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Short-term Exposure to Ambient Air Pollution and Acute Cardiorespiratory Biomarker Response in a Panel of Adults in Atlanta, GA

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By

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Abstract

Short-term Exposure to Ambient Air Pollution and Acute Cardiorespiratory Biomarker Response in a Panel of Adults in Atlanta, GA

By Cheryl Cornwell

***Background**:* Short-term exposure to ambient air pollution has been linked to numerous, adverse cardiorespiratory health outcomes. A growing body of research supports the hypothesis that acute, cardiorespiratory response is linked to inflammatory and oxidative stress mediated pathways. However, uncertainty remains around the specific biological mechanisms underlying these health effects.

***Aims*:** The aim of this study is to examine associations between short-term changes in ambient air pollutants and acute changes in cardiorespiratory biomarkers of inflammation and oxidative stress in a panel of adults with and without asthma. A secondary aim is to explore potential differences in the exposure-response relationship by asthma status and other subject-specific characteristics.

***Methods**:* This analysis uses baseline, biomarker data from the Atlanta Commuter Studies (ACE-1 and ACE-2), as well as ambient concentrations of NO_2, $PM_{2.5}$, and O_3 from a stationary ambient monitoring site located in Atlanta, GA. Mixed-effect linear models were used to explore associations between pollution concentrations and changes in biomarker levels. Associations were examined using several exposure windows, including the 1-day, 3-day and 7-day pollutant concentration averages prior to subjects' biomarker measurements.

***Results**:* Positive associations were found primarily between NO_2 and the endpoints eNO, CRP and SAA at the prior 1-day exposure window, as well as at the prior 3-day exposure average with CRP. No significant interaction was detected by asthma status in any of the models.

***Conclusions**:* This analysis builds on evidence from previous studies that have also found positive associations between short-term changes in ambient air pollution and markers of inflammation and oxidative stress. Further research should be conducted to confirm these results and to carry out additional analysis on potential interactions by asthma status and other factors.

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INTRODUCTION

Short-term exposure to air pollution has been linked to a variety of adverse cardiorespiratory health outcomes (Brook et al., 2010; Dockery et al., 1994; Mustafic et al., 2012). Studies have documented associations between daily pollution levels and increases in daily mortality, as well as in the number of asthma and other respiratory disease-related emergency visits and hospital admissions (Sava et al., 2012; Strickland et al., 2010; Tolbert et al., 2000). The American Heart Association (AHA) has concluded, based on a comprehensive review of evidence from time-series studies, that short-term elevations in daily PM concentrations increase the relative risk for daily cardiovascular mortality (Brook et al., 2010). The studies included in the AHA's review also linked increased rates of cardiovascular hospitalizations to daily changes in PM air pollution and noted significant differences observed between geographic regions of the risk relationship.

While recent findings support the underlying biological plausibility of observed air pollution related health effects (Brook et al., 2010), uncertainty remains concerning the specific mechanisms and modes of action underlying response. Broadly, a growing body of research supports the hypothesis that acute cardiorespiratory response is linked to inflammatory and oxidative stress mediated pathways. A number of studies have explored the association of different biomarkers, consistent with inflammation and oxidative stress, and exposure to air pollution (Chuang et al., 2007; Delfino et al., 2006; Delfino et al., 2009; Ghio et al., 2003; Hertel et al., 2010; Pope et al., 2004; Wu et al., 2012). These studies have reported positive associations between air pollution and several of these markers, including increases in C-reactive protein with increases in PM_{10}

(Chuang et al., 2007), $PM_{2.5}$ (Pope et al., 2004), particle number (Hertel et al., 2010), sulfate, nitrate and ozone (Chuang et al., 2007); tumor necrosis factor-α with $PM_{2.5}$ components (Wu et al., 2012); and fibrinogen with exposure to concentrated air particles (Ghio et al., 2008). The specific biomarkers and exposure metrics examined, the populations studied, and the relationships observed, including lagged response periods, differ substantially by study. More research is needed to determine the specific biological pathways most consistently associated with exposure - at different levels of exposure and among different populations.

Panel studies have proven useful in providing evidence of associations between short-term exposures and corresponding acute health effects, given their ability to provide highly accurate characterizations of both real-world exposure and response at the individual level. Several panel studies have contributed to knowledge in regard to mechanistic pathways elicited following short-term air pollution exposures. For example, a 2007 study of young, healthy students in Taiwan found positive associations of urban air pollution with markers of inflammation and oxidative stress, looking at pollutant averages over a 1-3 day period (Chuang et al., 2007). Additional panel studies have reported associations with acute respiratory endpoints among asthmatic children (Sarnat et al., 2012), and inflammatory markers among individuals with a pre-existing respiratory disease (Hildebrandt et al., 2009).

This current analysis includes a panel of 99 adults living in Atlanta, Georgia, half of who were previously diagnosed with asthma and half of who are healthy adults. This panel study is an opportunistic, secondary analysis of data collected from the Atlanta Commuters Exposure (ACE) panels; two studies conducted to examine the relationship

between car commuting and acute cardiorespiratory response. The aim of the current sub-analysis is to examine associations between ambient air pollution, measured at a stationary ambient site in Atlanta, GA, and cardiorespiratory biomarkers of inflammation and oxidative stress in the ACE participants, at their pre-commute, baseline measurement periods. It includes analysis of several different biological endpoints and ambient pollutant concentrations averaged over several, relevant exposure windows prior to the measurement period.

METHODS

Study Population

Data for this analysis is comprised of a subset of measurements collected originally, as part of the first and second Atlanta Commuters Exposure panel studies (ACE-1 and ACE-2, respectively) in Atlanta, Georgia (Greenwald et al., 2014; Sarnat et al., 2014; Mirabelli et al., 2015). Participant samples for the ACE-1 study were collected from December 2009 - April 2011 and for the ACE-2 study from May 2012- August 2013. Subjects were recruited largely through word of mouth and flyers posted at Emory University and the Centers for Disease Control and Prevnetion (CDC). Subjects were restricted to those living within a 15 minute drive from the laboratory facility at Emory University.

The total combined panel used in the present analysis includes 99 adults from the two studies (42 from ACE-1 and 60 from ACE-2). Of these, 50 were healthy adults and 49 had mild to moderate asthma. A participant was defined as having asthma if he/she self-reported ever having been diagnosed with asthma by a health care provider.

Individuals were excluded from the ACE studies if they smoked, were pregnant, or had any of the following: diabetes, a previous myocardial infarction, non-asthma pulmonary disease, any type of lung cancer, implantable cardioverter-defibrillators or pacemakers, used digoxin or beta blockers to treat hypertension or arrhythmias, or a forced expiratory volume in 1 s (FEV_1) less than 70% predicted at baseline.

Biomarker Measurements

In the orginal ACE studies, each study participant completed two exposure sessions, consisting of either a highway or surface street commute, or a controlled clinic visit. Biomarker measurements, conducted at laboratory facilities at Emory University, were taken at baseline, prior to these sessions, as well as during and after the exposure sessions. The current analysis examines associations between ambient air pollution and the baseline measurements, exclusively. Two baseline biomarker measurements were taken per subject, corresponding to each of their two sessions, for a total of 198 observations used in the present analysis. Biomarkers selected for the present analysis include those that were collected on subjects in both ACE study protocols and include, exhaled nitric oxide (eNO), forced expiratory volume in 1 second (FEV_1), and forced vital capacity (FVC). In addition, several biomarkers of systemic inflammation were analyzed in plasma for ACE-1 and in dried blood spot for ACE-2, including, C-reactive protein (CRP), serum amyloid A (SAA), interleukin 1 (IL-1β), tumor necrosis factor alpha (TNF-α), soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular adhesion molecule-1 (sVCAM-1). Interleukin 6 (IL-6) was also measured in both ACE studies, but was not included in the present analysis due to the presence of a large number of zero values for this endpoint. These specific biomarkers were selected as

markers of acute cardiorespiratory response consistent with current understanding of oxidative-stress and inflammatory mediated pathways (Brook et al., 2010; Ghio et al., 2003; Hertel et al., 2010).

Sampling methods for these markers have been described, in detail, elsewhere (Sarnat et al., 2014). Biomarker measurements were conducted on all subjects from both ACE studies at the clinic at roughly 6:30 AM. Prior to collection of each baseline biomarker measurement, subjects completed a questionnaire that collected information on age, gender, race, education, home and work addresses, and recent health status.

Ambient Air Pollution Measurements

For the current analysis, ambient pollutant measurements, from a stationary ambient monitoring site, were used as a primary exposure metric as a surrogate of individual air pollution exposure. Here, ambient measurements were used from the Jefferson Street air quality station in Atlanta, GA, a site used in our previous analyses examining short-term exposures to air pollution and acute health response (Darrow et al., 2009; Sarnat et al., 2010; Strickland et al., 2010; Tolbert et al., 2000). The pollutants examined in the study include $PM_{2.5}$ (particles ≤ 2.5 μm in aerodynamic diameter), ozone, and nitrogen dioxide. For $PM_{2.5}$ concentrations, a 24-hr average was used ($\mu g/m^3$) as a primary exposure averaging time; for ozone, an 8-hr max (ppb); and for nitrogen dioxide, the 1-hr max (ppb). These three pollutants were selected to provide a range of pollutants with local, regional, and mixed source contributions.

To explore the relationship between ambient pollution levels and corresponding changes in biomarker levels in subjects, we examined associations using several relevant exposure windows, including pollutant concentrations on the day prior to the day of each

subject's biomarker measurement, as well as the three-day and seven-day averages of each pollutant immediately prior to each subject's biomarker measurement. The dates of pollutant concentrations, therefore, ranged over the same period as the biomarker measurements, from December 2009 to August 2013. Additional air quality parameters used in the analysis included temperature, as the daily average, and relative humidity, using max relative humidity.

Epidemiologic analysis

Associations between the various pollution exposure windows and biomarkers of acute cardiorespiratory response were assessed using linear mixed-effect models (PROC MIXED in SAS, version 9.4; SAS Institute Inc., Cary, NC). Pollutants and other covariates were treated as fixed effects and subjects as random effects in these models. Each biomarker was log-transformed in the models given non-normality of these variables. The generalized form can be expressed as:

$$Ln(Y_{ij}) = \beta_0 + b_{0i} + \beta_1 \cdot pollutant_{ij} + \beta_2 \cdot temperature_j + \beta_3 \cdot humidity_j + \beta_4 (pollutant_{ij} \text{ x interaction term}_{ij}) + \varepsilon_{ij}$$

where Y_{ij} was the endpoint measurement for subject i at time j (baseline measurement 1 or 2), β_0 was the intercept term, $pollutant_{ij}$ was the pollutant concentration for time j (the 1, 3, or 7-day concentration average) prior to subject i's endpoint measurement, and $temperature_j$ and $humidity_j$ were the average temperature and max. relative humidity at the 1, 3, or 7-day moving averages (time j) prior to subject i's endpoint measurement. Interaction terms assessed in model selection included asthma status (yes vs. no), age,

sex, BMI, relative humidity, and temperature. Age, BMI, relative humidity, and temperature were treated as continuous variables. The model included a random intercept for subject (b_{0i}) and a compound symmetry covariance structure for the error term (ε_{ij}) that allows for correlation between biomarker values taken at different times for the same subject (in SAS Proc Mixed, type=cs).

All models controlled for average ambient temperature and max. relative humidity. Subject-specific characteristics asthma status (yes vs. no), age, sex, and BMI were considered as potential covariates in the models. These factors, as well as the meteorological factors of temperature and relative humidity, were considered as potential effect modifiers in secondary analyses by including a product term of each with a given air pollutant. To test for interaction, backwards elimination was carried out starting with all interaction terms in the model. In addition, each interaction term was tested separately in a model without the other interaction terms. Associations were compared among the different pollutants and different exposure windows.

Additional models, including models with fixed effects only, and models with alternative covariance structures, were also considered for this analysis, with the overall results and interpretations similar to the findings discussed here.

RESULTS

Exposure and health measurement from a total of 99 participants was included within this analysis. Due to missing measurements for some individuals (Table 1), the number of biomarker observations used in the final analysis ranged from 181 to 192 observations per biomarker endpoint. Missing measurements were determined to be

missing-at-random and not related to either the exposures or endpoints of interest. Descriptive characteristics of the study sample are shown in Table 1. The mean age of the study population was 27 years (range: 18-59) and 52% were male. Approximately 60% of the sample was Caucasian. Subjects without asthma had a higher median age (29 years) compared to those without asthma (25 years) ($p=0.0008$). Median FEV_1 was lower for subjects with asthma (89.5) than in subjects without asthma (93.0) ($p=0.01$). Median IL-1β was higher for subjects with asthma (0.8 pg/mL) than in those without asthma (0.3 pg/mL) ($p=0.0002$). Median baseline concentrations of CRP (2821 vs. 1712 ng/mL), SAA (5480 vs. 2853 ng/mL), sICAM (1664 vs. 687 ng/mL), and sVCAM (3228 vs. 1590 ng/mL) were higher for subjects without asthma compared to those with asthma ($p<0.05$ for all).

Summary statistics for measurements of ambient air pollutants for the prior 1-day, 3-day and 7-day moving averages collected over the study period, from December 2009 to August 2013, are shown in Table 2. The median for the 24-hr average of $PM_{2.5}$ ranged from 9.8 – 10.0 $\mu g/m^3$ for these exposure windows (min/max: 1.9-29.7); the median for the 1-hr max of NO_2 ranged from 28.4 – 29.0 ppb (min/max: 5.7-69.5); and the median for the 8-hr max of O_3 ranged from 43.2-43.5 ppb (min/max 2.9-94.0).

Epidemiologic Model Results

Associations between the selected pollutants and corresponding FEV_1, TNF-α, sICAM-1, and sVCAM-1 were all consistent with a null response, in the *a priori* models, controlling for temperature and relative humidity, at the $\alpha=0.05$ level (Table 3). Several positive associations were found between NO_2 and eNO, SAA, and CRP at varying exposure windows (Table 3; Figures 1 -3). For every unit increase in NO_2 exposure there

was an estimated increase in the log of eNO (ppb) of 0.005 at the prior 1-day exposure ($p_{1\text{-day}}$=0.03). The estimate was borderline significant at the 3-day moving average ($p_{3\text{-day}}$=0.07) and not significant at the 7-day moving average. There was an estimated increase in the log of CRP of 0.02 at all exposure windows for every unit increase in NO_2 exposure ($p_{1\text{-day}}$=0.003; $p_{3\text{-day}}$=0.01). The estimate included the null when the 7-day average was used to estimate exposure ($p_{7\text{-day}}$=0.08).

For SAA, there was an estimated increase of 0.02 at the 1-day exposure ($p_{1\text{-day}}$=0.03) and the 3-day average was borderline significant ($p_{3\text{-day}}$=0.08). As was the case with CRP, the estimated increase in SAA was not significant when the 7-day average was used ($p_{7\text{-day}}$=0.4). Outside of these associations found with NO_2 , there was a significant association found between ILβ-1 and the prior 3-day average of O_3 ($p_{3\text{-day}}$ =0.02), however there was no association at either the prior 1-day or prior 7-day exposure averages. Finally, we observed negative associations in some adjusted models, including: the prior 3-day and 7-day averages of O_3 with SAA ($p_{3\text{-day}}$=0.03; $p_{3\text{-day}}$=0.002), and the prior 1-day exposure of O_3 with FVC ($p_{1\text{-day}}$=0.03). These negative associations were not seen in the unadjusted models.

Models including interaction terms showed no significant interaction by asthma status between the exposures and endpoints examined. A marginally significant interaction by asthma status was found in the eNO 1-day NO_2 model ($p_{1\text{-day}}$=0.05), where the effect of NO_2 exposure was higher for individuals without asthma. For the change in the log of eNO by NO_2, there was significant interaction by age at the 1-day NO_2 exposure only ($p_{1\text{-day}}$=0.003), with a higher age associated with a greater effect of NO_2 exposure on the change in eNO. For the CRP models, significant interaction by sex was

observed at the 1-day NO_2 exposure only ($p_{1\text{-}day}$=0.04). Here, the effect was lower for females. No significant interaction by any of the other subject characteristics or meteorology factors was detected in any of the other models.

DISCUSSION

The purpose of this analysis was to assess the relationship between short-term changes in ambient air pollution and acute changes in cardiorespiratory endpoints, particularly biomarkers of inflammation and oxidative stress, in a panel of adults with and without asthma. Overall, the results showed a positive association between short-term changes in ambient NO_2 concentrations measured at a centrally-located ambient monitoring site and corresponding changes in the airway inflammation endpoint eNO, as well as with changes in two circulating markers of inflammation, CRP and SAA. For these associations, prior day NO_2 concentrations were significantly and positively predictive of changes in each of these endpoints, compared to the 3-day average, which was significant only in the CRP model, and the 7-day averages, which were not significant, but showed similar estimates as the 1-day and 3-day models.

This study analyzed three ubiquitous urban air pollutants, NO_2, $PM_{2.5}$, and O_3. The primary emission sources of NO_2 include motor vehicle exhaust and stationary sources such as coal-fired power plants. NO_2 has a life span of around a day and thus is concentrated around its source (NASA, 2011). $PM_{2.5}$ is composed of a complex mixture of particles, and the specific composition of these particles can vary significantly. Sources of $PM_{2.5}$ include power plants, industrial sources, motor vehicles and natural sources. In the Atlanta area, average $PM_{2.5}$ is largely composed of sulfate and organic

carbon, with smaller components of nitrates and crustal materials (EPA, 2004). $PM_{2.5}$ can exhibit seasonal pattern, with the highest levels typically seen between July-September in the southeastern United States. $PM_{2.5}$, due to their size, can be transported long distances by wind and weather (EPA, 2004). Emissions sources for precursor compounds of O_3, NOx and VOC's, include similar sources as described for the other two pollutants, such as vehicle exhaust and power plants. These emissions can also be carried miles from the original source and lead to high concentrations of ozone across large areas. Higher ambient ozone concentrations are seen during the summer months when the presence of heat and sunglight, on which ozone formation is dependent, is in abundance (EPA, 2016).

This study included several markers of acute cardiorespiratory response that reflect current understanding of oxidative-stress and and inflammatory mediated pathways. The specific respiratory endpoint that was used was eNO, which serves as a measure of airway inflammation and is used to assess asthma and other diseases that are characterized by pulmonary pathophysiology (Dweik et al., 2011). Two measurements of lung function were also included, FEV_1 and FVC. These endpoints relate to the amount of air that can be exhaled from a forced breath and individuals with asthma generally have lower values than healthy individuals. The last category of endpoints included in the study were circulating markers of inflammation in the body (CRP, SAA, IL-1β, TNF-α, sICAM-1, and sVCAM-1). The levels of these markers are seen to rise in response to inflammation in the body. As mentioned earlier, these markers have been used in other epidemiological studies to assess inflammatory response.

A limited number of epidemiological studies have found associations of these specific endpoints with increases in NO_2 under different pollutant levels and populations.

A study carried out in Beijing, China found that a substantial change in ambient NO_2 was associated with an increase in eNO in 125 healthy, young adults (Zhang et al., 2013). This study found associations at moving averages of 0, 1, 3, 4, 5, and 6 day moving averages ($p<0.05$). Similar to our current finding, the largest effect estimates observed in the study were seen at early lag days (0 and 1 day lags) compared to later lag days. Positive associations between NO_2 and eNO have also been found among children with asthma living in the U.S. (Delfino et al., 2006). In this study, an expected change of 1.63 (95% CI: 0.43 to 2.83) in eNO was associated with one IQR change in NO_2 ($p=0.008$). This association was found using a 2-day moving average of NO_2 . A later study carried out by Delfino et al. (2009) concluded that traffic-related air pollutants are associated with increased systemic inflammation. This study found increases in CRP, among 60 elderly subjects residing in Los Angeles, California, with increases in several traffic-related air pollutants using linear mixed-effect models. In this study, the authors used outdoor home air pollutant measurements and evaluated the 24-hr average prior to biomarker measurements and cumulative exposures up to 9 days. The authors found positive associations of CRP with NOx at all exposure averages. At the 1-day average, an expected change of 651 (ng/mL) in CRP was associated with one IQR change in NOx [95% CI: (295, 1008)] and an expected change of 566 [95% CI: (154, 978)] at the 3-day average.

Two previous studies reported no associations between short-term changes in NO_2 and changes in CRP, SAA, and other markers of inflammation (Johannesson et al., 2014; Rudez et al., 2009). In Johannesson et al. (2014), measured biomarker levels were analyzed from blood samples in 16 healthy adults, on days of both low (NO_2 < 35μg/m3)

and high pollution for comparison and had a median of 8 biomarker samples per individual. This study found no significant increase in blood levels of CRP or SAA, or other biomarkers used in the current analysis (sVCAM-1 and sICAM-1), in mornings after high air pollution days compared to low pollution days. Comparatively, in the current analysis carried out in Atlanta in which positive associations were found between NO_2 and CRP levels, the median concentration of NO_2 at the 1, 3 and 7-day moving averages ranged from 26.5-28.6 ppb with values ranging from 5.7 – 69.5 ppb. Rudez et al. (2009), looked at CRP and fibrinogen among 40 healthy individuals and observed no association between these markers and any air pollutants using time lags from 24 – 96 hours prior to blood sampling. Other studies have found associations between specific markers of inflammation and oxidative stress and pollutants not observed in the current analysis (Bind et al., 2012; Calderon-Garciduenas et al., 2009).

Another objective of the current analysis was to assess differences in the exposure-response relationship for individuals with and without asthma. Studies have shown associations between air pollution and asthma outcomes (Salam et al., 2008; Tzivian, 2011). However, more is needed to understand how asthma status in individuals may influence the extent of the effects of air pollution on inflammatory response. A previous ACE analysis looked specifically at traffic-related exposures among car commuters (Mirabelli et al., 2015), and it found the largest postcommute increases in eNO among individuals with below-median asthma control, compared to above-median asthma control (2 hr post-commute: 14.6% [95% CI: 5.7, 24.2]) (Mirabelli et al., 2015). Although no significant interaction was detected for asthma status in the models in the

current analysis, this may be due to a lack of sufficient power to detect differences in the study population.

We also conducted an exploratory assessment on potential interaction effects for additional subject-specific characteristics. This assessment suggested possible differences of the effect of air pollution changes by age and sex. However, this interaction was not consistently observed across the different exposure windows and averaging times. Significant interaction of exposure with age and sex was only observed at the 1-day exposure averages, each for one model – in the eNO and NO_2 model for age and in the CRP and NO_2 model for sex. Thus, no definitive conclusions can be drawn from this assessement and point to a need for further analysis on potential modification of the observed health responses.

As meteorological factors are known to impact changes in air pollution and have also been associated in epidemiological studies with cardiovascular morbidities (Ye et al., 2011), average temperature and max. relative humidity were automatically included in all models to account for potential confounding by these variables. Including these covariates in the models did not alter the primary model results, with the exception of several findings, where a significant result became insignificant when including these variables. The association that was present in the unadjusted models of the 3-day and 7-day moving averages of NO_2 with eNO became insignificant after including these factors, although the effect estimate did not change. Similar changes in model results existed for the effect of the 3-day moving average of NO_2 on SAA. Formal tests of interaction, however, again yielded no detectable, significant interaction by these meteorological factors.

A limitation of this analysis is potential exposure misclassification due to use of a central monitoring site to approximate individual exposure. Information on individuals' residential proximity to major roadways was not able to be incorporated into the current analysis. Since individuals were recruited through Emory University and resided in the nearby area, it is likely that any exposure misclassification would be non-differential across the study population. Future research into this area could explore use of personal exposure monitors and incorporation of other exposure metrics, such as proximity to major roadways, to minimize the possibility of exposure misclassification and error. Lastly, this study incorporated 2-repeated biomarker measurements per individual in its analysis. Incorporating a larger number of repeated measurements in the study could strengthen the ability to detect changes in biomarker levels.

Strengths of the study include a large sample size and inclusion of multiple measures of inflammation and oxidative response, as well as multiple exposure metrics. Including three different exposure windows of the prior 1, 3, and 7-day moving averages strengthened the robustness of the results observed in the study. Associations of NO_2 with each of the biomarkers (eNO, CRP, and SAA) were seen in all of the models using the prior 1-day exposure window, and, in the case of CRP, the association was seen across both the prior 1-day and 3-day exposure averages. These results provided insight into which exposure windows may be most associated with changes in biomarker levels. These results into relevant exposure windows need to be confirmed by further research.

CONCLUSION

Using adjusted, mixed-effect linear models, this analysis found evidence of positive associations between urban ambient air pollution and biomarkers of inflammation and oxidative stress in a relatively small panel of participants in Atlanta, GA. This is consistent with other, larger population-based studies, but among the first to report effects in a small study design. NO_2 was associated with increased levels of eNO, CRP, and SAA at the prior 1-day exposure window, among all study participants, as well as at the prior 3-day average with CRP. An additional significant association was found between ILβ-1 and the prior 3-day average of O_3. No further positive associations were found between any of the other air pollutants and biomarkers. In addition, no significant interaction by asthma status was detected in any of the models. Further research should be conducted in order to confirm these results and to carry out further analysis on potential interactions by asthma status and other factors. Future research can incorporate individual-level exposure measurements, continuous air pollution measurements, and a greater number of repeated biomarker samples in order to provide a more detailed look at the exposure-response relationship between air pollution exposure and inflammatory response in this population.

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Table 1.
Subject characteristics of adults in the ACE studies, combined panel (N=99 subjects, 198 biomarker observations[1])

Characteristic	All Subjects (N=99)	Subjects w/ asthma (N=50)	Subjects w/o asthma (N=49)	p-Value[2]
Age (years), median (range)	27.0 (18-59)	25.0 (18 – 59)	29.0 (21 – 57)	0.0008
Male, n (%)	52 (52.5%)	23 (46.0%)	29 (59.2%)	0.2
Caucasian, n (%)	59 (59.6%)	28 (56.0%)	31 (63.3%)	0.5
BMI (kg/m^2), mean (sd)	23.6 (3.6)	23.0 (3.1)	24.2 (4.1)	0.1
Respiratory endpoint, [median (range)]				
eNO (ppb),	18.0 (5-174)	19.0 (6 - 174)	18.0 (5 - 104)	0.06
Lung Function % of predicted value, [median (range)]				
FEV_1	91.7 (49 – 134)	89.5 (64 – 119)	93.0 (49 – 134)	0.01
FVC	91.2 (62 – 137)	92.5 (62 – 109)	90.0 (64 – 137)	0.8
Inflammation biomarkers, median (range)				
C-reactive protein (ng/mL)	2550 (16 - 137,618)	1712 (16 – 134,670)	2821 (209 – 137,618)	0.003
SAA (ng/ml)	3922 (145 -148,671)	2853 (145 – 128,036)	5480 (276 – 148,671)	0.002
sICAM-1 (ng/ml)	1122 (29 – 6,669)	687 (29 – 6669)	1664 (248 – 5903)	<0.0001
sVCAM-1 (ng/ml)	2450 (23 – 12,562)	1590 (23 – 9415)	3228 (833 – 12,562)	<0.0001
Interleukin 1-beta (pg/mL)	0.4 (0.03 - 27.6)	0.8 (0.03 – 27.6)	0.3 (0.05 – 13.3)	0.0002
TNF- α (pg/mL)	3.5 (0.1 – 9.9)	3.9 (0.1 – 9.9)	2.9 (0.1 – 8.6)	0.2

Abbreviations: BMI body mass index; eNO exhaled nitric oxide; FEV_1 Forced expiratory volume in 1 second; FVC Forced vital capacity; SAA Serum amyloid A; sICAM-1 Soluble intercellular adhesion molecule 1; sVCAM-1 Soluble vascular adhesion molecule-1.
[1]Missing information: eNO (6 subjects), FVC (7 subjects), FEV_1 (6 subjects), SAA (17 subjects), and CRP, sICAM-1, sVCAM, IL-1β, TNF-α (16 subjects each). [2]p-Values for Wilcoxon-Mann-Whitney tests for non-normal variables, t-test for BMI, and chi-square tests for categorical variables.

Table 2. Descriptive Statistics: ambient air pollution for the 1-day, 3-day, and 7-day moving averages prior to subject biomarker measurements (Dec 2009 – Aug 2013).

	Mean	SD	Median	Min/max	N
$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)					
Prior 1-day Avg.	10.0	4.6	8.9	1.9/29.7	197
Prior 3-day Avg.	9.8	3.4	9.4	2.9/25.0	197
Prior 7-day Avg.	10.0	2.9	9.4	3.8/21.4	197
NO_2, 1-hr max (ppb)					
Prior 1-day Avg.	28.5	12.5	26.5	5.7/69.5	186
Prior 3-day Avg.	28.4	9.7	27.9	8.8/56.0	193
Prior 7-day Avg.	29.0	7.7	28.6	10.4/50.4	197
O_3, 8-hr max (ppb)					
Prior 1-day Avg.	43.2	15.7	42.1	2.9/94.0	190
Prior 3-day Avg.	43.3	13.5	42.6	9.7/88.5	196
Prior 7-day Avg.	43.5	12.2	43.3	15.4/79.6	197

Table 3. Epidemiologic Model Results for all log-transformed biomarker endpoints and pollutant exposure averages, controlling for temperature and relative humidity.

Endpoint	Pollutant Exposure Avg.	Estimate (95 % CI)		P-Value
CRP (ng/mL)	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	0.02	(0.008, 0.04)	0.003
	Prior 3-day Avg.	0.02	(0.005, 0.04)	0.01
	Prior 7-day Avg.	0.02	(-0.003, 0.05)	0.08
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	0.04	(-0.002, 0.08)	0.06
	Prior 3-day Avg.	0.04	(-0.01, 0.1)	0.1
	Prior 7-day Avg.	0.07	(-0.008, 0.15)	0.08
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	0.008	(-0.007, 0.02)	0.3
	Prior 3-day Avg.	0.004	(-0.01, 0.02)	0.7
	Prior 7-day Avg.	0.0002	(-0.02, 0.03)	0.99
eNO (ppb)	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	0.005	(0.0005, 0.001)	0.03
	Prior 3-day Avg.	0.006	(-0.0005, 0.01)	0.07
	Prior 7-day Avg.	0.005	(-0.003, 0.01)	0.3
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	0.01	(-0.0007, 0.02)	0.06
	Prior 3-day Avg.	0.02	(-0.001, 0.03)	0.07
	Prior 7-day Avg.	-0.003	(-0.03, 0.02)	0.8
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	0.001	(-0.004, 0.006)	0.6
	Prior 3-day Avg.	-0.0004	(-0.006, 0.005)	0.9
	Prior 7-day Avg.	-0.0004	(-0.008, 0.007)	0.9
FEV_1	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	-0.0009	(-0.002, 0.0004)	0.2
	Prior 3-day Avg.	-0.0004	(-0.002, 0.001)	0.7
	Prior 7-day Avg.	0.0007	(-0.002, 0.003)	0.6
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	0.0002	(-0.003, 0.004)	0.9
	Prior 3-day Avg.	0.003	(-0.002, 0.008)	0.3
	Prior 7-day Avg.	0.002	(-0.005, 0.009)	0.5
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	-0.001	(-0.002, 0.0003)	0.1
	Prior 3-day Avg.	-0.0006	(-0.002, 0.001)	0.5
	Prior 7-day Avg.	-0.0007	(-0.003, 0.002)	0.6

FVC	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	-0.0008	(-0.002, 0.0004)	0.2
	Prior 3-day Avg.	-0.0004	(-0.002, 0.001)	0.6
	Prior 7-day Avg.	0.0002	(-0.002, 0.002)	0.8
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	-0.0002	(-0.003, 0.003)	0.9
	Prior 3-day Avg.	0.001	(-0.003, 0.006)	0.6
	Prior 7-day Avg.	0.0002	(-0.006, 0.006)	0.9
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	-0.001	(-0.002, -0.0001)	0.03
	Prior 3-day Avg.	-0.0007	(-0.002, 0.0008)	0.4
	Prior 7-day Avg.	-0.00007	(-0.002, 0.002)	0.9
ILβ-1 (pg/mL)	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	-0.01	(-0.02, 0.003)	0.1
	Prior 3-day Avg.	-0.004	(-0.02, 0.01)	0.7
	Prior 7-day Avg.	-0.007	(-0.03, 0.02)	0.6
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	-0.01	(-0.05, 0.02)	0.5
	Prior 3-day Avg.	-0.01	(-0.06, 0.03)	0.6
	Prior 7-day Avg.	-0.04	(-0.1, 0.03)	0.3
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	0.007	(-0.007, 0.02)	0.3
	Prior 3-day Avg.	0.02	(0.003, 0.04)	0.02
	Prior 7-day Avg.	0.02	(-0.003, 0.04)	0.08
SAA (ng/ml)	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	0.02	(0.002, 0.03)	0.03
	Prior 3-day Avg.	0.02	(-0.002, 0.04)	0.08
	Prior 7-day Avg.	0.01	(-0.01, 0.04)	0.4
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	0.009	(-0.03, 0.05)	0.7
	Prior 3-day Avg.	-0.009	(-0.06, 0.05)	0.8
	Prior 7-day Avg.	0.05	(-0.02, 0.13)	0.2
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	-0.002	(-0.02, 0.01)	0.8
	Prior 3-day Avg.	-0.02	(-0.04, -0.003)	0.03
	Prior 7-day Avg.	-0.04	(-0.06, -0.01)	0.002

sICAM-1 (ng/ml)	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	0.005	(-0.006, 0.02)	0.4
	Prior 3-day Avg.	0.008	(-0.006, 0.02)	0.3
	Prior 7-day Avg.	0.002	(-0.02, 0.02)	0.8
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	0.006	(-0.03, 0.04)	0.7
	Prior 3-day Avg.	-0.008	(-0.05, 0.04)	0.7
	Prior 7-day Avg.	0.02	(-0.04, 0.08)	0.5
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	0.006	(-0.006, 0.02)	0.3
	Prior 3-day Avg.	0.0001	(-0.01, 0.01)	0.99
	Prior 7-day Avg.	-0.0097	(-0.03, 0.008)	0.3
sVCAM-1 (ng/ml)	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	0.002	(-0.009, 0.01)	0.7
	Prior 3-day Avg.	0.006	(-0.008, 0.02)	0.4
	Prior 7-day Avg.	0.007	(-0.01, 0.03)	0.5
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	-0.01	(-0.04, 0.02)	0.5
	Prior 3-day Avg.	-0.02	(-0.06, 0.02)	0.4
	Prior 7-day Avg.	0.004	(-0.05, 0.06)	0.9
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	0.003	(-0.008, 0.01)	0.6
	Prior 3-day Avg.	-0.002	(-0.02, 0.01)	0.8
	Prior 7-day Avg.	-0.007	(-0.03, 0.01)	0.4
TNF-α (pg/mL)	**NO_2, 1-hr max (ppb)**			
	Prior 1-day Avg.	-0.003	(-0.02, 0.01)	0.7
	Prior 3-day Avg.	-0.006	(-0.03, 0.01)	0.5
	Prior 7-day Avg.	0.007	(-0.02, 0.03)	0.6
	$PM_{2.5}$, 24-hr avg. ($\mu g/m^3$)			
	Prior 1-day Avg.	-0.01	(-0.06, 0.03)	0.5
	Prior 3-day Avg.	-0.008	(-0.07, 0.05)	0.8
	Prior 7-day Avg.	0.02	(-0.05, 0.096)	0.2
	O_3, 8-hr max (ppb)			
	Prior 1-day Avg.	0.008	(-0.007, 0.02)	0.3
	Prior 3-day Avg.	-0.001	(-0.02, 0.02)	0.9
	Prior 7-day Avg.	0.01	(-0.01, 0.03)	0.4

Figure 1: Adjusted Mixed model estimates and 95% Confidence Intervals (eNO and NO_2).

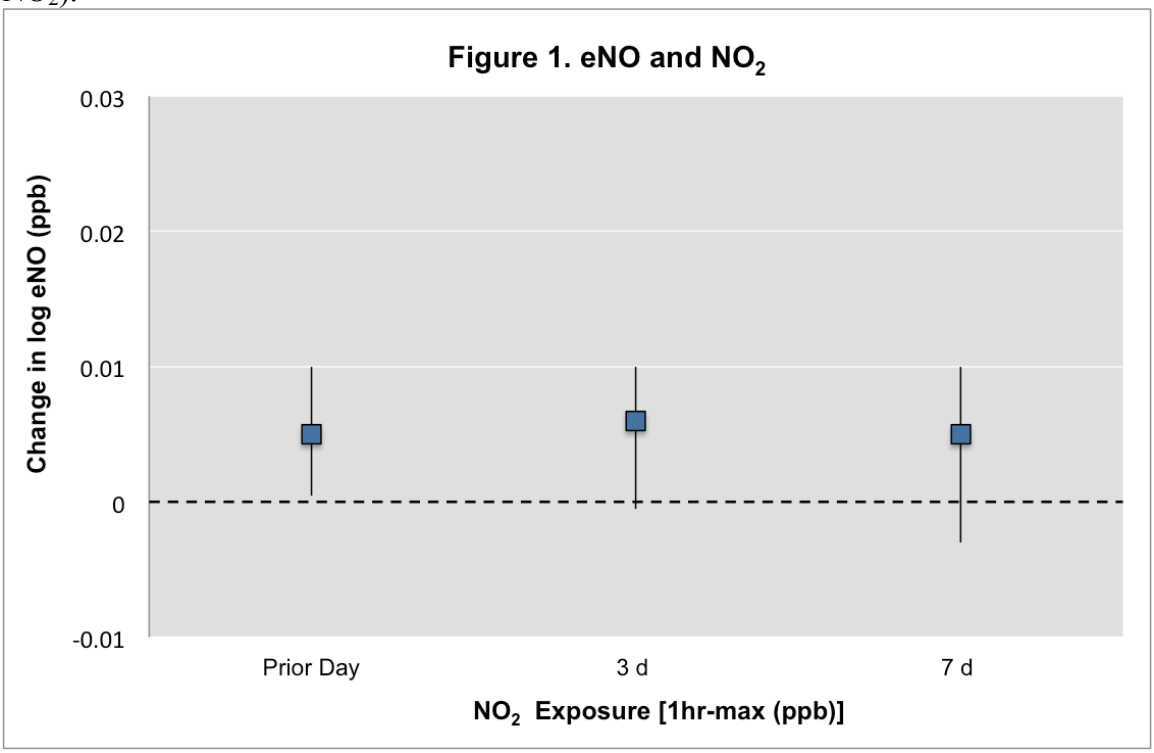

Figure 2: Adjusted Mixed model estimates and 95% Confidence Intervals (CRP and NO_2).

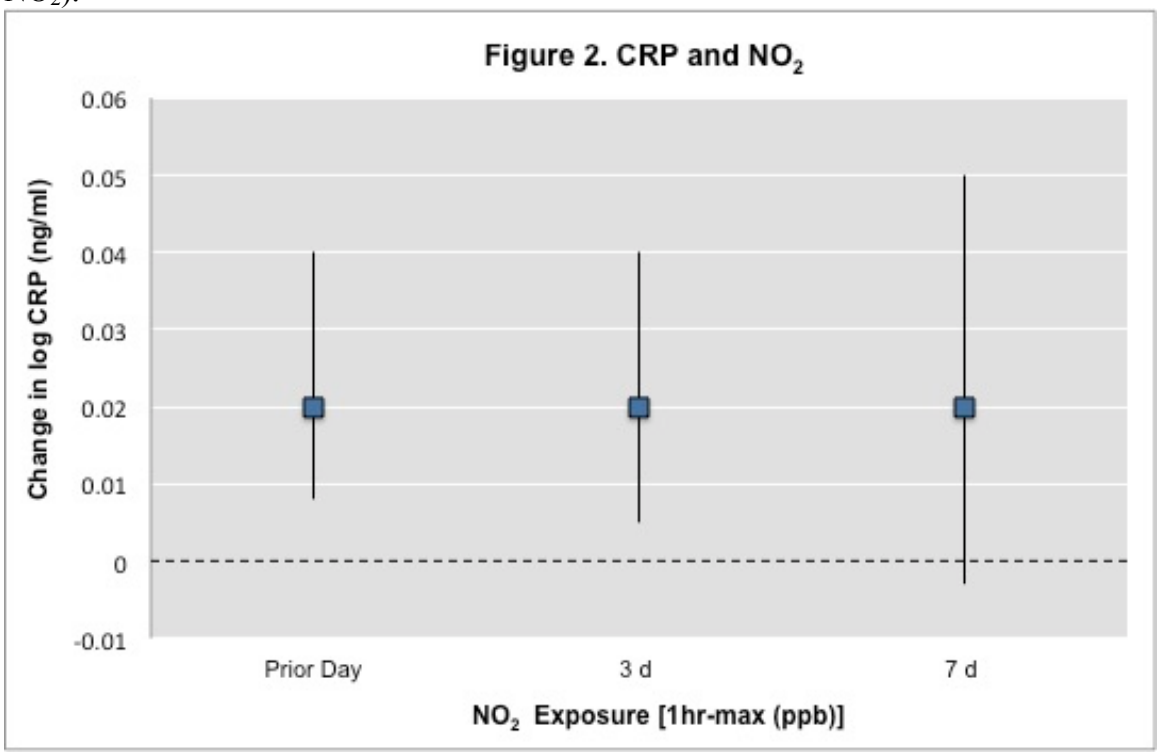

Figure 3: Adjusted Mixed model estimates and 95% Confidence Intervals (SAA and NO_2).