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Exposure to traffic pollution, acute inflammation and autonomic response in a panel of car commuters

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Abstract

Background—Exposure to traffic pollution has been linked to numerous adverse health endpoints. Despite this, limited data examining traffic exposures during realistic commutes and acute response exists. Objectives: We conducted the Atlanta Commuters Exposures (ACE-1) Study, an extensive panel-based exposure and health study, to measure chemically-resolved in-vehicle exposures and corresponding changes in acute oxidative stress, lipid peroxidation, pulmonary and systemic inflammation and autonomic response.

Methods—We recruited 42 adults (21 with and 21 without asthma) to conduct two 2-h scripted highway commutes during morning rush hour in the metropolitan Atlanta area. A suite of in-vehicle particulate components were measured in the subjects' private vehicles. Biomarker measurements were conducted before, during, and immediately after the commutes and in 3 hourly intervals after commutes.

Results—At measurement time points within 3 h after the commute, we observed mild to pronounced elevations relative to baseline in exhaled nitric oxide, C-reactive-protein, and exhaled malondialdehyde, indicative of pulmonary and systemic inflammation and oxidative stress initiation, as well as decreases relative to baseline levels in the time-domain heart-rate variability parameters, SDNN and rMSSD, indicative of autonomic dysfunction. We did not observe any detectable changes in lung function measurements (FEV1, FVC), the frequency-domain heart-rate

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Conflict of interest statement

The authors declare they have no competing financial interests.

variability parameter or other systemic biomarkers of vascular injury. Water soluble organic carbon was associated with changes in eNO at all post-commute time-points ($p < 0.0001$).

Conclusions—Our results point to measureable changes in pulmonary and autonomic biomarkers following a scripted 2-h highway commute.

Keywords

Car commute; Exhaled nitric oxide; Heart rate variability; Asthma

1. Introduction

There is considerable evidence from observational and controlled studies linking traffic-related pollution and adverse health (HEI, 2010). Although the etiology of traffic pollution health effects is complex and may be mediated via numerous pathways (Brook et al., 2010), it is possible that biological response to traffic pollution components or mixtures is elicited following very short-term exposures (Ghio et al., 2003; Peters et al., 2004). Daily commuters may be especially vulnerable given their proximity and enhanced exposures to traffic-related pollution, as well as other non-chemical stressors including noise and psychosocial stress. While time spent daily in traffic may be limited, exposure assessments measuring in-vehicle pollutant concentrations indicate that even short durations inside vehicles (~30 min) can contribute substantially to total daily exposures to particulate matter (PM) (Adams et al., 2001; Boogaard et al., 2009; Rodes et al., 1998; Sioutas et al., 2005; Zuurbier et al., 2010). Despite this, there is still considerable uncertainty concerning in-vehicle exposures during typical commuting scenarios and corresponding cardiorespiratory responses for daily commuters.

Panel-based exposure studies afford unique opportunities to investigate the impacts of commuting on health, given their ability to accurately measure both real world exposures and health response on an individual level. An important panel study of highway patrolmen in North Carolina reported associations between in-vehicle PM exposures over 8 h shifts and acute changes in systemic inflammation biomarkers and cardiac autonomic function (Riediker et al., 2004). Among the notable findings from this study was that sub-clinical biological changes in cardiorespiratory response were observed in young, healthy, active adults following exposures to traffic PM at commonly experienced levels. Subsequent in-vehicle panel studies have provided additional indication that exposures experienced during scripted car or bus commutes may be associated with measures of heart rate variability (Adar et al., 2007; Laumbach et al., 2010; Shields et al., 2013; Wu et al., 2010) and pulmonary inflammation (Zuurbier et al., 2011).

Although suggestive, results from these initial commuter panel studies provide inconsistent evidence concerning the specific factors most associated with response or specific biological pathways most associated with exposures. Some of this inconsistency is likely due to the complexity of the in-vehicle microenvironment, comprising a combination of chemical, physical and psychosocial stressors. A more complete understanding of in-vehicle exposures and health for commuters is becoming increasingly necessary, as commuting durations as well as roadway congestion have steadily increased throughout the U.S. during the last 20

years. Over 10 million Americans spend greater than two hours each day commuting to and from their place of work, with 61% of those commuters driving alone (U.S. Census Bureau, 2011 American Community Survey Reports, 2011 Out-of-State and Long Commutes: 2011 Brian McKenzie).

To investigate in-vehicle exposures among daily car commuters and provide additional insight into the potential health effects of this activity, we conducted two large, panel-based exposure and health assessment studies in the metropolitan Atlanta area, including adults with and without asthma. The current analysis presents results from the initial Atlanta Commuters Exposure (ACE) study, ACE-1, which included measurements collected for over 80 morning rush hour commutes. We examined the hypothesis that exposures occurring during rush hour car commuting lead to acute changes in cardiorespiratory response, consistent with oxidative-stress mediated pathways of injury.

2. Methods

In-vehicle pollutant exposures and corresponding biomarker measurements were collected for 21 adults with self-reported asthma and 21 non-asthmatic adults between December 2009 and April 2011. Subjects used their personal vehicles to conduct a scripted commute lasting approximately 2 h during the morning rush hour period (7–9 AM) in the metropolitan Atlanta area. Commute routes began and ended at our environmental health laboratory at the Rollins School of Public Health of Emory University. Routes were similar among commutes and were designed to include heavily used commuting roadways with both gasoline and diesel engine vehicles. Trained field technicians accompanied subjects throughout the entire commute. Each subject conducted two scripted commutes as part of the protocol, with the exception of 3 subjects who withdrew from the study after conducting a single commute. The repeat commutes for a given subject were scheduled at varying time intervals from the initial commute, ranging from 2 weeks to 17 months, with a median between-commute interval of 4 months.

The driver's side window was alternately opened for 15 min and then closed for 15 min throughout the commute except during rain or uncomfortably cold temperatures. Subjects were allowed to use the vehicle's air condition or ventilation system but were asked to use the outside air setting throughout the commute.

2.1. Exclusion criteria

Subjects for this study were recruited largely by word of mouth and flyers posted on the Emory University and Centers for Disease Control and Prevention (CDC) campuses. To limit exposure to traffic pollution prior to the study commute, we restricted subjects to those living within close proximity (within 15 min drive) of our laboratory facility and commute start point. One subject, who lived approximately 20 miles from our facility, was met by field staff at their residence and began the commute from that location. Participants were considered ‘Asthmatics” if they self -reported ever being diagnosed by a health provider of having asthma. All participants with asthma were instructed to continue normal medication regimens throughout their participation in the study.

We excluded individuals who were pregnant; had diabetes; a previous myocardial infarction; implantable cardioverter-defibrillators or pacemakers; used digoxin or beta blockers for treatment of hypertension or arrhythmias; or had non-asthma pulmonary disease such as COPD, emphysema, any type of lung cancer, or a forced expiratory volume in 1 s (FEV_1) less than 70% predicted at baseline. We excluded individuals who smoked. The study was approved by the Emory Institutional Review Board. Written informed consent was provided by all participants.

2.2. Biomarker measurements

Prior to sampling, each subject was administered a baseline questionnaire assessing factors related to both exposure and health, including proximity of subject residences to major roadways, potential exposures to indoor or outdoor pollution events, and recent health status. Approximately 30 min before each commute, a trained field technician and phlebotomist met with subjects at our laboratory facilities at Emory University to conduct initial baseline measurements (~6:30 AM). Biomarker measurements were also conducted during and immediately following the commute (0 h), as well as at hourly intervals for 3 hours after the commutes. In between measurements, participants were asked not to leave the surrounding area of the clinic.

The selected biomarker measurements were targeted primarily to assess acute response consistent with oxidative stress and inflammation pathways. The specific endpoints included those that have been shown in previous studies to be related to exposure to ambient particulate or gas-phase pollution (Brook et al., 2010; Ghio et al., 2003; Hertel et al., 2010; Mills et al., 2007; Park et al., 2010). For the current analysis, we examined lung function, exhaled nitric oxide (eNO), malondialdehyde (MDA) in exhaled breath condensate (EBC), C-reactive protein (CRP) and heart rate variability (HRV) parameters. Several additional circulating biomarkers of systemic inflammation including soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular adhesion molecule-1 (sVCAM1), interleukin 1 (IL-1β), interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor alpha (TNF-α) were analyzed in plasma, which was collected at the pre-commute baseline and 3 h post-commute time points only.

The concentration of NO in exhaled breath, an indicator of acute bronchial inflammation and oxidative stress (Alving and Malinovschi, 2010), was measured first, using the portable NIOX MINO analyzer (Aerocrine, New Providence, NJ, USA). Participants were asked not to consume foods with high levels of nitrates (i.e. spinach, beets, radishes, celery, cabbage and cured meats) the night before the study and throughout the day of the study, in order to eliminate the effect of nutrition on eNO measurements. They were asked not to eat 30 min prior to each biomarker measurement session. FEV1 and forced vital capacity (FVC) measurements were performed with the use of an OHD KoKo spirometer (Occupational Health Dynamics, Birmingham, AL, USA). Metrics of lung function are presented as percent of age-, sex-, and race-specific predicted values (Hankinson et al., 1999). EBC was collected during a tidal breathing protocol with the use of a standardized breath-condensate collector which was stored at −80 °C prior to sampling (RTube, Austin, T, USA). Concentrations of MDA in the expired droplets of respiratory tract lining fluid, a marker of

pulmonary lipid peroxidation in EBC were measured using a high-performance liquid chromatography (HPLC) technique to assess the progression of airway lipid peroxidation reactions (Lärstad et al., 2002). We measured CRP in blood obtained from finger prick samples collected at each of the measurement periods (Cholestech LDX system, Inverness Medical, Hayward, CA, USA). Blood was drawn by a trained phlebotomist at our clinical facility from an antecubital vein and immediately centrifuged to separate plasma. The suite of inflammation biomarkers in plasma were analyzed according to manufacturer's specifications at the National Health and Environmental Effects Research Laboratory of the US Environmental Protection Agency (Vascular Injury Panel II assay, Human Pro-inflammatory II 4-plex assay ultra-sensitive kit, MesoScale Discovery, Gaithersburg, MD). Blood pressure was measured using the Ambulo 2400 ABPM System (Tiba Medical, Portland, OR, USA).

Heart rate and heart rate variability (HRV) were recorded continuously throughout the commute and during the entire sampling day using a 5-lead Holter monitor (2010 Plus Philips Healthcare, Eindhoven, The Netherlands). For the current analyses, time and frequency domain HRV parameters were characterized, during a 10 min rest period at our clinical facility, performed in the sitting position, immediately prior to the collection of the other biomarker endpoints at each sampling time point. All normal-to-normal intervals from the 10-min recording windows were analyzed for time and frequency domain parameters in 10-min epochs using standard, validated algorithms on Zymed analysis software. The software automatically detected heart beats and labeled ectopic beats such as periventricular contractions or pretrial contractions. A trained technician working with an Emory cardiologist then visually viewed the ECG tracing, removing regions with noise, artifact and ectopy. Time domain parameters included the standard deviation of all normal to-normal intervals (SDNNs) and the square root of the mean squared difference between adjacent normal-to-normal intervals (rMSSDs); frequency domain parameters included power in the high frequency range (HF), power in the low frequency range (LF) and the LF/HF ratio. Average heart rate was also reported. Systemic inflammation (sICAM-1, sVCAM1, IL-1β,IL-6IL-8 and TNF-α were analyzed in plasma, using Vascular Injury Panel II assay, Human Proinflammatory II 4-plex assay ultra-sensitive kit (Meso Scale Discovery, Gaithersburg, MD).

Subjects were asked to complete a diary card to record the baseline incidence of respiratory-related symptoms, diets, time spent in traffic and exposures to other pollutant generating activities. Finally, subject stress levels were assessed by examining salivary cortisol concentrations, both before and immediately after the commute via ELISA analytical methods (ENZO life sciences Farmingdale, NY, USA).

2.3. Pollutant measurements

A range of size- and chemically-resolved particulate components were measured in each vehicle from filter samples collected during the 2-h scripted commutes using both continuous and time-integrated instrumentation. Inlets for all the instruments were situated in the passenger side of the front seat, no more than 1 m from the breathing zone of the driver with pump exhaust routed to the exterior of a rear window. We measured $PM_{2.5}$ mass

(AeroTrak model 9306 (TSI Inc., Shoreview, MN); particle number concentration (PNC) (CPC model 3007 (TSI Inc., Shoreview, MN); black carbon concentration (BC)(MicroAeth AE51 (AethLabs, San Francisco, CA); and particle-bound polycyclic aromatic hydrocarbons (pb PAH) (PAS 2000CE (EcoChem Analytics, League City, TX) continuously during the commute periods. Filter-based analyses were performed at Emory University, Georgia Tech, and the University of Wisconsin. For these analyses, we present results for a subset of pollutants which were selected, *a priori*, to reflect specific traffic sources or physiochemical categories. For the carbonaceous species, we included total organic carbon (OC), total elemental carbon (EC), water soluble organic carbon (WSOC), total pb-PAHs, *n*-alkanes, and total hopanes as markers of various internal combustion engine processes. For the elemental species, we included transition metal species (zinc, copper, nickel, vanadium, iron, manganese, chromium, and aluminum), as well as specific tracers of on-road source categories, including lead, antimony and sulfur. All of these chemical components were measureable, above their respective detection limits, in greater than 79% of the collected filter samples. In addition, in-cabin noise levels were measured continuously during the commutes using a noise decibel meter (Extech HD600, Extech Instruments, Nashua, NH). Noise has previously been suggested as a potential confounder of traffic-related health effects in epidemiologic studies of air pollution (Babisch, 2005; Boogaard et al., 2009). All collected data were assessed for bias, precision and completeness. Full details on the data quality parameters for all of the measured pollutants can be found elsewhere (Greenwald et al. 2014).

2.4. Epidemiologic analyses

Associations between exposures and response were examined using mixed-effects linear regression analyses. All endpoints were transformed logarithmically given non-normality in their respective distributions. We used two, complementary mixed-effects modeling approaches to examine biological changes associated with the commuting periods. First, time trends in the specific endpoints at pre- and various post-commute periods were examined as:

$$ln\left(Y_{ijk}\right)=\beta_0+b_{0i}+\sum_{j=1}^{4}\beta_j\left(\text{time}_j\right)+\gamma\left(\text{commute}_k\right)+\varepsilon_{ijk} \quad (1)$$

where Y_{ijk} was the endpoint measurement for subject i at time j (with time_1–time_4 being indicator variables for the baseline measurement, and measurements 0, 1, 2 and 3 h after the commute) on commute_k (1 or 2). This model included a random intercept for subject (b_{0i}), and a spatial power covariance structure for the error term (ε_{ijk}) allowing for additional correlation between biomarker values at different times for the same subject (in SAS Proc Mixed, type sp(pow)(*c-list*)). The coefficients (βs) for the various time points after the commute can interpreted as the extent to which the log of the outcome changed at time j=1,2,3, or 4 relative to time 0 (j=0). The effects were expressed as the average percent change relative to baseline with the percent change calculated as $(\exp(\beta_j)-1)\times 100$. It should be emphasized that this time trend model, which we refer to as the ‘commute as exposure’ models, may reflect changes due to one or multiple factors experienced during the commute, chemical or non-chemical, or even natural diurnal patterns.

We also examined the relationship between measured in-vehicle pollution and corresponding changes in the biomarker measurements between the baseline time point and post-commute time points. For these models, the outcome was the difference in log transformed in values between the baseline and post-commute time points. The models had the following form:

$$\Delta \quad ln\left(Y_{ijk}\right)=\beta_0+b_{0i}+\beta_1\left(\text{pollution}_{ik}\right)+\gamma\left(\text{commute}_k\right)+\delta\left(\text{baseline}Y_{ik}\right)+\varepsilon_{ijk} \quad (2)$$

where $\Delta\ln(Y_{ijk})$ is the difference between the natural log of the biomarker measurement for subject *i* on commute *k* (1 or 2) at time$_j$ (*j*=1,2,3 or 4 representing 0, 1, 2 and 3 h after the commute) and the natural log of the biomarker measurement for subject *i* on commute$_k$ before the commute; and pollution$_{ik}$ is a measure of the level of a given pollutant during the commute. We scaled effects associated with an approximate interquartile range (IQR) increase in concentration for the various pollutant metrics. The model included a random intercept for subject (b_{0i}). The model also controlled for the natural log of the baseline biomarker level for subject *i* on commute$_k$ (baseline ln*Y*). A separate model was run for each post-commute time point *j*.

Effect modification by asthma status (yes vs. no) and season (cold vs. warm, with cold season defined as October 15–April 15) was assessed using stratified models and product terms with the relevant exposure variables. As a sensitivity analysis, we included cortisol concentrations, a marker of psychosocial stress, and in-vehicle noise as covariates in Eq. (2), to assess the potential confounding of pollutant effects by these factors. All statistical analyses were conducted using SAS v9.3 (SAS Institute Inc., Cary, NC).

3. Results

In total, 42 subjects conducted 81 highway commutes as part of the ACE-1 study. Participant median age was 32 years (range: 20–58 years); 50% were women. Participants with asthma had higher baseline eNO and lower baseline FEV1 compared to participants without asthma (Table 1). Among the participants with asthma, half reported using asthma medications regularly and six were considered ‘poorly controlled”. Commutes were conducted during all seasons of the year and in all meteorological conditions. The majority of commutes were conducted in sedans or hatchbacks (52/81 or 64%), followed by SUVs (22 commutes), pickups (3), minivans (2), and station wagons (2). The median age of the vehicles was 5 years with a range of < 1–16 years.

Characteristics of in-vehicle pollutant concentrations are shown in Table 2. In-vehicle concentrations of BC, PNC, $PM_{2.5}$ and pb-PAHs were generally elevated during the commuting periods, relative to corresponding ambient pollutant concentrations (Greenwald et al. 2014). Mean concentrations for these pollutants were typically higher during the commutes for the non-asthmatic compared to the asthmatic participants ($p < 0.05$ for all) (data not shown). Strong in-vehicle correlations existed between both the BC and EC and corresponding pb-PAH concentrations (Spearman's $r > 0.76$), with weak to moderate correlations among the other the measured pollutant distributions (Table 3). Detailed

descriptive findings for the ACE-1 exposure measurements are detailed elsewhere (Greenwald et al. 2014).

3.1. Time trends in measure biological endpoints: Commute as exposure models

3.1.1. Respiratory endpoints—eNO levels measured after commuting were from 8.3 to 13.7% higher than baseline levels ($p < 0.001$) at all post-commute time periods (Table 4, Fig. 1). Peak eNO levels were measured at 1 h post commute, yet remained significantly elevated relative to baseline levels at all post commute measurement periods. For both asthmatic and non-asthmatic subjects, eNO levels exhibited modest declines at the final follow-up time point, 3h after the subjects' commutes (Fig. 1). MDA concentrations in exhaled breath were higher than baseline levels in both asthmatic and non-asthmatic subjects, albeit insignificantly, at the 0 h post-commute measurement time point (p=0.34) and lower than baseline levels at the three subsequent measurement time points. FEV_1 levels were slightly elevated relative to baseline levels among asthmatic subjects at the 1 h and 2 h post-commute time points (Table 4, Fig. 1). For all of the commute as exposure models, we observed no significant difference in strength of response by asthma/non-asthma health status.

3.1.2. Cardiovascular and other systemic endpoints—Slight elevations in CRP, corresponding to levels that were approximately 8% higher than those measured before the commute, were observed in the subjects at the 0 h post-commute time point (p=0.05)(Table 4, Fig. 1). This result was primarily driven by CRP response in the non-asthmatic subjects (11.2% post-commute CRP increase; p=0.06). CRP levels remained elevated relative to baseline levels for non-asthmatic subjects at 1 and 2 h post-commute, although none of these estimates were statistically significant. Time trends in sICAM, sVCAM, IL-1B, IL-6, IL-8, and TNF-α concentrations, measured only at the baseline and 3 h post-commute time points, were consistent with the null, in both asthmatic and non-asthmatic subjects (data not shown).

SDNN values were significantly lower at each of the post-commute measurement time points (Table 4), with the lowest levels measured at the 0 h post-commute time point (−31.2.4% change, $p < 0.0001$). For rMSSD in all subjects and SDNN in non-asthmatic subjects, levels were closer to baseline levels 1 h after the commutes, roughly corresponding to 10 AM for most subjects, which remained consistent until the end of the measurement protocol around noon (Fig. 1). We conducted sensitivity analyses examining model robustness associated with three extreme observations for SDNN. Results from models removing these observations did not change the overall direction or interpretation for the SDNN trend. At the 0 h post-commute time point, rMSSD levels were significantly lower than baseline (change in post-commute rMSSD: −21.6%; $p < 0.0006$), although this finding was largely driven by response in the asthmatic subjects. Model results examining changes in the frequency domain HRV parameters, including High Frequency (HF), Low Frequency (LF) and the ratio of the two measures, were all consistent with the null (data not shown).

A formal examination of interaction by health status showed significantly stronger decrements in the asthmatic compared with the non-asthmatic subjects in both SDNN at the 0 h post-commute time-point (−47 vs. −26% for the asthmatic and non-asthmatic subjects,

respectively, interaction: p=0.027) and rMSSD at the 0 h post-commute time-point (−43 vs. −2% for the asthmatic and non-asthmatic subjects, p=0.003). No other significant differences were detected between these two cohorts among all of the endpoints we examined. There was no observed effect measure modification by season (results not presented).

3.2. In-vehicle pollution as a predictor of response

Results from analyses using the in-vehicle pollutant concentrations as predictors of changes in the biomarker endpoints were highly variable, and largely consistent with the null. Of the pollutant models we considered, the clearest and most consistent positive associations existed between WSOC and changes in eNO relative to baseline at all post-commute time points ($p < 0.05$ during 0, 1, 2, and 3 h post commute) (Fig. 2). There were also positive associations between in-vehicle $PM_{2.5}$ mass and Fe levels and changes in eNO at the 0 h time point (Fig. 2). WSOC, $PM_{2.5}$ mass and Fe were all negatively associated with MDA at the 2 h post-commute time period. Associations between changes in eNO and WSOC and Fe levels remained significant during both seasons, with positive associations between BC and pb-PAHs and eNO in the cool season alone ($p < 0.0009$ for both, results not shown). All pollutant associations were robust to the inclusion of in-vehicle noise and cortisol levels as covariates in the models. Neither noise nor cortisol levels were independently associated with any of the measured endpoints.

4. Discussion

We conducted the ACE-1 study to examine whether car commuting during morning rush hour conditions is associated with acute, sub-clinical changes in markers of oxidative stress and inflammation. During the commutes, we measured substantially elevated in-vehicle particulate pollutant concentrations relative to ambient concentrations (Greenwald et al. 2014). At measurement time points within 3 h after the commute, we observed mild to pronounced elevations in eNO, CRP, and MDA relative to baseline in these subjects, indicative of pulmonary and systemic inflammation and oxidative stress initiation, as well as decreases relative to baseline levels in the time-domain HRV parameters, SDNN and rMSSD, indicative of autonomic dysfunction. For several of these endpoints, including eNO and SDNN, response occurred in subjects both with and without asthma. Further, several biomarkers exhibited trends indicating a return to approximate baseline levels within a 3 h follow up period. Since the participants were non-randomly selected volunteers, these results may not be general-izable to individuals outside of this panel.

Among the pulmonary response endpoints, the most pronounced effects were seen in eNO, which we hypothesized as potentially most temporally sensitive to air pollution insult. Peak eNO levels were observed at 1 h post-commute and exhibited modest declines at the final follow-up period, 3 h after the subjects' commutes. Given the relatively short follow-up period for this study, however, inferences relating to the temporality of any of the endpoints should be viewed cautiously. Numerous studies have reported associations between air pollution and acute eNO response utilizing panel based designs (Adamkiewicz et al., 2004; Buonanno et al., 2013; Delfino et al., 2006; Greenwald et al., 2013; Sarnat et al., 2012).

Notably, the elevated eNO response was similar in subjects both with and without asthma, with slightly stronger associations existing for the subjects with asthma. As expected, baseline eNO levels were higher in asthmatic subjects (Table 1), so percent increases from baseline levels also denote larger absolute increases in measured eNO concentrations. We did not expect a response of this magnitude in the non-asthmatic subjects. There is limited information concerning the use of eNO as a biomarker of acute pulmonary inflammation in individuals without preexisting respiratory disease (Alving and Malinovschi, 2010). In both asthmatic and non-asthmatic subjects, eNO is produced in the upper and peripheral airways and alveoli by the expression of inducible nitric oxide synthase (iNOS) in epithelial cells (Alving and Malinovschi, 2010; Barnes and Kharitonov, 1996). Regulation of iNOS is influenced by the cytokines IL-4 and IL-13 on a pathway involving the transcription factors STAT-6 and AP-1 which in turn are responsive to airway oxidative status (Alving and Malinovschi, 2010). The results presented here are consistent with this model of eNO production; namely, that highway commuters are exposed to inhaled oxidants, which alter redox balance after rapid dissolution in the airway epithelium. This may lead to upregulation of systemic cytokines and increased expression of iNOS in both non-asthmatic and asthmatic subjects. The expression of iNOS is elevated in the bronchial epithelium of asthmatics leading to higher baseline eNO, which may also result in a higher absolute change in eNO. While biologically plausible, the eNO trends may also indicate exposures to stress in the subjects. A recent clinical study showed eNO in asthmatic and non-asthmatics to be associated with psychological stress, also expressed as changes in the subjects' salivary cortisol levels (Ritz et al., 2011). While cortisol was not independently predictive of any of the current health responses, at the very least, the role of psychosocial stress as an additional biologically-plausible driver of respiratory response should be considered.

MDA levels in EBC were slightly, albeit insignificantly, elevated in both asthmatic and non-asthmatic subjects at the measurement period immediately following the commutes (8.6% increase relative to baseline levels), and were not elevated relative to baseline during later measurement periods. While statistically insignificant, the trends are suggestive of acute oxidative stress and inflamma-tory processes occurring in the lung. This interpretation is supported by our eNO findings as well as similar results from a recent natural intervention study showing lagged associations between MDA in exhaled breath and several ubiquitous urban air pollutants, at lags of 1–4 days, in a panel of 125 healthy adults living in Beijing during the 2008 Olympics (Gong et al., 2013). While methods for measuring EBC biomarkers of oxidative stress and associated processes are still novel (Effros et al., 2004; Horvath et al., 2005), the ability to characterize these processes in exhaled breath is important for elucidating mechanistic pathways of air pollution toxicity.

We did not see anticipated decrements in either FEV1 or FVC at the post commute measurement time points, as has been reported in a previous panel study examining exposures in a heavy traffic emission environments (McCreanor et al., 2007). The current results showing FEV1, specifically, to be slightly and significantly elevated at the post-commute time points may be an artifact of our repeated measure design, and improved subject performance, over time, in completing the spirometry protocol. Among the non-respiratory endpoints we analyzed, CRP was slightly elevated in the 0 h post commute measurement period only. Previous panel studies have shown similar increases in CRP

following exposures to traffic pollution at longer time lags (~hours-to-days) (Brook et al., 2010; Chuang et al., 2007; Riediker et al., 2004), presumably reflecting a lengthy cascade of inflammation-mediated steps in its production (Ruckerl et al., 2006). It is conceivable, however, that a more rapid acute phase response in CRP, similar in magnitude to that reported here, can occur following insult (Ghio et al., 2003; Pepys and Hirschfield, 2003; Seo, 2012). Admittedly, the lack of measureable post-commute elevations in the other vascular inflammation biomarkers, including some known to be precursors of CRP production (i.e., IL-6); in addition to apparent complete clearance of CRP at the 1 h post-commute time point, for a protein with a reported plasma half-life of 8–18 h (Pepys and Hirschfield, 2003), complicates the interpretation of these results. Overall, we view these CRP findings as intriguing and supportive of future investigation.

SDNN in all subjects and rMSSD in the asthmatic subjects, exhibited marked post-commute changes, mainly at the measurement period immediately following the commutes. Among the time- and frequency-domain HRV metrics we measured, these were the only two for which responses were observed. Reviews examining the link between short-term exposures to air pollution and HRV note the variability of results (Brook et al., 2010), with studies reporting decreases in primarily the frequency-domain HRV parameters (Adar et al., 2007; Laumbach et al., 2010; Riediker et al., 2004; Shields et al., 2013), and decreases in time-domain HRV parameters, similar to the current results (Liao et al., 1999; Shields et al., 2013). In a panel study examining traffic pollution, Shields et al (2013) recently found associations between traffic-related PM exposures and acute reductions in HRV in a middle-aged Mexico City population. A recent study of 21 subjects with type-2 diabetes also found reductions in HF mainly one day after subjects completed 90- to 110-min car rides on a busy highway (Laumbach et al., 2010). Similarly, Adar et al (2007) reported reductions in frequency-domain HRV associated with in-vehicle pollutant exposures in 44 non-smoking senior adults during highway commutes on a diesel-powered mini-bus (Adar et al., 2007). The stronger SDNN and rMSSD response that we observed in the asthmatic subjects is intriguing. While speculative, the observed discrepancy in autonomic response may reflect enhanced underlying sensitivity to inflammation-mediated processes in asthmatic subjects which, in turn may trigger reduced vagal function (Rhoden et al., 2005; Simkhovich et al., 2008).

We cannot rule out the explanation that the SDNN results, in particular, reflect diurnal patterns in autonomic function, mediated via circadian rhythmicity or other endogenous mechanisms (Vandewalle et al., 2007), rather than the effect of external stressors experienced during the commute itself. Interestingly, most of the subjects exhibited some degree of post-commute decline in SDNN, with only 10 of 60 observations (17%) showing mean SDNN readings higher at the post-commute period compared to baseline measurements. Although limited information exists regarding HRV diurnal patterns, it has been suggested that SDNN substantially decreases after waking and may exhibit modest elevations during the late morning or early afternoon (Burger et al., 1999; Vandewalle et al., 2007), which is roughly consistent with the trends we observed in the ACE-1 subjects.

An important limitation of this quasi-experimental design was the lack of a comparison commute with substantially lower exposure levels; this would have afforded time trend

comparisons between commutes with larger differences in exposure, such as “exposed” vs. “non-exposed” conditions. Barring this element of control, we cannot preclude an explanation that normal diurnal variability, in any of the measured biomarkers, is truly responsible for the observed time-trend model results (Eq. (1)). It is improbable that the time-trend results, which reflect changes in multiple endpoints, processes and biological systems, are solely expressions of normal diurnal patterns. Moreover, indications of an acute return to baseline levels for many of the endpoints shortly after the commutes, argue against this interpretation. Similar to other on-road exposure study designs (Adar et al., 2007; Riediker et al., 2004), the strategy for designing the ACE-1 study to include two highway commutes was intentional and, and intended as a means of enhancing intra-individual variability in exposure and improving our ability to detect changes in response associated with different within-commute levels of specific pollutant components (using the analyses described by Eq. (2)). We note that the recently completed ACE-2 panel study follows an additional 60 subjects during both a highway commute, similar to ACE-1, as well as a control, non-highway traffic exposure session for all participants. This second study will provide opportunities to directly examine the presence of biomarker diurnality and the potential that other forms of confounding are responsible for the observed time trend results.

By design, the models examining changes in response as a function of in-vehicle pollution (Eq. (2)) are not subject to this form of potential temporal confounding, since diurnal variability in the endpoints should not be correlated with the in-vehicle pollutant measurements. Results from models including individual pollutants or classes of pollutants (e.g., pb-PAH's, transition metal species) as predictors of response were largely consistent with the null, with the exception of a few notable associations. In this study, in-vehicle WSOC and, to a lesser degree $PM_{2.5}$ and Fe, were predictors of corresponding changes in eNO. The WSOC finding agrees with previous results from a panel study of 60 older adults with coronary artery disease, in which eNO was also associated with WSOC, as well as organic acids (Delfino et al., 2010). In that study conducted in Los Angeles, WSOC was attributed to photo-chemically-produced, secondary organic aerosol in the ultrafine particle range. The origin of the WSOC we measured, either biogenic or anthropogenic, primary or secondary, is not known. Although speculative, it is worth noting that the strong eNO-WSOC associations existed in both warm and cool seasons, perhaps indicating a contribution from non-photochemically produced components of WSOC. We did not find any indication that in-vehicle noise or cortisol levels, a marker of psychosocial stress, either confounded these observed pollutant effects or were independent predictors of any biomarker variability. Future analyses examining alternative ways of characterizing biologically relevant noise and stress metrics may provide additional information about the potential role of these commuting-related exposures.

The general lack of observed associations from the pollutant models may be due to several factors, including errors associated with analytical imprecision and uncertainty stemming from the measurement of trace pollutant species at their limits of detection. It is also possible that the extensive suite of particulate pollutants we measured was not causally associated with the selected endpoints, or at least not associated with measureable response within this acute timeframe. Further, unmeasured gaseous or non-chemical environmental

factors (Zappulla, 2008), which are also present during commuting, may be the true drivers of biomarker variability.

Clearly, the in-vehicle microenvironment is a highly dynamic exposure setting. During their participation in this study protocol, subjects were exposed to multiple exogenous and endogenous stressors that can elicit similar physiological response via numerous oxidative stress and inflammation pathways. While commuting, individuals may be cumulatively exposed to elevated particulate and gaseous chemical pollution, noise, and psychosocial stress. It is possible, and perhaps probable, that these stressors, as well as elements of the commuting protocol itself, contribute a *t* varying degrees to the biological responses we observed in this panel. Traditional health effects modeling involving single pollutants or even pollutant categories (e.g., total PAHs), thus, may be inadequate for capturing variability attributable to this rich mixture of stressors. This challenge necessitates the development of novel exposure metrics that better reflect the multiplicity of exposures occurring during typical commuting. For this type of setting, it is possible that our 'commute as exposure' models (in which we did observe clear differences in our outcome measures after the commute compared with baseline) best represents the biologically-relevant mix of exposures one typically experiences during commuting.

In spite of these areas of uncertainty, we believe that these results collectively point to measureable changes in pulmonary, autonomic and other systemic biomarkers following the scripted 2 h highway driving protocol. A thorough characterization of in-vehicle PM exposure and acute health response represents a key environmental health challenge given that the duration of the average commute to work in the United States has increased steadily in recent decades to a national average of 25.5 min. The U. S. Census Bureau reports that over 8.1% of Americans spend at least an hour each day commuting to and from their place of work with almost 600,000 people commuting at least three hours per day (U. S. Census Bureau report March 2013).

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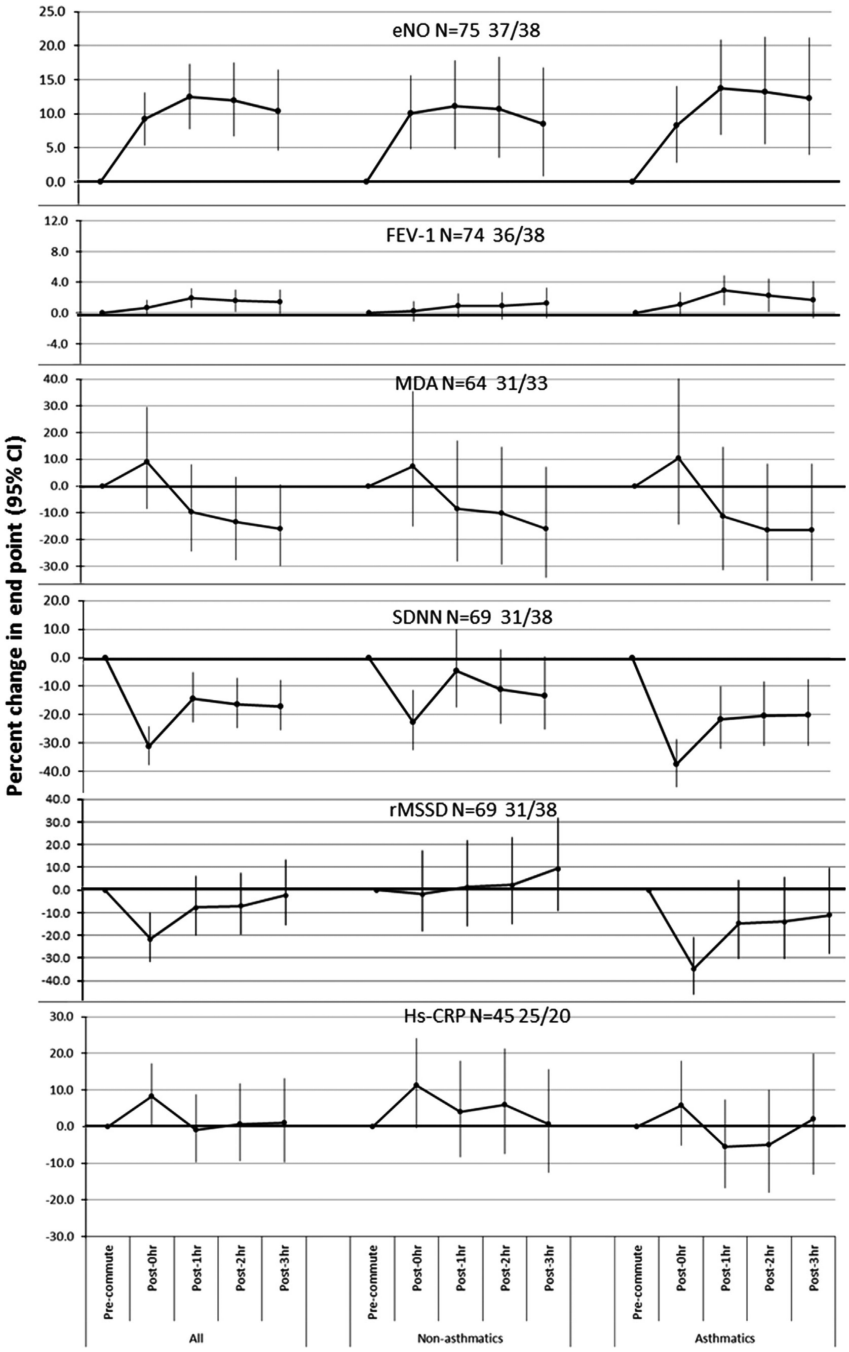

Fig. 1.

Percent change, over time, in selected health endpoints for the entire panel (left), subjects without asthma (center), and subjects with asthma (right). Abbreviations: eNO exhaled nitric oxide; FEV1 forced expiratory volume in 1 s; MDA malondialdehyde; SDNN standard deviation of normal-to-normal intervals; RMSSD square root of the mean squared difference between adjacent normal-to-normal intervals; CRP C-reactive protein.

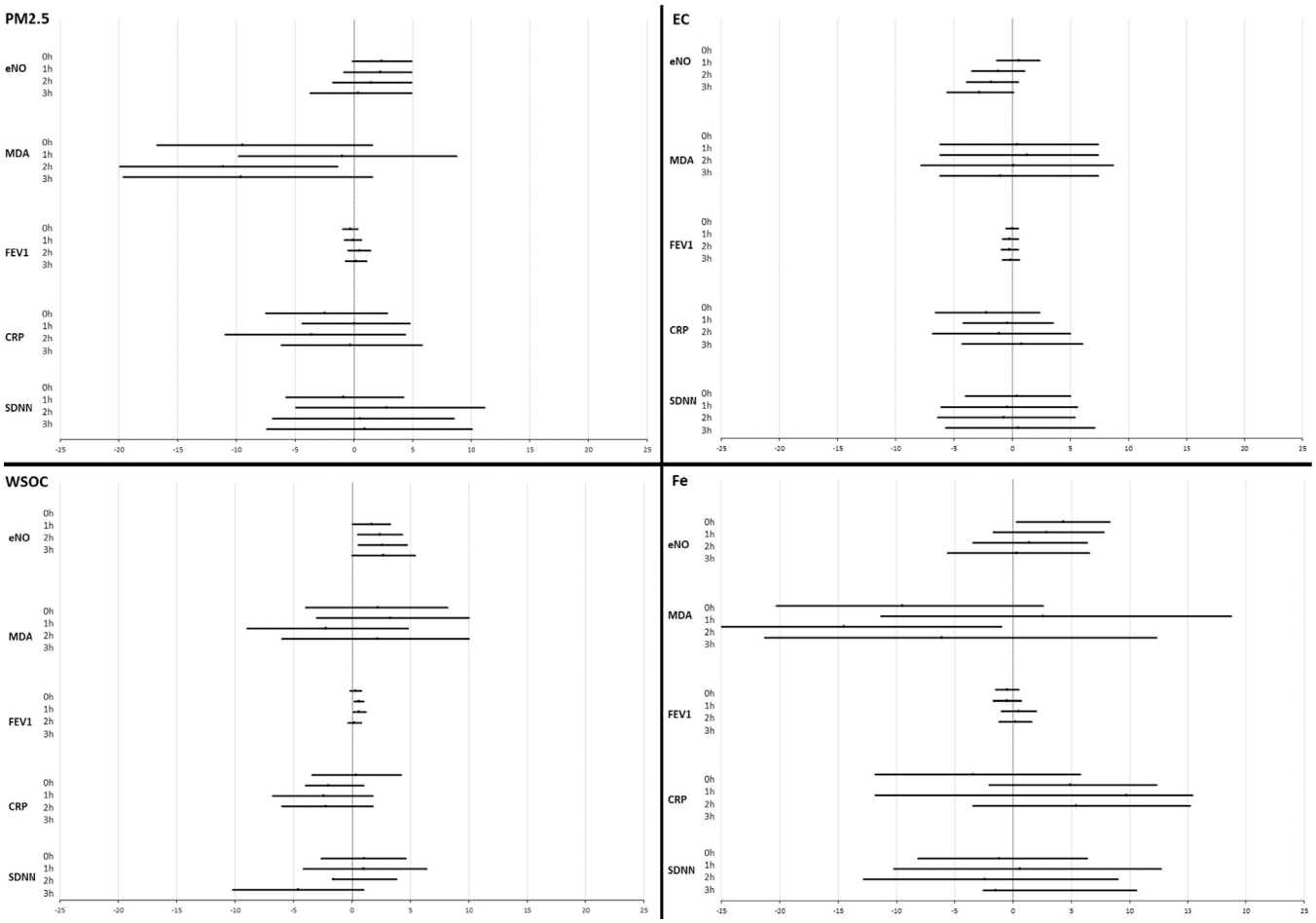

Fig. 2.

Percent change in biomarker per change in selected pollutant. Coefficient scaling for $PM_{2.5}$: per 10 μ/m^3; EC: per 1 $\mu g/m^3$; WSOC: 2 $\mu g/m^3$; Fe: 250 ng/m^3. Abbreviations: $PM_{2.5}$=fine particulate matter; EC=elemental carbon; WSOC=water soluble organic carbon; Fe=iron.

Table 1

Baseline characteristics of study population by health status.

Characteristics	All participants (*N*=42)	Subjects with asthma (*N*=21)	Subjects w/o asthma (*N*=21)	*p* Value[a]
Number of commutes	81	41	40	
Female (%)	50	62	38	0.12
Age, years [median (range)]	32.4 (20-58)	29.9 (20-58)	35.0 (22-57)	0.20
Caucasian (%)[b]	61.9	61.9	57.1	0.75
BMI (kg/m^2) (mean ± SD)	23.5 (3.6)	22.8 (2.1)	24.4 (4.6)	0.42
Treatment with inhaled corticosteroids *n* (%)		6 (28.6)	-	
Treatment with beta-agonist *n* (%)		12 (57.1)	-	
Respiratory endpoints				
eNO, ppb [median (range)]	24 (9-174)	28 (10-174)	18 (9-104)	0.02
Malondialdehyde (MDA) μM (mean ± SD)	0.097 ± 0.06	0.10 ± 0.6	0.09 ± 0.05	0.4
Lung function % of predicted value (mean ± SD)[c]				
FVC	96.4 ± 14.1	95.3 ± 11.3	97.4 ± 16.5	0.52
FEV1	94.8 ± 15.6	91.4 ± 14.2	98.3 ± 16.3	0.05
Cardiovascular endpoints (mean ± SD)				
Blood pressure				
Systolic blood pressure (mmHg)	115.1 ± 14.5	114.8 ± 15.0	115.7 ± 14.3	0.82
Diastolic blood pressure (mmHg)	78.4 ± 9.3	75.9 ± 8.6	81.1 ± 9.5	0.04
Heart rate (bpm)	77.3 ± 16.3	76.7 ± 16.5	78.1 ± 16.6	0.75
Heart rate variability				
SDNN “10 min” (mm^2)	94.1 ± 32.4	85.5 ± 34.0	85.5 ± 34.0	0.36
RMSSD “10 min” (mm^2)	61.5 ± 36.6	71.7 ± 39.8	50.2 ± 29.6	0.45
HF LF ratio “10 min”	1.03 ± 0.5	1.2 ± 0.6	0.9 ± 0.2	0.13
Inflammation biomarkers [mean (SD)]				
C-reactive protein, (mg/L)	1.72 ± 2.0	1.57 ± 1.6	1.86 ± 2.4	0.6
Soluble Intercellular adhesion molecule 1(ng/mL)	1804 ± 1445	1021 ± 1361	2544 ± 1100	< 0.0001
Soluble vascular cell adhesion molecule-1(ng/mL)	2928 ± 2314	1624 ± 1917	4162 ± 1917	< 0.0001
Interleukin 1-beta (pg/mL)	0.31 ± 0.4	0.41 ± 0.5	0.23 ± 0.2	0.07
Interleukin 6 (pg/mL)	0.93 ± 0.5	0.77 ± 0.4	1.1 ± 0.6	0.015
Interleukin 8 (pg/mL)	6.1 ± 6.7	8.45 ± 8.8	3.89 ± 2.1	0.005
Other endpoints	2.4 ± 1.0	3.05 ± 1.0	1.85 ± 0.5	< 0.0001
Salivary cortisol pg/mL [mean (SD)]				

Abbreviations: BMI body mass index; eNO exhaled nitric oxide; FVC Forced vital capacity; FEV1 Forced expiratory volume in 1 s; SDNN standard deviation of normal-to-normal intervals; RMSSD square root of the mean squared difference between adjacent normal-to-normal intervals.

[a] *p*-Values for *t*-tests for continuous variables and chi-square tests (when all cell values > 5) or Fisher's exact test for categorical variables.

[b] Race was self-reported.

[c] Metrics of lung function are reported as percent of age-, sex-, and race-specific predicted values (Hankinson et al., 1999).

Table 2

Descriptive statistics for in-vehicle $PM_{2.5}$ concentrations, PNC, and concentrations of organic components and transition metals from 2-h commutes.

Pollutant	*n* Commutes	Mean	SD	Median	Min/max
$PM_{2.5}$ mass (mg/m^3)	72	19.2	13.6	15.2	3.08/85.5
Particle number (n/cm^3)	76	26,067	12,211	24,218	4,936/68,951
Black carbon ($\mu g/m^3$)	78	6.6	3.3	6.00	1/16
Element carbon[a] ($\mu g/m^3$)	76	2.8	1.8	2.3	0.34/8.3
Organic carbon[a] ($\mu g/m^3$)	75	19.2	6.9	18.6	6.04/38.4
WSOC ($\mu g/m^3$)	72	6.2	3.9	5.5	0.4/26.7
pb-PAHs (ng/m^3)	78	118.8	32.3	116	50/207
Hopanes (pg/m^3)	72	822.3	631.0	642.3	67.59/4,218
n-Alkanes (pg/m^3)	75	54996	143,840	32,598	3062/1257,613
Noise (dB)	69	71.6	3.6	72.0	62.2/81.9
Elements (ng/m^3)					
V	76	0.6	0.8	0.4	0.012/7.1
Cr	66	1.3	1.3	0.9	0.008/7.3
Mn	76	2.4	2.2	1.7	0.008/12.0
Fe	75	247.4	232.3	193.4	0.52/1358
Ni	66	1.5	3.1	0.6	0.056/23.1
Cu	73	39.8	57.0	20.7	0.094/3255
Zn	74	19.4	30.1	8.2	0.14/170
Al	71	39.1	46.5	23.5	0.41/265
S	74	381.6	488.1	252.5	17.50/2784
Sb	72	2.9	2.8	2.2	0.023/17.1
Pb	76	1.7	2.8	0.8	0.02/16.7

Abbreviations:$PM_{2.5}$ Particle matter 2.5; WSOC water soluble organic carbon; pb-PAHs particle-bound polycyclic aromatic hydrocarbons; Alkanes C23 to C27 = sum of *n*-alkanes with 23-27 carbons. V Vanadium; Cr Chromium; Mn Manganese; Fe Iron; Ni Nickel; Cu Copper; Zn Zinc; Al Aluminum; S Sulfur Sb Antimony Pb Lead; Max, maximum; Min, minimum.

[a]Measured using filter-based thermal-optical transmittance.

Table 3

Pearson correlation coefficient matrix among select in-vehicle particulate components.

Pollutant	PNC	BC	EC	OC	WSOC	Pb-PAHs	Hopanes	*n*-Alkanes	Noise
$PM_{2.5}$ mass	0.36*	0.50*	0.39*	0.43*	0.01	0.44*	0.37*	0.04	0.27
PNC		0.25*	0.26*	0.29*	–0.01	0.31*	0.34*	0.06	0.27
BC			0.65*	0.21	–0.11	0.85*	0.33*	–0.08	–0.005
EC				0.53*	–0.09	0.76*	0.36*	–0.03	0.11
OC					–0.04	0.30*	0.35*	0.05	0.36
WSOC						–0.14	0.32*	0.25*	0.08
Pb-PAHs							0.46*	–0.07	0.03
Hopanes								0.13	0.22
***n*-Alkanes**									0.07

Abbreviations:$PM_{2.5}$ Particle matter 2.5; BC black carbon; OC organic carbon; WSOC water soluble organic carbon; pb-PAHs particle-bound polycyclic aromatic hydrocarbons *n*-Alkanes = sum of *n*-alkanes with 23-27 carbons.

*$p < 0.05$.

Table 4

Association between commute and biomarker changes, linear mixed model (N = 80).

		Parameter estimate	SE	df	*t* Value	*p* Value	Percent change
eNO							
Post commute	All	8.8	1.8	327	4.84	< 0.0001	9.2
Non-asthmatics		9.6	2.5	159	3.85	0.0002	10.1
Asthmatics		8.0	2.6	163	3.04	0.002	8.3
1 h post commute	All	11.7	2.2	327	5.36	< 0.0001	12.4
Non-asthmatics		10.6	3.0	159	3.51	0.0006	11.1
Asthmatics		12.9	3.1	163	4.10	< 0.0001	13.7
2 h post commute	All	11.3	2.5	327	4.55	< 0.0001	11.9
Non-asthmatics		10.2	3.4	159	2.98	0.003	10.7
Asthmatics		12.4	3.5	163	3.48	0.0006	13.2
3 h post commute	All	9.9	2.7	327	3.62	0.0003	10.4
Non-asthmatics		8.2	3.8	159	2.18	0.03	8.5
Asthmatics		11.6	3.9	163	2.96	0.003	12.3
FEV1							
Post commute	All	0.7	0.5	328	1.35	0.18	0.7
Non-asthmatics		0.3	0.6	157	0.40	0.69	0.2
Asthmatics		1.1	0.8	166	1.43	0.15	1.1
1 h post commute	All	1.9	0.6	328	3.09	0.002	1.9
Non-asthmatics		0.9	0.8	157	1.22	0.22	1.0
Asthmatics		2.9	0.9	166	3.03	0.003	2.9
2 h post commute	All	1.6	0.7	328	2.26	0.02	1.6
Non-asthmatics		1.0	0. 9	157	1.02	0.31	0.9
Asthmatics		2.3	1.0	166	2.13	0.03	2.3
3 h post commute	all	1.5	0.8	328	1.91	0.06	1.5
Non-asthmatics		1.3	0.9	157	1.31	0.19	1.3
Asthmatics		1.7	1.2	166	1.44	0.15	1.7
FVC							
Post commute	All	–0.2	0.4	323	–0.36	0.72	–0.16
Non-asthmatics		–0.04	0.6	157	–0.08	0.94	–0.04
Asthmatics		–0.3	0.6	161	–0.42	0.67	–0.27
1 h post commute	all	0.5	0.5	323	0.98	0.33	0.5
Non-asthmatics		0.2	0.7	157	0.30	0.76	0.2
Asthmatics		0.8	0.8	161	1.08	0.28	0.8
2 hour post commute all		0.3	0.6	323	0.45	0.65	0.3
Non-asthmatics		–0.1	0.8	157	–0.17	0.87	–0.14
Asthmatics		0.7	0.9	161	0.79	0.43	0.7
3 h post commute	all	0.4	0.	323	0.66	0.51	0.4
Non-asthmatics		0.3	0.9	157	0.33	0.74	0.3
Asthmatics		0.6	0.9	161	0.61	0.54	0.6

		Parameter estimate	SE	df	*t* Value	*p* Value	Percent change
MDA							
Post commute	All	8.6	8.9	285	0.96	0.34	8.9
Non-asthmatics		7.1	11.8	125	0.60	0.55	7.3
Asthmatics		10.0	13.0	155	0.77	0.44	10.5
1 h post commute	All	–10.1	9.1	285	–1.11	0.27	–9.6
Non-asthmatics		–8.7	12.5	125	–0.70	0.48	–8.4
Asthmatics		–12.0	13.1	155	–0.91	0.36	–11.3
2 h post commute	All	–14.4	9.1	285	–1.58	0.11	–13.4
Non-asthmatics		–10.6	12.3	125	–0.86	0.39	–10.0
Asthmatics		–17.8	13.2	155	–1.35	0.18	–13.6
3 h post commute	All	–17.4	9.2	285	–1.90	0.06	–15.9
Non-asthmatics		–17.5	12.5	125	–1.40	0.16	–16.0
Asthmatics		–17.9	13.2	155	–1.36	0.18	–16.4
CRP							
Post commute	All	8.0	4.1	201	1.96	0.05	8.3
Non-asthmatics		10.6	5.6	103	1.89	0.06	11.2
Asthmatics		5.6	5.6	93	1.00	0.32	5.7
1 h post commute	All	–0.9	4.8	201	–0.19	0.85	–0.9
Non-asthmatics		3.9	6.4	103	0.62	0.54	4.0
Asthmatics		–5.6	6.5	93	–0.87	0.39	–5.5
2 h post commute	All	0.6	5.4	201	0.12	0.91	0.6
Non-asthmatics		5.9	6.9	103	0.85	0.40	6.0
Asthmatics		–5.1	7.5	93	–0.68	0.50	–4.9
3 h post commute	All	1.0	5.8	201	0.18	0.86	1.0
Non-asthmatics		0.6	7.1	103	0.09	0.93	0.6
Asthmatics		2.1	8.2	93	0.25	0.80	2.1
Heart rate							
Post commute	All	–6.0	3.5	236	–1.73	0.08	–5.8
Non-asthmatics		1.1	4.7	103	0.24	0.81	1.1
Asthmatics		–10.8	4.8	128	–2.24	0.03	–10.2
1 h post commute	All	–0.1	3.5	236	–0.03	0.97	–0.1
Non-asthmatics		2.9	4.9	103	0.59	0.56	2.9
Asthmatics		–2.2	4.8	128	–0.45	0.65	–2.2
2 h post commute	All	–3.1	3.6	236	–0.86	0.39	–3.0
Non-asthmatics		–0.6	5.0	103	–0.12	0.91	–0.6
Asthmatics		–4.8	5.1	128	–0.95	0.34	–4.7
3 h post commute	All	–0.9	3.6	236	–0.25	0.80	–0.9
Non-asthmatics		–1.0	5.1	103	–0.19	0.85	–1.0
Asthmatics		–0.7	5.0	128	–0.15	0.88	–0.7
SDNN10							
Post commute	All	–37.4	5.0	263	–7.54	< .0001	–31.2
Non-asthmatics		–25.7	6.9	117	–3.72	0.0003	–22.6

		Parameter estimate	SE	df	*t* Value	*p* Value	Percent change
Asthmatics		–47.0	6.9	141	–6.86	< .0001	–37.5
1 h post commute	All	–15.5	5.2	263	–2.99	0.003	–14.3
Non-asthmatics		–4.8	7.3	117	–0.66	0.51	–4.7
Asthmatics		–24.5	7.1	141	–3.47	0.0007	–21.7
2 h post commute	All	–17.8	5.3	263	–3.35	0.0009	–16.3
Non-asthmatics		–11.7	7.4	117	–1.57	0.12	–11.0
Asthmatics		–22.9	7.2	141	–3.17	0.002	–20.5
3 h post commute	All	–18.7	5.4	263	–3.46	0.0006	–17.0
Non-asthmatics		–14.3	7.5	117	–1.90	0.0596	–13.3
Asthmatics		–22.5	7.4	141	–3.05	0.003	–20.2
rMSSD10							
Post commute	All	–24.3	7.0	262	–3.48	0.0006	–21.6
Non-asthmatics		–1.8	9.2	116	–0.20	0.84	–1.8
Asthmatics		–42.5	9.9	141	–4.29	< .0001	–34.6
1 h post commute	All	–8.1	7.2	262	–1.12	0.27	–7.6
Non-asthmatics		1.4	9.4	116	0.15	0.88	1.4
Asthmatics		–15.9	10.3	141	–1.54	0.12	–14.7
2 h post commute	All	–7.2	7.4	262	–0.98	0.33	–7.0
Non-asthmatics		2.2	9.5	116	0.23	0.81	2.3
Asthmatics		–15.1	10.5	141	–1.44	0.15	–14.0
3 h post commute	All	–2.3	7.5	262	–0.30	0.76	–2.2
Non-asthmatics		9.0	9.5	116	0.94	0.35	9.4
Asthmatics		–11.7	10.8	141	–1.08	0.28	–11.0

Abbreviations: eNO exhaled nitric oxide; FVC forced vital capacity; FEV1 forced expiratory volume in 1 s; MDA malondialdehyde; CRP C-reactive protein; SDNN standard deviation of normal-to-normal intervals; RMSSD square root of the mean squared difference between adjacent normal-to-normal intervals.