.5} and oxidative stress is confounded by O₃. The degree of correlation between PM_{2.5} and O₃ is moderate, with Pearson's $r=0.67$. This suggests that multi-pollutant models may be more appropriate for evaluating the separate effects of each air pollutant, holding all other pollutant levels constant. However, this information comes with a cost, namely, the increased number of parameters in multi-pollutant models sacrifices power and increases the chance of a type II error. Thus, it is also possible that both types of air pollution have relationships with oxidative stress, but we were not able to measure them. Furthermore, in the multi-pollutant models explored in this analysis, BC was not included because of the large amount of missing data for this pollutant. In addition to BC, other types of air pollutants and

interactions between pollutants were not examined. Future research that is adequately powered to examine a wider range of pollutants and interactions between pollutants in a single multivariable model would help to determine the individual effects of each pollutant.

Fifth, in the present study, an increase in PNT was associated with an apparent increase in the antioxidant CYS and with airway inflammation marked by an increase in eNO, suggesting that high doses may have overwhelmed the antioxidant response. The present study considered only the total dose over a period of several hours, and, as such, ignored variability in dose concentration over the exposure period. However, someone who inhales a high cumulative dose despite a low activity level is likely breathing in a higher concentration of air pollution in a shorter period of time than a person who receives the same dose with a high activity level. Thus, the differences seen according to activity level may reflect differences in dose concentration over time. The moderating effects of activity level on eNO and %CYSS suggest that peaks of high concentration inhaled doses of air pollution may overwhelm the endogenous redox balance of cells, resulting in increased airway inflammation. Further research that examines the relationships between dose peaks at the minute level and oxidative stress and inflammation over time could help to determine whether a high concentration dose over a short period of time has a different effect than a lower concentration dose over a longer period of time.

CONCLUSIONS

We hypothesized that interactions exist between physical activity and air pollution, and that, when controlling for physical activity; increased inhaled doses of air pollutants would be associated with a decrease in measures of lung function; an increase in eNO; and an increase in the %GSSG, %CYSS, and %Oxidized, as GSH and CYS are oxidized during the course of exposure. In keeping with the hypothesis, we found that, in both single and multi-pollutant models, an increase in the particle number total in the inhaled dose (PNT) was associated with a decrease in lung function, FEF₂₅₋₇₅, and, in multi-pollutant models only, an increase in airway inflammation marked by eNO. Furthermore, we saw that, in multi-pollutant models, the relationship between PNT and eNO, as well as the relationship between O₃ and

%CYSS were attenuated by activity level. Contrary to our hypothesis, in multi-pollutant models, an increased inhaled dose of O₃ was associated with a decrease in %CYSS and %Oxidized. Likewise, in single pollutant models, increasing inhaled doses of O₃ and BC were associated with a decrease in %CYSS and %Oxidized. An increasing inhaled dose of PM_{2.5}, however, was associated with a decrease in %CYSS, but attenuated an increase in %Oxidized, and, at doses higher than 41 µg, was associated with a decrease in %Oxidized. In multi-pollutant models, no significant relationships were found between any type of air pollution and %GSSG, FEV₁, or FVC. In single pollutant models, BC was associated with a decrease in FEV₁.

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APPENDIX

Table 4. Multi-pollutant Models*Associations between air pollution, physical activity, and respiratory response*

Outcome	Predictor	Estimated β Coefficient	Standard error	95% CI		Δ for IQR increase in dose	P- value
Log of eNO (n=369*)							
	time	-0.1227	0.1317	-0.3820	0.13660	--	0.352
	PM _{2.5} Dose x time	0.000616	0.001988	-0.0033	0.00453	-9.84%	0.757
	PNT Dose x time	0.00011	0.000047	1.8E-05	0.00020	14.77% [†]	0.019
	O ₃ Dose x time	-0.00008	0.000226	-0.0005	0.00036	-13.96%	0.719
	Activity Level x time	0.001112	0.004786	-0.0083	0.01054	10.06%	0.817
	PNT Dose x Activity x time	-0.00000282	1.424E- 06	-6E-06	-1E-08	--	0.049
Log of %GSSG (n=201*)							
	time	-0.187	0.4881	1.15410	0.78010	--	0.702
	PM _{2.5} Dose x time	0.009758	0.01126	0.01255	0.03206	12.21%	0.388
	PNT Dose x time	-0.00016	0.000102	0.00036	4.1E-05	43.21%	0.116
	O ₃ Dose x time	-0.00074	0.001184	0.00308	0.00161	35.77%	0.535
	Activity Level x time	0.019	0.01726	0.01520	0.05319	10.27%	0.273
Log of %CYSS (n=203*)							
	time	0.2196	0.2348	0.24550	0.68480	--	0.352
	PM _{2.5} Dose x time	0.00102	0.004131	0.00716	0.00920	28.56%	0.805
	PNT Dose x time	0.000027	0.000038	0.00005	0.00010	32.78%	0.480
	O ₃ Dose x time	-0.00263	0.000971	0.00455	-0.0007	49.81% [†]	0.008
	Activity Level x time	-0.00682	0.00883	0.02431	0.01067	12.46%	0.441
	O ₃ Dose x Activity x time	0.000053	0.000025	3.4E-06	0.00010	--	0.036
Log of %Oxidized (n=203*)							
	time	0.1668	0.2915	0.41050	0.74410	--	0.568
	PM _{2.5} Dose x time	0.004897	0.006613	0.00820	0.01800	37.50%	0.461
	PNT Dose x time	-0.00004	0.000062	0.00016	0.00009	7.47%	0.551
	O ₃ Dose x time	-0.00148	0.000717	0.00290	-6E-05	29.16%	0.041
	Activity Level x time	0.002249	0.01044	0.01844	0.02294	22.20%	0.830

Outcome	Predictor		Estimated β Coefficient	Standard error	95% CI		Δ for IQR increase in dose	P- value
FEF ₂₅₋₇₅ (n=317*)								
	time		-0.00228	0.2444	-0.48410	0.47960	--	0.993
	PM _{2.5} Dose x time		-0.00252	0.004778	-0.01194	0.00690	-0.0803	0.598
	PNT Dose x time		-0.00007	0.000036	-0.00014	-1E-06	-0.168	0.047
	O ₃ Dose x time		0.00005	0.000575	0.00108	0.00118	0.015	0.930
	Activity Level x time		0.004851	0.008432	0.01177	0.02148	0.07042	0.566
FEV ₁ (n=310*)								
	time		-0.00204	0.09936	-0.19800	0.19390	--	0.984
	PM _{2.5} Dose x time		0.0013	0.001935	-0.00252	0.00512	0.03823	0.502
	PNT Dose x time		0.000001272	0.000014	-0.00003	0.00003	0.00097	0.930
	O ₃ Dose x time		-0.00023	0.000232	-0.00069	0.00023	-0.0815	0.323
	Activity Level x time		-0.00072	0.003417	-0.00746	0.00602	-0.0128	0.833
FVC (n=318*)								
	time		-0.07345	0.09686	-0.26440	0.11750	--	0.449
	PM _{2.5} Dose x time		0.001413	0.001896	-0.00233	0.00515	-0.0297	0.457
	PNT Dose x time		0.000019	0.000014	-0.00001	0.00005	-0.0285	0.193
	O ₃ Dose x time		-0.0002	0.000228	-0.00065	0.00025	-0.1426	0.382
	Activity Level x time		0.000437	0.003345	-0.00616	0.00703	-0.0669	0.896

Observations with missing data were excluded from the analysis. For each outcome, the model includes terms for all predictors listed beneath the outcome as well as sex, age and BMI.

*n represents the number of measurements included in the analysis out of 494 total measurements.

† For models that include dose x activity x time interactions, the dose x time interaction can only be interpreted as the effect of dose on change in outcome when activity level is zero.

Abbreviations: %CYSS, percent oxidized cysteine; %GSSG, percent oxidized glutathione; %Oxidized, total percent oxidized of measured antioxidants; BMI, body mass index; CI, confidence interval; CYSS, cystine; eNO, exhaled nitric oxide; FEF₂₅₋₇₅, forced expiratory flow; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; GSSG, glutathione disulfide; IQR, interquartile range; O₃, ozone; n, number; PM_{2.5}, particulate matter 2.5; PNT, particle number total; SE, standard error;

Table 5. Single Pollutant Models*Associations between air pollution and respiratory response*

Outcome	Predictor	n*	Estimated β Coefficient	Standard error	95% CI		Δ for IQR increase in dose	P-value
Log of eNO								
	PM _{2.5} Dose x time	418	0.000147	0.001	-0.0020	0.002324	10.37	0.894
	PNT Dose x time	392	0.000022	1E-05	-4.35E-06	0.00005	12.13	0.102
	O ₃ Dose x time	395	0.000057	1E-04	-0.00021	0.000326	10.07	0.675
	BC x time	348	0.000015	1E-05	-0.00001	0.000042	8.36	0.274
Log of %GSSG								
	PM _{2.5} Dose x time	222	-0.00344	0.007	-0.01673	0.009847	-7.96	0.609
	PNT Dose x time	218	-0.00018	9E-05	-0.00036	1.29E-06	-49.21	0.052
	O ₃ Dose x time	205	-0.00104	7E-04	-0.00247	0.000392	-17.03	0.153
	BC x time	183	-0.00019	2E-04	-0.00053	0.000145	-26.52	0.262
Log of %CYSS								
	PM _{2.5} Dose x time	224	-0.00709	0.003	-0.01218	-0.002	-6.90	0.007
	PNT Dose x time	221	-0.00002	4E-05	-0.0001	0.000046	-14.73	0.495
	O ₃ Dose x time	206	-0.00087	3E-04	-0.0014	-0.00035	-31.42	0.001
	BC x time	184	-0.00014	7E-05	-0.00028	-5.98E-07	-23.35	0.049
Log of %Oxidized								
	PM _{2.5} Dose x time	224	-0.00808	0.004	-0.01544	-0.00072	9.68	0.032
	PNT Dose x time	221	-0.00009	5E-05	-0.0002	0.000011	-12.38	0.08
	O ₃ Dose x time	206	-0.00135	4E-04	-0.00221	-0.0005	-18.16	0.002
	BC x time	184	-0.00023	9E-05	-0.00041	-0.00004	-12.67	0.017
FEF ₂₅₋₇₅								
	PM _{2.5} Dose x time	362	-0.00116	0.003	-0.00633	0.003997	-0.158	0.657
	PNT Dose x time	339	-0.00006	3E-05	-0.00011	-7.86E-06	-0.179	0.025
	O ₃ Dose x time	340	-0.00033	3E-04	-0.00101	0.000353	-0.227	0.345
	BC x time	311	-0.00005	3E-05	-0.00011	0.000011	-0.263	0.106
FEV ₁								
	PM _{2.5} Dose x time	355	-0.00023	0.001	-0.0023	0.001839	0.019	0.826
	PNT Dose x time	332	-5.68E-06	1E-05	-0.00003	0.000016	-0.004	0.599
	O ₃ Dose x time	333	-0.00005	1E-04	-0.00032	0.000229	0.012	0.746
	BC x time	304	-0.00002	1E-05	-0.00005	-8.63E-07	-0.028	0.042
FVC								
	PM _{2.5} Dose x time	363	-0.00006	0.001	-0.00207	0.001945	-0.0026	0.95
	PNT Dose x time	340	0.00001	1E-05	-0.00001	0.000031	-0.0146	0.339
	O ₃ Dose x time	341	0.000028	1E-04	-0.00024	0.000296	-0.0035	0.836
	BC x time	311	-0.00002	1E-05	-0.00004	7.32E-06	-0.0590	0.181

Observations with missing data were excluded from the analysis. For each outcome, four separate models were run. The models include the single pollutant predictor term listed as well as activity level, sex, age and BMI.

*n represents the number of measurements included in the analysis out of 494 total measurements.

Abbreviations: %CYSS, percent oxidized cysteine; %GSSG, percent oxidized glutathione; %Oxidized, total percent oxidized of measured antioxidants; BC, black carbon; BMI, body mass index; CI, confidence interval; CYSS, cystine; eNO, exhaled nitric oxide; FEF₂₅₋₇₅, forced expiratory flow; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; GSSG, glutathione disulfide; IQR, interquartile range; O₃, ozone; n, number; PM_{2.5}, particulate matter 2.5; PNT, particle number total; SE, standard error;