**UNIVERSITY OF CALIFORNIA,**
**IRVINE**

# Cardiovascular, Oxidative Stress, and Inflammatory Consequences of Short-term Exposure to Chemically-characterized Air Pollution in an Elderly Cohort

DISSERTATION

Submitted in partial satisfaction of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

In Epidemiology

By

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# DEDICATION

To

My parents, Xiaorong Wu and Ruquan Zhang,

whose encouragement and support provided me the strength and perseverance;

and my son, Charles Zhang,

whose innocence smiles taught me the beauty of life.

以此论文谨致我的父母 (张汝全，吴晓蓉)

感谢他们一直以来的鼓励与支持。

同时，也感谢我的儿子 (张谨诚)，

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## ABSTRACT OF THE DISSERTATION

Cardiovascular, Oxidative Stress, and Inflammatory Consequences of Short-term Exposure to Chemically-characterized Air Pollution in an Elderly Cohort

By

Xian Zhang

Doctor of Philosophy in Epidemiology

University of California, Irvine, 2016

Professor Ralph J. Delfino, Chair

BACKGROUND: Cardiovascular disease is the leading cause of death in the United States. Short-term exposures to air pollution have been associated with acute increases in cardiovascular hospitalization and mortality. However, the causative chemical components and underlying pathophysiological mechanisms remain to be clarified.

METHODS: We conducted a cohort panel study involving 97 elderly subjects living in the Los Angeles metropolitan area. Weekly, we measured microvascular function, represented by reactive hyperemia index (RHI), and airway and circulating biomarkers of oxidative stress and inflammation, including exhaled breath condensate malondialdehyde (EBC MDA), fractional exhaled nitric oxide, oxidized low-density lipoprotein, and plasma interleukin-6 (IL-6) over 12 weeks. Exposures included 7-day personal nitrogen oxides ($NO_X$), daily ambient pollutants, including particulate matter size <0.25 μm in aerodynamic diameter ($PM_{2.5}$), ozone, carbon monoxide (CO), black carbon (BC), and $NO_x$, five-day average PM measured in three size-fractions and

characterized by chemical components including transition metals, PM oxidative potential, and electrophilic potential. CAlifornia LINE Source Dispersion Model, version 4 (CALINE4) was used to estimate individual exposure to local traffic-generated $NO_x$. Associations between clinical outcomes and pollutants were assessed using linear mixed effects regression models. Potential effect modification by demographic characteristics and CALINE4-modeled NOx was assessed by adding interaction terms to these models.

RESULTS: RHI was inversely associated with daily ambient traffic-related air pollutants (BC, $NO_X$, and CO), other mobile-source components/tracers (polycyclic aromatic hydrocarbons, elemental carbon, and hopanes), PM oxidative potential, and transition metals, and was positively associated electrophilic potential. We found significant positive associations of airway oxidative stress (EBC MDA), and inflammation (IL-6) with traffic-related air pollutants, ultrafine particles, and transition metals. The observed associations were generally stronger among subjects at higher local traffic-generated NOx (estimated by CALINE4). Associations between systemic oxidative stress and inflammation biomarkers and air pollutants were mostly non-significant, expect for positive associations that observed for IL-6 and traffic-related air pollutants at 1-day averages.

CONCLUSIONS: Short-term exposure to pollutants with high oxidative potential (traffic-related pollutants, ultrafine particles, and transition metals) was associated with impaired microvascular function and increased airway oxidative stress and inflammation

in elderly adults. Chronic exposure to residential $NO_x$ may further strengthen the observed associations.

# Chapter 1. Introduction

## 1.1 Background

### 1.1.1 Source and components of air pollutants

Air pollution consists of gases [e.g., carbon monoxide (CO), nitrogen monoxide (NO), nitrogen dioxide ($NO_2$) and ozone ($O_3$)], volatiles (e.g., hydrocarbons, aldehydes), and particulate matter (PM) coming from different sources. PM is a dynamic mixture of different-sized particles and can be defined by their size: ultrafine particles (size <0.1 μm in aerodynamic diameter, $PM_{0.1}$), fine particles (size <0.25 μm in aerodynamic diameter, $PM_{2.5}$) and coarse particles (size < 10 μm μm in aerodynamic diameter, $PM_{10}$). In addition, “mode” is a different way to define PM to emphasize both size and formation (Englert 2004). The nucleation mode refers to particle size smaller than 0.1 μm, formed by nucleation. These small particles can rapidly coagulate and convert to the accumulation mode (0.1 μm < particle size < 2.5 μm) while the particle number decreases (Dennekamp et al. 2001). This process is formed in the atmosphere by photochemical reactions. Particles in the accumulation mode rarely convert into the coarse mode (particle size > 2.5 μm). Therefore, the accumulation mode has a relatively long lifetime in the atmosphere. The coarse mode refers to large particles that are normally generated by mechanical forces, such as wind and erosion. Because of their large size, these particles rapidly settle out with a very short lifetime (with few hours) in

stable atmospheres. The nucleation and the accumulation modes together consist of fine particles and are in urban areas primarily products of fossil fuel combustion, e.g., vehicle traffic and coal-fired power plants.

Particle size is important because it determines entrance, penetration, body deposition, and clearance. The coarse particles can only enter and deposit in the upper respiratory tract of the lung. In contrast, the fine and ultrafine PM can reach the alveolar region of the lungs. Moreover, ultrafine particles can pass through the alveolar epithelium and accumulate in the lung interstitium and might even be translocated from the lung into systemic circulation (Elder et al. 2006). In addition, smaller particles have a greater surface area with a given mass and therefore carry high concentrations of pro-oxidant chemical components, such as polycyclic aromatic hydrocarbons (PAHs) and transition metals, each of which has been shown to induce oxidative stress.

However, size alone is insufficient to completely explain the toxicity of air pollution because of the complex composition of PM. The chemical composition of PM highly varies spatially and temporally, and can have different harmful health effects. The composition can vary due to the source, temperature, wind and humidity. In urban cities, such as Los Angeles, PM components can be dominated by traffic-related air pollutants including PAHs, hopanes, element carbon, and transition metals (Pant et al. 2013). Meteorological conditions can be a big effect as well. For example, in the Los Angeles area, cool marine air onshore from ocean breezes and the cool air from sub-tropical pressure on the top create a warm layer call “inversion layer”. This layer acts as a lid to trap air pollutants near the ground. As discussed below, rather substantial

epidemiological evidence shows that traffic-related air pollutants increase the risk of cardiorespiratory diseases.

### 1.1.2 Biological mechanisms linking between cardiovascular disease and air pollutants

It has long been recognized that exposure to air pollution has been implicated as a risk factor in the pathogenesis of respiratory and cardiovascular diseases (Brook et al. 2010; Franklin et al. 2015). However, the precise underlying pathway and biological mechanisms have yet to be fully elucidated. Existing evidence suggests potential pathological mechanisms include systemic inflammation, oxidative stress, and vascular (including endothelial) dysfunction (Gold et al. 2013). Despite the lack of direct evidence, it has been suggested that associations between air pollution and cardiopulmonary disease could be stronger with PM in a smaller range than a larger range. Traffic-related air pollutants have been shown to be associated with asthma (Delfino et al. 2006), markers of inflammation and oxidative stress, heart rate variability (Shields et al. 2013; Mirowsky et al. 2015), and endothelial dysfunction (Alexeeff et al. 2011). However, with limited evidence, the particular biological mechanism under these association is still unclear.

One hypothesis is that the oxidative stress and inflammation are playing the role of the pathogenesis of respiratory and cardiovascular disease (Vaziri et al. 2006; Schnabel et al. 2007; Forstermann 2008; Crowley 2014). Oxidative stress results when reactive oxygen species (ROS) production overwhelms cells' ability to neutralize and

eliminate the reactive intermediates, leading to damage in tissue and cellular components. This process involves a potent vasodilator product: nitric oxide. It is synthesized from the amino acid L – arginine by the enzyme nitric oxide synthase (NOS) generated by stimulus agonists and shear stress (Furchgott et al. 1980; Luscher et al. 1997). Exposure to air pollutant chemicals capable of generating ROS may result in oxidative stress that reduce the bioavailability of nitric oxide (Utell et al. 2002; Miller et al. 2012). Inhaled particles can provoke an inflammatory response in the lung directly or through oxidative stress, with the consequent release of prothrombotic and inflammatory cytokines into circulation. Once circulating, these cytokines would have direct effect on alterations in autonomic tone and vascular function. For example, toxicological studies have provided evidence that transition metals influence the toxicity of PM. These redox-active metals can induce or catalyse chemical change leading to production of free raicals such as the hydroxyl radical, which have a known ability to cause tissue inflmmation (Harrison et al. 2000). Another hypothesis is that inhaled gas and insoluble fine or ultrafine particles may be capable of migrating across the alveolar membrane into the circulation, with the potential for direct effects on hemostasis and cardiovascular integrity. The third hypthesis is that PM could activate the autonomic nervous system following stimulation of sensory receptors in the lung. Autonomic actionation may alter baroreceptor sensitivity, by which PM can influence the response to systemic vasodilation and other aspects of the cardiovascular system (Miller et al. 2012). However, this process is rather a rapid change and normally takes place within few hours, thus this menchanism is beyond the scope of the present study, which focuses on longer term effects from days to one week.

## 1.2. Hypothesis and aims

Cardiovascular disease is the leading cause of death in the US (Mozaffarian 2016). Short-term exposure to air pollution has been associated with acute increases in cardiovascular hospitalization and mortality. However, the causative chemical components and underlying pathophysiological mechanisms remain to be clarified. Toxicological data suggest pro-oxidant particulate matter chemical species from fossil fuel combustion are causative factors in oxidative stress, inflammation, and vascular dysfunction, which have the potential to precipitate adverse events such as myocardial infarction. The overall objective of this study is to examine the relations of microvascular function, oxidative stress and inflammation in both the airways and circulation to air pollution exposure in an elderly cohort, and to identify subpopulations with potentially increased risk.

In the following chapters, several hypotheses will be tested. These include:

(1) Cardiovascular outcomes are associated with air pollution concentration via alteration of endothelial homeostasis as well as an elevation in airway and systemic oxidative stress and inflammation.

(2) Various air pollutant sources and components will impact these outcomes differently, and that stronger associations will be observed for pollutants linked to vehicular traffic sources and for ultrafine particles.

(3) Adverse cardiopulmonary effects of air pollution will be modified by residential traffic exposure level.

The hypotheses will be tested with the following three aims:

**Aim 1.** To assess the relations of microvascular endothelial function with short-term air pollution exposures.

**Aim 2.** To assess the relations of airway and systemic oxidative stress and inflammation biomarkers with short-term air pollution exposures.

**Aim 3.** To investigate the potential modifying effects of residential traffic exposure level on the relations of microvascular function, and airway and systemic oxidative stress and inflammation biomarkers with short-termair pollution exposures that are established in Aim 1 and Aim 2.

This study assesses the effects of well-characterized air pollution on multiple airway and systemic biomarkers and physiological outcome, and offers a novel approach to air pollution research in human populations. Along with previous toxicological studies, this research can provide new insights into the underlying mechanisms linking adverse cardiovascular effects with air pollution exposure. The investigation of cardiorespiratory responses to air pollution exposure (characterized by size, toxicity and source) is challenging and can add evidence to the existing literature. Results will not only inform regulatory decisions regarding air pollutants that aim to protect sensitive populations, but may also translate into innovative personalized interventions.

# Chapter 2. Methods

## 2.1. Overall study design and population

This study utilized, and added to, data from the parent study: Cardiovascular Health and Air Pollution Study 2 (CHAPS 2). CHAPS 2 is a cohort panel study with up to 12 repeated measurements of cardiovascular outcomes and exposures in 97 elderly people (age > 65 years) who are non-smoking individuals living in the Los Angeles metropolitan area (Zhang et al. 2016b). The research targeted two regions of the Los Angeles metropolitan area: downtown Los Angeles and Anaheim. A total of 8 South Coast Air Quality Management District (SCAQMD) air quality monitoring stations and two University of Southern California (USC) monitoring sites are located in those two areas (Figure 2.1).

Subjects were recruited from study area and the inclusion and exclusion criteria were listed as below:

*Inclusion Criteria*:

1) Age > 65;

2) Live in selected regions;

3) Sufficiently ambulatory to attend the weekly follow-up study visits.

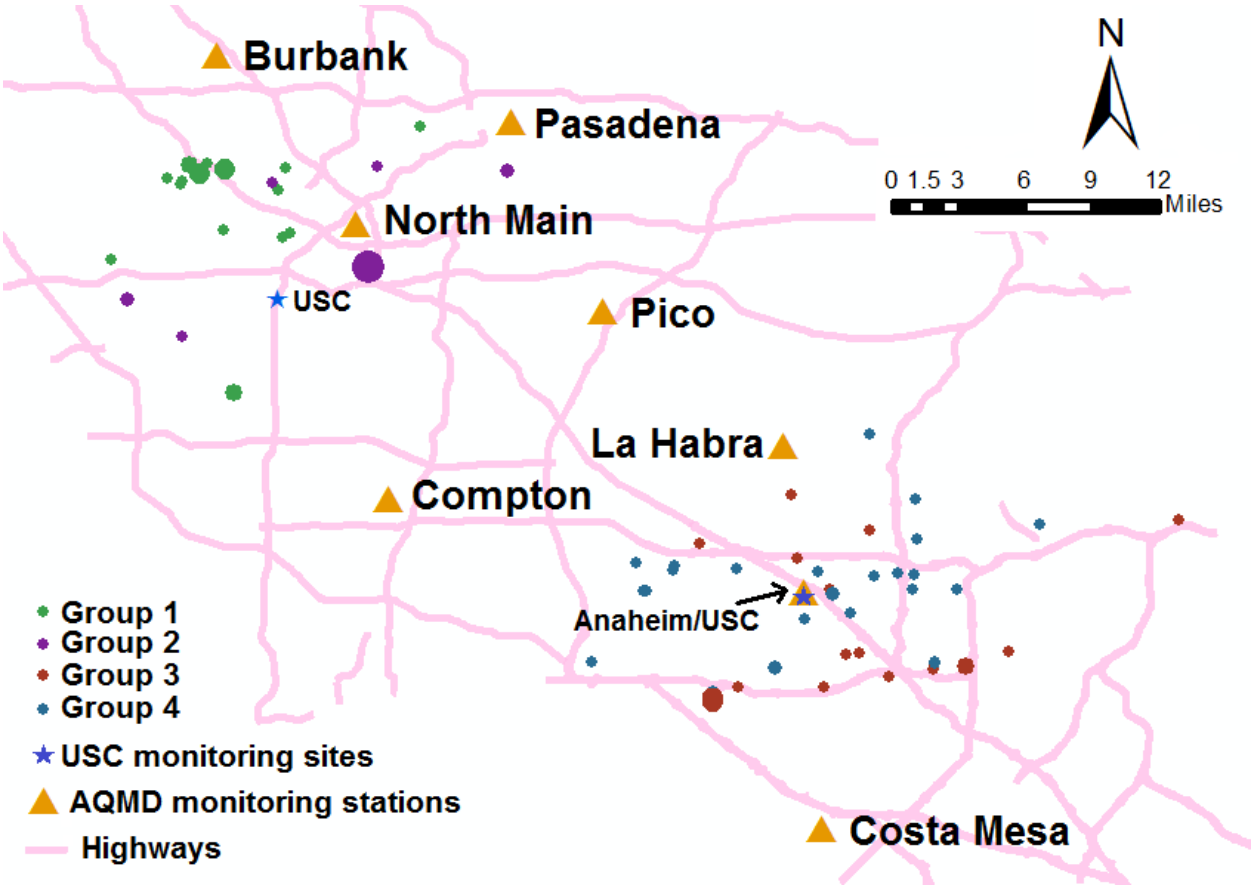


**Figure 2.1. Geographic locations of central monitoring stations and subjects' residential addresses in the study area.**
Size of circles reflect number of subjects.

*Exclusion Criteria:*

1) Employment outside of the monitored community;

2) Smoking within the last 12 months;

3) Exposure to environmental tobacco smoke at home or on a regular basis at other locations;

4) Psychiatric disorder, dementia, alcohol or drug abuse;

5) Dialysis treatment or renal failure;

6) Daily oral corticosteroids;

7) Active cancer;

8) Medical conditions that prevent the subject from giving blood;

9) Medical and physical condition that prevent the subject from the procedure to measure microvascular endothelial function using the EndoPAT device (Itamar Medical, Caesarea, Israel).

This population was selected because previous studies have suggested that the elderly may be particularly susceptible to the adverse cardiovascular effects of air pollution exposure (Brook et al. 2010).

Exclusion criteria 1 is to increase the validity of exposures measured at the nearby community air monitoring sites.

Exclusion criteria 2 and 3 are intended to reduce the influence of this risk factor on associations between health outcomes and personal exposures to air pollution of outdoor origin, which is the primary exposure of interest. Smoking as well as passive smoke are well-known cardiovascular risk factors (Glantz et al. 1991).

Exclusion criteria 4 is to ensure subjects are able to complete the clinic follow-ups, including filling out daily diaries, wearing the personal air sampling badge and global positioning system (GPS), and coming to a community location each week at the same time for blood draws and exhaled breath measurements.

Exclusion criteria 5-7 are to control the overriding influence of these factors on outcomes, especially the biomarker responses.

Exclusion criteria 8 is to protect subjects from health problems related to phlebotomy, including hemophilia, serious anemia, or nutritional disorders.

Exclusion criteria 9 is to protect subjects from the use of the EndoPAT. Subjects who were using an anticoagulant (Coumadin/warfarin) and subjects whose fingers have deformities or injuries that cannot perform the test were excluded from the EndoPAT test.

Each subject was followed weekly with measurements of vascular function and airway inflammation, and blood draws for protein biomarkers of systemic inflammation and oxidative stress.

The subject follow-up flow results are shown in Figure 2.2. Subjects who were not retained were either discovered later to be ineligible or they decided to not participate before or shortly after the start of the weekly panel follow-up. Infections have a profound confound factor on the analysis of exposure-response relations and weeks that subjects reported acute infection thus were excluded *a priori*.

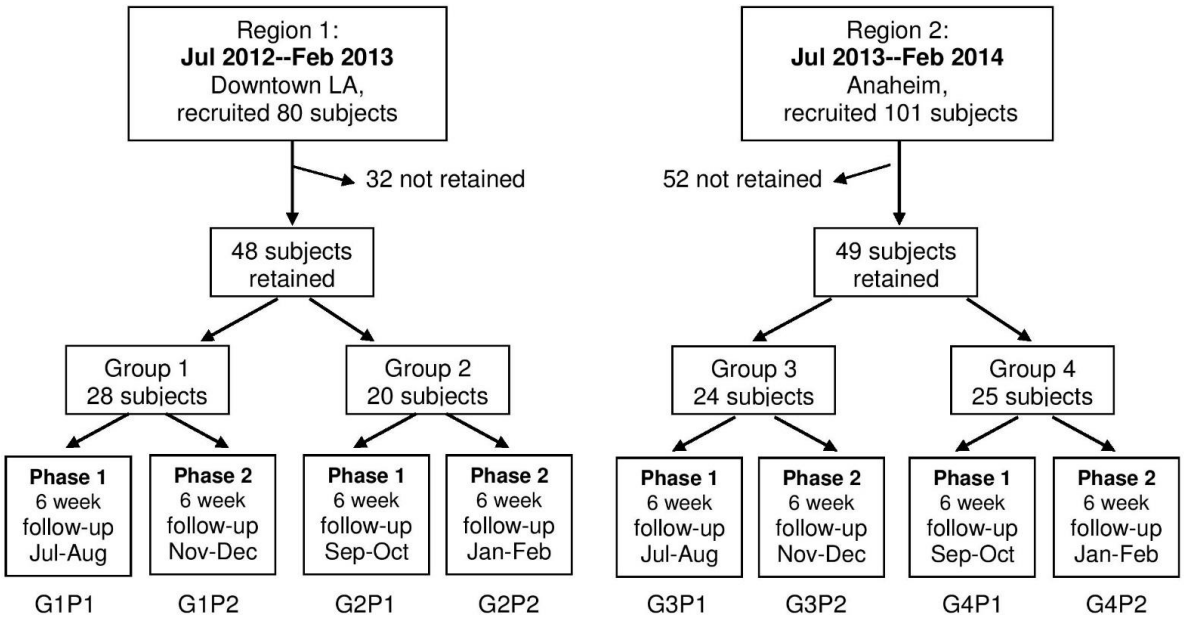


**Figure 2.2. CHAPS 2 study flow chart.**

## 2.2. Baseline measurements

A background questionnaire was administered at the beginning of the study that included medical history, socioeconomic status (SES), medications, history of active smoking, and environmental exposure profile. A fasting blood sample was taken to obtain for plasma lipid and glucose profiles.

## 2.3. Follow-up

After baseline measurements were taken, subjects began weekly visits to local community clinics at fixed days of the week (Fridays in the first year and Saturdays in the second year) and fixed times of the day for each subject to control for circadian and day-of-week variation in outcomes. Two groups of subjects in each study area were followed alternatively in two discrete 6-week phases (Figure 2.2). This design ensures sufficient variation in air pollutants by season, and allows for a subject rest period between the two 6-week follow-up phases. Before each weekly clinical follow-up, subjects completed a diary once per day to report on health status, medication use, passive smoke exposure and travel in motor vehicles. A daily home environment questionnaire also was used to assess indoor exposures to gas cooking-related nitrogen oxides ($NO_x$: $NO+NO_2$) that may influence measured personal $NO_x$ exposures. The compliance rate for diary completion was 88.9%.

## 2.4. Outcome Measurements

In this study, we measured one cardiovascular outcome: microvascular function and a panel of airway and systemic biomarkers. These biomarkers include an airway oxidative stress biomarker: malondialdehyde in exhaled breath condensate (EMB MDA), an airway inflammation biomarker: fractional concentration of exhaled nitric oxide (FeNO), a systemic oxidative stress biomarker: oxidized low-density lipoprotein (oxLDL) and a systemic inflammation biomarker: Interleukin-6 (IL-6). Details for outcome measurements are described in Chapters 4 and Chapters 5.

## 2.5. Exposure measurements

### 2.5.1. Overview of air pollution exposure data measurements

Air pollutant exposure data for this study were obtained from 1) the SCAQMD, which reports data to the U.S. Environmental Protection Agency (EPA), as measured at 8 monitoring stations in the study area, 2) Two USC sites located in the study areas, and 3) personal exposure to $NO_x$. A summary of measured exposures is presented in Table 2.1. A map with the locations of the SCAQMD monitoring stations and the USC sites is presented in Figure 2.1.

### 2.5.2. Data from the SCAQMD monitoring station

Ambient hourly concentrations of U.S. EPA criteria air pollutants ($PM_{2.5}$ mass, CO, NOx and $O_3$) were retrieved from the 8 SCAQMD monitoring stations. However, primary air pollution data were obtained from one site for each study year/region, which was to serve as the main monitoring station (the Los Angeles monitoring station on North Main Street for the first year, and the Anaheim monitoring station for the second year (Figure 2.1). Daily (24-hr) averages were computed from hourly data and required at least 18 (of 24 possible) hourly values (availability of ≥75%). If data were available for less than 18 hours in a given day, the daily value of this day was considered as missing. Hourly metrological data including temperature, relative humidity, wind direction and wind speed were only obtained from two main monitoring stations. Hourly heat index, which aims to capture the combined experience of both temperature and relative humidity, was calculated according to a previous study (Anderson et al. 2013).

*Missing values imputation*

Exposure data were not obtained from the nearest air pollution monitoring stations according to subjects' residential addresses. This is because it is difficult to compare ambient data obtained from multiple stations to size-fractionated PM concentrations, which were measured only at one station each year. Therefore, for SCAQMD exposure data, we only utilized data from the two main stations which have minimal distance to all subjects. If there were missing data from the main stations, other stations were used to impute missing data using a linear regression model. To impute

missing values, the correlations for each ambient air pollutant between the main stations and other monitoring stations in the study area were calculated during the study period. If missing data for exposures were observed in the two main stations, the site used to impute missing data was selected by the highest correlation and availability of the exposure data. Missing data were imputed using a linear regression model:

$$Y = \beta_0 + \beta_1 X_1 + \varepsilon$$

Where $Y$ is the vector of the predicted variable

$\beta_0$ is the estimate of the intercept

$\beta_1$ is the estimate of the slope

$X_1$ is the exposure variable from the closest station

$\varepsilon$ is the error term

All imputation linear models had $R^2$ >0.8. If there were no other available data from nearby stations in the study area, then linear interpolation was used to impute missing data. We had a maximum of three continuous days of missing (except for BC in Los Angeles had one 8-day period that was missing). Less than 4% of the exposure data were linearly interpolated

Ambient air pollutant concentrations for 1-day, 3-day, 5-day and 7-day averages preceding clinic follow-ups were calculated from the daily data. We preselected these averaging times to represent effect estimation for up to 7 days of exposure while avoiding an excessive presentation of data using all seven daily averaging times.

### 2.5.3. Data from the USC sites

We also collected hourly $PM_{2.5}$ black carbon (BC, Aethalometer model AE22, Magee Scientific, Berkeley, CA) and 5-day integrated concentrations of $PM_{0.18}$, $PM_{0.18\text{-}2.5}$ and $PM_{2.5\text{-}10}$ (MOUDI, model 100-1, MSP, Inc., Minneapolis, MN) at the USC monitoring sites near downtown Los Angeles and in Anaheim. The three particle size ranges represent the ultrafine mode ($PM_{0.18}$), the accumulation mode ($PM_{0.18\text{-}2.5}$), and the coarse mode ($PM_{2.5\text{-}10}$). Although the typical size range for ultrafine particles has been stated in studies to be < 0.1 μm, the reported upper size cutoff point for particles that dominate the particle number concentration (which may be considered in the ultrafine mode) has varied temporally and spatially from 0.1 to 0.2 μm (Sioutas et al. 2005). We considered five days of continuous particle collection because it was necessary to obtain a sufficient amount of PM mass loading for the chemical and oxidative potential assays described below and this duration had been previously demonstrated to be associated with cardiovascular and biomarker outcomes (such as IL-6) in in our previous studies (Delfino et al. 2008; Delfino et al. 2010b; Delfino et al. 2010c). The USC monitoring site for Los Angeles was approximately 3 km southwest of the SCAQMD monitoring station where criteria air pollutants were measured, and was at the same location as the SCAQMD station in Anaheim (Figure 2.1). Carbonaceous species were measured in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$, and we included as exposure variables the sum of PAHs, the sum of hopanes (Table 2.3) and elemental carbon (EC) as markers of various fossil fuel combustion products. Organic carbon (OC) was measured and is representative of both primary and secondary organic carbon sources. We also used the sum of organic (n-alkanoic) acids as a tracer of secondary organic aerosols

(Rogge et al. 1993). For elemental species, we included the sum of available transition metals (V, Cr, Mn, Ni, Cu, and Fe) in all three size-fractions because they are known to participate in catalyzing Fenton's reaction, which can generate oxidative stress (Ghio et al. 2012). Composites were analyzed for oxidative potential [alveolar macrophage reactive oxygen species (ROS) assay and dithiothreitol (DTT) activity], and electrophilic potential (glutathione peroxidase-1, GPx-1) (Staimer et al. 2012). The pollutant air sampling measurements and instrumentation are summarized in Table 2.1. The detailed measurements of PM carbonaceous aerosol, oxidative potential, and electrophilic potential are described below.

*Measures of PM carbonaceous aerosol*

Following gravimetric measurements using a precision microbalance (Mettler Toledo Inc., Columbus, OH, USA) ($\pm$ 0.001 mg), filters were sectioned and particles extracted for chemical characterization. EC and OC were quantified from a 1.5 $cm^2$ punch taken from the quartz/aluminum filter according to the National Institute for Occupational Safety and Health Thermal Optical Transmission method (Schauer et al. 2003). PAHs and hopanes were analyzed using gas chromatography mass spectrometry (Stone et al. 2008). The total elemental composition of the three size fractions was measured by digestion of a section of the Teflon filter-collected PM and subsequently analyzed by high-resolution sector-field inductively-coupled plasma mass spectrometry (SF-ICPMS).

*Measures of PM oxidative potential*

Alveolar macrophage ROS assay represents the biotic oxidative potential of particle mixtures. Details of these methods are described elsewhere (Landreman et al. 2008). Briefly, biotic ROS production was quantified by extracting the filter with 1.00 ml of Milli-Q water. Both unfiltered (total ROS) and filtered (water-soluble ROS) (0.22 μm polypropylene syringe filter) PM extracts were then exposed to rat alveolar macrophage cells (NR8383, American Type Culture Collection) in a 96-well plate. The fluorescent probe DCFH-DA (2',7'-dichloroluorescein diacetate) was used and the fluorescence intensity was measured using a plate reader, thus representing the cell-based oxidative generating capacity of PM. A model of the microbial particles, un-opsonized Zymosan (a β-1,3-polysachharide of D-glucose) served as a positive ROS control as it binds to Toll-like receptor-2 on macrophage cells and then activates a strong respiratory burst and ROS production. ROS results are reported in μg Zymosan equivalent units per $m^3$ air.

DTT activity represents the capacity of the PM extract to generate abiotic chemically-produced ROS (electron transfer from DTT to oxygen). DTT activity was quantified using a well-established method (Cho et al. 2005) on extracts of 5-day composites of the three different size-fractionated PM from quartz filters.

*Measures of electrophilic potential*

Highly reactive electrophiles are expected to add to the endogenous burden of oxidative stress. Since GPx-1 is an important antioxidant and vulnerable to electrophilic attack, we used GPx-1 as a bioassay probe in a high throughput 96-well microtiter plate format to screen for the inhibitory electrophilic potential of the aqueous $PM_{0.18}$ fraction on antioxidant enzyme activities. This antioxidant enzyme is irreversibly inactivated by electrophile-derived covalent modifications (oxidative cross-linking) of the selenocysteine and cysteine residue at the catalytic center of GPx-1 that would be expected to increase oxidative stress. Details are described previously (Staimer et al. 2012). Although the $PM_{18\text{-}2.5}$ fraction was also of interest given the potential electrophilic chemicals in that fraction, the samples could not be assayed due to insufficient dilution.

### 2.5.4. Personal exposure measurements

The Ogawa passive badge sampler (Ogawa & Co. USA, Inc. Pompano Beach, FL) was used to collect seven-day average personal exposures to $NO_X$. Subjects were instructed to wear the sampler clipped outside of their clothing and placed near their bedside at night. Personal $NO_X$ was collected on cellulose fiber filters and concentrations were determined by a spectrophotometer at a wavelength of 545 nm following the manufacturer's instructions (Ogawa & Company 2006).

## 2.6. Statistical analysis

To account for the clustering of longitudinal repeated measurements taken on

each subject, we performed repeated measurement analysis to investigate the association between outcomes and air pollutants using a linear mixed effect model that included a random subject intercept to account for the correlation of repeated measures. The general form of this model is given by:

$$Y_{i,j} = a_{i,} + \alpha Z_{i,} + \beta X_{i,j} + \gamma W_{i,j} + \varepsilon_{i,j}$$

Here, *i* indexes the subject (i =1,…, 97), *j* indexes the outcome measurement on each subject (*j* =1,…,12). $Y_{i,j,}$ is the outcome measurement, $a_i$ is the random subject intercept, $Z_i$ is a vector of time-invariant subject characteristics (e.g. sex or medical conditions), $X_{i,j}$ is a vector of time-variant air pollutant exposure levels (of primary interest), $W_{i,j}$ is a vector of time- variant covariates (time trend, medications, and weather), and $\varepsilon_{i,j}$ denotes random within-person error in the outcome measurement. The best covariance structure of $\varepsilon_{i,j}$ was selected using Akaike's information criterion (AIC) and was the autoregressive moving average (ARMA) (1,1) covariance structure. To compare associations across different pollutants, the magnitude of effect was expressed relative to an interquartile range (IQR, 25$^{th}$ to 75$^{th}$ percentile) difference in each pollutant concentrations. All statistical analyses were performed using R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria) or SAS 9.3 software (SAS, Cary, NC).

Table 2.1. Multi-Pollutant Air Sampling Measurements and Instrumentation

| Air Pollutant Measurement (Sampling Monitor) | Sampling Frequency |
|---|---|
| **PM Measurements** | |
| Size-fractionated $PM_{2.5-10}$, $PM_{0.18-2.5}$, & $PM_{0.18}$ mass (MOUDI) | 5-day |
| Size-fractionated $PM_{0.18-2.5}$ & $PM_{0.18}$ EC and total OC (MOUDI) | 5-day |
| Size-fractionated $PM_{0.18-2.5}$ & $PM_{0.18}$ speciated organics (MOUDI) | 5-day |
| Size-fractionated $PM_{2.5-10}$, $PM_{0.18-2.5}$, & $PM_{0.18}$ metals and inorganic ions (MOUDI) | 5-day |
| Size-fractionated $PM_{2.5-10}$, $PM_{0.18-2.5}$, & $PM_{0.18}$ ROS and DTT | 5-day |
| Size-fractionated $PM_{0.18}$ GPx-1 | 5-day |
| $PM_{2.5}$ mass measurement [Beta Attenuation Monitor (Model 1400, R&P, Inc.)][a] | Hourly |
| Black carbon [Aethalometer (model AE22, Magee Scientific, Berkeley, CA)] | Hourly |
| **Gaseous Pollutant Measurements** | |
| Nitrogen Oxides [$NO_X$, chemiluminescence (Teledyne API model 200E)] [a] | Hourly |
| Carbon Monoxide [CO, infrared absorption (Horiba model APMA-360)] [a] | Hourly |
| Ozone [$O_3$, UV photometer (Teledyne API model 400E)] [a] | Hourly |
| **Personal nitrogen oxides ($NO_x$)** (Ogawa passive badge sampler) | Weekly |
| **Meteorology:** Temperature, Relative Humidity | Hourly |

[a] Data are provided by South Coast Air Quality Management District monitoring stations in study areas.
DTT: dithiothreitol; EC: elemental carbon; GPx-1: glutathione peroxidase-1; OC: organic carbon; PM: particulate matter; ROS: reactive oxygen species.

Table 2.2. Daily ambient exposure missing rates and results of imputation.

| **Downtown Los Angeles n=228** | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Pollut-ants** | Primary Method | Variable | Missing Rate | Inter-cept | Slope | $R^2$ | Secondary Method | Missing Rate | Dependent Variable | Inter-cept | Slope | $R^2$ |
| **BC** | Linear interpolation[a] | | 3.95% | | | | | | | | | |
| **$PM_{2.5}$** | Linear interpolation | | 2.19% | | | | | | | | | |
| **O3** | linear regression | Burbank | 6.14% | 2.55 | 0.79 | 0.86 | | | | | | |
| **$NO_X$** | linear regression | Burbank | 27.19% | 2.38 | 0.91 | 0.87 | Linear regression | 8.33% | Pasadena | -8.27 | 1.99 | 0.84 |
| **CO** | linear regression | Burbank | 4.47% | 0.23 | 0.76 | 0.88 | | | | | | |
| **Anaheim n=229** | | | | | | | | | | | | |
| **BC** | Linear interpolation | | 2.62% | | | | | | | | | |
| **$PM_{2.5}$** | linear regression | Long Beach | 8.30% | 4.55 | 0.79 | 0.81 | Linear interpolation | 3.93% | | | | |
| **O3** | linear regression | La Habra | 10.91% | 2.66 | 1.00 | 0.90 | Linear interpolation | 2.18% | | | | |
| **$NO_X$** | linear regression | La Habra | 9.61% | 0.48 | 0.97 | 0.92 | Linear interpolation | 2.18% | | | | |
| **CO** | linear regression | La Habra | 4.80% | 0.16 | 0.79 | 0.87 | Linear interpolation | 2.18% | | | | |

[a]Linear interpolation was applied where no other available data from nearby stations;

Table 2.3. Measured organic components in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$.

**Total polycyclic aromatic hydrocarbons**
Fluoranthene
Pyrene
Benzo(ghi)fluoranthene
Benz(a)anthracene
Chrysene
1-Methylchrysene
Retene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(e)pyrene
Benzo(a)pyrene
Perylene
Indeno(1,2,3-cd)pyrene
Benzo(g,h,i)perylene
Coronene

**Hopanes**
17α(H)-22,29,30-Trisnorhopane
17α(H)21$\beta$(H)-30-Norhopane
17α(H)21$\beta$(H)-hopane
22S-Homohopane
22R-Homohopane
22S-Bishomohopane
22R-Bishomohopane

**Selected organic acids**
Heptadecanoic acid
Hexadecanoic acid
Octadecanoic acid
Pentadecanoic acid
Tetradecanoic acid
Phthalic acid

# Chapter 3. Microvascular function analysis

## 3.1. Background

### 3.1.1. Endothelial function

The endothelium, a barrier between the vessel lumen and surrounding tissues, is a thin layer of cells that line the interior surface of blood vessels. Endothelial cells are able to synthesize and secrete an important vasodilator molecule, namely: nitric oxide, a gas that is generated from the metabolism of L-arginine by endothelial nitric oxide synthase (eNOS) (Flammer et al. 2011). Endothelial dysfunction, the inability of blood vessels to dilate fully in response to an appropriate stimulus, is the earliest stage when the endothelium undergoes functional and structural alteration in becoming a pro-atherosclerotic structure (Cai et al. 2000; Yeboah et al. 2007; Flammer et al. 2011). The fundamental feature of this condition is impaired nitric oxide bioavailability with the consequence of either a reduced production of eNOS or of an increased depletion caused by ROS (Flammer et al. 2011). Recent studies support the hypothesis that the state of endothelial dysfunction is an independent prognostic predictor for the risk of developing cardiovascular disease (Struijker-Boudier et al. 2007). Therefore, the endothelium represents a potentially valuable endpoint to a therapeutic approach to cardiovascular risk management.

### 3.1.2. Measurements of endothelial function

The first measurement of endothelial dysfunction used intracoronary infusion of acetylcholine and quantitative coronary angiography in 1986 (Ludmer et al. 1986). Flow-mediated dilatation (FMD), a non-invasive technique, was developed in 1992, using branchial artery as a surrogate for coronary arteries (Celermajer et al. 1992). Since then, FMD has been widely used in clinics and considered as a “gold standard” for the demonstration of endothelial dysfunction. However, there are several limitations of this procedure. First, it requires extensive sonographer training and use of high-resolution ultrasound equipment. Second, the analysis of images is costly and time-consuming and operator-dependent. Third, it represents only macrovascular function and may not capture all dimensions of endothelial dysfunction. Given these limitations, assessment of microvascular function using the EndoPAT device (Itamar Medical, Caesarea, Israel) is emerging as an alternative to FMD. The EndoPAT device tests the reactive hyperemia response via measurement of peripheral blood volume, yielding the reactive hyperemia index (RHI) score. A low RHI score indicates impaired endothelial function. The basic principle of FMD and EndoPAT is similar. The healthy coronary, branchial or microvascular arteries dilate in response to reactive hyperemia (flow-mediated vasodilation) or after pharmacological stimuli via release of nitric oxide, while the impaired arteries have decreased ability in this regard. However, it is important to note that vasodilator responses that are examined may have different clinical significance (Flammer et al. 2012). Emerging research shows that the microvascular circulation may be an important dimension of many cardiovascular conditions (Rubinshtein et al. 2010;

Matsuzawa et al. 2013) and many studies have demonstrated that using EndoPAT to assess microvascular function is an informative method (Moerland et al. 2012). EndoPAT has been used to monitor microvascular function with different lifestyle modifications, such as smoking cessation, physical activity, supplement intake and dietary change (Bard et al. 2010; Skulas-Ray et al. 2011; Goldstein et al. 2012; Flammer et al. 2013; Lerman et al. 2014). Recent studies have also been carried out to test the reliability of EndoPAT to evaluate acute changes in microvascular function in individuals with metabolic syndrome. These studies demonstrated that EndoPAT can be used to assess microvascular dysfunction in adults with morbidity as reliably as in other populations. This device was validated and used in the Framingham Study, where a significant inverse relation was observed between microvascular function as determined by EndoPAT and multiple cardiovascular risk factors (i.e., male sex, body mass index, total to high-density lipoprotein (HDL) cholesterol ratio, diabetes, and smoking) (Hamburg et al. 2008). A meta-analysis of three EndoPAT studies for cardiovascular events demonstrated a pooled relative risk of 0.85 (95% CI: 0.78, 0.93) for every 0.1 increase in RHI (Xu et al. 2014). In a large community-based cohort study, microvascular dysfunction assessed by EndoPAT provided additional prognostic information to the Framingham Risk Score (Lind et al. 2011). In a study with 270 subjects, a lower RHI value was associated with a greater number of adverse cardiac events during a 7-year follow-up in a cohort of asymptomatic patients, highlighting the role of RHI as an independent predictor (Rubinshtein et al. 2010). A more recent study from Japan also reported that, in subjects with coronary artery disease, RHI was an independent predictor of adverse events in addition to Framingham risk score and the

SYNTAX score (a unique tool to score complexity of coronary artery disease) (Matsuzawa et al. 2013). In addition, it has been suggested that EndoPAT data can identify patients in the early stages of coronary artery disease (Bonetti et al. 2004).

### 3.1.3. Air pollution and endothelial function

Previous studies have reported positive associations between cardiovascular morbidity and mortality with short-term exposure to air pollutions (Brook et al. 2010). While precise pathways underlying these associations have yet to be clarified, it has been hypothesized that the short-term cardiovascular effect of air pollution exposure may be mediated by induction of abnormal vascular responses characterized by reduced endothelium-mediated vasodilation and vessel constriction (Miller et al. 2012; Gold et al. 2013). Most previous studies have focused on macrovasculature endothelial function assessed using FMD of the brachial artery. However, recent studies show that dysfunction in the microvascular circulation can be an important dimension of many cardiovascular conditions (Rubinshtein et al. 2010; Matsuzawa et al. 2013). Several epidemiological studies investigated the effect of air pollution exposure on microvascular function. A recent cross-sectional study investigated microvascular function using peripheral arterial tonometry, and reported associations between baseline pulse amplitude and short-term exposure to ambient air pollutants, including $PM_{2.5}$, BC, and particle number concentrations, but not with vasodilator response (Ljungman et al. 2014). A cohort panel study examined microvascular function in the retinal blood vessels and suggested that short-term exposure to higher levels of $PM_{10}$

and BC may be associated with damage to the retinal microvasculature (Louwies et al. 2013). In a cross-over study among 53 healthy non-smoking women, ultrafine particle exposure was associated with a decrease in microvascular function that was measured by EndoPAT during physical activity (Weichenthal et al. 2014). To our knowledge, no cohort panel studies have evaluated relationships between peripheral microvascular function and air pollution, and there are no data on the importance of particle oxidative potential or specific particle components to microvascular function.

It is important to note in this regard that ambient air particles are a complex mixture of numerous components originating from different sources (Brook et al. 2010), each with complex particle size distributions. This may result in different adverse health effects. An increasing literature suggests that ultrafine particles (aerodynamic diameter smaller than approximately 0.1-0.2 μm), as compared with larger particles, may have greater adverse cardiovascular effects because of higher deposition efficiency and larger surface area (Delfino et al. 2005), as well as higher redox activity (Araujo et al. 2008)

## 3.2. Methods

### 3.2.1. Study design and population

This analysis was performed in the CHAPS 2 cohort. This cohort panel study is described in detail in Chapter 2. Briefly, subjects were recruited from two areas in the

Los Angeles metropolitan area. To be eligible, a person had to be age 65 years or older, a nonsmoker, not working outside of the monitored areas, and without medical conditions that may interfere with measured outcomes. Two groups were studied in 2012-2013 and another two groups were studied in 2013-2014. Each subject was followed for up to 12 weeks. Each group was studied in two discrete 6-week seasonal periods in order to increase the variability in primary and secondary organic aerosols and to provide a subject rest period of around 2-3 months. Ninety-three of 97 subjects were included in this analysis because three subjects could not tolerate the microvascular function measurement and one subject had less than four valid microvascular function measurements, which was an *a priori* criterion for inclusion in the repeated measures analysis.

This study was approved by the institutional Review Board of the University of California, Irvine. All subjects provided written informed consent before participation.

### 3.2.2. Outcome measurements

Microvascular function was measured weekly with forearm blood flow dilatation response to brachial artery occlusion using a noninvasive plethysmograph (EndoPAT 2000, Itamar Medical, Israel), yielding the RHI score. During the 15 min test, each subject was supine and in a relaxed condition. The EndoPAT device employs finger probes with inflatable air cuffs placed on the index finger of each hand, one being the control side and the other the test side. It records finger arterial pulsatile volume changes with plethysmographic bio-sensors that impart a uniform sub-diastolic pressure

(60 mmHg above diastolic blood pressure up to 300 mmHg) in order to prevent distal venous blood pooling and veno-arteriolar vasoconstriction reflex. After recording 5-minute baseline data, a 5-minute occlusion of the brachial artery is performed using a standard blood pressure cuff on the arm of the test finger. After cuff release, the surge of blood flow causes an endothelium-dependent, flow-mediated dilatation. This reactive-hyperemia response in the hyperemic finger is then measured by EndoPAT over another 5 minutes. The RHI is the outcome variable, which is calculated as the increase in peripheral arterial tone signal amplitude (post-occlusion to pre-occlusion ratio), normalized by a baseline correction factor [$K$ = 0.52397×log (mean baseline amplitude) − 0.2], as calculated by the EndoPAT software (Figure 3.1). This measurement was used to represent microvascular function, the lower RHI score indicates impaired endothelial function.

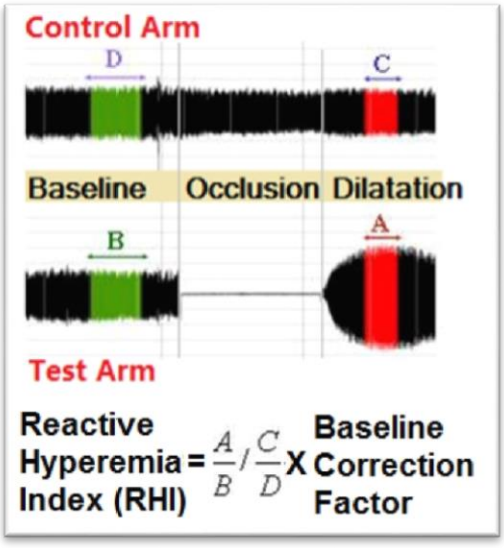


**Figure 3.1.** Microvascular function measurement using EndoPAT 2000

Systolic and diastolic blood pressures were measured before the EndoPAT using the Omron 7015IT (Omron Health Care, Kyoto, Japan) with direct computer linkage (Mengden et al. 2010). Due to space limitations in our clinics, our blood pressure measurements were taken under non-standard conditions, namely insufficient time for subjects to rest (< 5 min), as well as noisy and potentially stressful level of social activity in the common areas. Therefore, blood pressure measurements were only used to assist in setting the cuff inflation pressure for the EndoPAT.

### 3.2.3. Exposure measurement

We collected exposure data for the week preceding the microvascular measurement. Detailed descriptions of exposure measurement are given in Chapter 2. Briefly, we obtained hourly exposures of EPA-criteria air pollutants ($PM_{2.5}$, CO, $NO_x$ and $O_3$) from SCAQMD monitoring stations. We collected size-fractionated PM ($PM_{0.18,}$ $PM_{0.18\text{-}2.5,}$ and $PM_{2.5\text{-}10}$) on filters over 5 days before microvascular measurements and hourly exposure of BC from the USC monitoring sites. We also evaluated concentration of 5-day average organic components (PAHs, Hopanes and organic acids) in $PM_{0.18}$ and $PM_{0.18\text{-}2.5,}$ and composites for OC/EC, and transition metals in all three size-fractionated PM samples. We are particularly interested in traffic-related air pollutants because previous studies have shown strong associations between cardiovascular outcomes and exposure to traffic-related air pollutants. In order to assess PM oxidative potential, we measured *in vitro* redox activity by two different methods: alveolar macrophage ROS assay (Landreman et al. 2008), representing the biotic oxidative potential of particle

mixtures, and DTT activity (Li et al. 2003), representing the abiotic oxidative potential. Electrophilic potential, represented by GPx-1, was measured in $PM_{0.18}$. Finally, 7-day average personal exposures to $NO_x$ were collected using the Ogawa passive badge sampler.

### 3.2.4. Statistical analysis

We analyzed pollutant-outcome relationships using a linear mixed model to accounting for the correlation of repeated measures with subjects. The models were described in detail in Chapter 2.

*Selection of covariates*

Time-invariant subject characteristics were controlled by study design and the specified repeated measures model, and hence were not included as adjustment covariates. An *a priori* covariate was heat index with the same lag as the pollutant to adjust for the effects of weather. Other time-variant potential confounders were tested in the models. Exercise (Green et al. 2004) and/or food intake (Gokce et al. 2001) can change peripheral blood flow and potentially affect microvascular function. In the model, we adjusted exercise and/or food intake within an hour before subject came to the clinic as a potential confounding factor for microvascular function. Days of gas stove use per week were significantly associated with increased personal $NO_x$ and were adjusted in the models of personal $NO_x$ because the exposure of interest was outdoor fossil fuel

sources of $NO_x$. Long-term temporal trend was tested by including cubic splines using different knots for day of study. However, it did not significantly change the estimation or improve model fit. As a result, adjustment for temporal trend was not included in our final model.

*Influential observations*

The impact of influential observations was assessed using the Cook's D statistic and standardized residual diagnostics, at both the individual observations level and clustered subjects level (Cook 1977). No evidence to suggest a departure from normality was observed and no significant influential observations were detected.

*Sensitivity analysis*

Several sensitivity analyses were conducted. First, to investigate potential exposure error, we restricted the analysis to the subjects who lived within the 90$^{th}$ percentile of subjects' residential distance to the stations (11.3 km for the SCAQMD monitoring stations and 13.1 km for the USC monitoring sites). Second, we limited the analysis to measured ambient exposure, rather than exposure including imputed data. Third, we excluded days with extreme heat index (< 52.5 and > 75.04, 10$^{th}$ percentile and 90$^{th}$ percentile of the heat index during the study period, respectively) because the decreased ventilation at subjects' residential buildings during those days may lead to an increased exposure error. Finally, we tested for the independent effects of

photochemically-related and primary air pollutants by using two-pollutant models with $O_3$ and another primary pollutant.

*Effect modifications*

Risk factors for cardiovascular disease may be potential effect modifiers of the association between microvascular function and air pollution. Accordingly, we tested them by incorporating multiplicative interactions with air pollutants in exploratory analyses. These risk factors included age (> 75 years old), sex, body mass index (BMI ≥ 30 kg/cm$^2$), measured hypertension (systolic blood pressure > 140 or diastolic blood pressure > 90), diabetes mellitus, hypercholesterolemia by history, high cholesterol (total cholesterol > 200 mg/dL), high low-density lipoprotein (LDL) concentration (≥ 140 mg/dL), total cholesterol/HDL > 3.5, history of cardiovascular disease, and former smokers. We also tested differences in association between the cohort in Los Angeles and the cohort in Anaheim. Evidence of significant interaction was considered at a nominal product term *p*-value < 0.1 to avoid increased type II errors in these hypothesis-generating analyses.

## 3.3. Results

### 3.3.1. Descriptive data

One hundred and ninety-one subjects (191) were recruited and 87 subjects dropped out or become ineligible (cancer, smoker, or moved out of the study area) before or soon after the start of follow-up, resulting 104 subjects. We additionally required *a priori* that subjects have at least 4 repeated outcome measures to provide sufficient within-subject exposure-response data. Seven subjects were excluded from the study because < 4 repeated measures and four additional subjects were excluded because they can not perform EndoPAT measurement. Therefore, 93 subjects were retained in microvascular function analysis. Detailed information on the characteristics of study subjects are listed in Table 3.1. The subjects were 65 to 96 years old, and two thirds of the subjects were female. All were currently non-smokers, though approximately 40% were former smokers. Among the 93 subjects, there were 60 non-Hispanic Whites, 9 Hispanics, 10 African Americans, 9 Asians and 5 other race/ethnicities. Approximately one-third of subjects were obese (BMI ≥ 30 $kg/m^2$) and one-third were overweight (25 $kg/m^2$ ≤ BMI < 30 $kg/m^2$). More than 60% of subjects had history of a hypertension, and over half had a history of dyslipidemia. Sixteen percent had diabetes. The mean RHI score was 1.94, which was in the normal endothelial function range (> 1.67) according to the EndoPAT manufacturer's manual (Itamar Medical 2015).

Table 3.2 provides metrics for the 24-hour-average concentrations of ambient air pollutants, 7-day average personal $NO_X$, and 5-day average size-fractionated PM air pollutants. Data for specific transition metals is presented in the Table 3.3. For 24-hour ambient pollutants at the central sites, $NO_X$ had the highest missing rates (29.95%), and

all other pollutants had less than 10% of missing data. Personal $NO_X$ had 15.91% missing data due to measurement errors where $NO_X$ concentration was less than $NO_2$, subject noncompliance, or incorrect sampling times. For size-fractionated PM components, up to 4 of the planned 48 weeks were missing due to equipment failure or power outages at the Anaheim site. Ninety-seven percent of the study period had average 24-hour $PM_{2.5}$ below the National Air Quality Standard recommended upper limit of 35 $\mu g/m^3$. Total PAHs and hopanes were higher in $PM_{0.18\text{-}2.5}$ than in $PM_{0.18}$. Both DTT and ROS were highest in $PM_{0.18\text{-}2.5}$, and were higher in $PM_{2.5\text{-}10}$ than in $PM_{0.18}$. For most transition metals (V, Cr, Mn, Cu and Fe), the highest mass concentrations were observed in $PM_{2.5\text{-}10}$ (Table 3.3).

As expected, concentrations of traffic-related air pollutants (BC, NOx, CO, EC, PAHs, and hopanes) were higher in the more densely urban Los Angeles region than in the more suburban Anaheim (Table 3.4). The correlation between 5-day averages of $PM_{2.5}$ BC and EC were much lower in Los Angeles (R = 0.54) than in Anaheim (R = 0.93). This is likely because the sampling sites were different in Los Angeles but were the same location in Anaheim (Figure 2.1). The correlation between personal NOx and ambient NOx, BC, and CO was much stronger in Anaheim than in Los Angeles (Table 3.5). This could be because of a greater influence of micro-environmental exposures, including local traffic, in Los Angeles than in Anaheim (discussed in Chapter 5). Given these regional differences, we present correlations for combined regions in Table 3.6 and Table 3.7 after mean-centering exposures by region. Spearman correlations of ambient air pollutants showed strong positive correlations among the traffic-related air pollutants (BC, $NO_X$, CO, R > 0.87) (Table 3.6). These pollutants correlated weakly to

$PM_{2.5}$, especially in Los Angeles (Table 3.5). Moderate to strong inverse correlations were observed for traffic-related air pollutants and $O_3$, with stronger correlations in Anaheim than in Los Angeles. For 5-day size-fractionated PM, total mass in $PM_{0.18}$ was not correlated with total mass in $PM_{0.18\text{-}2.5}$ and inversely correlated with total mass in $PM_{2.5\text{-}10}$ (Table 3.7). Total PAHs and hopanes were strongly correlated (R = 0.89-0.91), suggesting that the primary source of PAHs was traffic since hopanes are unique tracers of vehicular emissions in the Los Angeles basin and found in the lubricant oils of diesel and gasoline vehicles (Schauer et al. 1996). Correlations between total PAHs and OC were strong in both $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$ while the correlations between total PAHs and EC were stronger in $PM_{0.18}$ than in $PM_{0.18\text{-}2.5}$. Correlation between DTT and OC/EC was strong in $PM_{0.18}$, and weak in $PM_{0.18\text{-}2.5}$ and $PM_{2.5\text{-}10}$, suggesting that oxidative potential may have different chemical determinants in these size fractions. Transition metals (except for V) were strongly correlated with OC/EC, total ROS and DTT in $PM_{2.5\text{-}10}$ (R= 0.59-0.72). GPx-$1_{0.18}$ was not correlated with PM components in $PM_{0.18}$ (Table 3.7).

### 3.3.2. Microvascular function and air pollution association

Figure 3.2 presents the relationships between RHI, as a measure of microvascular function, and an interquartile range increases in ambient air pollutants measured daily and 5-day size-fractionated PM components. RHI was inversely associated with ambient BC and $NO_X$ for 1- through 7-day averaging times, and with CO for 3- and 5-day averages, indicating impaired endothelial function with exposure to

these pollutants. RHI was not significantly associated with ambient $PM_{2.5}$ or $O_3$ at any averaging time, or with 7-day average personal $NO_x$ (Figure 3.2.A). The strongest associations with ambient daily exposures were observed for 5-day averages. For example, an interquartile range increase in 5-day average BC (1.06 μg/$m^3$) was associated with an RHI decrease of -0.093 (95% CI: -0.151, -0.035). We observed no significant regional differences of the associations between RHI and ambient exposures, except for $PM_{2.5}$ at 7-dayaverage (product term $p$=0.08), with a positive association in Los Angeles and a negative association in Anaheim (95% CI for both regional associations were wide and contained 0.00) (Figure 3.3).

RHI was not significantly associated with 5-day total mass of $PM_{0.18}$ or $PM_{0.18\text{-}2.5}$, but was marginally associated with total mass in $PM_{2.5\text{-}10}$ (-0.046, 95% CI: -0.095, 0.004; Figure 3.2.B). We observed that RHI was inversely associated with total PAHs, hopanes, and DTT in $PM_{0.18}$, marginally inversely associated with $PM_{0.18}$ EC, total and water-soluble ROS, but not $PM_{0.18}$ organic acids or OC (representing a mixture of primary and secondary organic aerosols). RHI was associated more strongly (inversely) with EC, total and water-soluble ROS in $PM_{0.18\text{-}2.5}$ than in the other particle sizes. RHI was also inversely associated with OC in $PM_{2.5\text{-}10}$ (a minor fraction of that particle size), but not in the other particle sizes, suggesting that the fraction of primary and secondary OC may be different in $PM_{2.5\text{-}10}$ or that OC is correlated with other components (e.g. metals) associated with RHI (Table 3.8). We found that RHI was inversely associated with all transition metals in $PM_{2.5\text{-}10}$, with the transition metals Cr, Mn, Ni, Cu and Fe, but not Ni or V in $PM_{0.18\text{-}2.5}$, and with the transition metals Cr, Mn, Ni, Cu, and Ni but not V in $PM_{0.18}$ ( Figure 3.4). Of great interest, we found that RHI was positively associated with

GPx-1 in $PM_{0.18}$ (estimated change: 0.038, 95% CI: 0.020, 0.048), indicating that high electrophilic potential that resulted by decreased GPx-1 activity may have an adverse effect on microvascular function.

### 3.3.3. Sensitivity analysis

For two-pollutant models, the magnitude of the non-significant associations of $PM_{2.5}$ and personal NOx were largely unchanged with $O_3$ in the model (Figure 3.5). Estimations of association for ambient BC, CO, $NO_X$ showed even greater decreases in microvascular function by 45-113% in RHI after adjusting for $O_3$ than in single-pollutant models (Figure 3.2.A), although the confidence intervals were wider. We also noted that adjusting for BC in the model with $O_3$, the estimations of association for $O_3$ changed from positive in single-pollutant models (Figure 3.2.A) to negative in two-pollutant models (Figure 3.5.A), and became significant for 1- and 3-day averages. To address the possibility of an interaction between primary and secondary air pollutants (BC and $O_3$, respectively), we further tested a model with the product term of BC and $O_3$ and found significant positive interactions ($p$ <0.1) of BC and $O_3$ on RHI at 3-day and 5-day averages, suggesting synergism (Figure 3.6). The estimates of association for 5-day size-fractionated PM and components remained relatively unchanged after adjusting for $O_3$ (Figure 3.5.B).

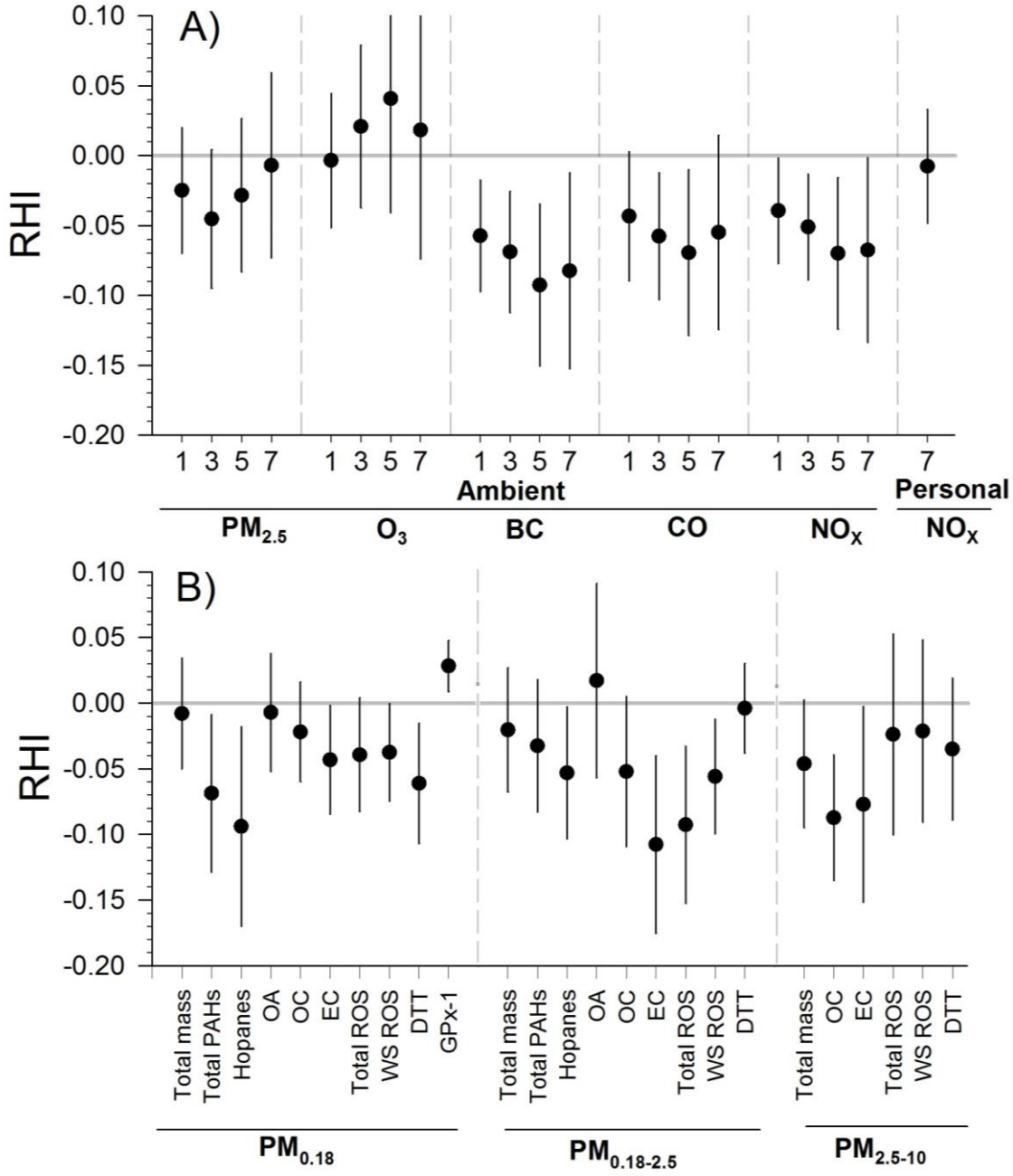


**Figure 3.2. Association of microvascular function with a one interquartile range increase of ambient and personal air pollutants**.
Exposures were averaged across 1 day, 3 days, 5 days, and 7 days preceding each subject's reactive hyperemia index (RHI) measurement (A) and the PM components in three different size-fractions for exposures averaged across 5 days preceding each subject's RHI measurement (B). BC: black carbon; DTT: dithiothreitol; EC: elemental carbon; OA: Organic acids; OC: organic carbon; PAHs: polycyclic aromatic hydrocarbons; ROS: reactive oxygen species; WS: water-soluble.

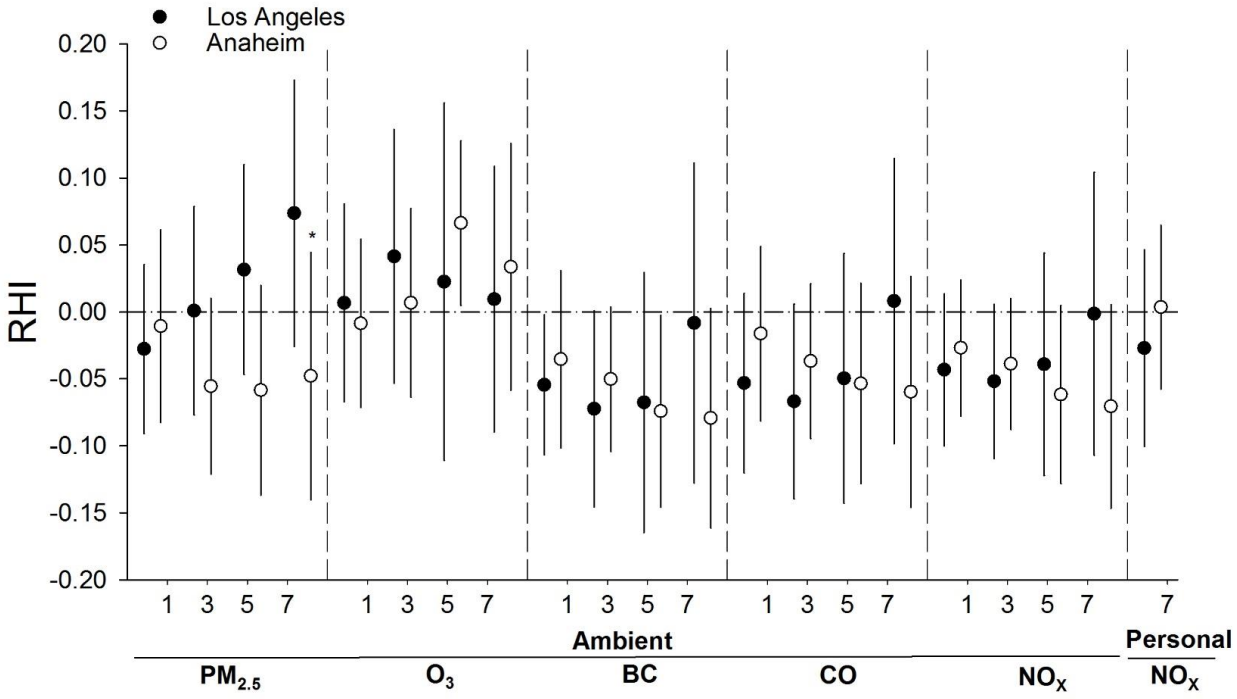


**Figure 3.3. Association of microvascular function with a one interquartile range increase of air pollutants by study region.**
RHI: reactive hyperemia index. Numbers on X axis refer to the exposure averaging time: 1-day, 3-day, 5-day and 7-day. *p < 0.1, compared with no effect modification by obesity status. BC: black carbon.

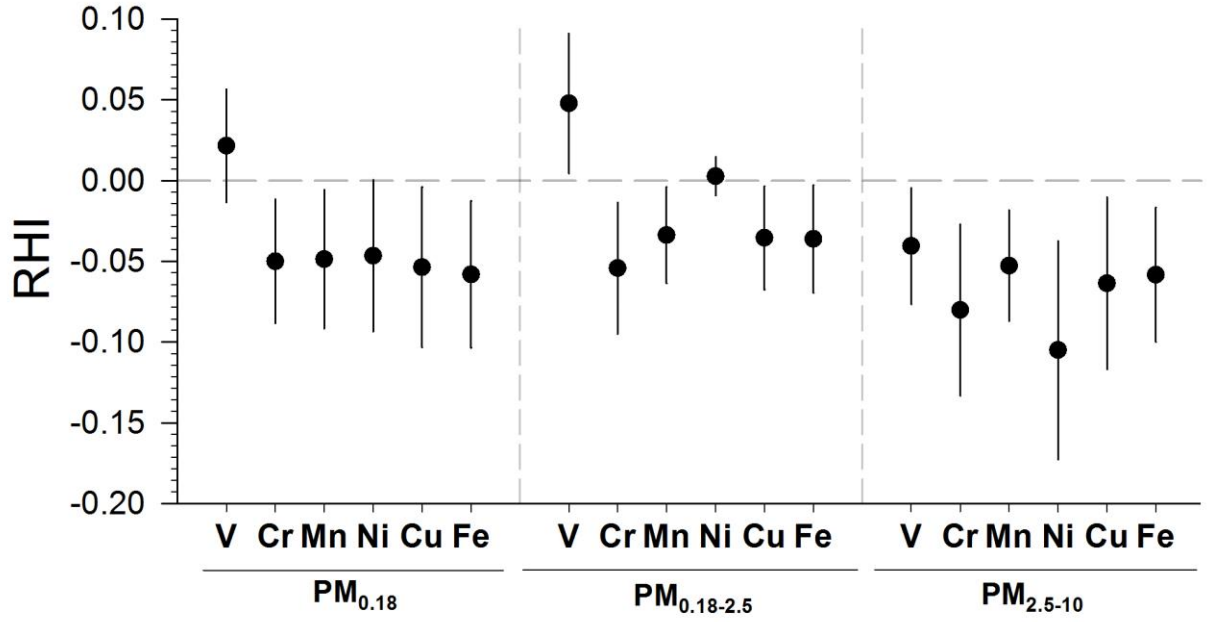


**Figure 3.4. Association of microvascular function with a one interquartile range increase of selected transition metals**.
Transition metals were measured in three size-fractions for exposures averages

Associations also persisted when we restricted the analysis to subjects living within the 90$^{th}$ percentile of all subjects' residential distance to monitoring stations (Figure 3.7). When we excluded imputed ambient exposure data, the associations for $PM_{2.5}$, $O_3$ and BC remained similar. Conversely these associations became slightly weaker for $NO_X$ and CO (Figure 3.8). We did not observe significant changes when we excluded days with extreme heat index (data not shown).

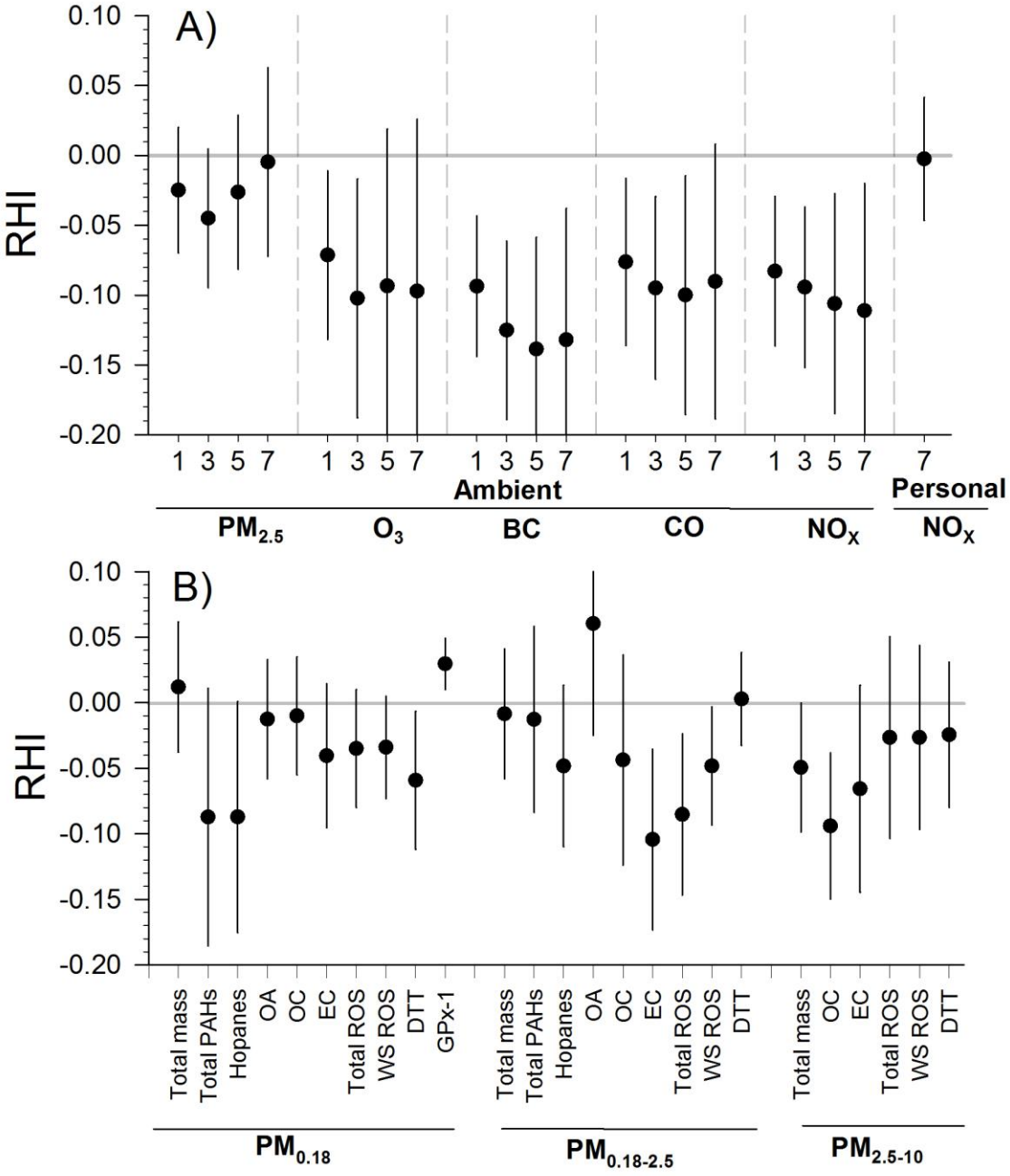


**Figure 3.5. Sensitivity analysis of relations between microvascular function and air pollution: two-pollutant models.**
Association of reactive hyperemia index (RHI) with a one interquartile range increase of ambient and personal air pollutants for exposures averaged across 1 day, 3 days, 5 days, and 7 days preceding each subject's measurement (A) and the PM components in three different size-fractions for exposures averaged across 5 days preceding each subject's RHI measurement (B). The sensitivity analysis is adjusting for ozone with the same averaging time, except for the ozone model, which adjusts for black carbon with the same averaging time.

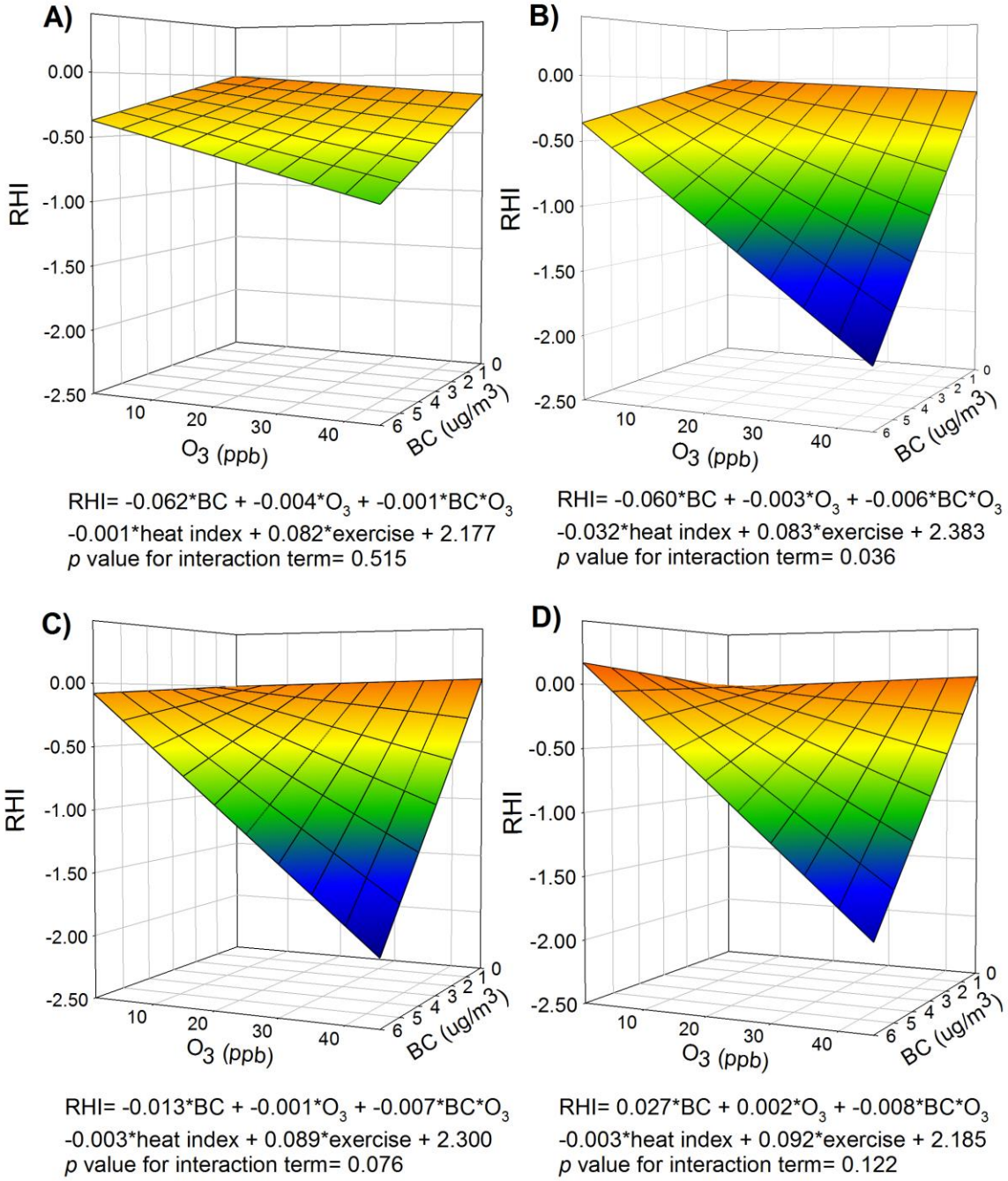


**Figure 3.6. Relation of microvascular function to interaction between black carbon and $O_3$.**

Changes in reactive hyperemia index (RHI) per unit increase of BC, $O_3$ and their interaction across 1 day (A), 3 days (B), 5 days (C), and 7 days (D) averaged before each subject's measurement. BC: black carbon;

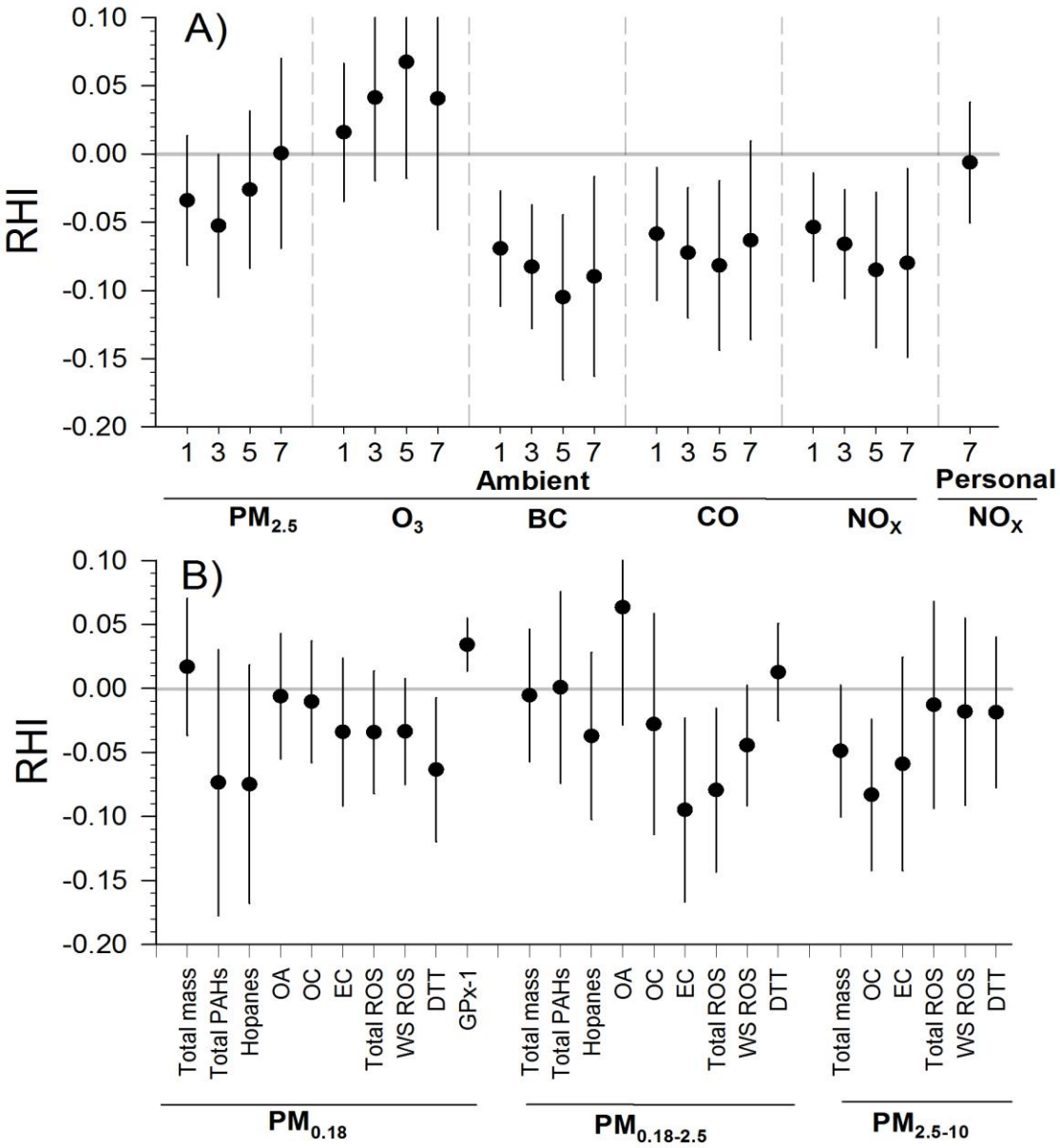


**Figure 3.7. Sensitivity analysis of relations between microvascular function and air pollution restricted to subjects living within the 90$^{th}$ percentile of subjects' residential distance to the stations.**

Association of reactive hyperemia index (RHI) with a one interquartile range increase of ambient and personal air pollutants Exposures were averaged across 1 day, 3 days, 5 days, and 7 days preceding each subject's measurement with sensitivity analysis restricted to subjects living within 13.75 km (A); and the PM components in three different size-fractions for exposure averages across 5 days preceding each subject's RHI measurement with sensitivity analysis restricted to subjects living within 11.41 km (B). BC: black carbon; DTT: dithiothreitol; EC: elemental carbon; OA: Organic acids; OC: organic carbon; PAH: polycyclic aromatic hydrocarbons; ROS: reactive oxygen species; WS: water-soluble.

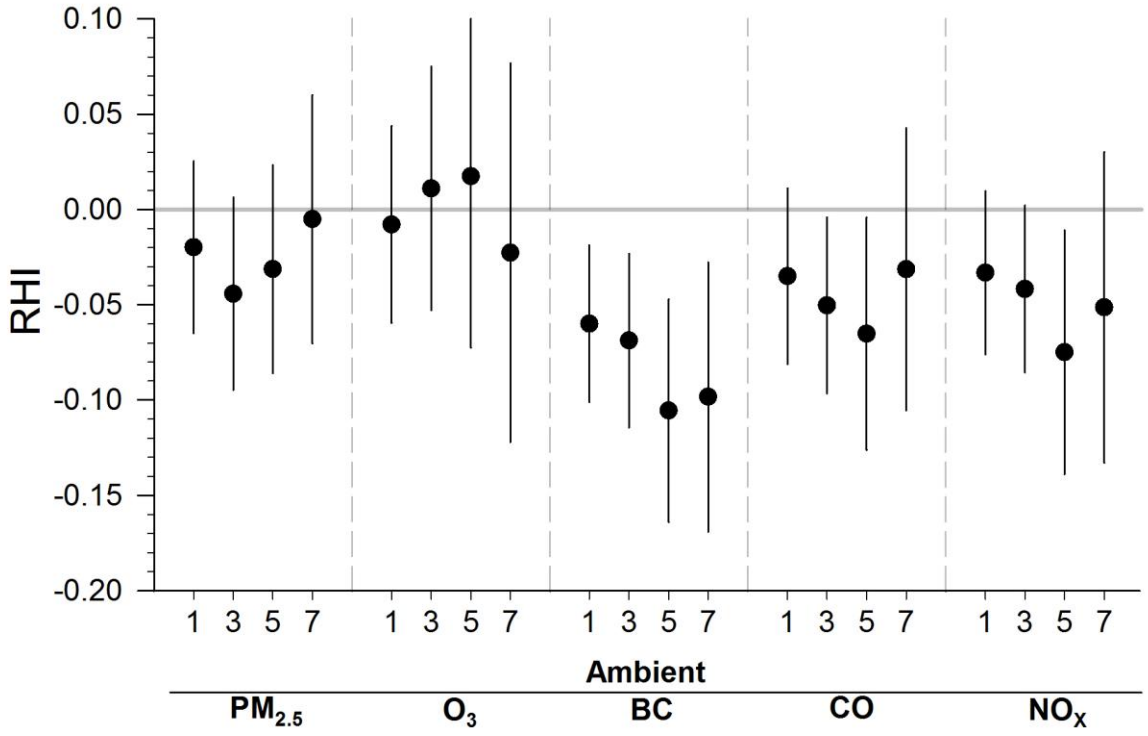


**Figure 3.8. Sensitivity analysis of relations between microvascular function and air pollution excluding imputed exposure values**.
Association of reactive hyperemia index (RHI) with a one interquartile range increase of ambient and personal air pollutants for exposures averaged across 1 day, 3 days, 5 days, and 7 days preceding each subject's measurement. BC: black carbon.

### 3.3.4. Effect modification

We evaluated effect modification of associations between RHI and 5-day average air pollutants (except for personal $NO_X$ since only 7-day averages were available). Five-day averages were selected because we found that the largest and most consistent associations with RHI were for 5-day averages. Also, this is the averaging time for the PM components. We observed evidence of effect modification by smoking status on the association between RHI and exposures to traffic-related air

pollutants (ambient BC, CO, NOx, personal $NO_X$, PAHs, hopanes, EC) with stronger inverse associations estimated for former smokers than for individuals who never smoked (Figure 3.9). Subjects who were obese generally had stronger inverse associations of RHI with exposure to air pollutants, except for secondary pollutants (Figure 3.10). Significant modifying effects ($p < 0.1$) were observed for exposures to ambient $PM_{2.5}$ BC, and EC in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$. We observed no evidence of statistically significant interaction between exposures and age, sex, study region, diabetes status, lipid factors, hypertension or history of cardiovascular diseases (data not shown).

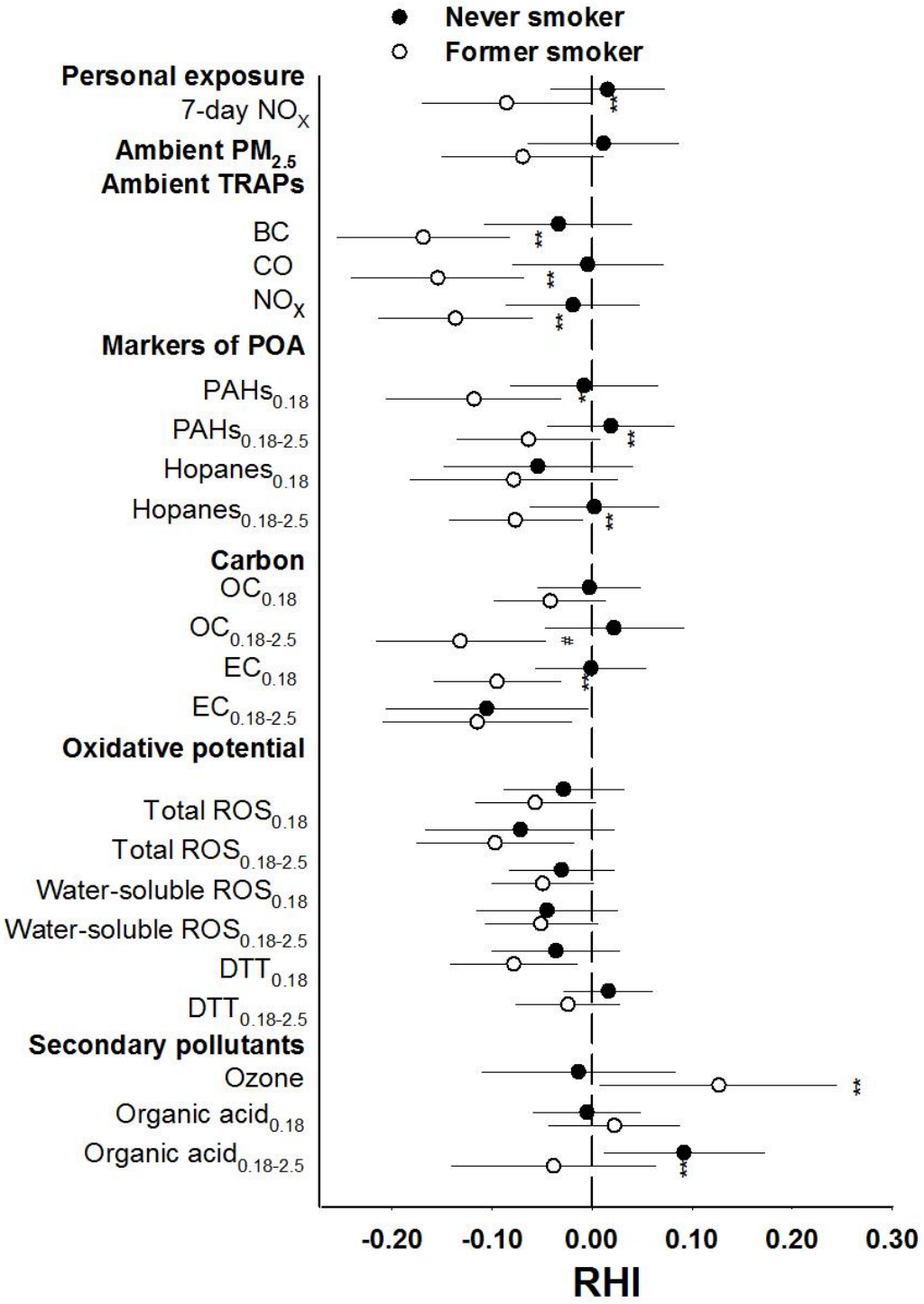


**Figure 3.9. Effect modification of relations between microvascular function and air pollution by never smoker and former smoker.**
Association of reactive hyperemia index (RHI) with one interquartile increase of selected air pollutant exposures averaged across 5 days preceding each subject's RHI measurement. *$p$ < 0.1, **$p$ < 0.05, $^{\#}p$ < 0.01, compared with no effect modification by smoking status.

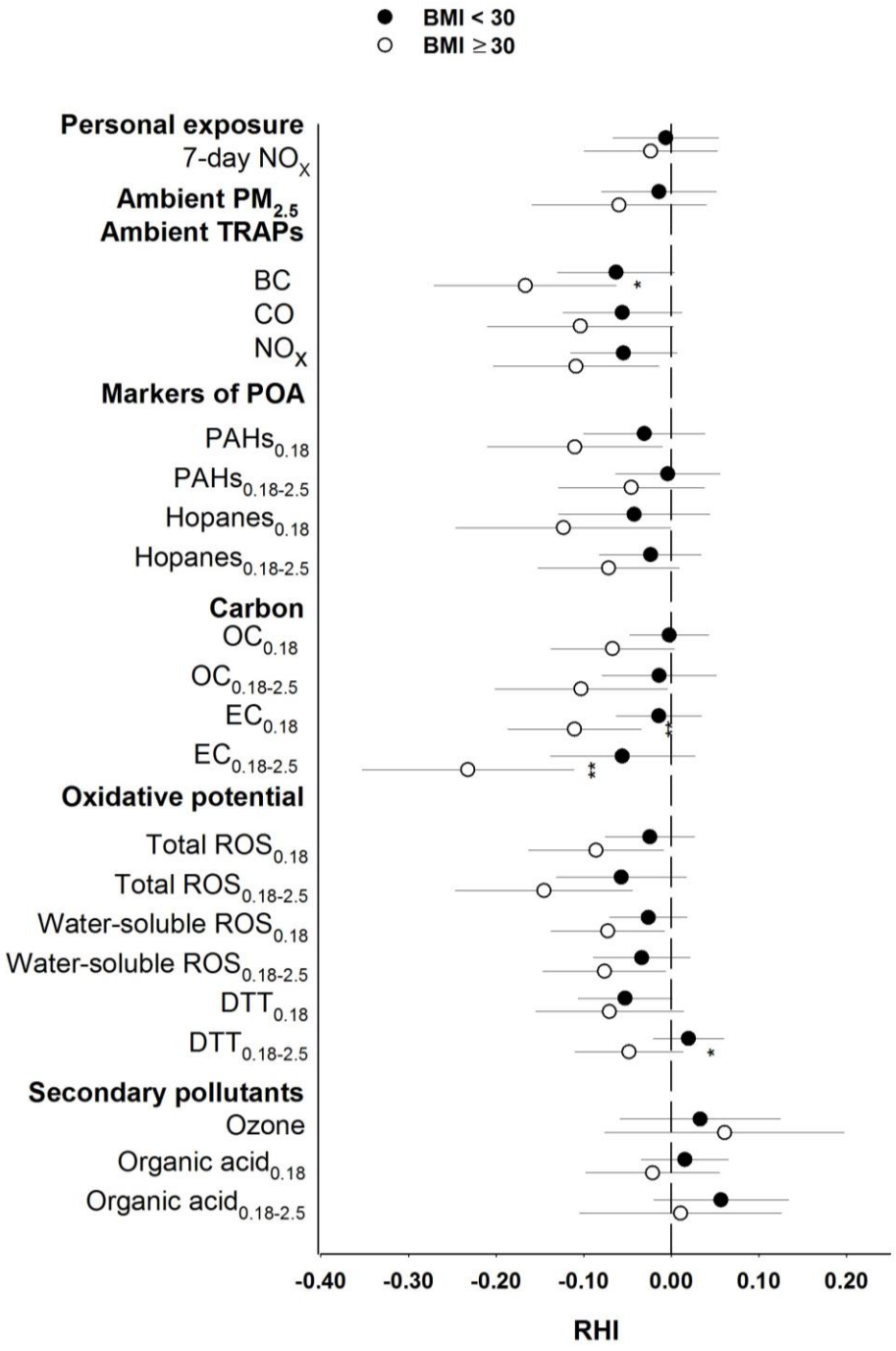


**Figure 3.10. Effect modification of relations between microvascular function and air pollution by obesity status**.
Association of RHI score with one interquartile range increase of selected air pollutant exposures averaged across 5 days preceding each subject's reactive hyperemia index (RHI) measurement: *$p < 0.1$, **$p < 0.05$, compared with no effect modification by obesity status. BMI: body mass index.

## 3.4. Discussion

We found decreased microvascular endothelial function in relation to increases in short-term exposure to air pollution. These associations were observed primarily for markers of primary fossil fuel combustion sources (EC, BC, CO, $NO_X$, PAHs, and hopanes). One of the strongest associations was observed for 5-day average BC with an interquartile range increase associated with a RHI decrease of -0.093. There is no standardized guideline for the clinical relevance of changes in RHI. However, previous studies indicate that a low RHI score is independently associated with adverse cardiovascular outcomes. For example, the hazard ratio for cardiovascular events per 0.1 ln(RHI) increase (1.1 increase of RHI) showed a significant decrease in risk 0.761 (95% CI: 0.691, 0.832) after controlling for other risk factors in a high risk cohort of 528 subjects (Matsuzawa et al. 2013).

We used hourly ambient air monitoring data over the seven days preceding outcome measurements and found that the strongest associations were for 5-day averages. Several controlled exposure studies have found that diesel exhaust is associated with impaired vascular function from as early as 2 hours after the exposure and up to 24 hours (Mills et al. 2005; Tornqvist et al. 2007). Comparisons with the present study are difficult because previous studies used experimental study designs incorporating higher exposure levels (i.e. 300 $\mu g/m^3$ particulate mass concentrations).

We found stronger associations of RHI with PAHs and hopanes in $PM_{0.18}$ than in the $PM_{0.18\text{-}2.5}$, which includes larger particles. In the Los Angeles metropolitan area, most outdoor PAHs in $PM_{0.18}$ are expected to be from mobile sources (Schauer et al.

2000). The strong correlation of PAHs (R=0.89) with hopanes (source markers of vehicular emissions) is consistent with this expectation. PAHs were more strongly correlated with DTT in $PM_{0.18}$ than in $PM_{0.18-2.5}$ indicating that PAHs in $PM_{0.18}$ were more redox active. This is consistent with our findings that even though the concentration of PAHs and hopanes were higher in $PM_{0.18-2.5}$ than in $PM_{0.18}$ (Table 3.2), we observed stronger associations of RHI with PAHs and hopanes in $PM_{0.18}$. Our previous panel study did not have $PM_{0.18}$ components but did find that circulating biomarkers of inflammation were associated with PAHs and hopanes measured in quasi-ultrafine $PM_{0.25}$ (Delfino et al. 2010b). The biomarkers were not associated with other organic components or transition metals in the $PM_{0.25}$. Furthermore, PAHs confounded nominal associations of biomarkers with $PM_{0.25}$ mass. In a follow-up panel study with organic components also measured in the accumulation mode size fraction ($PM_{0.25-2.5}$), we found that exposure markers of combustion-related air pollutants including $PM_{0.25-2.5}$ PAHs and/or $PM_{0.25}$ PAHs were positively associated with expression of genes in oxidative stress and inflammatory pathways, including *NFE2L2, Nrf2*-mediated genes (*HMOX1, NQO1*, and *SOD2*), *CYP1B1, IL1B*, and *SELP* (Wittkopp et al. 2015). In toxicological studies, it has been demonstrated that ultrafine particles have high levels of organic compounds and metals, and were more capable of generating ROS (Cho et al. 2005) and pro-inflammatory responses (Li et al. 2010).

This is the first epidemiological study reporting a decrease in microvascular function in relation to markers of PM oxidative potential. This is consistent with our previous novel findings for associations with chemical components such as PAHs with known pro-oxidant effects in cell cultures (Bonvallot et al. 2001). As noted by Higashi et

al. (Higashi et al. 2009), the underlying mechanisms may be increased production of ROS during oxidative stress that inactivates nitric oxide production. Decreased nitric oxide impairs endothelial function leading to an imbalance in microvascular function.

Specifically, we observed inverse associations of microvascular function with PM oxidative potential both in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$ with DTT associations stronger in $PM_{0.18}$ and macrophage ROS associations stronger in $PM_{0.18\text{-}2.5}$. Estimated associations of microvascular function with total ROS was stronger than with water-soluble ROS in $PM_{0.18\text{-}2.5}$ while no such difference was observed in $PM_{0.18}$. The DTT assay is a chemical (acellular) assay based on the ability of redox-active compounds to transfer electrons from DTT to oxygen (Ayres et al. 2008a) while the ROS assay represents cellular production of ROS measured using rat alveolar macrophage cells exposed to particle extracts (Landreman et al. 2008). The highest oxidative potential measured by both assays were in $PM_{0.18\text{-}2.5}$. However, the correlations between the two assays in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$ were weak (R= 0.37 to 0.42), and not correlated in $PM_{2.5\text{-}10}$ (-0.11 and -0.20 for DTT with total ROS and water-soluble ROS, respectively). Together with the observation of different associations for DTT and macrophage ROS across the size-fractions, this finding may indicate that these two assays are sensitive to different components of PM and those components lead to health effects that vary depending on particle size distribution and source. Therefore, these two assays complement each other and are informative of the importance of PM under different exposure conditions. Further studies are needed to better understand the relationships between PM components and the associated DTT and macrophage ROS activities.

We measured GPx-1 activity of PM in the ultrafine mode, which is a novel marker of electrophilic potential and have not reported to be used in studying health effect of air pollution. In previous study, the presence of electrophilic substances has been demonstrated in samples of diesel particles and ultrafine particles collected at the Los Angeles area (Shinyashiki et al. 2008). Along with the present result of positive association between RHI and GPx-1 (inactivated by electrophilic potential), it suggests that the observed association of microvascular function with exposure to traffic-related air pollutants may be at least in part attributable to the potential of air pollutant components to generate oxidative stress by increasing electrophilic potential. Our study showed successful use of the GPx-1 bioassay to measure the potential inhibitory effect of electrophilic pollutants in a cohort panel study setting.

Few cohort panel studies of within-subject exposure-response relations have examined effects on endothelial function by exposure to ambient air pollution (Schneider et al. 2008; Williams et al. 2012; Louwies et al. 2013; Zanobetti et al. 2014). Some (Schneider et al. 2008; Williams et al. 2012) but not all (Zanobetti et al. 2014) found that short-term exposure to $PM_{2.5,}$ sulfate, and BC were associated with FMD of the brachial artery. Most previous studies have investigated the function of forearm conduit arteries. However, the peripheral microvasculature shares more similarities in development and anatomy with the microvasculature of the heart than with conduit arteries and may be an early cardiovascular risk indicator (Struijker-Boudier et al. 2007; Flammer et al. 2012).

A few air pollution studies have evaluated microvascular function. For example, one cross-sectional study of the Framingham Heart Study Offspring Cohorts, Ljungman

et al (Ljungman et al. 2014), found no consistent associations between the air pollution exposures, including BC, and microvascular response. However, a cross-sectional study design may not efficiently capture acute air pollution effects given the high temporal and spatial variation in ambient air pollution levels and the variation of outcome and confounders between subjects. Only one previous study was conducted to investigate acute within-subject responses of microvascular function (Louwies et al. 2013). They examined retinal microvasculature using fundus image analysis in a panel of 84 healthy adults, 22-63 years of age. Consistent with our results, they found that impaired microvascular responses were associated with exposure to BC but with a more acute response (at lag 1 and lag 2 day BC). Many reasons may contribute to the inconsistency among panel studies including: different methods to assess microvascular function, different study population, exposure error, different composition of pollutants, and duration and frequency of exposure assessment. Compared with these above panel studies, our sample size is large, with more repeated measures (N=845), and with more detailed characteristics of the air pollutants.

We found that “protective” effects of $O_3$ were confounded by BC, a marker of primary combustion sources. However, the inverse associations between BC and microvascular function persisted after adjusting for $O_3$ indicating a robust estimate for BC. This may be attributable partly to meteorological determinants and partly to the observation that high concentrations of a correlated primary pollutant like NO is associated with a reduction of $O_3$ (Rodes et al. 1981), both causing the inverse correlation between primary pollutants and $O_3$. Therefore, the “protective” effect of $O_3$ may be attributed to low levels of primary air pollutants. Another explanation is $O_3$

exposure error, given that indoor $O_3$ is generally much lower than outdoor concentrations, especially when windows are closed and air conditioning is in use (Weschler 2000).

Unexpectedly, our measurement of personal exposures to $NO_x$ was not associated with microvascular function. One explanation may be subject non-compliance, although we have no direct evidence of this. It is possible that the device was not worn as instructed (exposed to outside air and not held in a pocket or purse), or not worn for the 7-day time period requested. In addition, the device is a passive sampler subject to face velocity effects from variations in airflow. Lastly, even though our personal exposure models adjusted for indoor origin of $NO_X$, it was possible that this was not fully addressed since the source of adjustment data was from self-administered daily diaries.

We found that microvascular function was significantly inversely associated with OC in $PM_{2.5-10}$, but not in $PM_{1.8}$ or $PM_{1.8-2.5}$. This maybe because of the strong correlation between OC and inorganic species such as total Cr, Mn, Ni, Cu and Fe (R=0.74-0.82) in $PM_{2.5-10}$ (Table 3.8). These metals had the highest concentrations in $PM_{2.5-10}$ (Table 3.3) and they can generate ROS by Fenton-type reactions, resulting in adverse health effects (Ntziachristos et al. 2007). Indeed, we found that decreased RHI was significantly associated with Cr, Mn, Ni, Cu and Fe in $PM_{2.5-10}$ with the strongest association observed with Ni (-0.156, 95% CI: -238, -0.074,). However, co-regression of $PM_{2.5-10}$ OC with $PM_{2.5-10}$ Ni showed that OC was still significantly associated with decreased RHI, and although both exposures showed weaker associations when co-regressed, the confounding was much smaller for OC than for Ni (21% decrease for

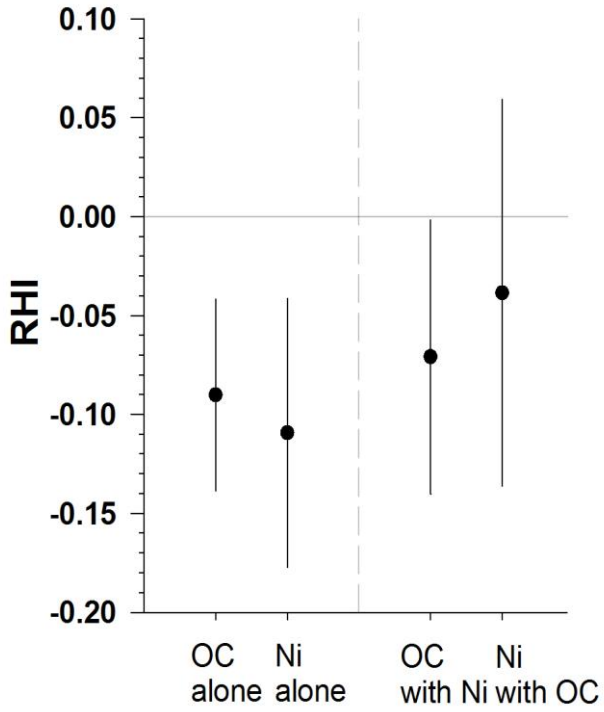


**Figure 3.2. Association of microvascular function with a one interquartile range increase of OC co-regressed with Ni in $PM_{2.5-10}$.**

Air pollutants are averaged across 5 days preceding each subject's measurement of reactive hyperemia index (RHI). OC: organic carbon.

OC, 65% decrease for Ni, Figure 3.11). An unmeasured determinant of the OC fraction may be important, perhaps endotoxins. Compared with smaller PM size fractions, there is more endotoxin in the coarse than fine PM fractions, and consequently greater inflammatory responses by alveolar macrophages (Becker et al. 2003).

This study enabled us to demonstrate for the first time that associations of impaired microvascular function with primary markers of fossil fuel combustion were stronger for subjects who were former smokers. Given the reduced sample size among subgroups, it is difficult to further explore explanations for the observed effect modification by former smokers. We infer from the present results that former smokers may be more susceptible to exposure to air pollution. It is also possible that being a former smoker is an indicator of other unmeasured co-morbidities or lifestyle habits that place them at increased risk. The finding that subjects who were obese showed stronger associations of impaired microvascular function with air pollutants needs further investigation. However, this result agrees with the previous studies showing greater response of FMD with exposure to $PM_{2.5}$ among obese diabetic adults (O'Neill et al. 2007; Schneider et al. 2008). It is possible that obesity is associated with enhancement of the pro-inflammatory effect of air pollution (Dubowsky et al. 2006; O'Neill et al. 2007). Additionally, obese individuals have a higher inhalation rate compared to their normal weight counterparts (Brochu et al. 2014). Thus, elderly subject with higher BMI is a potentially susceptible population.

Growing evidence (Brook et al. 2010) supports the view that air pollutants with higher pro-oxidant potential are more capable of generating oxidative stress and inducing inflammation at both respiratory and systemic sites (Delfino et al. 2010a; Delfino et al. 2013) in human population studies. This process may alter the function of the vascular endothelium and initiate endothelial dysfunction. In the present study, we measured the effects of short-term exposure but it is possible that the observed acutely-impaired endothelial function can result in long-term effects following repeated insults as

evidenced by recent cohort studies of long-term exposure to traffic-related air pollutants and the development of atherosclerosis (Rivera et al. 2013; Hajat et al. 2015).

One limitation of our study is that most of our exposure data were obtained from central monitoring stations ranging from 1.58 km to 16.37 km to the subject's residential address. However, any exposure misclassification is likely to be non-differential, leading to an underestimation of the health effects of air pollution (Zeger et al. 2000). This is validated by our sensitivity analysis in that we did not observe notable changes by restricting the analysis to subjects living within a smaller radius around the air monitoring stations (Figure 3.7). Another limitation of our study was that all of the health measures were collected in clinical setting rather than in the home, resulting in non-ambient exposures on the way to the clinic. Further, the lack of data on daily personal exposure prevented us from directly comparing associations with ambient daily data. We also did not assess daily PM composition, which could have provided information on the lag effect of chemical components. Finally, we did not collect information on subjects' daily diet, which may affect vascular function, and as with any observational study there is the possibility of unmeasured confounding factors in the relationships of interest. Strengths of this study include the repeated measures study design, which enabled control of potentially confounding personal characteristics, the use of non-invasive and relatively technician-independent measures of microvascular function, and the detailed exposure estimation of PM composition and oxidative potential of different particle size-fractions.

## 3.5. Conclusion

In summary, our results show that microvascular endothelial dysfunction is associated with ambient air pollutants and that these pollutants are linked to primarily mobile sources. PM oxidative potential, measured both by abiotic DTT and macrophage ROS assays, was associated with microvascular dysfunction further highlighting their potential roles in the overall associations between air pollutants and vascular function. Lowing residual GPx-1 activity resulted by increased electrophilic potential of traffic-related pollutants may, in part, play the role in impairing microvascular function. European Union National Emission Ceilings and U.S. EPA regulated ambient $PM_{2.5}$ mass measurements may not adequately represent risk for cardiovascular diseases because they are uncharacterized by composition, source or oxidative potential. Further data are needed using measurements of organic components and oxidative potential across several PM size-fractions and with personal exposures. However, our findings provide clues to the potential mechanisms behind the effects of air pollution on cardiovascular disease and provide further justification of the importance of simultaneously measuring particulate air pollution composition, toxicity, and source tracers in assessing adverse cardiovascular health effects.

Table 3.1. Characteristics of subjects (N=93).

| **Characteristic** | **Mean ± SD or N (%)** |
|---|---|
| Age (years) ± SD | 74.9 ± 7.6 |
| Body mass index (kg/m$^2$) ± SD | 27.8 ± 5.5 |
| Overweight (25 - 29.9) (%) | 35 (37.6) |
| Obesity (≥ 30) (%) | 28 (30.1) |
| Male (%) | 25 (26.9) |
| Former smoker (%) | 39 (41.9) |
| Reactive hyperemia index (RHI) score | 1.95 (0.4) |
| Cardiovascular history | |
| Coronary artery disease (%) | 16 (17.2) |
| Congestive heart failure (%) | 8 (8.6) |
| Stroke (%) | 9 (9.7) |
| Hypertension (%) | 62 (66.7) |
| Hypercholesterolemia (by history) (%) | 50 (53.8) |
| Lipid Profile | |
| Total cholesterol > 200 mg/dL (%) | 37 (39.8) |
| LDL-C > 130 mg/dL (%) | 29 (31.2) |
| HDL-C < 50 mg/dL for women; < 40 mg/dL for men (%) | 25 (26.9) |
| Adult-onset diabetes mellitus (%) | 21 (22.6) |
| Medications: | |
| Anti-hypertensive medications (%) | 62 (66.7) |
| HMG-CoA reductase inhibitors (statins) (%) | 45 (45.2) |

Table 3.2. Descriptive statistics of air pollutant measurements.

| | N (Missing) | Mean (SD) | IQR | Min | Max |
|---|---|---|---|---|---|
| **Personal Exposures (7-day average)** | | | | | |
| $NO_x$ (ppb) | 729 (116) | 28.07 (19.66) | 20.87 | 2.21 | 160.13 |
| **Ambient Exposures (24-hr Averages)** | | | | | |
| Black Carbon (μg/m$^3$) | 320 (16) | 1.28 (0.85) | 1.06 | 0.21 | 5.21 |
| $PM_{2.5}$(μg/m$^3$) | 309 (27) | 17.56 (7.70) | 9.62 | 3.83 | 49.23 |
| CO (ppm) | 311 (25) | 0.54 (0.24) | 0.32 | 0.11 | 1.59 |
| Ozone (ppb) | 303 (33) | 23.16 (9.25) | 12.66 | 1.33 | 48.51 |
| $NO_x$ (ppb) | 241 (95) | 35.42 (27.69) | 31.02 | 3.6 | 175.63 |
| Heat Index (F°) | 366 (0) | 66.09 (8.39) | 12.56 | 42.57 | 84.33 |
| **Size-fractionated PM (5-day average)** | | | | | |
| Mass (μg/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 2.41 (0.86) | 1.13 | 1.17 | 4.81 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 8.64 (3.21) | 4.01 | 4.40 | 19.43 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 14.91 (6.78) | 7.80 | 4.45 | 35.36 |
| Total PAHs (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 0.32 (0.22) | 0.30 | 0.03 | 0.77 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 0.46 (0.45) | 0.46 | 0.21 | 1.98 |
| Hopanes (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 0.17 (0.12) | 0.19 | 0.01 | 0.44 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 0.21 (0.20) | 0.21 | 0.00 | 0.82 |
| OA (μg/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 26.21 (10.84) | 14.45 | 9.64 | 57.98 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 18.12 (13.99) | 16.76 | 0.56 | 61.43 |
| OC (μg/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 1.18 (0.39) | 0.42 | 0.47 | 2.35 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 1.54 (0.78) | 1.11 | 0.53 | 3.48 |
| $PM_{2.5} – PM_{10}$ | 45 (3) | 0.67 (0.22) | 0.30 | 0.28 | 1.12 |
| EC (μg/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 0.26 (0.13) | 0.16 | 0.09 | 0.60 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 0.15 (0.12) | 0.18 | 0.01 | 0.45 |
| $PM_{2.5} – PM_{10}$ | 45 (3) | 0.04 (0.03) | 0.05 | 0.00 | 0.09 |
| Total ROS (μg Zym/m$^3$)[a] | | | | | |
| $PM_{0.18}$ | 45 (3) | 19.8 (12.21) | 16.60 | 2.10 | 53.20 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 136.69 (94.62) | 125.90 | 26.20 | 394.00 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 60.19 (49.73) | 76.45 | 8.70 | 181.00 |
| Water-soluble ROS (μg Zym/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 16.74 (11.49) | 13.60 | 1.50 | 50.60 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 117.05 (87.66) | 95.60 | 18.60 | 421.90 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 25.04 (22.63) | 34.05 | 2.40 | 84.20 |
| GPx-$1_{0.18}$ (% activity/100 μg) | 45 (3) | 30.51 (13.46) | 8.79 | 10.88 | 100 |
| Dithiothreitol (nmol/min/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 0.10 (0.05) | 0.06 | 0.02 | 0.24 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 0.26 (0.08) | 0.08 | 0.12 | 0.48 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 0.24 (0.11) | 0.14 | 0.10 | 0.53 |
| Transition metals[b] (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 49.20 (40.43) | 56.24 | 7.22 | 153.39 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 72.02 (52.86) | 46.02 | 12.89 | 237.11 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 396.57 (171.18) | 219.95 | 111.97 | 914.57 |

Abbreviations: IQR: interquartile range; CO: carbon monoxide; GPx: glutathione peroxidase; PM: particulate matter; PAHs: polycyclic aromatic hydrocarbons; OC: organic carbon; EC: elemental carbon; ROS: Reactive oxygen species;

[a]Zym: μg Zymosan equivalent units;

[b]Total sum of transition metals include V, Cr, Mn, Ni, Cu and Fe.

Table 3.3. Descriptive statistics of the selected transition metals.

| Metals | N (Missing) | Mean (SD) | IQR | Min | Max |
|---|---|---|---|---|---|
| V (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 0.18 (0.08) | 0.08 | 0.07 | 0.49 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 0.52 (0.26) | 0.32 | 0.18 | 1.37 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 0.57 (0.19) | 0.16 | 0.25 | 1.16 |
| Cr (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 0.31 (0.17) | 0.23 | 0.07 | 0.72 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 0.46 (0.27) | 0.31 | 0.10 | 1.28 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 1.18 (0.53) | 0.76 | 0.30 | 2.63 |
| Mn (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 0.79 (0.61) | 0.75 | 0.15 | 2.41 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 1.54 (1.09) | 0.92 | 0.32 | 5.23 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 4.77 (1.82) | 1.89 | 1.63 | 11.35 |
| Ni (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 0.19 (0.1) | 0.14 | 0.03 | 0.45 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 0.97 (1.2) | 0.41 | 0.19 | 5.78 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 0.49 (0.29) | 0.46 | 0.00 | 1.11 |
| Cu (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 3.21 (2.78) | 3.62 | 0.35 | 12.09 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 3.86 (2.78) | 2.42 | 0.65 | 14.19 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 16.06 (9.28) | 12.14 | 2.46 | 39.05 |
| Fe (ng/m$^3$) | | | | | |
| $PM_{0.18}$ | 45 (3) | 47.79 (36.84) | 52.21 | 6.11 | 140.63 |
| $PM_{0.18} – PM_{2.5}$ | 45 (3) | 64.67 (49.01) | 46.46 | 10.42 | 217.46 |
| $PM_{2.5} – PM_{10}$ | 44 (4) | 373.50 (159.73) | 204.41 | 107.29 | 861.48 |

Table 3.4. Descriptive statistics of air pollutant measurements by region.

| | Los Angeles | | | | Anaheim | | | |
|---|---|---|---|---|---|---|---|---|
| | **Mean (SD)** | **IQR** | **Min** | **Max** | **Mean (SD)** | **IQR** | **Min** | **Max** |
| **Personal Exposures (7-day average)** | | | | | | | | |
| $NO_x$ (ppb) | 31.20 (20.60) | 20.87 | 3.99 | 154.9 | 27.02 (22.36) | 22.45 | 2.21 | 160.13 |
| **Ambient Exposures (24-hr Averages)** | | | | | | | | |
| Black Carbon (μg/m$^3$) | 1.58 (0.86) | 1.10 | 0.31 | 4.08 | 1.14 (0.84) | 0.96 | 0.13 | 5.21 |
| $PM_{2.5}$(μg/m$^3$) | 19.17 (9.55) | 10.98 | 3.83 | 80.58 | 16.26 (7.77) | 7.03 | 2.68 | 46.58 |
| CO (ppm) | 0.62 (0.25) | 0.37 | 0.11 | 1.33 | 0.51 (0.25) | 0.28 | 0.16 | 1.59 |
| Ozone (ppb) | 22.03 (8.03) | 12.84 | 5.22 | 40.76 | 21.25 (9.19) | 14.14 | 1.33 | 43.52 |
| $NO_x$ (ppb) | 42.69 (29.05) | 34.69 | 3.60 | 175.6 | 33.38 (28.57) | 35.04 | 4.20 | 142.08 |
| Heat Index (F°) | 63.84 (9.64) | 83.13 | 42.57 | 14.85 | 64.59 (7.89) | 84.33 | 44.79 | 12.25 |
| **Size-fractionated PM (5-day average)** | | | | | | | | |
| Mass (μg/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 2.25 (0.74) | 1.11 | 1.27 | 3.68 | 2.59 (0.97) | 1.03 | 1.17 | 4.81 |
| $PM_{0.18} – PM_{2.5}$ | 10.21 (3.21) | 4.51 | 5.39 | 19.43 | 6.84 (2.13) | 2.57 | 4.4 | 13.25 |
| $PM_{2.5} – PM_{10}$ | 17.32 (7.5) | 9.37 | 6.92 | 35.36 | 12.02 (4.45) | 3.46 | 4.45 | 25.26 |
| Total PAHs (ng/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 0.37 (0.20) | 0.31 | 0.12 | 0.77 | 0.25 (0.23) | 0.26 | 0.03 | 0.74 |
| $PM_{0.18} – PM_{2.5}$ | 0.57 (0.47) | 0.50 | 0.07 | 1.98 | 0.33 (0.39) | 0.37 | 0.00 | 1.21 |
| Hopanes (ng/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 0.21 (0.11) | 0.17 | 0.06 | 0.44 | 0.12 (0.12) | 0.10 | 0.01 | 0.39 |
| $PM_{0.18} – PM_{2.5}$ | 0.29 (0.21) | 0.36 | 0.08 | 0.82 | 0.12 (0.15) | 0.19 | 0.00 | 0.48 |
| OA (μg/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 28.01 (9.69) | 15.09 | 12.85 | 46.14 | 24.06 (11.90) | 11.90 | 9.64 | 57.98 |
| $PM_{0.18} – PM_{2.5}$ | 21.58 (16.41) | 18.36 | 8.31 | 61.43 | 14.17 (15.32) | 15.32 | 0.56 | 43.22 |
| OC (μg/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 1.05 (0.34) | 0.5 | 0.47 | 1.69 | 1.34 (0.4) | 0.45 | 0.80 | 2.35 |
| $PM_{0.18} – PM_{2.5}$ | 1.71 (0.68) | 0.97 | 0.91 | 3.48 | 1.35 (0.86) | 1.16 | 0.53 | 3.48 |
| $PM_{2.5} – PM_{10}$ | 0.77 (0.19) | 0.28 | 0.35 | 1.06 | 0.55 (0.19) | 0.23 | 0.28 | 1.12 |
| EC (μg/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 0.29 (0.10) | 0.13 | 0.17 | 0.55 | 0.23 (0.15) | 0.13 | 0.09 | 0.60 |
| $PM_{0.18} – PM_{2.5}$ | 0.24 (0.10) | 0.16 | 0.12 | 0.45 | 0.05 (0.04) | 0.03 | 0.01 | 0.13 |
| $PM_{2.5} – PM_{10}$ | 0.05 (0.03) | 0.04 | 0.00 | 0.09 | 0.02 (0.02) | 0.03 | 0.00 | 0.06 |
| Total ROS (μg Zym/m$^3$)[a] | | | | | | | | |
| $PM_{0.18}$ | 21.98 (12.90) | 21.15 | 3.9 | 53.2 | 17.3 (11.15) | 12.8 | 2.1 | 46.7 |
| $PM_{0.18} – PM_{2.5}$ | 196.68 (91.54) | 135.4 | 39.7 | 394 | 68.13 (28.4) | 29.5 | 26.2 | 132.2 |
| $PM_{2.5} – PM_{10}$ | 92.15 (47.20) | 77.55 | 15.6 | 181 | 21.83 (7.75) | 12.95 | 8.7 | 33.1 |
| Water-soluble ROS (μg Zym/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 19.12 (12.44) | 17.95 | 3.8 | 50.6 | 14.03 (9.89) | 10.8 | 1.50 | 39.6 |
| $PM_{0.18} – PM_{2.5}$ | 165.69 (93.85) | 122.1 | 34.8 | 421.9 | 61.46 (26.29) | 19.4 | 18.6 | 131.4 |
| $PM_{2.5} – PM_{10}$ | 37.02 (24.58) | 41.6 | 2.4 | 84.2 | 10.66 (5.39) | 7.60 | 3.40 | 22.5 |
| Dithiothreitol (nmol/min/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 0.1 (0.05) | 0.06 | 0.04 | 0.24 | 0.09 (0.05) | 0.06 | 0.02 | 0.21 |
| $PM_{0.18} – PM_{2.5}$ | 0.24 (0.07) | 0.08 | 0.12 | 0.39 | 0.28 (0.10) | 0.10 | 0.12 | 0.48 |
| $PM_{2.5} – PM_{10}$ | 0.3 (0.12) | 0.17 | 0.13 | 0.53 | 0.17 (0.05) | 0.04 | 0.10 | 0.29 |
| GPx-$1_{0.18}$ (% activity/100) | 27.82 (9.31) | 9.84 | 10.88 | 47.66 | 30.68(14.13) | 12.02 | 18.13 | 100.00 |
| Total metals[b] (ng/m$^3$) | | | | | | | | |
| $PM_{0.18}$ | 47.79 (31.96) | 46.17 | 7.22 | 116.7 | 57.84 (47.14) | 78.91 | 12.05 | 153.39 |
| $PM_{0.18} – PM_{2.5}$ | 75.59 (51.43) | 30.39 | 25.76 | 237.1 | 67.94 (55.44) | 63.14 | 12.89 | 203.44 |
| $PM_{2.5} – PM_{10}$ | 478.31 (122.92) | 139.10 | 246.60 | 729.3 | 298.49 (171.63) | 97.58 | 112.0 | 914.57 |

Abbreviations: CO: carbon monoxide; EC: elemental carbon; GPx: glutathione peroxidase; IQR: interquartile range; OC: organic carbon; PAHs: polycyclic aromatic hydrocarbons; ROS: Reactive oxygen species;
[a]Zym: μg Zymosan equivalent units;
[b]Total sum of transition metals include V, Cr, Mn, Ni, Cu and Fe.

Table 3.5. Spearman correlation matrix of ambient, personal air pollutants and heat index by region.

| | Los Angeles | | | | | | Anaheim | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BC | CO | $NO_X$ | $PM_{2.5}$ | $O_3$ | Heat Index (F◦) | BC | CO | $NO_X$ | $PM_{2.5}$ | $O_3$ | Heat Index (F◦) |
| 24-hr averages of ambient exposures | | | | | | | | | | | | |
| BC (μg/$m^3$) | 0.88 | 0.90 | 0.23 | -0.63 | -0.13 | | 0.85 | 0.91 | 0.47 | -0.76 | -0.45 | |
| CO (ppm) | | 0.91 | 0.12 | -0.68 | -0.31 | | | 0.87 | 0.46 | -0.78 | -0.32 | |
| $NO_x$ (ppb) | | | 0.17 | -0.69 | -0.20 | | | | 0.25 | -0.85 | -0.46 | |
| $PM_{2.5}$ (μg/$m^3$) | | | | 0.02 | 0.26 | | | | | -0.17 | -0.15 | |
| $O_3$ (ppb) | | | | | 0.56 | | | | | | 0.54 | |
| 7-day averages of personal exposure [a] | | | | | | | | | | | | |
| Personal NOx (ppb) | 0.25 | 0.24 | 0.26 | -0.07 | -0.21 | -0.06 | 0.66 | 0.63 | 0.66 | 0.30 | -0.64 | -0.44 |

Abbreviations: BC: black carbon; CO: carbon monoxide;
[a]Correlations for personal $NO_x$ were calculated with 7-day average of ambient pollutants and heat index.

Table 3.6. Spearman correlation matrix of ambient, personal air pollutants and heat index.[a]

| | BC | CO | $NO_X$ | $PM_{2.5}$ | $O_3$ | Heat Index (F°) |
|---|---|---|---|---|---|---|
| **24-hr averages of ambient exposures** | | | | | | |
| BC (μg/m$^3$) | | 0.87 | 0.90 | 0.33 | -0.69 | -0.27 |
| CO (ppm) | | | 0.90 | 0.27 | -0.73 | -0.32 |
| $NO_x$ (ppb) | | | | 0.20 | -0.77 | -0.32 |
| $PM_{2.5}$(μg/m$^3$) | | | | | -0.06 | 0.09 |
| $O_3$ (ppb) | | | | | | 0.54 |
| **7-day averages of personal exposure[b]** | | | | | | |
| Personal NOx (ppb) | 0.47 | 0.45 | 0.47 | 0.16 | -0.43 | -0.25 |

Abbreviations: BC: black carbon; CO: carbon monoxide;
[a]Pollutants are mean-centered by region.
[b]Correlations for personal NOx were calculated with 7-day average of ambient pollutants and heat index

Table 3.7. Spearman correlation matrix of 5-day average of components in size-fractionated PM.[a]

| | **$PM_{0.18}$** | | | | | | | | | | | $PM_{0.18-2.5}$ | | | | | | | | | | | **$PM_{2.5-10}$** | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Pollutants | T PAHs | Hopanes | OA | OC | EC | T ROS | WS ROS | DTT | T Metal | GPx-1 | T mass | T PAHs | Hopanes | OA | OC | EC | T ROS | WS ROS | DTT | T Metal | T mass | OC | EC | T ROS | WS ROS | DTT | T Metal |
| **$PM_{0.18}$** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mass | 0.58 | **0.63** | 0.29 | **0.74** | **0.62** | 0.47 | 0.45 | **0.72** | **0.70** | 0.47 | 0.00 | **0.50** | 0.44 | 0.24 | 0.52 | 0.24 | 0.18 | 0.14 | 0.38 | 0.57 | -0.32 | 0.26 | 0.11 | -0.40 | -0.36 | 0.34 | 0.23 |
| T PAHs | | **0.89** | 0.51 | **0.80** | **0.75** | 0.10 | 0.11 | **0.76** | **0.81** | 0.25 | 0.13 | **0.94** | **0.86** | **0.71** | **0.87** | 0.37 | 0.18 | 0.21 | 0.52 | **0.68** | -0.60 | 0.55 | 0.51 | -0.30 | -0.42 | 0.57 | **0.61** |
| Hopanes | | | **0.60** | **0.82** | **0.72** | 0.17 | 0.16 | **0.79** | **0.78** | 0.15 | 0.13 | **0.85** | **0.78** | 0.58 | **0.80** | 0.42 | 0.16 | 0.20 | 0.56 | **0.68** | -0.47 | 0.49 | 0.46 | -0.27 | -0.38 | 0.58 | **0.61** |
| OA | | | | 0.52 | 0.27 | 0.09 | 0.11 | 0.42 | 0.30 | 0.21 | 0.21 | 0.54 | **0.65** | 0.55 | 0.54 | 0.25 | 0.08 | 0.23 | 0.45 | 0.37 | -0.33 | 0.32 | 0.40 | -0.10 | -0.15 | **0.66** | 0.35 |
| OC | | | | | **0.83** | 0.45 | 0.44 | **0.86** | **0.80** | 0.27 | 0.08 | **0.73** | **0.72** | 0.43 | **0.76** | 0.46 | 0.26 | 0.28 | **0.61** | **0.70** | -0.36 | 0.53 | 0.36 | -0.25 | -0.29 | 0.49 | 0.57 |
| EC | | | | | | 0.42 | 0.42 | **0.77** | **0.68** | 0.03 | 0.19 | **0.64** | 0.51 | 0.36 | **0.76** | 0.58 | 0.39 | 0.28 | 0.57 | **0.66** | -0.23 | 0.57 | 0.31 | -0.07 | -0.07 | 0.29 | **0.67** |
| T ROS | | | | | | | **0.98** | 0.41 | 0.25 | -0.05 | 0.01 | -0.02 | -0.02 | -0.15 | 0.12 | 0.34 | 0.30 | 0.28 | 0.17 | 0.18 | 0.28 | 0.20 | -0.04 | 0.12 | 0.13 | -0.01 | 0.09 |
| WS ROS | | | | | | | | 0.42 | 0.23 | -0.07 | -0.01 | -0.01 | 0.00 | -0.12 | 0.13 | 0.35 | 0.28 | 0.27 | 0.15 | 0.16 | 0.26 | 0.21 | 0.00 | 0.12 | 0.15 | 0.00 | 0.11 |
| DTT | | | | | | | | | **0.81** | 0.14 | 0.00 | **0.68** | 0.59 | 0.30 | **0.63** | 0.50 | 0.14 | 0.17 | 0.44 | **0.68** | -0.42 | 0.45 | 0.25 | -0.27 | -0.37 | 0.40 | 0.45 |
| T Metals[b] | | | | | | | | | | 0.26 | 0.00 | **0.76** | **0.61** | 0.33 | **0.64** | 0.32 | 0.13 | 0.18 | 0.48 | **0.80** | -0.46 | 0.50 | 0.33 | -0.40 | -0.51 | 0.42 | 0.51 |
| **$PM_{0.18-2.5}$** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mass | | | | | | | | | | | | 0.18 | 0.12 | 0.21 | 0.36 | 0.19 | **0.65** | **0.67** | 0.48 | 0.24 | 0.26 | 0.15 | 0.29 | 0.47 | 0.43 | 0.32 | 0.24 |
| T PAHs | | | | | | | | | | | | | **0.90** | **0.73** | **0.85** | 0.32 | 0.09 | 0.22 | 0.49 | **0.63** | -0.54 | **0.60** | 0.56 | -0.29 | -0.44 | **0.67** | **0.65** |
| Hopanes | | | | | | | | | | | | | | **0.77** | **0.81** | 0.23 | 0.08 | 0.18 | 0.47 | 0.52 | -0.60 | 0.55 | 0.51 | -0.34 | -0.43 | **0.71** | 0.52 |
| OA | | | | | | | | | | | | | | | **0.70** | 0.15 | -0.03 | 0.14 | 0.35 | 0.27 | -0.53 | 0.31 | 0.47 | -0.20 | -0.30 | **0.61** | 0.37 |
| OC | | | | | | | | | | | | | | | | 0.47 | 0.31 | 0.35 | **0.62** | **0.63** | -0.42 | 0.59 | 0.56 | -0.09 | -0.16 | **0.63** | **0.67** |
| EC | | | | | | | | | | | | | | | | | 0.41 | 0.46 | 0.25 | 0.27 | 0.15 | 0.31 | 0.33 | 0.27 | 0.29 | 0.18 | 0.52 |
| T ROS | | | | | | | | | | | | | | | | | | **0.84** | 0.37 | 0.30 | 0.27 | 0.11 | 0.14 | 0.33 | 0.47 | 0.11 | 0.14 |
| WS ROS | | | | | | | | | | | | | | | | | | | 0.40 | 0.32 | 0.22 | 0.16 | 0.26 | 0.43 | 0.44 | 0.30 | 0.25 |
| DTT | | | | | | | | | | | | | | | | | | | | 0.57 | -0.16 | 0.42 | 0.35 | 0.14 | 0.01 | 0.41 | 0.53 |
| T Metals[b] | | | | | | | | | | | | | | | | | | | | | -0.37 | 0.51 | 0.23 | -0.11 | -0.20 | 0.32 | 0.53 |
| **$PM_{2.5-10}$** | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mass | | | | | | | | | | | | | | | | | | | | | | -0.04 | -0.01 | **0.60** | **0.67** | -0.31 | -0.06 |
| OC | | | | | | | | | | | | | | | | | | | | | | | 0.55 | 0.06 | 0.00 | 0.32 | **0.75** |
| EC | | | | | | | | | | | | | | | | | | | | | | | | 0.19 | 0.11 | 0.39 | 0.51 |
| T ROS | | | | | | | | | | | | | | | | | | | | | | | | | **0.87** | -0.11 | 0.23 |
| WS ROS | | | | | | | | | | | | | | | | | | | | | | | | | | -0.20 | 0.09 |
| DTT | | | | | | | | | | | | | | | | | | | | | | | | | | | 0.39 |

Abbreviations: DTT: dithiothreitol; EC: elemental carbon; GPx: glutathione peroxidase, OA: Organic acids; OC: organic carbon; PM: particulate matter; ROS: reactive oxygen species; T: total; WS: water-soluble;
Bold numbers indicate correlation values ≥ 0.60 and P < 0.05;
[a]Pollutants are mean-centered by region;
[b]Total sum of transition metals include V, Cr, Mn, Ni, Cu and Fe.

Table 3.8. Spearman correlations of selected PM components and transition metals in three size-fractions

| | OC | EC | T ROS | WS ROS | DTT | OC | EC | T ROS | WS ROS | DTT | OC | EC | T ROS | WS ROS | DTT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **$PM_{0.18}$** | | | | | | | | | | | | | | | |
| V | 0.15 | 0.02 | 0.25 | 0.24 | 0.24 | -0.19 | -0.28 | -0.20 | -0.13 | 0.05 | -0.31 | -0.40 | -0.27 | -0.19 | -0.37 |
| Cr | 0.41 | 0.49 | 0.17 | 0.16 | 0.48 | 0.45 | 0.18 | 0.16 | 0.12 | 0.23 | 0.36 | 0.19 | -0.12 | -0.34 | 0.30 |
| Mn | 0.74 | 0.60 | 0.25 | 0.25 | 0.79 | 0.57 | 0.16 | 0.02 | 0.05 | 0.41 | 0.34 | 0.25 | -0.32 | -0.54 | 0.31 |
| Ni | 0.21 | 0.44 | 0.34 | 0.29 | 0.28 | 0.26 | 0.23 | 0.26 | 0.14 | 0.03 | 0.21 | 0.10 | -0.01 | -0.13 | 0.07 |
| Cu | 0.60 | 0.69 | 0.24 | 0.23 | 0.71 | 0.73 | 0.35 | 0.29 | 0.32 | 0.48 | 0.50 | 0.43 | -0.02 | -0.30 | 0.55 |
| Fe | 0.76 | 0.63 | 0.28 | 0.27 | 0.81 | 0.58 | 0.18 | 0.03 | 0.04 | 0.42 | 0.34 | 0.26 | -0.32 | -0.54 | 0.31 |
| **$PM_{0.18-2.5}$** | | | | | | | | | | | | | | | |
| V | -0.54 | -0.17 | 0.07 | 0.06 | -0.42 | -0.25 | 0.07 | 0.27 | 0.31 | -0.26 | -0.29 | -0.20 | 0.36 | 0.55 | -0.15 |
| Cr | 0.34 | 0.73 | 0.28 | 0.27 | 0.56 | 0.67 | 0.51 | 0.51 | 0.50 | 0.43 | 0.46 | 0.38 | 0.31 | 0.11 | 0.47 |
| Mn | 0.56 | 0.73 | 0.24 | 0.24 | 0.70 | 0.68 | 0.37 | 0.34 | 0.33 | 0.55 | 0.48 | 0.33 | 0.09 | -0.11 | 0.39 |
| Ni | -0.21 | 0.22 | 0.17 | 0.15 | -0.18 | 0.23 | 0.34 | 0.53 | 0.55 | 0.12 | 0.08 | 0.15 | 0.52 | 0.54 | 0.29 |
| Cu | 0.46 | 0.76 | 0.20 | 0.20 | 0.64 | 0.73 | 0.53 | 0.48 | 0.47 | 0.50 | 0.56 | 0.46 | 0.23 | -0.01 | 0.55 |
| Fe | 0.56 | 0.69 | 0.21 | 0.22 | 0.69 | 0.66 | 0.38 | 0.31 | 0.30 | 0.51 | 0.50 | 0.37 | 0.07 | -0.14 | 0.43 |
| **$PM_{2.5-10}$** | | | | | | | | | | | | | | | |
| V | -0.30 | 0.24 | 0.24 | 0.22 | -0.15 | 0.06 | 0.38 | 0.43 | 0.46 | 0.09 | 0.35 | 0.24 | 0.60 | 0.63 | 0.06 |
| Cr | 0.15 | 0.74 | 0.24 | 0.25 | 0.38 | 0.70 | 0.75 | 0.62 | 0.65 | 0.31 | 0.82 | 0.71 | 0.58 | 0.32 | 0.69 |
| Mn | 0.07 | 0.67 | 0.22 | 0.23 | 0.30 | 0.62 | 0.74 | 0.58 | 0.59 | 0.30 | 0.81 | 0.68 | 0.61 | 0.39 | 0.64 |
| Ni | -0.08 | 0.62 | 0.25 | 0.26 | 0.21 | 0.53 | 0.76 | 0.67 | 0.69 | 0.15 | 0.74 | 0.63 | 0.70 | 0.47 | 0.59 |
| Cu | 0.30 | 0.83 | 0.18 | 0.21 | 0.50 | 0.83 | 0.76 | 0.61 | 0.61 | 0.32 | 0.81 | 0.75 | 0.46 | 0.18 | 0.72 |
| Fe | 0.22 | 0.78 | 0.24 | 0.26 | 0.41 | 0.74 | 0.75 | 0.58 | 0.60 | 0.34 | 0.85 | 0.74 | 0.54 | 0.28 | 0.68 |

Abbreviations: DTT: dithiothreitol; EC: elemental carbon; OC: organic carbon; PM: particulate matter; T: total; ROS: reactive oxygen species; WS: water-soluble

# Chapter 4. Systemic and airway oxidative stress and inflammation analysis

## 4.1. Background

Epidemiological studies have shown associations between short-term exposures to air pollutants and cardiopulmonary morbidity and mortality as reviewed by Franklin et al. (Franklin et al. 2015). The particular mechanisms linking air pollution to acute respiratory and cardiovascular events are not completely understood, but oxidative stress and inflammation induced by pro-oxidant components of the air pollution mixture are believed to play key roles. As discussed previously, particle size is an important determinant of deposition in the respiratory tract, and an indicator of chemical composition and source (Delfino et al. 2005; Sioutas et al. 2005). Smaller particles have a higher pulmonary deposition fraction and penetrate deeper in the lung (Lippmann 1977). The large surface area of ultrafine particles also carries high concentrations of pro-oxidant chemical components, such as PAHs and transition metals, each of which has been shown to induce oxidative stress responses (Li et al. 2003)and can translocate from pulmonary sites to the circulatory system (Elder et al. 2006). Experimental studies have provided evidence that ultrafine particles can induce the greatest amount of oxidative stress and inflammation per unit of PM mass (Li et al. 2002; Cho et al. 2005). This may in-turn impact physiologic responses that ultimately

increase the risk of acute cardiorespiratory morbidity (Weichenthal 2012). In epidemiological studies, some have shown that ultrafine PM air pollution is more strongly associated with adverse health effects when compared to larger PM diameters (Delfino et al. 2009; Franck et al. 2011) while other studies have shown $PM_{2.5}$ to have stronger or as strong associations as ultrafine PM (Ruckerl et al. 2014; Lanzinger et al. 2016). The inconsistent epidemiological evidence may be due to the fact that the sources and components of pollutants vary from study to study. In urban cities, such as Los Angeles, traffic-related pollutants are the main source for PM mass concentrations (Pant et al. 2013). Those traffic-related pollutants have been shown to contain redox active chemicals that are able to generate ROS responsible for increases in oxidative stress (Ayres et al. 2008b). By quantifying the inherent capacity of PM to oxidize target molecules, oxidative potential has proposed to be a more attractive and biologically more relevant exposure metric than PM mass (Borm et al. 2007). Therefore, the direct measurement of PM oxidative potential may show stronger or more precise associations than either ultrafine PM or traffic-related air pollutants.

Most previous epidemiological studies have used ambient air monitoring data from central air monitoring stations, and focused primarily on the U.S. EPA's criteria air pollutants, namely, $PM_{2.5}$, $PM_{10}$, $O_3$, $NO_x$, and CO. Several research groups including ours have been investigating the associations between biomarkers of effect and size-fractionated and/or chemically-characterized particulate air pollutants (Delfino et al. 2008; Delfino et al. 2009; Delfino et al. 2010a; Chen et al. 2015; Wu et al. 2015). However, there is still a need to systematically explore oxidative stress and

inflammatory biomarkers in both pulmonary and circulatory systems in relation to exposures to chemically-characterized PM.

To better understand the underlying mechanism of the association between cardiorespiratory morbidity and short-term exposure to air pollution, we investigated the potential roles of air pollutant components and pollution source tracers on both airway and systemic biomarkers of oxidative stress and inflammation in an elderly cohort. We hypothesize that chemicals from fossil fuel combustion, related ultrafine particles, and PM oxidative potential would have stronger adverse health effects than other air pollutant variables. To our knowledge, this is the first study to relate biomarkers of airway and systemic oxidative stress responses to exposure markers of PM oxidative potential in an elderly population.

## 4.2. Methods

### 4.2.1. Study design

This analysis was performed using the cohort panel design described in detail in Chapter 2. Briefly, repeated measures of outcomes and exposures were captured for 97 elderly non-smoking adults (age ≥ 65) living in two Los Angeles California metropolitan areas (downtown Los Angeles and Anaheim, CA) between 2012 and 2014. The repeated measures design can effectively allow for the control of between and within-subject variability in regression models. Two groups of subjects in each area were

followed alternatively for up to 12 weeks in two discrete 6-week periods in order to incorporate seasonal differences in air pollution levels in the Los Angeles metropolitan area (Daher et al. 2013; Hasheminassab et al. 2014b). Specifically, one 6-week period took place during the warm season (July-October) and the other took place in the cool season (November-February). Weekly clinical visits for study participants were scheduled on the same day of the week and at the same time of day in order to minimize potential biases induced by weekly and circadian variation (94% of the subjects arrived to clinic with a variability of ± 2 hours).

Subjects were excluded from study participation if they lived or were employed outside of the monitored community (18 km radius), smoked within the last 12 months, abused drugs or alcohol, or reported exposure to environmental tobacco smoke at home or on a regular basis at other locations. Additional health criteria for exclusion included the presence of psychiatric disorders or dementia that would prevent the subject from full participation, dialysis treatment or renal failure, daily oral corticosteroids, and active cancer. Observations following the previous 7 days when subjects reported any acute infection (7.62%) were excluded *a priori* given the known major impact of infections on systemic inflammation.

This study was approved by the institutional Review Board of the University of California, Irvine. All subjects provided written informed consent before participation.

### 4.2.2. Markers of airway oxidative stress and inflammation

*EBC sampling and MDA analysis*

We collected EBC samples during normal breathing with the RTube™ Collection System (Respiratory Research, Inc., Austin, TX) using standard procedures recommended by the American Thoracic Society and European Respiratory Society (Holvoet et al. 2003). Room air was inhaled through a one-way valve and the exhaled air was directed into a collection chamber (solid aluminum tube pre-chilled on dry ice) where vapors, aerosols and moisture in the breath condense. EBC was then collected from the walls of the condenser. The samples were transported to our laboratory on dry ice and then frozen at –80ºC until analysis. We analyzed EBC samples for MDA using High Performance Liquid Chromatography (HPLC) analysis by modifying the Laerstad *et al.* 2002 protocol (Larstad et al. 2002). Different MDA standards or EBC samples (50 µL) were derivatized with 12.25 mM 2-thiobarbituric acid (TBA) in 450 µl phosphoric acid-KOH buffer (60-minutes incubation at 95◦C). A Shimadzu HPLC autosampler (SIL-20A/AC) was then loaded after cooling down the samples in an ice bath for 5 minutes and adapting to room temperature. HPLC analysis was performed under the following conditions: Quaternary LC-20AT pump and RF-20AXS Fluorescence Detector (Shimadzu Scientific Instruments), excitation: 532 nm; emission: 553 nm; mobile phase: acetonitrile -20 mM phosphate buffer, pH: 6.8; (20:80, v/v); flow-rate: 1.3 ml/min; column: Kinetex 5u C18 100A, 150x60 mm; injection volume: 20 ul). Linearity was evaluated by the analysis of duplicate MDA standards ranging from 3.0 to 25.0 nM. To

calculate more accurately of the MDA concentrations in the airway fluid lining, the respiratory tract based on those measured in EBC needs an independent method of determining the dilution of the EBC by water vapor (Corradi et al. 2003). However, dilution of these nonvolatile biomarkers by water vapor can vary dramatically, and to date, there is no gold standard for assessing the dilution of airway lining fluid biomarkers in the EBC (Liang et al. 2012). The estimated limit of quantification (LOQ) for MDA in EBC samples is 3 nM. All values < LOQ were set to 1.5 nmol. We excluded MDA results if the concentration was greater than the lower limit of quantification (12.5 nM) and coefficient of variation (CV) > 25% (occurring in 1.16% of samples) (Zhang et al. 2016a).

*FeNO measurement*

We used the NIOX MINO (Aerocrine Inc, New Providence, NJ) to noninvasively measure FeNO. Based on previous research (ATS/ERS 2005), a questionnaire was administered to obtain the following information prior to the FeNO measurement: (1) did the subject have a meal (breakfast if in the morning, lunch if in the afternoon) or exercise within an hour before the test; (2) was the subject's previous meal high in fat or sugar. An affirmative answer to any of these questions was tested as independent variables for their influence on FeNO and on air pollutant regression parameters. An NO scrubbing filter was used at the air intake to control for indoor NO.

### 4.2.3. Markers of systemic oxidative stress and inflammation

*OxLDL and IL-6*

At each follow-up visit, venous peripheral blood samples were drawn by a trained phlebotomist using anti-coagulant PPT BD Vacutainer® tubes and immediately centrifuged to separate plasma and transported at -20 ºC before storage in our laboratory at -80 ºC until analysis. The blood samples on one week (n=21) were lost due to improper storage procedures. The systemic oxidative stress biomarker oxLDL was measured in plasma by standardized ELISA using monoclonal antibodies directed against a neo-epitope in the aldehyde-substituted apoB-100 moiety of LDL (oxLDL-4E6 assay, Mercodia AB, Sweden). The lower limit of detection of oxLDL was 28.8 U/L and the results were considered invalid if the estimated CV was > 25% (occurring in 0.34% of samples). Systemic inflammation biomarker IL-6 was measured in plasma using 96-well immunoassay kits (Quantikine High Sensitivity, Minneapolis, MN). The lower and upper limit of detection of IL-6 were 0.156 pg/ml and 10 pg/ml, respectively. IL-6 values were considered invalid if the estimated CV was > 25%. This occurred in 15.09% of samples.

### 4.2.4. Air pollution and meteorology

Details of exposure measurement are outlined in Chapter 2. Briefly, hourly $PM_{2.5,}$ BC, CO, $NO_X$, and ozone, 7-day personal $NO_{X,}$ and 5-day PM mass and components

(such as PAHs, hopanes, EC/OC, oxidative potential, organic acid and transition metals) were collected in different size-fractions. Of particular importance for this analysis, *in vitro* oxidative potential was measured for particle extracts in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$ size fractions using two different methods: macrophage ROS and DTT. Macrophage ROS was measured by a rat alveolar macrophage reactive oxygen species production assay (Landreman et al. 2008), while DTT was quantified by the capacity of the PM extract to generate abiotic chemically-produced ROS (i.e., electron transfer from DTT to oxygen) (Cho et al. 2005). These novel exposure metrics capture both biotic and abiotic potential for these particulate matter size fractions to induce possible oxidative stress. Hourly meteorological data (temperature and relative humidity) were collected as well.

### 4.2.5. Statistical analysis

Details of statistical analysis are given in Chapter 2. Briefly, data were analyzed using a linear mixed effect model to account for the clustering of longitudinal repeated measurements taken on each subject. All models include a random subject intercept to account for the correlation of repeated measures. We selected an ARMA (1,1) covariance structure based on AIC for the covariance matrix to model the correlation between repeated measures in each subject. The magnitude of the effect was expressed relative to an IQR ($25^{th}$ to $75^{th}$ percentile) difference in each pollutant concentration to compare associations across different pollutants.

*Covariates*

Time-invariant subject characteristics were controlled by the study design and the specified repeated measures model, and hence were not included as adjustment covariates. However, oxLDL levels were found to be correlated to LDL level. In order to test for the independent effect of oxLDL, we controlled for LDL level in the model of oxLDL. An *a priori* adjustment variable was heat index with the same lag average as the pollutant and was included in the model as a covariate to adjust for the potential confounding effect of weather. Time-variant potential confounders were tested in the models, and were included in the model if they improved model fit. Days of gas stove use per week were significantly associated with increased personal $NO_X$ and were adjusted in the models of personal $NO_X$ *a priori* because the exposure of interest was outdoor fossil fuel sources of $NO_X$. All biomarker outcome variables were log-transformed before the analyses to fulfill the model assumption of residual normality. The results of biomarkers are given as the percent change in geometric mean.

*Influential observations*

The impact of influential observations was assessed using the Cook's D statistic and standardized residual diagnostics, at both the individual observations level and clustered subjects level (Cook 1977). One influential subject (out of 88 subjects) was identified for EBC MDA, and after excluding that subject the positive associations

became stronger and more significant for BC, $NO_x$, CO, and ultrafine mass and components (Table 4.1).Three influential observations out of 719 observations for IL-6 were detected by restricted likelihood distance >1 or Cook's D value> 4/n (n=number of observations) (Cook et al. 1982; Fox et al. 1990). The estimated of regression coefficients for covariates of interest were generally unchanged but the CI became smaller after excluding these three influential observations (Table 4.2).

*Effect modification*

In exploratory analyses, we considered whether the following variables may be potential effect modifiers of the associations between biomarkers and air pollution: age (≤ 75 years, > 75 years), sex (male, female), obesity (BMI ≥ 30 kg/$m^2$), history of hypertension (yes, no), intake of anti-hypertensive medication (yes, no), diabetes mellitus (yes, no), hypercholesterolemia by history (yes, no), total cholesterol/HDL ratio (≥ 3.5, < 3.5) cardiovascular risk score (≥ 0.2, < 0.2), intake of statin (yes, no), study area (Los Angeles, Anaheim), and former smokers (yes, no). Additionally, for airway biomarkers, we assessed effect modification by asthma and/or chronic obstructive pulmonary disease (COPD). Evidence of a significant interaction was considered at a nominal product term *p*-value<0.1 to avoid increased type II errors in these hypothesis-generating analyses.

*Sensitivity analysis*

Several sensitivity analyses were conducted. First, to investigate potential exposure error, we restricted the analysis to the subjects who lived within the 90$^{th}$ percentile of subjects' residential distance to the stations (11.3 km for the SCAQMD monitoring stations and 13.1 km for the USC monitoring sites). Second, we limited the analysis to measured ambient exposures, rather than exposures including imputed data from the next nearest air monitoring station or interpolated from the adjacent day's data. Third, we excluded days with extreme heat index (< 52.5 and > 75.04, 10$^{th}$ percentile and 90$^{th}$ percentile of the heat index during the study period, respectively) because the decreased ventilation at subjects' residential buildings during those days may lead to increased exposure error for the representation of personal exposures by ambient measurements. Lastly, in order to test for the independent effects of primary and photochemically-produced air pollutants, we applied two-pollutant models by including a primary air pollutant (BC, NOx and PAHs) and $O_3$, which showed unexpected inverse associations with some biomarkers. Note that this two-pollutant analysis is exploratory since $O_3$ and the primary pollutants were moderately to strongly inversely correlated (Spearman R ≥ 0.6). Interactions are also of interest because the highly oxidizing capability of $O_3$ gives it the potential to react with primary pollutant components to produce other pro-oxidant components that can adversely affect the airways. In order to address this in an exploratory analysis we tested interactions between the same primary air pollutants and $O_3$ on the airway biomarkers (FeNO and EBC MDA).

All statistical analyses were performed using R version 3.0.3 (R Foundation for Statistical Computing, Vienna, Austria) or SAS 9.3 software (SAS, Cary, NC).

## 4.3. Results

### 4.3.1. Descriptive data

One hundred and ninety-one subjects (191) were recruited and 87 subjects dropped out or become ineligible (cancer, smoker, or moved out of the study area) before or soon after the start of follow-up, resulting 104 subjects. The median repeated measures for FeNO, EBC MDA, IL-6, and oxLDL are 11, 8, 8, and 10, respectively. Those excluded from regression analyses due to lack of sufficient repeated outcomes (< 4) measures were 16 subjects for EBC MDA, 8 subjects for FeNO, 11 subjects for IL-6, and 11 subjects for oxLDL. Among these listed above, 7 subjects were completely excluded for all outcomes leaving 97 with at least some outcome measurements. Among 97 subjects, 48 were from the Los Angeles area and 49 were from the Anaheim area. Descriptive characteristics of 97 subjects and descriptive data for the biomarker measurements are shown in Table 4.3 and the descriptive data by region are shown in Table 4.4. The subjects were between 65 and 96 years old (mean, 74.8 years), with two-thirds of the subjects being female. Among the 97 subjects, there are 64 non-Hispanic Whites, 9 Hispanics, 10 African Americans, 9 Asians and 5 other race/ethnicities. All were non-smokers and around 40% were former smokers. Eight subjects had asthma and 12 subjects had COPD. Approximately 60% of study subjects were taking antihypertensive medications and over half of the subjects were taking statins. Twenty-two subjects had diabetes. Depending on the biomarker outcomes, the numbers of observations without any report of infection in the previous week ranged from 643 to 901 (Table 4.3). Spearman correlations showed no correlations (-0.04 to

0.11) among these 4 biomarkers. Additionally, we assessed the relationships among 4 biomarker outcomes in linear mixed models. No significant associations were observed (data not shown). Descriptive statistics of the exposures are given in Chapter 3.

### 4.3.2. Airway biomarkers and air pollutant associations

Since daily ambient traffic-related pollutants (BC, CO, and NOx) were strongly correlated and showed similar results, we only display results of BC in the result figures. The detailed information can be seen in the result tables (Table 4.1, Table 4.2, Table 4.5, and Table 4.6). The biomarker of airway oxidative stress, EBC MDA, was positively associated with daily ambient traffic-related air pollutants (represent by BC in the figure) and 7-day personal $NO_x$, but not with BAM (Beta Attenuation Monitor) $PM_{2.5}$ (Figure 4.1 and Table 4.1). For ambient traffic-related air pollutants, the strongest positive associations were observed for 7-day averages. For example, MDA had an estimated 7.4% increase (95% CI: 1.7%, 13.4%) per interquartile increase of 7-day average BC. For 5-day PM components, MDA was positively associated with total $PM_{0.18}$ mass, and carbonaceous aerosol components [more strongly and significantly ($p < 0.05$) with the $PM_{0.18}$ fraction]. MDA was also positively associated with total transition metals and significantly so for $PM_{0.18}$ and $PM_{2.5-10}$ fractions. The associations of MDA with individual transition metals were, in general, significant in all size-fractions, except for V (Figure 4.2.A). The associations of MDA with $O_3$ were negative. We did not observe any significant associations of MDA with PM oxidative potential (ROS and DTT), GPx-1, or organic acids. The estimates of association for water-soluble ROS and total ROS were

similar for all biomarkers, therefore, we did not present water-soluble ROS results in the figures.

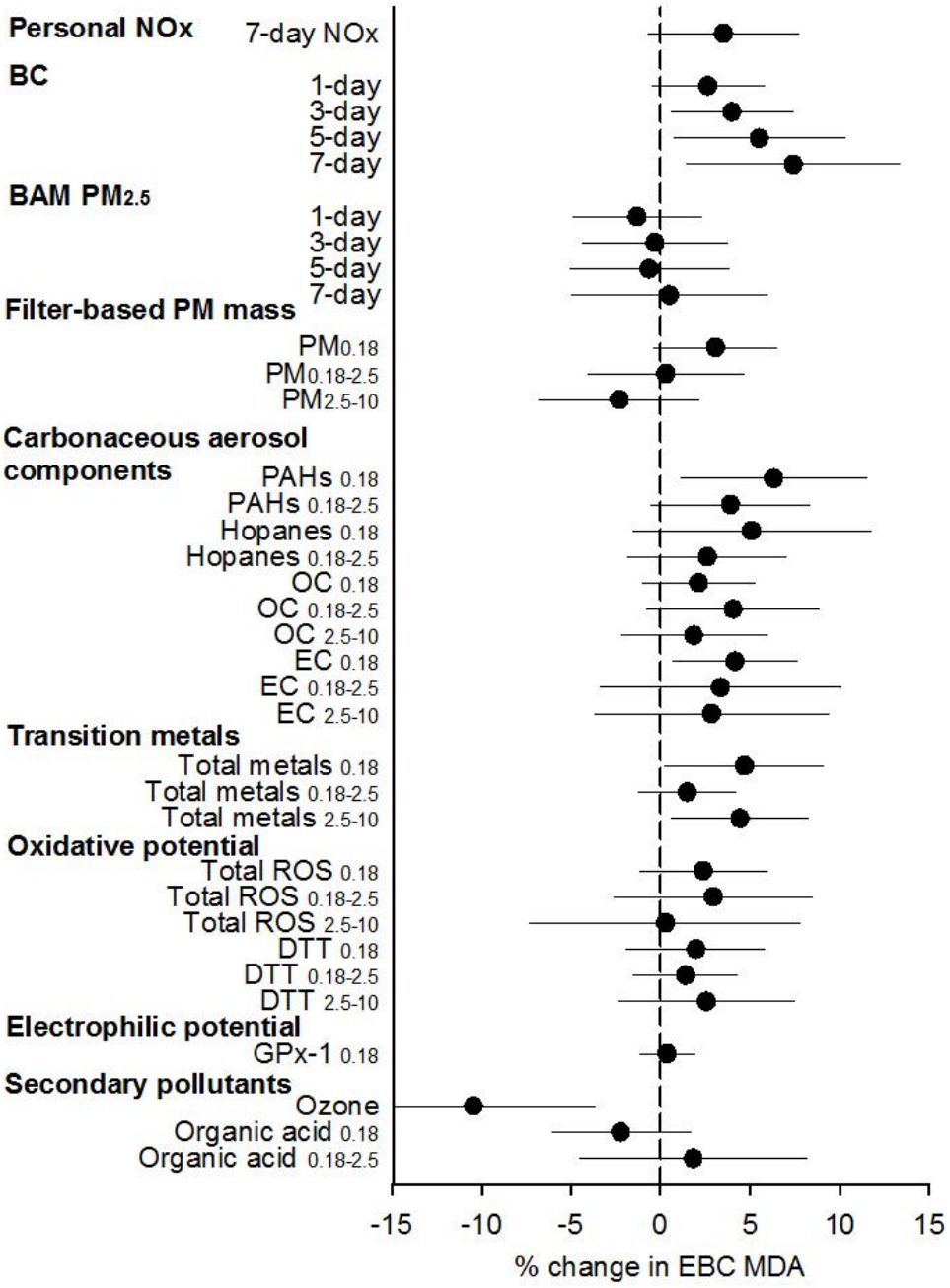


**Figure 4.1. Percent change (mean and 95 % confidence intervals) in airway oxidative stress biomarker EBC MDA with a one interquartile range increase of ambient and personal air pollutants.**

Exposures were averaged across 5 days except as specified. BC: black carbon; CO: carbon monoxide; DTT: dithiothreitol; EBC MDA: malondialdehyde in exhaled breath condensate; EC: elemental carbon; FeNO: exhaled nitric oxide; $NO_X$: nitrogen oxides; OC: organic carbon; PAHs: polycyclic aromatic hydrocarbons; ROS: reactive oxygen species.

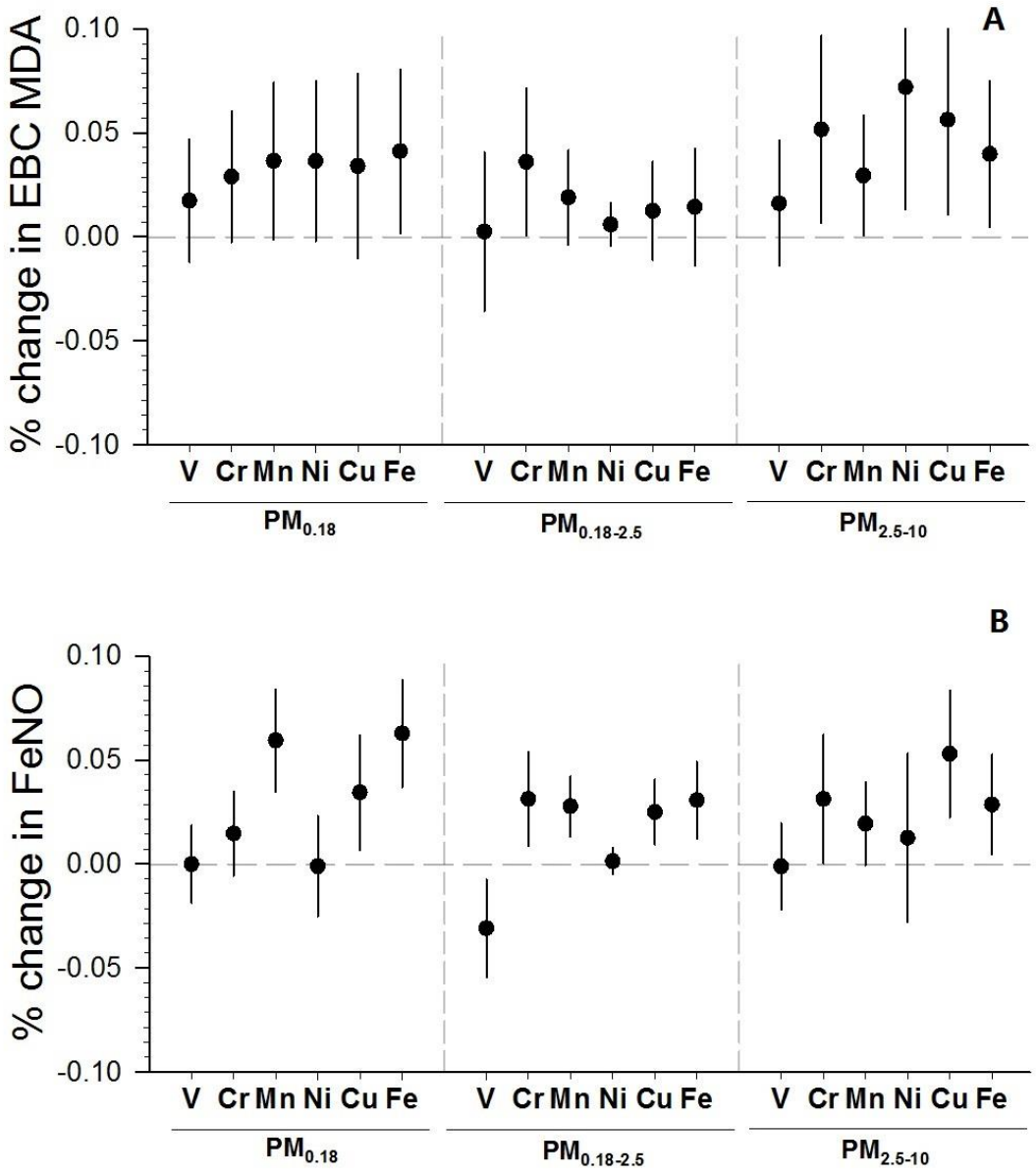


**Figure 4.2. Association of airway oxidative stress biomarker EBC MDA (A) and airway inflammation biomarker FeNO (B) with a one interquartile range increase of selected transition metals.**
Transition metals were measured in three size-fractions for exposures averages

The biomarker of airway inflammation, FeNO, was positively associated with markers of traffic-related air pollutants (BC, CO, NOx, all carbonaceous aerosol

components including OC), ultrafine $PM_{0.18}$ mass, and total transition metals (Figure 4.3 and Table 4.5). Further analysis in individual transition metal found significant association with Cr, Mn, Cu, and Fe in three size fractions (Figure 4.2.B). Similar to MDA, the strongest association for daily ambient measurements of traffic-related air pollutants (BC, CO, and $NO_x$) were observed for 7-day averages. For example, BC at 7-day average had the strongest estimated association (7.5%, 95% CI: 3.6%, 11.5%). For the 5-day average PM components, we observed stronger estimated associations in $PM_{0.18}$ than in the larger size-fractions for total mass for PAHs, hopanes, organic acid, and total transition metals. Associations between FeNO and DTT in all three size-fractions are positive, although not significant. Unexpectedly, FeNO was estimated to be inversely associated with total ROS in the coarse fraction ($PM_{2.5-10}$) and with $O_3$. No association was observed for FeNO with 7-day personal $NO_X$, total ROS in $PM_{0.18}$ and $PM_{0.18-2.5}$, GPx-1 in $PM_{0.18}$, or organic acid in $PM_{0.18-2.5}$.

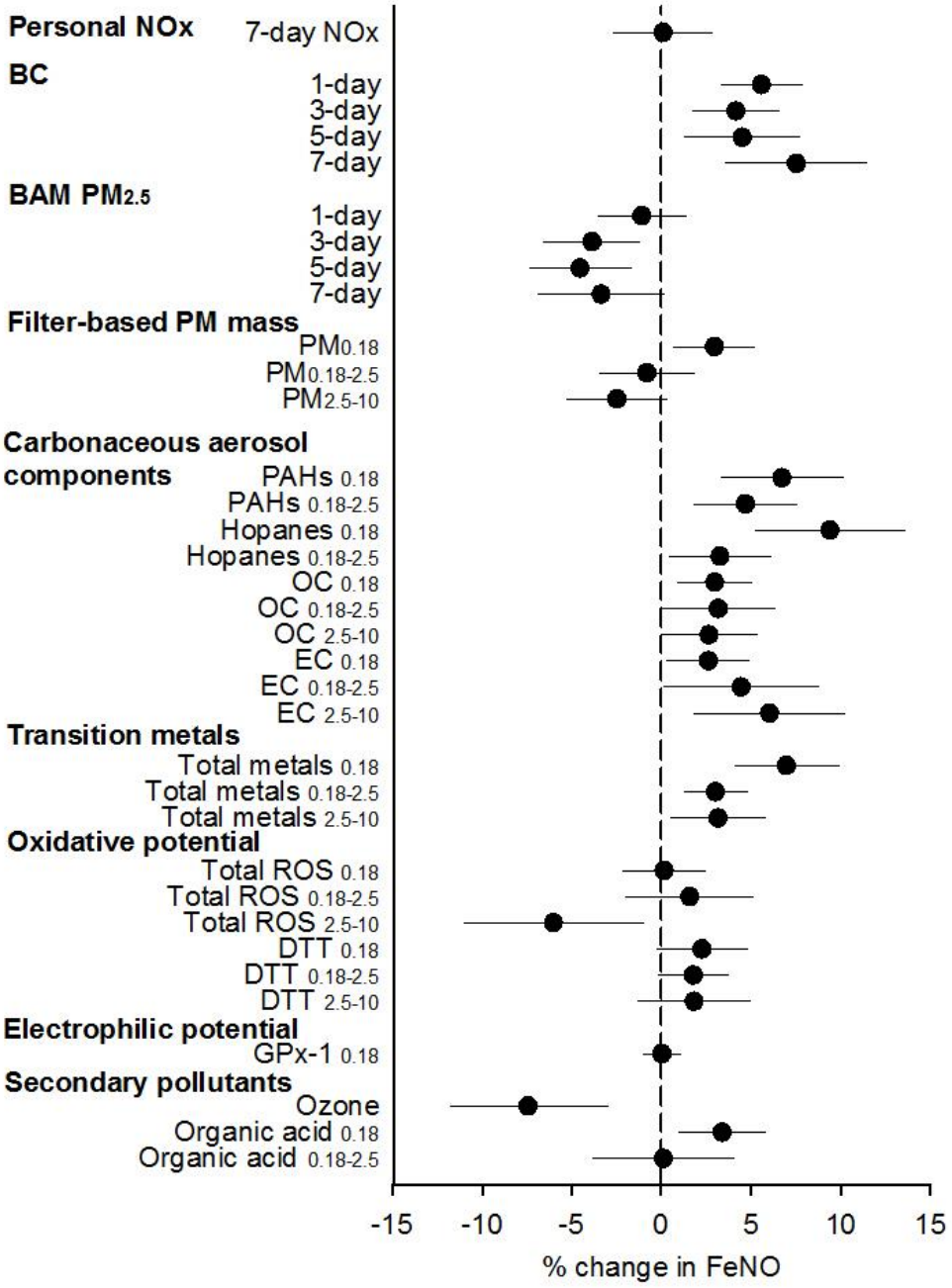


**Figure 4.3. Percent change (mean and 95 % confidence intervals) in airway inflammation biomarker FeNO with a one interquartile range increase of ambient and personal air pollutants.**

Exposures were averaged across 5 days except as specified. BC: black carbon; CO: carbon monoxide; DTT: dithiothreitol; EBC MDA: malondialdehyde in exhaled breath condensate; EC: elemental carbon; FeNO: exhaled nitric oxide; $NO_X$: nitrogen oxides; OC: organic carbon; PAHs: polycyclic aromatic hydrocarbons; ROS: reactive oxygen species.

### 4.3.3. Systemic biomarkers and air pollutant associations

We did not observe significant associations between oxLDL and PM air pollutant components except an unexpected positive association with GPx-10.18 (decreased GPx-1 is expected with electrophilic inactivation) (Figure 4.4 and Table 4.6). However, positive (expected) but non-significant associations of oxLDL with traffic-related pollutants (BC, PAHs, hopanes), total transition metals in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$, ROS in $PM_{0.18\text{-}2.5}$, EC in $PM_{2.5\text{-}10}$, and organic acids in $PM_{0.18\text{-}2.5}$ were observed. The systemic inflammation biomarker IL-6 was significantly and positively associated with daily ambient traffic-related air pollutants at 1-day averages and then gradually became weaker and non-significant at 3-day, 5-day and 7-day averages (Figure 4.5 and Table 4.2). We observed unexpected inverse associations of IL-6 with EC and with ROS in $PM_{0.18\text{-}2.5}$. The associations of IL-6 with 7-day personal $NO_X$ and other 5-day average PM components were nonsignificant. The associations of systemic biomarkers with individual selected transition metals were present in Figure 4.6. Positive associations between oxLDL and transition metal were observed for all metals except for V in $PM_{0.18}$. However, the associations were found significant only for Cr and Cu (Figure 4.6.A). The associations between IL-6 and transition metals were generally non-significant except for the unexpected inverse association between IL-6 and V in $PM_{0.18\text{-}2.5}$ (Figure 4.6.B).

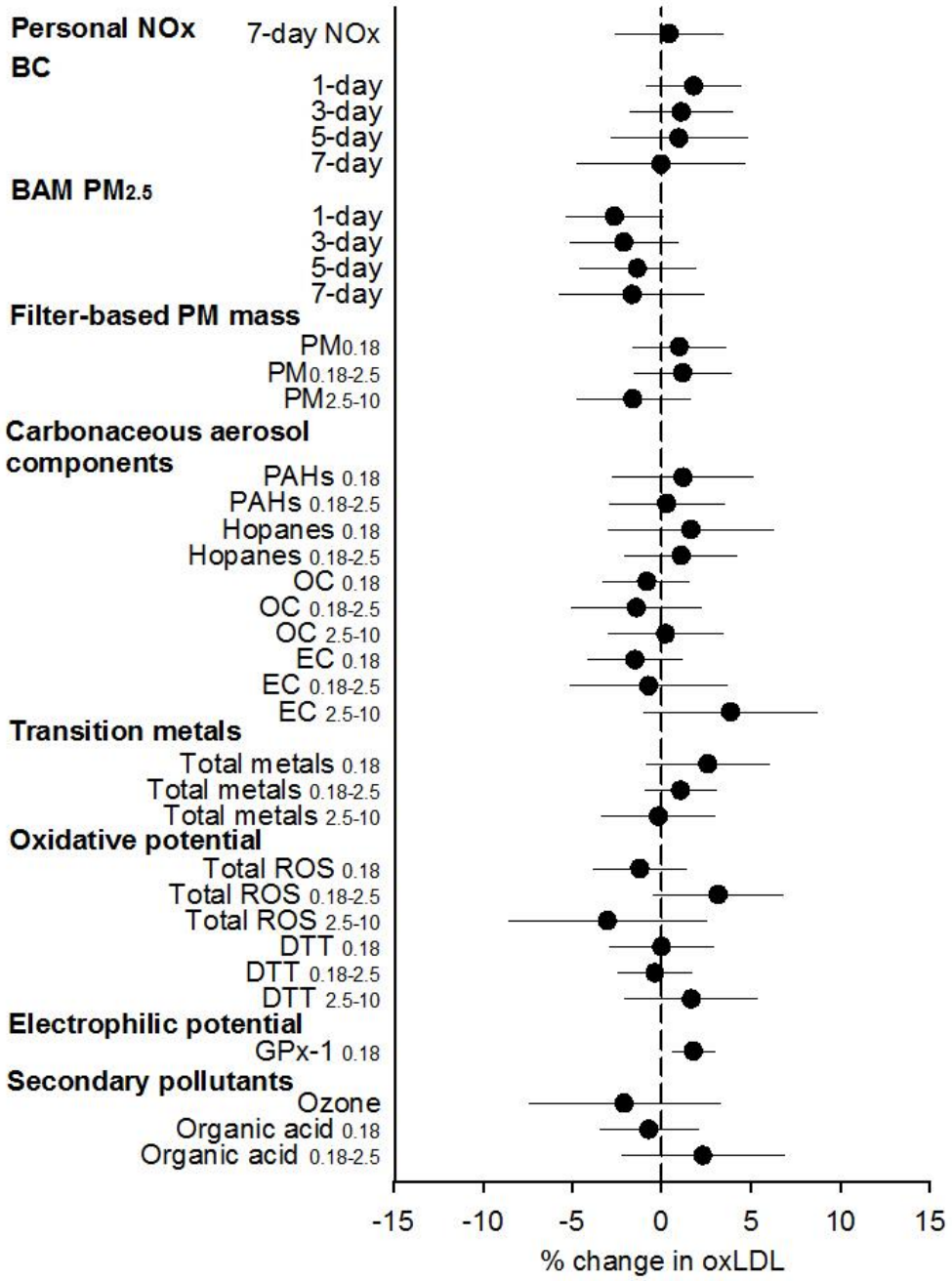


**Figure 4.4. Percent change (mean and 95 % confidence intervals) in systemic oxidative stress biomarker oxLDL with a one interquartile range increase of ambient and personal air pollutants.**

Exposures were averaged across 5 days except as specified. BC: black carbon; CO: carbon monoxide; DTT: dithiothreitol; EBC MDA: malondialdehyde in exhaled breath condensate; EC: elemental carbon; $NO_X$: nitrogen oxides; OC: organic carbon; OxLDL: Oxidized low-density lipoprotein PAHs: polycyclic aromatic hydrocarbons; ROS: reactive oxygen species.

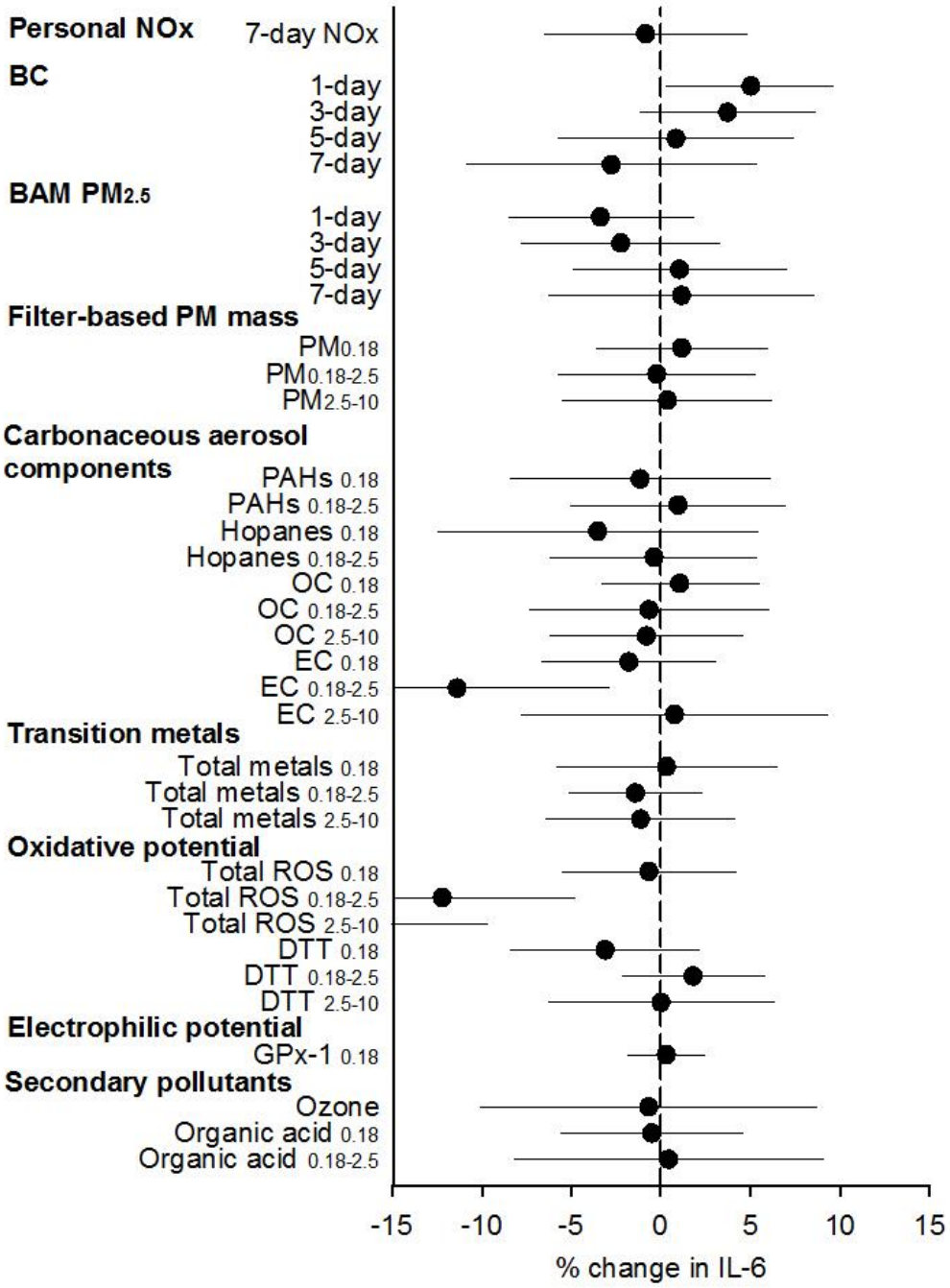


**Figure 4.5. Percent change (mean and 95 % confidence intervals) in systemic inflammation biomarker IL-6 with a one interquartile range increase of ambient and personal air pollutants.**

Exposures were averaged across 5 days except as specified. BC: black carbon; CO: carbon monoxide; DTT: dithiothreitol; EBC MDA: malondialdehyde in exhaled breath condensate; EC: elemental carbon; IL-6: Interleukin-6; $NO_X$: nitrogen oxides; OC: organic carbon; PAHs: polycyclic aromatic hydrocarbons; ROS: reactive oxygen species.

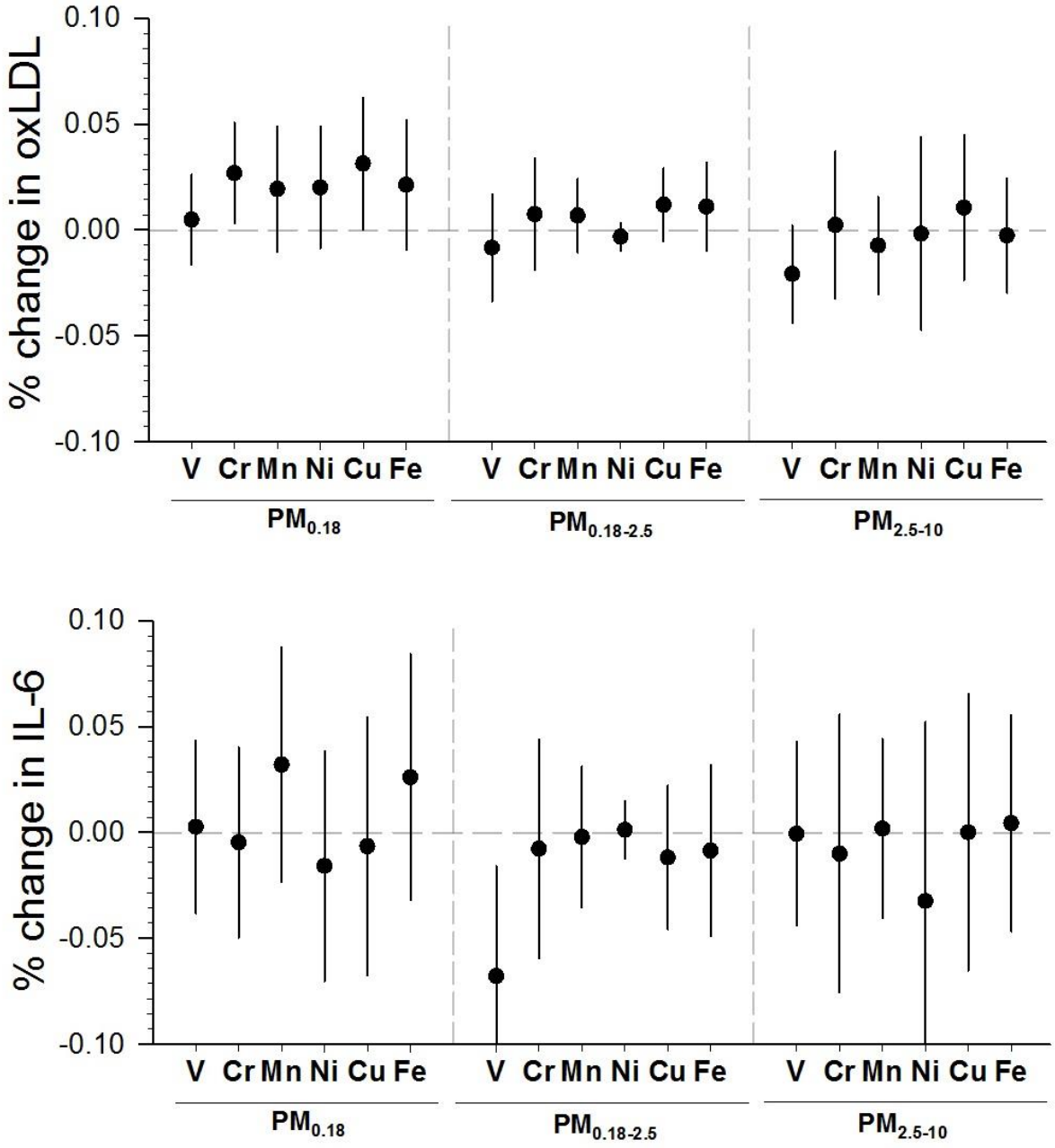


**Figure 4.6. Association of systemic oxidative stress biomarker oxLDL (A) and systemic inflammation biomarker IL-6 (B) with a one interquartile range increase of selected transition metals.**
Transition metals were measured in three size-fractions for exposures averages

### 4.3.4. Effect modification and sensitivity analysis

We found evidence of potential effect modification by cardiovascular risk score on the associations between EBC MDA and air pollutants. Subjects with higher cardiovascular risk score (≥ 0.2) were more likely to have positive associations (Figure 4.7). Consistent with this finding, the components of cardiovascular risk score: age (Figure 4.8) and total cholesterol to HDL ratio (Figure 4.9) also had similar modifying effects on the associations. The positive associations were pronounced in subjects who were older or had greater total cholesterol to HDL ratio. However, this effect modification by cardiovascular risk score was not observed in other outcome biomarkers (data not shown).

We also found regional differences of the associations between IL-6 and 1-day average traffic-related air pollutants and 7-day average personal $NO_X$ (Figure 4.10 A). Stronger positive associations were observed for traffic-related air pollutants in Los Angeles region than in Anaheim, while IL-6 was inversely associated with personal $NO_X$ in Anaheim and positively but non-significantly associated with personal $NO_X$ in Los Angeles. For $O_3$, significant inverse associations were observed in the Los Angeles, while positive but non-significant associations were observed in Anaheim. Regional differences were also observed between EBC MDA and daily traffic-related air pollutants and personal NOx. Positive associations were stronger in Anaheim than in Los Angeles (Figure 4.10 B). However, these differences were not observed for the 5-day PM components. Other independent variables showed no statistically significant effect modification for any outcomes (data not shown).

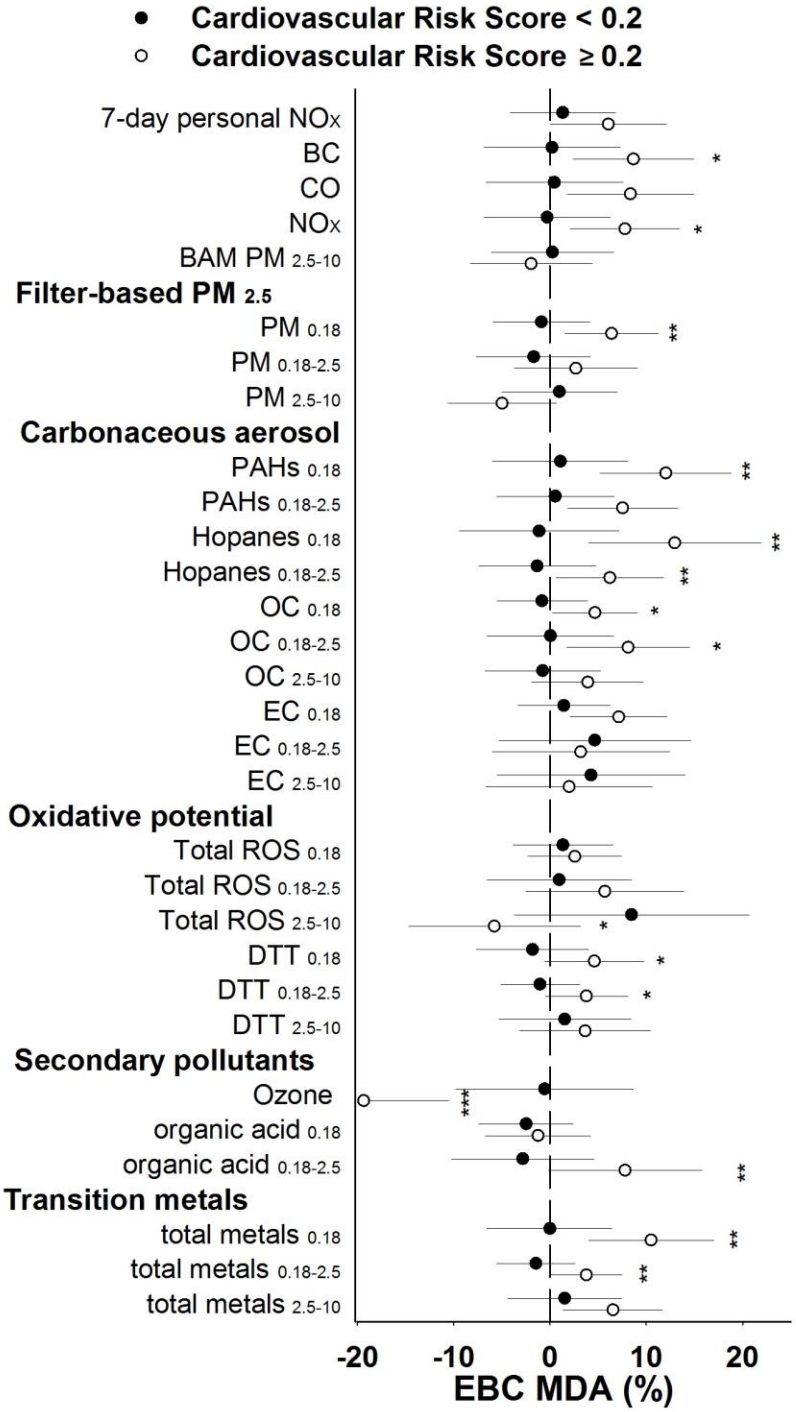


**Figure 4.7. Effect modification of relations between exhaled breath condensate malondialdehyde (EBC MDA) and air pollution by cardiovascular score.**
Percent Change (mean and 95 % confidence intervals) in EBC MDA with one interquartile range increase of selected air pollutant exposures averaged across 5 days (7 days for personal nitrogen oxides; $NO_X$) preceding each subject's clinic visits. *p < 0.1, **p < 0.05, compared with no effect modification by cardiovascular score.

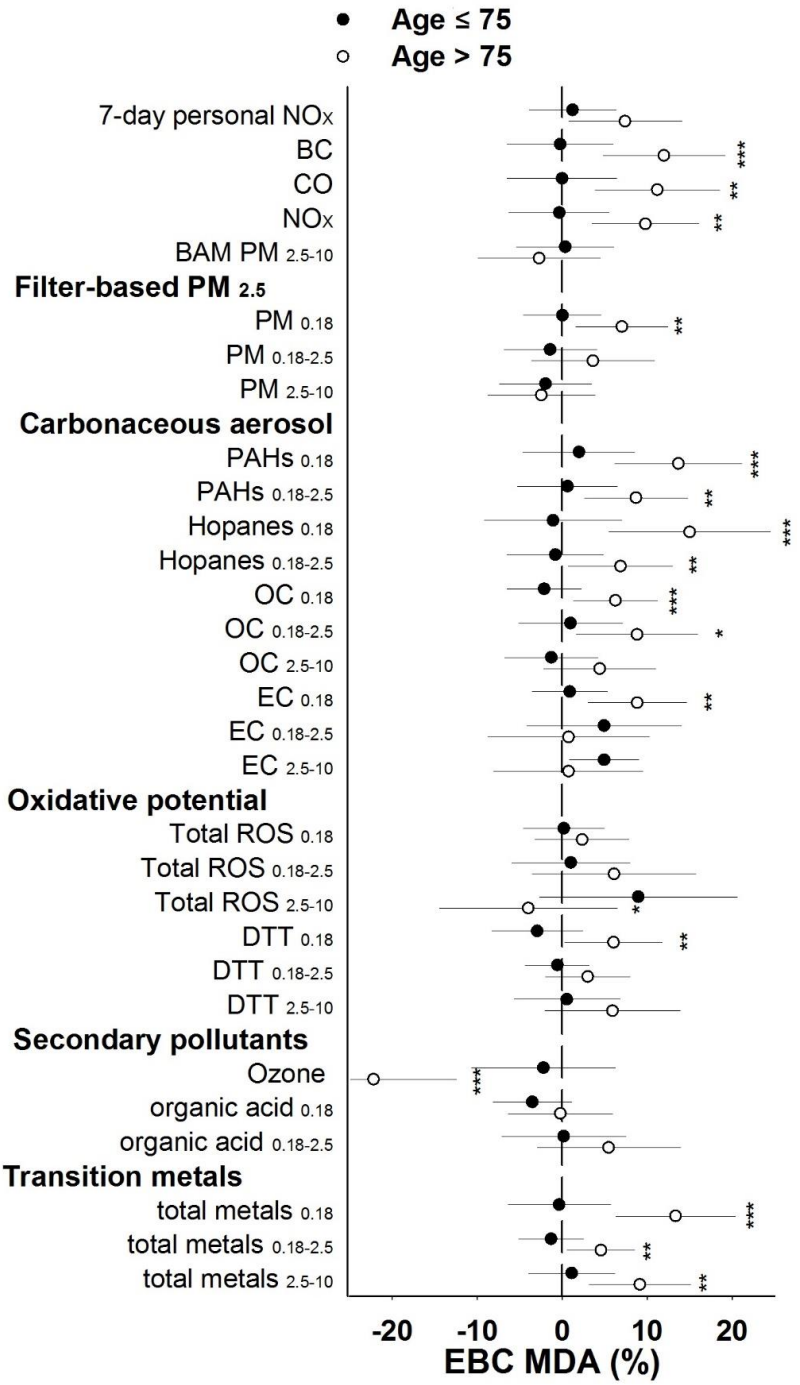


**Figure 4.8. Effect modification of relations between exhaled breath condensate malondialdehyde (EBC MDA) and air pollution by age.**
Association of EBC MDA with one interquartile range increase of selected air pollutant exposures averaged across 5 days (7 days for personal nitrogen oxides; $NO_X$) preceding each subject's clinic visits. *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$, compared with no effect modification by age.

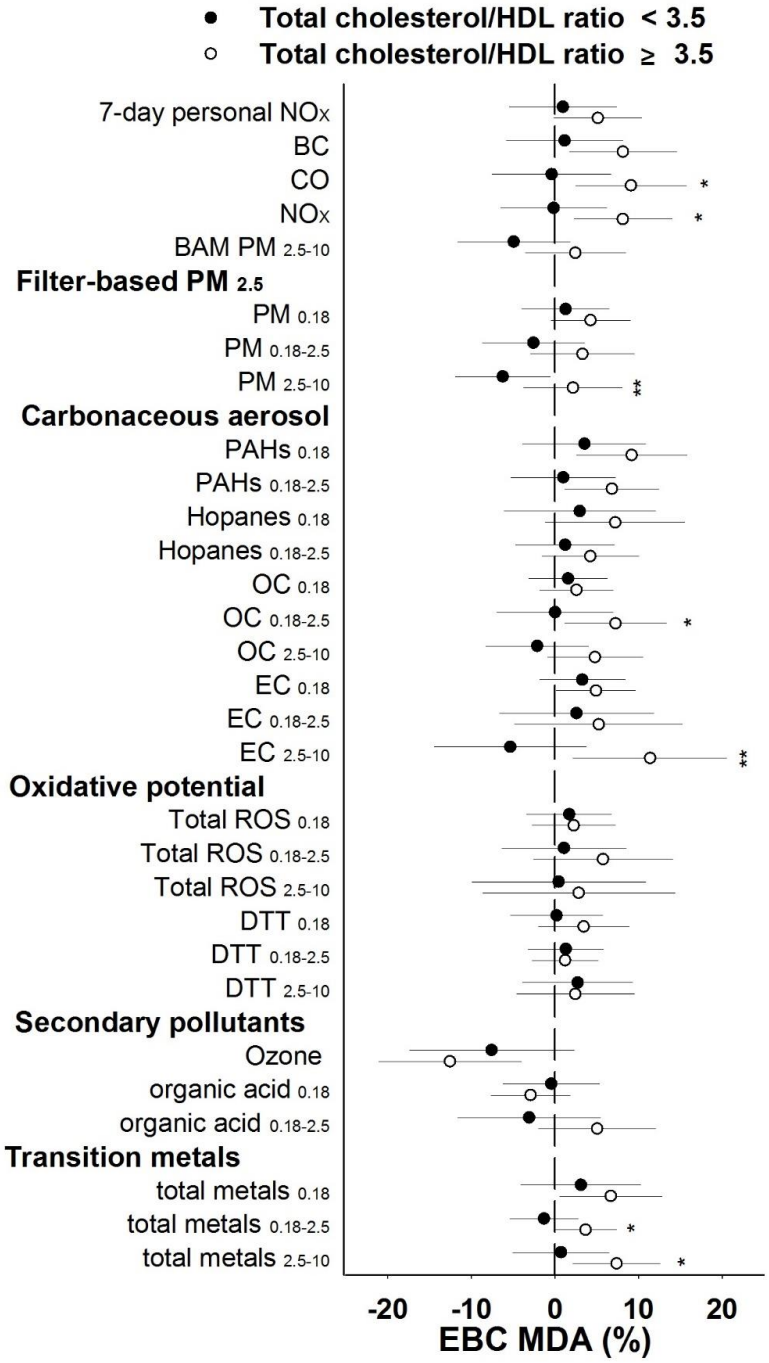


**Figure 4.9. Effect modification of relations between exhaled breath condensate malondialdehyde (EBC MDA) and air pollution by total cholesterol to high-density lipoprotein (HDL) ratio.**
Association of EBC MDA with one interquartile range increase of selected air pollutant exposures averaged across 5 days (7 days for personal nitrogen oxides; $NO_X$) preceding each subject's clinic visits. *$p < 0.1$, **$p < 0.05$, compared with no effect modification by total cholesterol to HDL ratio.

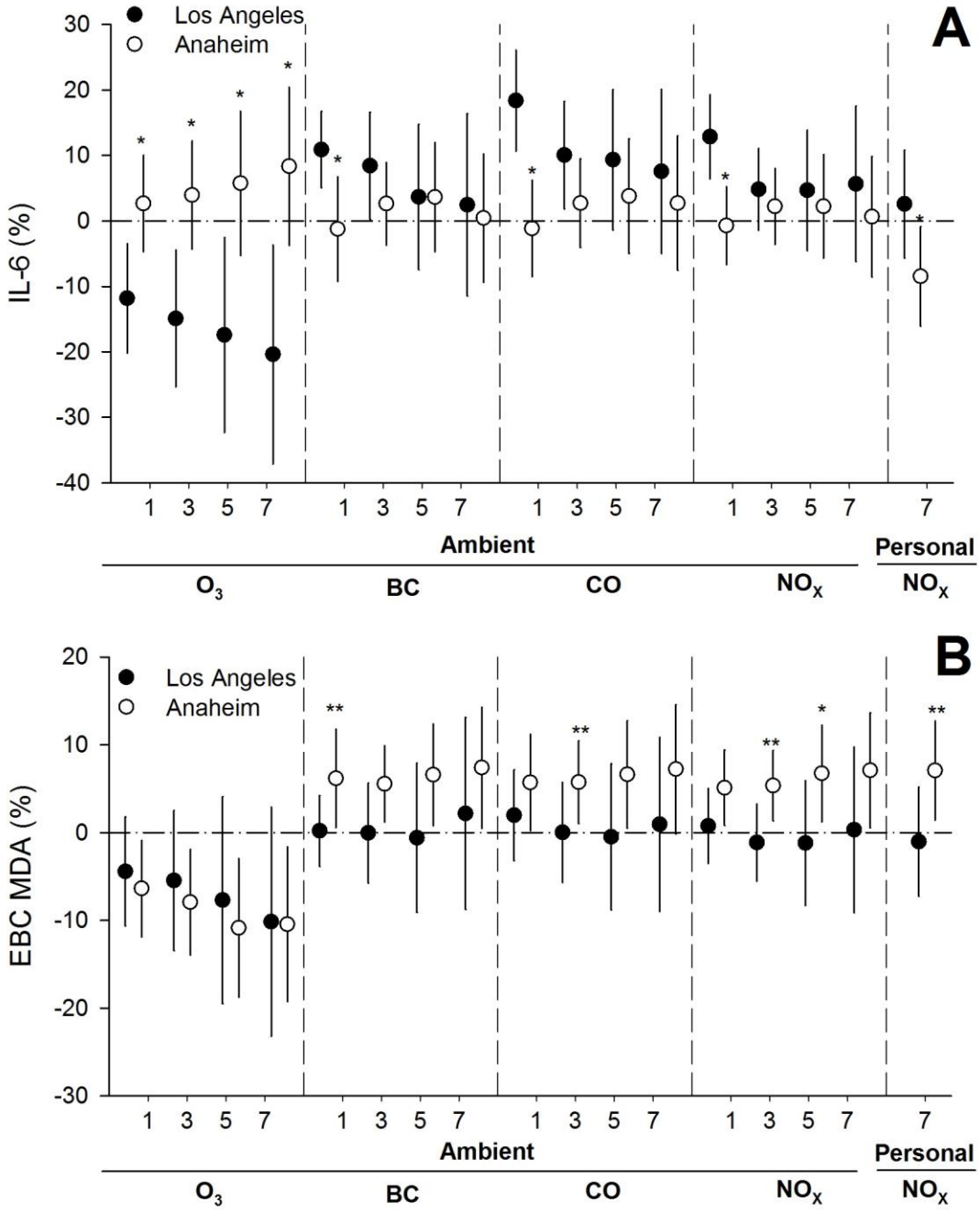


**Figure 4.10. Effect modification of relations between interleukin-6 (IL-6) and air pollution (A), and exhaled breath condensate malondialdehyde (EBC MDA) and air pollution (B) by region.**

Percent Change (mean and 95 % confidence intervals) in EBC MDA with one interquartile range increase of selected air pollutant exposures averaged across 1-day, 3-day, 5-day, and 7-day (7-day for personal nitrogen oxides; $NO_X$) preceding each subject's clinic visits. *p < 0.1, **p < 0.05, compared with no effect modification by region.

Results from the sensitivity analyses did not qualitatively change the main findings when restricting subjects to a smaller study area radius around central air monitors, excluding extreme weather conditions, or excluding imputed ambient exposure data. Specifically, after excluding imputed BC exposure data, the estimated percent change of MDA per interquartile increase of 7-day average BC slightly decreased from 7.4% (95% CI: 1.7%, 13.4%) to 7.2% (95% CI: 1.1%, 13.4%). Results were similar for FeNO.

The estimates of association from two-pollutant models of $O_3$ with a primary air pollutant (BC, NOx and PAHs) became largely non-significant with effect estimates attenuating toward the null for airway biomarkers, except for associations between FeNO and PAHs in $PM_{0.18}$ that remained significant. The results for the systemic biomarkers were similar using two-pollutant models and single-pollutant models (data not shown).

In the product term models testing interactions between ambient 1-day, 3-day, 5-day and 7-day averages of BC and $O_3$ on the airway biomarkers (FeNO and EBC MDA) we found significant positive interactions between the pollutants across all averaging times in relation to FeNO, with the strongest effect at the 5-day BC average (Figure 4.11). Similar interactions on FeNO were also observed between $O_3$ and PAHs in both $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$ (data not shown). However, this interaction effect was not observed for EBC MDA.

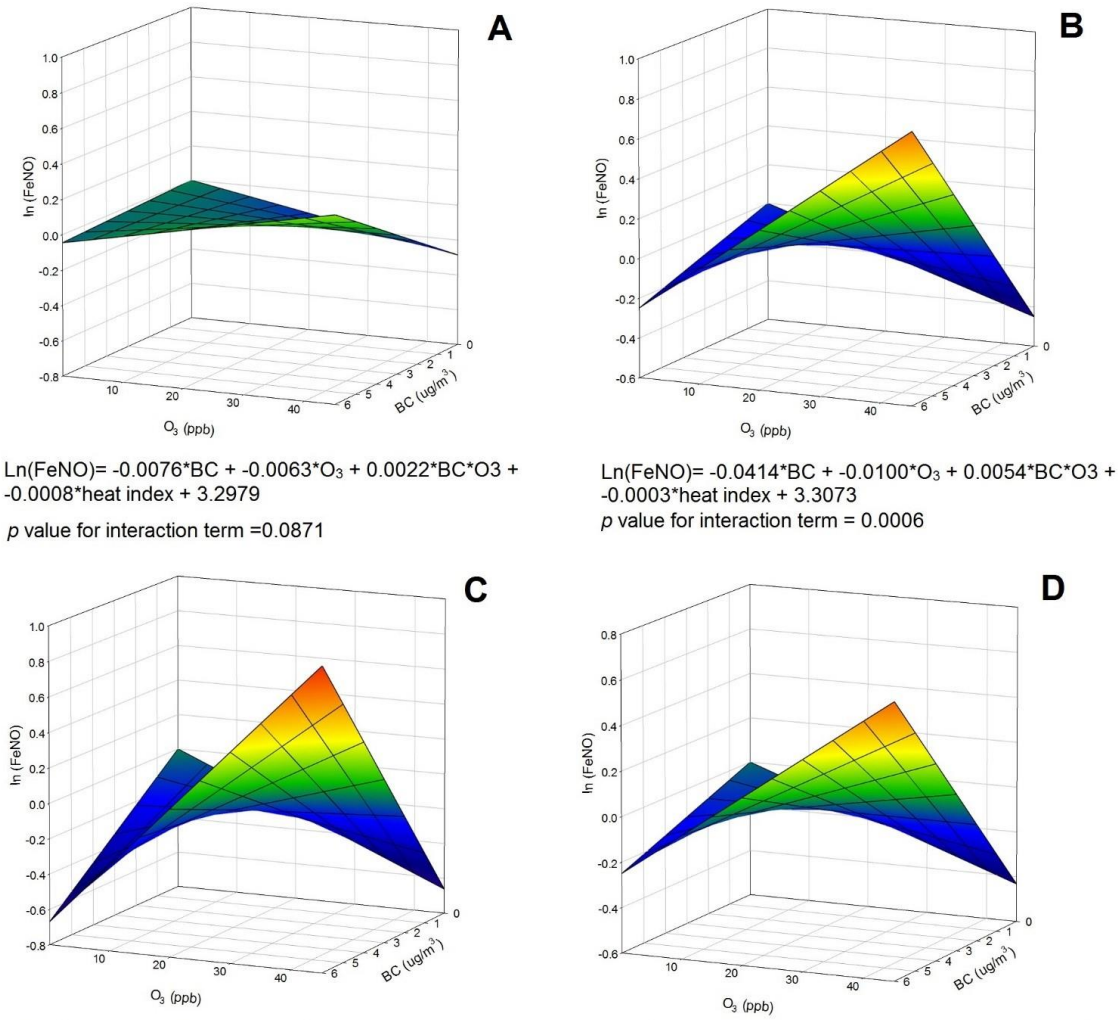


**Figure 4.11. Relation of FeNO (log value) to interaction between black carbon and $O_3$.** Changes in log FeNO per unit increase of BC, $O_3$ and their interaction across 1 day (A), 3 days (B), 5 days (C), and 7 days (D) averaged before each subject's outcome measurement. BC: black carbon; FeNO: exhaled nitric oxide; $O_3$: ozone.

## 4.4. Discussion

### 4.4.1. Summary

We investigated the effects of short-term exposure to personal and ambient air pollutants, including their chemically-characterized components, on biomarkers of oxidative stress and inflammation in the airways and circulation for a susceptible elderly cohort living in the Los Angeles metropolitan area. We found evidence that pollutants with high oxidative potential, including markers of traffic-related pollutants, ultrafine particles ($PM_{0.18}$), and transition metals, were associated with elevated airway oxidative stress (EBC MDA) and inflammation (FeNO). This is further evidenced by the positive, albeit non-significant, association between airway biomarkers and measurements of *in vitro* oxidative potential (ROS and DTT). In general, the associations for both of the airway biomarkers were stronger for traffic-related air pollutants in the ultrafine range than the larger particle size range. Moreover, we found acute positive associations between ambient traffic-related pollutants (1-day and 3-day pollutant averages of BC, $NO_x$ and CO) and the biomarker of systemic inflammation (IL-6).

### 4.4.2. Airway biomarkers and air pollutant associations

MDA is a lipid peroxidation end-product and has been recognized as a biomarker of oxidative stress (Nordenhall et al. 2000). Higher levels of MDA in EBC have been observed in subjects with diseases characterized by oxidative stress, such as COPD

(Lee et al. 2014) and diabetes (Dierckx et al. 2003). Few air pollution epidemiological studies have used EBC MDA as the biomarker of airway oxidative stress (Romieu et al. 2008; Gong et al. 2013; Sarnat et al. 2014). A study during the Beijing Olympic Games showed that a significant decrease in EBC MDA concentration in healthy young adults was associated with a substantial improvement in air quality (Gong et al. 2013). In a more recent study, EBC MDA was estimated to be higher three hours after the highway commutes of subjects during morning rush hours in the metropolitan Atlanta area in both asthmatic and non-asthmatic adults (Sarnat et al. 2014). However, to our knowledge, the present study is the first epidemiologic study that has shown positive associations of EBC MDA with components of aerosols with potentially high oxidative potential (ultrafine particle mass, PAHs, and transition metals) and with direct measures of PM oxidative potential (ROS and DTT).

Nitric oxide is produced in the respiratory tract as a part of the inflammatory process. It has previously been shown that nitric oxide is increased in exhaled air with exposure to air pollution among patients with asthma (Delfino et al. 2006), COPD (Brindicci et al. 2005), other respiratory conditions (Mar et al. 2005), and coronary artery disease (Delfino et al. 2010a). Previous epidemiological studies have shown that FeNO is associated with increases in traffic-related air pollutants in children (Delfino et al. 2006) and in young adults (Huang et al. 2012). However, this association has rarely been investigated in the general elderly population. We previously reported null associations of FeNO with traffic-related air pollutants (including markers of primary organic aerosols) in subjects with coronary artery disease, but found positive associations of FeNO with markers of secondary organic aerosols (Delfino et al. 2010a).

In the present study, the estimated associations between FeNO and primary organic aerosols were positive, while we also found associations of FeNO with the one marker of secondary organic aerosols (organic acids) in $PM_{0.18,}$ but not in $PM_{0.18\text{-}2.5}$. It is important to note that our previous study (Delfino et al. 2010a) had better exposure characterization including modeled hourly secondary organic carbon as a marker of secondary organic aerosol (Polidori et al. 2007). Another study of a group of elderly subjects in Steubenville, Ohio showed that FeNO was positively associated with exposure to 1-day $PM_{2.5}$ mass concentrations (not chemically characterized). However, in our current study, we did not find a positive association between FeNO and $PM_{2.5}$. Instead, we found FeNO was positively associated with exposure to 5-day ultrafine $PM_{0.18}$ mass, but not accumulation mode $PM_{0.18\text{-}2.5}$ mass concentrations. This may be due to the different chemical components in ultrafine versus accumulation mode size fractions of $PM_{2.5}$ in Los Angeles as compared with Steubenville. Consistent with our current results, controlled human exposure studies have shown that acute exposure to diesel exhaust particles can transiently increase airway inflammation (Behndig et al. 2006; Bosson et al. 2008).

### 4.4.3. Systemic biomarkers and air pollutant associations

Our findings of increased systemic inflammation with exposure to traffic-related air pollutants are generally consistent with previous studies (Rückerl et al. 2006; Rückerl et al. 2007; Delfino et al. 2009; Fang et al. 2012). However, the averaging time showing the strongest association has varied from hours to 9-days in these studies. It is unclear

why different studies have shown different pollutant averaging times in association with biomarkers of systemic inflammation, but some combination of varying exposure error and population susceptibilities may be at play here.

Our previous study in elderly subjects with coronary artery disease reported significant associations of both airway and systemic inflammation with contrasting PM air pollutant characteristics (secondary organic aerosols and primary organic aerosols, respectively) (Delfino et al. 2010a). Notably, the air pollutant species that were significantly associated with airway versus systemic outcomes differed with the present study as discussed above. In contrast, our current study also revealed associations of biomarkers in the airways with markers of combustion-related air pollutants that were less clear for biomarkers of inflammation in the circulation. This may due to the fact that study subjects in the current cohort are relatively healthier than the subjects in the previous study. Healthy subjects may have stronger antioxidant defense mechanisms in their circulation that combats the effects of air pollution and chemical components that may spillover from the lungs. Furthermore, with considerably greater resources than the present study, the previous study measured air pollutants at the residential location (retirement communities) of each subject group that likely greatly reduced exposure error. Another possible explanation for why our current results for the systemic biomarkers are not as definitive as our previous studies could be due to the fact that the air pollutant levels, especially ultrafine PM, EC, OC, and tracers of mobile sources measured by our research group, have decreased over the last decade in the Los Angeles area (Shirmohammadi et al. 2016). This resulted from more stringent

regulations on mobile source emissions by the US EPA, California Air Resources Board and SCAQMD (Hasheminassab et al. 2014a).

### 4.4.4. Plausible biological mechanisms

In response to these pro-oxidant components in PM (traffic-related pollutants, ultrafine PM, and transition metals), alveolar macrophages produce nitric oxide, which can combine with superoxide anion to produce peroxynitrite, a potent oxidizing compound, to generate ROS (Laumbach et al. 2010). Subsequently, excessive ROS can induce oxidative stress and inflammation in the airways. This is partly supported by our finding of positive, albeit nonsignificant, associations of both airway outcomes with *in vitro* macrophage ROS and DTT from ultrafine and accumulation mode PM extracts. This finding is consistent with our report of significant positive associations of FeNO with macrophage ROS and DTT from $PM_{2.5}$ extracts in children and adolescents with asthma living in the Los Angeles air basin (Delfino et al. 2013)

The associations of PM oxidative potential (ROS and/or DTT) with airway and systemic biomarkers were less strong than we expected. Nevertheless, the observed non-significant positive associations may be mediated by ROS-induced activation of *Nrf2* which is the major regulator of over 250 antioxidant and detoxifying enzymes and related proteins (Li et al. 2009). This potential mechanism is supported by our previous study that exposure markers of combustion-related air pollutants were positively associated with expression of the Nrf2 gene (NFE2L2) and *Nrf2*-mediated genes

(*HMOX1, NQO1*, and *SOD2*) in a cohort of elderly subjects with coronary artery disease (Wittkopp et al. 2015).

Previous epidemiological studies have shown that impaired vascular function is associated with short-term exposure to air pollutants, especially with pollutants linked to traffic sources (Ljungman et al. 2014; Provost et al. 2016). However, in our current study, we found relatively weak evidence of an elevation in systemic (plasma) oxidative stress and inflammation biomarkers with exposure to traffic-related pollutants. It is possible that these pollutants are translocated from the airways into the systemic circulation, where they may directly impact the endothelium and promote cardiovascular events without prior induction of systemic oxidative stress or inflammatory responses (Yamawaki et al. 2006; Wallenborn et al. 2007).

### 4.4.5. Effect modification

In assessing effect modification, we found suggestive evidence that subjects with a high cardiovascular risk score, as well as older subjects, and subjects with higher total cholesterol/HDL ratio experienced stronger and more significant positive associations of EBC MDA with air pollutants compared with healthier subjects. With the current study design, it is not possible to disentangle the independent effect of cardiovascular risk score, age or total cholesterol/HDL ratio. The stronger observed associations may simply be driven by single components of the cardiovascular risk score, such as age.

The observed regional differences for EBC MDA and IL-6 may be due to the fact that on average the baseline levels were different for the subjects in these two regions. For IL-6, the concentration in Los Angeles (1.95 pg/ml) is lower than in Anaheim (2.75 pg/ml) (Table 4.4). While for EBC MDA, the average concentration in Los Angeles is 10.47 nmol, which was higher than in Anaheim (6.39 nmol). From the known medical conditions, we cannot explain different levels of these two biomarkers in Los Angeles and Anaheim, but the higher levels may be due to unmeasured factors that confound air pollution associations, which may explain the stronger associations of IL-6 and EBC MDA in regions where their plasma concentrations were lower. It is important to note that in these exploratory analyses, we tested many interactions, hence multiple testing bias is a concern and the significant effect modification may be due to chance. However, we did not adjust for multiple comparisons as it is not explicitly required in exploratory analyses (Bender et al. 2001).

### 4.4.6. Pollutant interactions

The weakened associations for airway biomarkers with primary pollutants in the two-pollutant models with $O_3$ are likely due to the high inverse correlations between primary pollutants and $O_3$ (BC, $NO_x$ and PAHs correlations with $O_3$, $R^2 \geq 0.6$). However, interactions may be of more interest because the highly oxidizing capability of $O_3$ give it the potential to react with primary pollutant components (such as those indicated by BC, $NO_x$ and PAHs) leading to synergistic or additive adverse effects on human health. It was evidenced in an *in vitro* experimental study that PM and $O_3$ act synergistically in

generating a sustained production of oxidative stress (Valavanidis et al. 2009). Consistent with these concepts we found positive interactions between primary pollutants and $O_3$ on FeNO. This may indicate that the underlying effect of air pollutant mixtures may not be fully explained by only one air pollutant. Based on this observation, we built a model using ROS or DTT as the dependent variable and tested for interaction effects between BC/PAHs and $O_3$. For BC and $O_3$, the interaction effect was significant in Los Angeles but not in Anaheim. While for PAHs and $O_3$, the interaction effect was significant in both areas, but stronger in Los Angeles than in Anaheim. This indicates that there may be other unmeasured secondary pollutant species that are formed during periods with both high primary air pollutants, and high $O_3$ that lead to higher oxidative potential of the particle mixtures.

### 4.4.7. Personal $NO_X$

Our results for personal $NO_X$ were nonsignificant. The association between personal $NO_X$ and EBC MDA was borderline significant but weaker than ambient 7-day $NO_X$ (Figure 4.1 and Table 4.1). Meanwhile, we observed the strongest association of FeNO with ambient $NO_X$ at 7-day average, but the relation was null with 7-day personal NOx (Figure 4.3 and Table 4.5). The lack of association for personal $NO_X$ may be due to subject non-compliance, face velocity effects on the passive $NO_X$ badge from variations in airflow, and unadjusted indoor sources of $NO_X$.

### 4.4.8. Strengths and limitations

The strengths of our study include the comprehensive characterization of PM components, including directly measuring PM oxidative potential using *in vitro* macrophage ROS and DTT, the repeated measurements of biomarkers over time, which minimize confounding from time-invariant between-subject and within-subject factors, and the evaluation of biomarkers in both the airways and the circulation.

One of the limitations is that different biomarker outcomes were collected approximately at the same time. Therefore, we cannot investigate the relationships with respect to the time-sequence of oxidative stress and subsequent inflammatory responses to air pollution. Our size-fractionated PM composition data were collected across five-day integrated periods rather than daily limiting comparisons with ambient daily exposures that were measured continuously (BC and air pollutant gases). For example, we observed significant associations for IL-6 with continuously measured air pollutants at 1-day averages but this could not be assessed for PM components. Second, time-varying subject characteristics, such as daily physical activity and diet, can change associations between biomarkers and air pollution. However, we did not have this information in any detail resulting in potential unmeasured confounding. Third, measurement errors in exposure may have attenuated true associations. Most of our exposure data were obtained from central monitoring stations, which may not account for variations in personal exposure levels or variations in indoor air quality. Given that our elderly subjects spent most of their time indoors, our ambient outdoor exposure data

may have introduced exposure error. Finally, external validity of the findings is limited to elderly subjects living in the selected regions of Los Angeles and Orange County.

Table 4.1. Associations between EBC MDA and air pollutants as % change per increase in the interquartile range of air pollutants, comparing exclusion versus no exclusion of an influential subject.

| Pollutant | % Change in EBC MDA | | | |
|---|---|---|---|---|
| | No exclusion | | Excluding 1 influential subject | |
| **Personal exposure** | | | | |
| 7-day NOx | 0.027 (-0.016, 0.069) | | 0.035(-0.007, 0.077) | |
| **BC** | | | | |
| 1-day | 0.018 (-0.014, 0.051) | | 0.027 (-0.005, 0.058) | |
| 3-day | 0.026 (-0.009, 0.060) | | 0.040 (0.005, 0.074) | ** |
| 5-day | 0.035 (-0.013, 0.083) | | 0.055 (0.007, 0.103) | ** |
| 7-day | 0.050 (-0.011, 0.110) | | 0.074 (0.014, 0.134) | ** |
| **CO** | | | | |
| 1-day | 0.031 (-0.006, 0.069) | | 0.041 (0.004, 0.078) | ** |
| 3-day | 0.026 (-0.010, 0.063) | | 0.039 (0.003, 0.075) | ** |
| 5-day | 0.035 (-0.015, 0.085) | | 0.052 (0.002, 0.102) | ** |
| 7-day | 0.046 (-0.015, 0.107) | | 0.065 (0.005, 0.126) | ** |
| **NOx** | | | | |
| 1-day | 0.023 (-0.008, 0.053) | | 0.031 (0.001, 0.060) | ** |
| 3-day | 0.016 (-0.013, 0.046) | | 0.027 (-0.003, 0.056) | * |
| 5-day | 0.031 (-0.014, 0.076) | | 0.047 (0.002, 0.092) | ** |
| 7-day | 0.043 (-0.014, 0.100) | | 0.063 (0.006, 0.119) | ** |
| **BAM $PM_{2.5}$** | | | | |
| 1-day | -0.015 (-0.052, 0.023) | | -0.013 (-0.050, 0.024) | |
| 3-day | -0.010 (-0.051, 0.031) | | -0.003 (-0.044, 0.038) | |
| 5-day | -0.014 (-0.059, 0.031) | | -0.006 (-0.051, 0.039) | |
| 7-day | -0.005 (-0.061, 0.051) | | 0.005 (-0.050, 0.060) | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | |
| $PM_{0.18}$ | 0.022 (-0.012, 0.057) | | 0.031 (-0.004, 0.065) | * |
| $PM_{0.18-2.5}$ | -0.000 (-0.044, 0.044) | | 0.003 (-0.041, 0.047) | * |
| $PM_{2.5-10}$ | -0.025 (-0.070, 0.020) | | -0.023 (-0.068, 0.022) | |
| **Carbonaceous aerosol components, 5-day** | | | | |
| $PAHs_{0.18}$ | 0.045 (-0.008, 0.098) | * | 0.063 (0.011, 0.116) | ** |
| $PAHs_{0.18-2.5}$ | 0.027 (-0.018, 0.072) | | 0.039 (-0.006, 0.084) | |
| $Hopanes_{0.18}$ | 0.038 (-0.030, 0.105) | | 0.051 (-0.016, 0.117) | |
| $Hopanes_{0.18-2.5}$ | 0.014 (-0.031, 0.058) | | 0.026 (-0.018, 0.070) | |
| $OC_{0.18}$ | 0.011 (-0.022, 0.043) | | 0.021 (-0.011, 0.053) | |
| $OC_{0.18-2.5}$ | 0.024 (-0.025, 0.073) | | 0.041 (-0.008, 0.089) | |
| $OC_{2.5-10}$ | 0.004 (-0.037, 0.046) | | 0.019 (-0.023, 0.060) | |
| $EC_{0.18}$ | 0.030 (-0.005, 0.066) | * | 0.042 (0.007, 0.077) | ** |
| $EC_{0.18-2.5}$ | 0.028 (-0.041, 0.096) | | 0.034 (-0.034, 0.101) | |
| $EC_{2.5-10}$ | 0.020 (-0.046, 0.087) | | 0.029 (-0.037, 0.094) | |
| **Oxidative potential, 5-day** | | | | |
| Total $ROS_{0.18}$ | 0.014 (-0.022, 0.050) | | 0.024 (-0.012, 0.060) | |
| Total $ROS_{0.18-2.5}$ | 0.024 (-0.032, 0.080) | | 0.030 (-0.026, 0.085) | |
| Total $ROS_{2.5-10}$ | 0.004 (-0.074, 0.081) | | 0.003 (-0.073, 0.079) | |
| $DTT_{0.18}$ | 0.010 (-0.029, 0.050) | | 0.020 (-0.019, 0.059) | |
| $DTT_{0.18-2.5}$ | 0.007 (-0.023, 0.037) | | 0.014 (-0.016, 0.044) | |
| $DTT_{2.5-10}$ | 0.021 (-0.030, 0.071) | | 0.026 (-0.024, 0.075) | |
| **Electrophilic potential, 5-day** | | | | |
| GPx-1 $_{0.18}$ | 0.005 (-0.007, 0.017) | | 0.004 (-0.012, 0.019) | |
| **Secondary pollutants** | | | | |
| 1-day ozone | -0.048 (-0.089, -0.008) | ** | -0.055 (-0.095, -0.015) | # |
| 3-day ozone | -0.060 (-0.109, -0.011) | ** | -0.072 (-0.120, -0.024) | # |
| 5-day ozone | -0.085 (-0.154, -0.015) | ** | -0.105 (-0.173, -0.036) | # |
| 7-day ozone | -0.086 (-0.166, -0.007) | ** | -0.107 (-0.186, -0.029) | # |
| organic $acid_{0.18}$ | -0.023 (-0.061, 0.016) | | -0.022 (-0.061, 0.016) | |
| organic $acid_{0.18-2.5}$ | 0.016 (-0.048, 0.081) | | 0.018 (-0.046, 0.082) | |
| **Transition metals, 5-day** | | | | |
| Total $metals_{0.18}$ | 0.033 (-0.012, 0.079) | | 0.047 (0.002, 0.092) | ** |
| Total $metals_{0.18-2.5}$ | 0.008 (-0.020, 0.037) | | 0.015 (-0.013, 0.043) | * |
| Total $metals_{2.5-10}$ | 0.028 (-0.011, 0.067) | | 0.044 ( 0.005, 0.083) | |

*$^{*}p < 0.1$, $^{**}p < 0.05$, $^{\#}p < 0.01$*

Table 4.2. Associations between IL-6 and air pollutants as % change per increase in the interquartile range of air pollutants, comparing exclusion versus no exclusion of three influential observations.

| Pollutant | % Change in IL-6 | | | |
|---|---|---|---|---|
| | No exclusion | | Excluding 3 influential observations | |
| **Personal exposure** | | | | |
| 7-day NOx | -0.021 (-0.081, 0.038) | | -0.008 (-0.065, 0.048) | |
| **BC** | | | | |
| 1-day | 0.049 (-0.000, 0.098) | * | 0.050 (0.004, 0.097) | ** |
| 3-day | 0.038 (-0.015, 0.091) | | 0.037 (-0.012, 0.087) | |
| 5-day | 0.011 (-0.059, 0.081) | | 0.008 (-0.057, 0.074) | |
| 7-day | -0.029 (-0.116, 0.058) | | -0.028 (-0.109, 0.054) | |
| **CO** | | | | |
| 1-day | 0.068 (0.010, 0.125) | ** | 0.064 (0.010, 0.118) | ** |
| 3-day | 0.040 (0.015, 0.095) | | 0.040 (-0.011, 0.092) | |
| 5-day | 0.020 (-0.051, 0.092) | | 0.025 (-0.043, 0.092) | |
| 7-day | -0.014 (-0.100, 0.073) | | 0.000 (-0.081, 0.081) | |
| **NOx** | | | | |
| 1-day | 0.051 (0.005, 0.097) | ** | 0.046 (0.002, 0.090) | ** |
| 3-day | 0.025 (-0.019, 0.070) | | 0.024 (-0.018, 0.066) | |
| 5-day | 0.006 (-0.059, 0.072) | | 0.004 (-0.057, 0.065) | |
| 7-day | -0.019 (-0.100, 0.062) | | -0.014 (-0.090, 0.062) | |
| **BAM $PM_{2.5}$** | | | | |
| 1-day | -0.053 (-0.107, 0.001) | * | -0.034 (-0.085, 0.018) | |
| 3-day | -0.053 (-0.111, 0.006) | * | -0.022 (-0.078, 0.034) | |
| 5-day | -0.019 (-0.082, 0.044) | | 0.011 (-0.050, 0.071) | |
| 7-day | -0.030 (-0.108, 0.049) | | 0.012 (-0.063, 0.086) | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | |
| $PM_{0.18}$ | 0.018 (-0.034, 0.070) | | 0.012 (-0.036, 0.060) | |
| $PM_{0.18-2.5}$ | -0.009 (-0.067, 0.050) | | -0.002 (-0.058, 0.053) | |
| $PM_{2.5-10}$ | -0.004 (-0.067, 0.058) | | 0.004 (-0.055, 0.062) | |
| **Carbonaceous aerosol components, 5-day** | | | | |
| $PAHs_{0.18}$ | 0.002 (-0.076, 0.080) | | -0.011 (-0.084, 0.061) | |
| $PAHs_{0.18-2.5}$ | 0.017 (-0.047, 0.082) | | 0.010 (-0.050, 0.070) | |
| $Hopanes_{0.18}$ | -0.024 (-0.119, 0.071) | | -0.035 (-0.124, 0.054) | |
| $Hopanes_{0.18-2.5}$ | 0.007 (-0.054, 0.069) | | -0.004 (-0.061, 0.054) | |
| $OC_{0.18}$ | 0.027 (-0.020, 0.074) | | 0.011 (-0.034, 0.055) | |
| $OC_{0.18-2.5}$ | -0.010 (-0.083, 0.062) | | -0.007 (-0.074, 0.061) | |
| $OC_{2.5-10}$ | -0.008 (-0.067, 0.050) | | -0.008 (-0.062, 0.046) | |
| $EC_{0.18}$ | -0.014 (-0.066, 0.039) | | -0.018 (-0.067, 0.031) | |
| $EC_{0.18-2.5}$ | -0.109 (-0.199, -0.019) | ** | -0.114 (-0.199, -0.029) | # |
| $EC_{2.5-10}$ | 0.003 (-0.089, 0.095) | | 0.008 (-0.078, 0.094) | |
| **Oxidative potential, 5-day** | | | | |
| Total $ROS_{0.18}$ | -0.000 (-0.052, 0.052) | | -0.007 (-0.055, 0.042) | |
| Total $ROS_{0.18-2.5}$ | -0.124 (-0.203, -0.046) | # | -0.122 (-0.196, -0.048) | # |
| Total $ROS_{2.5-10}$ | -0.180 (-0.282, -0.078) | # | -0.191 (-0.286, -0.096) | # |
| $DTT_{0.18}$ | -0.016 (-0.074, 0.043) | | -0.031 (-0.084, 0.022) | |
| $DTT_{0.18-2.5}$ | 0.026 (-0.017, 0.069) | | 0.018 (-0.022, 0.058) | |
| $DTT_{2.5-10}$ | 0.018 (-0.050, 0.086) | | 0.000 (-0.063, 0.064) | |
| **Electrophilic potential, 5-day** | | | | |
| GPx-1 $_{0.18}$ | 0.001 (-0.017, 0.019) | | 0.003 (-0.019, 0.025) | |
| **Secondary pollutants** | | | | |
| 1-day ozone | -0.055 (-0.113, 0.004) | * | -0.034 (-0.089, 0.021) | |
| 3-day ozone | -0.035 (-0.106, 0.037) | | -0.030 (-0.097, 0.037) | |
| 5-day ozone | -0.003 (-0.104, 0.098) | | -0.007 (-0.101, 0.088) | |
| 7-day ozone | 0.013 (-0.102, 0.128) | | -0.001 (-0.109, 0.106) | |
| organic $acid_{0.18}$ | -0.005 (-0.060, 0.051) | | -0.005 (-0.057, 0.047) | |
| organic $acid_{0.18-}$ | 0.002 (-0.091, 0.094) | | 0.005 (-0.082, 0.091) | |
| **Transition metals, 5-day** | | | | |
| Total $metals_{0.18}$ | 0.029 (-0.037, 0.096) | | 0.003 (-0.058, 0.065) | |
| Total $metals_{0.18-}$ | -0.009 (-0.049, 0.031) | | -0.014 (-0.052, 0.023) | |
| Total $metals_{2.5-10}$ | 0.005 (-0.052, 0.062) | | -0.011 (-0.064, 0.042) | |

*$^{*}p < 0.1$, $^{**}p < 0.05$, $^{\#}p < 0.01$*

Table 4.3. Characteristics of 97 subjects (Max n= 946)

| | **Mean ± SD or N (%)** |
|---|---|
| Age (years) ± SD | 74.81 ± 7.50 |
| BMI (kg/m$^2$) ± SD | 28.09 ± 5.59 |
| Overweight (25-29.9) (%) | 34 (35.05) |
| Obesity (≥30) (%) | 31 (31.96) |
| Male (%) | 25 (25.77) |
| Former smoker (%) | 42 (43.3) |
| Cardiovascular Disease (%) | 16 (16.49) |
| Hypertension (%) | 66 (68.04) |
| Hypercholesterolemia (by history) (%) | 52 (53.61) |
| Lipid Profile | |
| Total cholesterol ± SD | 188.66 ± 44.73 |
| > 200 mg/dL (%) | 47 (48.45) |
| LDL-C ± SD | 116.36 ± 36.68 |
| > 130 mg/dL (%) | 30 (30.93) |
| HDL-C ± SD | 55.23 ± 18.46 |
| Female <50 mg/dL, male <40 mg/dL (%) | 32 (32.99) |
| Adult-onset Diabetes Mellitus (%) | 22 (22.68) |
| COPD (%) | 8 (8.25) |
| Asthma (%) | 12 (12.37) |
| Medications (%) | |
| Anti-hypertensive medication (%) | 63 (64.95) |
| HMG-CoA reductase inhibitors (statins) (%) | 45 (46.39) |
| Biomarkers | |
| IL-6 (pg/mL; n=716) ± SD | 2.35 ± 1.69 |
| OxLDL (U/L; n=841) ± SD | 59.19 ± 20.22 |
| FeNO (ppb; n=901) ± SD | 26.59 ± 13.36 |
| EBC MDA (nmol; n=643) ± SD | 8.37 ± 5.07 |

Abbreviations: BMI, body mass index; COPD: chronic obstructive pulmonary disease; EBC: exhaled breath condensate; FeNO: exhaled nitric oxide; MDA: malondialdehyde.

Table 4.4. Characteristics of 97 subjects by region.

| Characteristic | Mean ± SD or N (%) | |
|---|---|---|
| | Los Angeles (N=48) | Anaheim (N=49) |
| Age (years) ±SD | 75.63 ± 8.12 | 74.02 ± 6.84 |
| BMI (kg/m$^2$) ±SD | 28.22 ± 5.61 | 27.96 ± 5.63 |
| Overweight (25-29.9) (%) | 13 (27.08) | 21 (42.86) |
| Obesity (≥30) (%) | 15 (31.25) | 16 (32.65) |
| Male (%) | 16 (33.33) | 9 (18.37) |
| Former smoker (%) | 27 (56.25) | 15 (30.61) |
| Cardiovascular Disease (%) | 8 (16.67) | 8 (16.33) |
| Hypertension (%) | 32 (66.67) | 34 (69.39) |
| Hypercholesterolemia (by history) (%) | 26 (54.17) | 26 (53.06) |
| Lipid Profile | | |
| Total cholesterol ± SD | 184.9 ± 44.57 | 192.35 ± 45.03 |
| > 200 mg/dL (%) | 18 (37.5) | 29 (59.18) |
| LDL-C ± SD | 110.5 ± 32.96 | 122.1 ± 39.47 |
| > 130 mg/dL (%) | 11 (22.92) | 19 (38.78) |
| HDL-C ± SD | 58.4 ± 20.71 | 52.12 ± 15.56 |
| Female <50 mg/dL, male <40 mg/dL (%) | 13 (27.08) | 19 (38.78) |
| VLDL ± SD | 11.69 ± 11.89 | 14.94 ± 11.09 |
| ≥ 30 mg/dL (%) | 4 (8.33) | 4 (8.16) |
| Triglycerides ± SD | 111.88 ± 67.61 | 136.86 ± 69.98 |
| > 150 mg/dL (%) | 8 (16.67) | 19 (38.78) |
| Adult-onset Diabetes Mellitus (%) | 12 (25) | 10 (20.41) |
| COPD (%) | 2 (4.17) | 6 (12.24) |
| Asthma (%) | 6 (12.5) | 6 (12.24) |
| Medications: | | |
| Anti-hypertensive medication (%) | 32 (66.67) | 31 (63.27) |
| HMG-CoA reductase inhibitors (statins) | 25 (52.08) | 20 (40.82) |
| Biomarkers | | |
| IL-6 (pg/mL) ± SD | 1.95 ± 1.4 | 2.75 ± 1.87 |
| OxLDL (U/L) ± SD | 63.05 ± 19.42 | 55.41 ± 20.47 |
| eNO (ppb) ± SD | 26.22 ± 10.39 | 26.95 ± 26.96 |
| EBC MDA (nmol) ± SD | 10.47 ± 5.81 | 6.39 ± 3.26 |

Abbreviations: BMI, body mass index; COPD: chronic obstructive pulmonary disease; EBC: exhaled breath condensate; MDA: malondialdehyde.

Table 4.5. Associations between FeNO and air pollutants as percent change per increase in the interquartile range of air pollutants.

| Pollutant | | % Change in FeNO | |
|---|---|---|---|
| **Personal exposure** | 7-day NOx | 0.001 (-0.027, 0.029) | |
| **BC** | | | |
| | 1-day | 0.056 (0.033, 0.079) | # |
| | 3-day | 0.042 (0.017, 0.066) | # |
| | 5-day | 0.045 (0.013, 0.078) | # |
| | 7-day | 0.075 (0.036, 0.115) | # |
| **CO** | | | |
| | 1-day | 0.048 (0.021, 0.074) | # |
| | 3-day | 0.030 (0.005, 0.055) | ** |
| | 5-day | 0.030 (-0.003, 0.062) | * |
| | 7-day | 0.044 (0.005, 0.082) | ** |
| **NOx** | | | |
| | 1-day | 0.047 (0.025, 0.068) | # |
| | 3-day | 0.032 (0.010, 0.053) | # |
| | 5-day | 0.039 (0.009, 0.069) | ** |
| | 7-day | 0.057 (0.020, 0.093) | # |
| **BAM $PM_{2.5}$** | | | |
| | 1-day | -0.011 (-0.036, 0.014) | |
| | 3-day | -0.039 (-0.065, -0.012) | # |
| | 5-day | -0.045 (-0.074, -0.016) | # |
| | 7-day | -0.033 (-0.069, 0.002) | * |
| **Filter-based $PM_{2.5}$, 5-day** | | | |
| | $PM_{0.18}$ | 0.030 (0.007, 0.053) | ** |
| | $PM_{0.18\text{-}2.5}$ | -0.008 (-0.035, 0.019) | |
| | $PM_{2.5\text{-}10}$ | -0.025 (-0.053, 0.004) | * |
| **Carbonaceous aerosol components, 5-day** | | | |
| | $PAHs_{0.18}$ | 0.067 (0.033, 0.102) | # |
| | $PAHs_{0.18\text{-}2.5}$ | 0.047 (0.018, 0.076) | # |
| | $Hopanes_{0.18}$ | 0.094 (0.052, 0.136) | # |
| | $Hopanes_{0.18\text{-}2.5}$ | 0.033 (0.004, 0.061) | # |
| | $OC_{0.18}$ | 0.030 (0.008, 0.051) | # |
| | $OC_{0.18\text{-}2.5}$ | 0.032 (-0.000, 0.064) | * |
| | $OC_{2.5\text{-}10}$ | 0.027 (-0.001, 0.054) | * |
| | $EC_{0.18}$ | 0.026 (0.003, 0.050) | ** |
| | $EC_{0.18\text{-}2.5}$ | 0.045 (0.001, 0.088) | ** |
| | $EC_{2.5\text{-}10}$ | 0.060 (0.018, 0.103) | # |

*$^{*}p < 0.1$, $^{**}p < 0.05$, $^{\#}p < 0.01$*

Table 4.5. (Continued) Associations between FeNO and air pollutants as percent change per increase in the interquartile range of air pollutants.

| Pollutant | | % Change in FeNO | |
|---|---|---|---|
| **Oxidative potential, 5-day** | | | |
| | Total $ROS_{0.18}$ | 0.002 (-0.022, 0.025) | |
| | Total $ROS_{0.18-2.5}$ | 0.016 (-0.020, 0.051) | |
| | Total $ROS_{2.5-10}$ | -0.060 (-0.111, -0.010) | ** |
| | $DTT_{0.18}$ | 0.023 (-0.003, 0.049) | * |
| | $DTT_{0.18-2.5}$ | 0.018 (-0.002, 0.038) | * |
| | $DTT_{2.5-10}$ | 0.018 (-0.014, 0.050) | |
| **Electrophilic potential, 5-day** | | | |
| | GPx-1 $_{0.18}$ | 0.000 (-0.010, 0.011) | |
| **Secondary pollutants** | | | |
| | 1-day ozone | -0.066 (-0.092, -0.040) | # |
| | 3-day ozone | -0.069 (-0.100, -0.037) | # |
| | 5-day ozone | -0.074 (-0.118, -0.030) | # |
| | 7-day ozone | -0.088 (-0.138, -0.038) | # |
| | organic $acid_{0.18}$ | 0.034 (0.009, 0.059) | # |
| | organic $acid_{0.18-2.5}$ | 0.001 (-0.039, 0.041) | |
| **Transition metals, 5-day** | | | |
| | Total $metals_{0.18}$ | 0.070 (0.041, 0.099) | # |
| | Total $metals_{0.18-2.5}$ | 0.030 (0.012, 0.048) | # |
| | Total $metals_{2.5-10}$ | 0.032 (0.005, 0.058) | ** |

*$*p < 0.1$, $**p < 0.05$, $^{\#}p < 0.01$*

BAM: Beta Attenuation Monitor; BC: black carbon; CO: carbon monoxide; DTT: dithiothreitol; EC: elemental carbon; OC: organic carbon; PAHs: polycyclic aromatic hydrocarbons; ROS: reactive oxygen species.

Table 4.6. Associations between oxLDL and air pollutants as percent change per increase in the interquartile range of air pollutants.

| Pollutant | | % Change in oxLDL | |
|---|---|---|---|
| **Personal exposure** | | | |
| | 7-day NOx | 0.004 (-0.026, 0.035) | |
| **BC** | | | |
| | 1-day | 0.018 (-0.009, 0.045) | |
| | 3-day | 0.011 (-0.018, 0.040) | |
| | 5-day | 0.010 (-0.029, 0.048) | |
| | 7-day | -0.000 (-0.048, 0.047) | |
| **CO** | | | |
| | 1-day | 0.015 (-0.016, 0.046) | |
| | 3-day | 0.008 (-0.022, 0.038) | |
| | 5-day | 0.008 (-0.031, 0.046) | |
| | 7-day | 0.001 (-0.045, 0.046) | |
| **NOx** | | | |
| | 1-day | 0.014 (-0.011, 0.039) | |
| | 3-day | 0.008 (-0.017, 0.033) | |
| | 5-day | -0.000 (-0.036, 0.035) | |
| | 7-day | -0.017 (-0.061, 0.026) | |
| **BAM $PM_{2.5}$** | | | |
| | 1-day | -0.026 (-0.054, 0.002) | * |
| | 3-day | -0.021 (-0.052, 0.010) | |
| | 5-day | -0.014 (-0.047,0.020) | |
| | 7-day | -0.016 (-0.057, 0.025) | |
| **Filter-based $PM_{2.5}$, 5-day** | | | |
| | $PM_{0.18}$ | 0.010 (-0.017, 0.037) | |
| | $PM_{0.18-2.5}$ | 0.012 (-0.016, 0.039) | |
| | $PM_{2.5-10}$ | -0.011 (-0.041, 0.019) | |
| **Carbonaceous aerosol components, 5-day** | | | |
| | $PAHs_{0.18}$ | 0.012 (-0.028, 0.052) | |
| | $PAHs_{0.18-2.5}$ | 0.003 (-0.030, 0.035) | |
| | $Hopanes_{0.18}$ | 0.016 (-0.030, 0.063) | |
| | $Hopanes_{0.18-2.5}$ | 0.011 (-0.020, 0.042) | |
| | $OC_{0.18}$ | -0.008 (-0.032, 0.016) | |
| | $OC_{0.18-2.5}$ | -0.014 (-0.051, 0.023) | |
| | $OC_{2.5-10}$ | 0.001 (-0.029, 0.031) | |
| | $EC_{0.18}$ | -0.015 (-0.042, 0.012) | |
| | $EC_{0.18-2.5}$ | -0.007 (-0.052, 0.037) | |
| | $EC_{2.5-10}$ | 0.039 (-0.007, 0.085) | * |

Table 4.6. (Continued) Associations between oxLDL and air pollutants as percent change per increase in the interquartile range of air pollutants.

| Pollutant | | % Change in oxLDL | |
|---|---|---|---|
| **Oxidative potential, 5-day** | | | |
| | Total $ROS_{0.18}$ | -0.012 (-0.038, 0.014) | |
| | Total $ROS_{0.18-2.5}$ | 0.032 (-0.005, 0.069) | * |
| | Total $ROS_{2.5-10}$ | -0.015 (-0.065, 0.036) | |
| | $DTT_{0.18}$ | 0.000 (-0.029, 0.029) | |
| | $DTT_{0.18-2.5}$ | -0.004 (-0.025, 0.017) | |
| | $DTT_{2.5-10}$ | 0.014 (-0.020, 0.048) | |
| **Electrophilic potential, 5-day** | | | |
| | GPx-1 $_{0.18}$ | 0.018 (0.006, 0.030) | # |
| **Secondary pollutants** | | | |
| | 1-day ozone | -0.032 (-0.062, -0.001) | ** |
| | 3-day ozone | -0.025 (-0.062, 0.013) | |
| | 5-day ozone | -0.021 (-0.074, 0.033) | |
| | 7-day ozone | -0.004 (-0.066, 0.057) | |
| | organic $acid_{0.18}$ | -0.007 (-0.035, 0.021) | |
| | organic $acid_{0.18-2.5}$ | 0.023 (-0.023, 0.069) | |
| **Transition metals, 5-day** | | | |
| | Total metals $_{0.18}$ | 0.026 (-0.009, 0.061) | |
| | Total metals $_{0.18-2.5}$ | 0.011 (-0.010, 0.031) | |
| | Total metals $_{2.5-10}$ | -0.002 (-0.032, 0.028) | |

**p < 0.1, **p < 0.05, #p < 0.01*

BAM: Beta Attenuation Monitor; BC: black carbon; CO: carbon monoxide; DTT: dithiothreitol; EC: elemental carbon; OC: organic carbon; PAHs: polycyclic aromatic hydrocarbons; ROS: reactive oxygen species.

# Chapter 5. Cardiorespiratory outcomes and ambient air pollution: effect modification by residential traffic-related air pollution.

## 5.1. Introduction

Sufficient evidence has shown that short-term exposure to ambient air pollutants has adverse effect on pulmonary and cardiovascular function (Brook et al. 2010; Rice et al. 2013). This is consistent with our previous analyses, which have shown acute adverse effect on microvascular function (Chapter 3) and respiratory biomarkers of oxidative stress and inflammation (Chapter 4) among an elderly cohort from short-term exposures to ambient air pollutants, especially for traffic-related air pollutants such as BC, EC, $NO_X$, PAHs, and hopanes. Findings by particle size fraction showed that the strongest associations were for ultrafine particle components, which in the Los Angeles study region are primarily from mobile sources. However, the exposure data were obtained from two central monitoring stations in the two study areas and were assigned to each subject depending on his or her residence. Air pollution from local sources, such as ultrafine particles from traffic, have great spatial variation (Saffari et al. 2015). High concentrations of primary vehicle emissions are found on and near major roadways and decrease exponentially from major roadways (Zhu et al. 2002). During periods of high region-wide ambient air pollution, homes in high traffic area may have much higher

increases in traffic-related air pollution exposures than other homes. This is because meteorological conditions such as air stagnation reduce atmospheric dispersion and mixing, which may substantially increase pollutant concentrations near the sources. Under such conditions, homes in high traffic areas is likely to be more impacted than those away from the emission sources (Delfino et al. 2014). In addition, chronic exposures to primary traffic emissions at high traffic areas may change a person's underlying airway inflammation, oxidative stress, or other mechanisms that increase susceptibility to short-term exposures (Brauner et al. 2007; Riediker 2007).

A recent review determined that there is sufficient evidence of a causal association between near-road traffic-related air pollution, represented by residential proximity to roadways, and cardiorespiratory morbidity and mortality (HEI 2010). However, proximity is at best a crude surrogate of exposure with no quantitative exposure estimates. It does not account for the nature of emission sources, effects of meteorology, geographic features and other factors that may affect pollutant dispersion. Other model-based methods have also been used in estimating long-term traffic-related air pollutant level. For example, long-term exposure to traffic-related air pollution, estimated from a land-use regression model, was associated with increased cardiovascular mortality (Chen et al. 2013). CAlifornia LINE Source Dispersion Model, version 4 (CALINE4), a line-source dispersion model, was also used in estimating traffic-related air pollution level (Batterman et al. 2010). CALINE4 estimated traffic emissions were found to be associated with childhood asthma prevalence (Gauderman et al. 2005) but not with airway inflammation (measured by FeNO) in asthmatic children (Eckel et al. 2011). However, only limited data are available that have explored whether

variations in residential exposure to traffic-related air pollutant modify associations of cardiorespiratory outcomes with regional ambient air pollution. Our previous study investigating effect modification by residential traffic-related air pollution found that associations of asthma outcomes with CO, $NO_x$, and $PM_{2.5}$ were stronger among subjects living at residences with above-median versus at or below median traffic-related air pollutant exposures (Delfino et al. 2014).

In the present study, we hypothesize that higher chronic exposures to primary traffic emissions near the subjects' home enhances the observed associations between short-term cardiorespiratory outcomes and ambient air pollution.

## 5.2. Methods

### 5.2.1. Study Design

The study design in this chapter adds residential $NO_x$ to the parent study CHAPS 2 described in previous chapters. The overall study design and population are also described in detail in Chapter 2. Briefly, we measured air pollution exposures and health-related outcomes for 97 elderly nonsmoking subject living in the Los Angeles metropolitan area. One panel was studied in downtown Los Angeles from July 2012 through February 2013 (48 subjects) and one panel was studied in Anaheim from July 2013 through February 2014 (49 subjects). Each subject was followed for up to 12

weeks with weekly measurements of microvascular function, airway oxidative stress and inflammation biomarkers.

The Institutional Review Board of the University of California Irvine approved the research protocol. Informed written consent was obtained from subjects.

### 5.2.2. Outcome measurements

Previous analyses found evidences of significant associations of microvascular function and airway biomarkers with ambient air pollution, especially traffic-related pollutants (Chapter 3 and Chapter 4). The current study only focuses on these outcomes that were significantly and consistently associated with ambient air pollutants including 5-day average particle components. They include microvascular function (measured by EndoPAT), the airway oxidative stress biomarker (EBC MDA), and the airway inflammation biomarker (FeNO). The detailed methods for these measurements are described in Chapter 3 and Chapter 4. Briefly, endothelial function was measured by EndoPAT via plethysmography using beat-to-beat finger plethysmographic recordings of the finger arterial pulse wave amplitude captured with pneumatic probes. Endothelial function was represented by the RHI score, which is an index of plethysmography of the control arm and the post-ischemic arm. EBC samples were collected during normal breathing with the RTube$^{TM}$ Collection System (Respiratory Research, Inc, Austin, TX) using standard procedures (Holvoet et al. 2003). EBC MDA was then analyzed using modified HPLC analysis protocol (Larstad et al. 2002). FeNO

was measured noninvasively using the NIOX MINO (Aerocrine Inc, New Providence, NJ).

### 5.2.3. Exposures

Ambient air pollution exposures were obtained from ambient monitoring stations and USC monitoring sites. Detailed exposure assessment methods are given in Chapter 2. Briefly, we obtained hourly ambient concentrations of $PM_{2.5}$, $NO_x$, CO, and $O_3$ from SCAQMD monitoring stations. Hourly temperature and relative humidity were also obtained at these stations. At USC monitoring sites, we measured hourly BC and 5-day PM components of PAHs, hopanes, EC/OC, transition metals, organic acids, markers of PM oxidative potential (*in vitro* macrophage ROS and DTT), and electrophilic potential (GPx-1). However, analyses in this chapter only focuses on key traffic-related air pollutants (BC, CO, NOx, PAHs, hopanes, and EC/OC), total transition metals, marker of electrophilic potential (GPx-1), and measures of oxidative potential (*in vitro* macrophage ROS and DTT) since they are more directly linked to exposures near residential roadways. In addition, these pollutants were observed to have significant associations with the cardiorespiratory outcomes in our previous analyses. Therefore, we did not analyze $O_3$, organic acids, and PM components in $PM_{2.5-10}$.

Residential $NO_x$, which serves as tracer of local traffic emissions, was estimated using a modified CALINE4 at each residence, as previously described (Wu et al. 2009; Delfino et al. 2014). All subjects' residential locations were geocoded in ArcGIS 9.3 (ESRI, Redlands, CA). Major inputs to CALINE4 include roadway geometry and traffic

activities (annual average traffic counts), vehicle emission factors (obtained from the California Air Resources Board's EMFAC2011 vehicle emissions model), and meteorology (wind speed, wind direction, and temperature). We estimated $NO_x$ in the years when subjects participated in the study (i.e., subjects in Los Angeles in 2012-2013, subjects in Anaheim in 2013-2014). However, it is important to note that the difference in concentration estimates was relatively small by year because of small differences in the yearly-changing input variables (e.g., traffic counts, emission factors, and long-term meteorology). The estimation should be regarded as indicators of long-term exposures of primary emissions from local vehicular traffic on top of background ambient levels.

### 5.2.4. Statistical analysis

Subjects were stratified above and below median CALINE4-modeled $NO_x$. Student t-test or chi-square test were used to compare the baseline characteristics between low and high exposure groups. As described in Chapter 2, linear mixed models were used to analyze the associations between cardiorespiratory outcomes and air pollution. In order to evaluate whether residential traffic exposure is an effect modifier of the relation between cardiorespiratory outcomes and short-term ambient air pollution exposure, we included an interaction product term between each air pollutant and binary traffic exposure group. We incorporated random subject intercepts to allow for differential mean outcome levels across individuals. We selected an ARMA covariance structure for random within-subject errors in dependent variables. A nominal product term p-value <

0.1 was considered as significant evidence of interaction. This was selected to avoid substantial type II errors, while still allowing identification of effect modification. In order to compare associations across different pollutants, results are presented per interquartile range increase in pollutant concentration. Time-invariant subject characteristics were controlled by study design and statistical model. Therefore, these variables were not adjusted in the main models.

### 5.2.5. Secondary analyses

It has been shown that the air pollution effects estimated are modified by climate (Bobak et al. 1997), and this has been supported by observed seasonal differences in previous studies (Katsouyanni et al. 2001; Delfino et al. 2014). To test seasonal difference in the effect modification of dispersion-modeled exposures, we conducted the analysis within the warm season (July - October) and the cold season (November - February).

Although fixed subject characteristics are not likely to confound the associations in cohort panel study design and with the linear mixed model, it is possible that residential dispersion-modeled $NO_x$ strata could function as a surrogate of demographic differences that vary with traffic. For example, subjects living in downtown Los Angeles may have higher estimated $NO_x$ level than subjects living in Anaheim. Therefore, we conducted a series of secondary analyses to explore confounding factors by fixed subject characteristics that potentially vary with traffic, including age, sex, race, study region, and socioeconomic status (SES). First, we assessed the confounding effect of

these covariates by including them to the models. Second, we explore their effect modification on the associations between cardiorespiratory outcomes and short-term exposure to air pollutants. Lastly, we investigated the effect modification by CALINE4-modeled $NO_X$ exposure within different strata of age (≤75, >75 years old), sex (male, female), race/ethnicity (Non-Hispanic White, others), study region (Los Angeles, Anaheim), and SES (low, high) using 3-way interaction terms among CALINE4-modeled NOx classification, short-term exposure to air pollutant, and each characteristic. Subjects with annual income above $30,000 or had at least high school education (N=50) were assigned as high SES, otherwise were defined as low SES (N=45).

Some of the subjects were living in the same retirement community and thus residential address may also share similar lifestyle factors that affect the associations. Therefore, we performed a sensitivity analysis to exclude subjects living in three retirement communities (N=24). Each of three communities had at least 5 subjects living together.

## 5.3. Results

### 5.3.1. Descriptive data

Overall characteristics of 97 subjects are presented in Table 4.3. Table 5.1 describes subject characteristics for the low and high exposure groups of the CHAPS 2 cohort for local traffic emissions. A map of dispersion modeled $NO_x$ concentration at each residence address is shown in Figure 5.1. The estimated concentrations ranged

from 0.30 ppb to 16.59 ppb, with median concentration at 2.15 ppb. Subjects were categorized into two groups by the median concentration. We found that male subjects were more likely to live in residences with higher estimated traffic $NO_x$ (Table 5.1). As we expected, significantly more subjects living in the Los Angeles study region were in the high residential traffic exposure group than subjects living in the Anaheim study region. Non-Hispanic Whites were more likely to live in residences with higher CALINE4-modeled NOx, while Hispanic and African American subjects were more likely to live in residences with lower CALINE4-modeled NOx. On average, there were no significant differences in RHI, EBC MDA, and FeNO in subjects with higher estimated residential traffic exposure compared to those with lower estimated exposure.

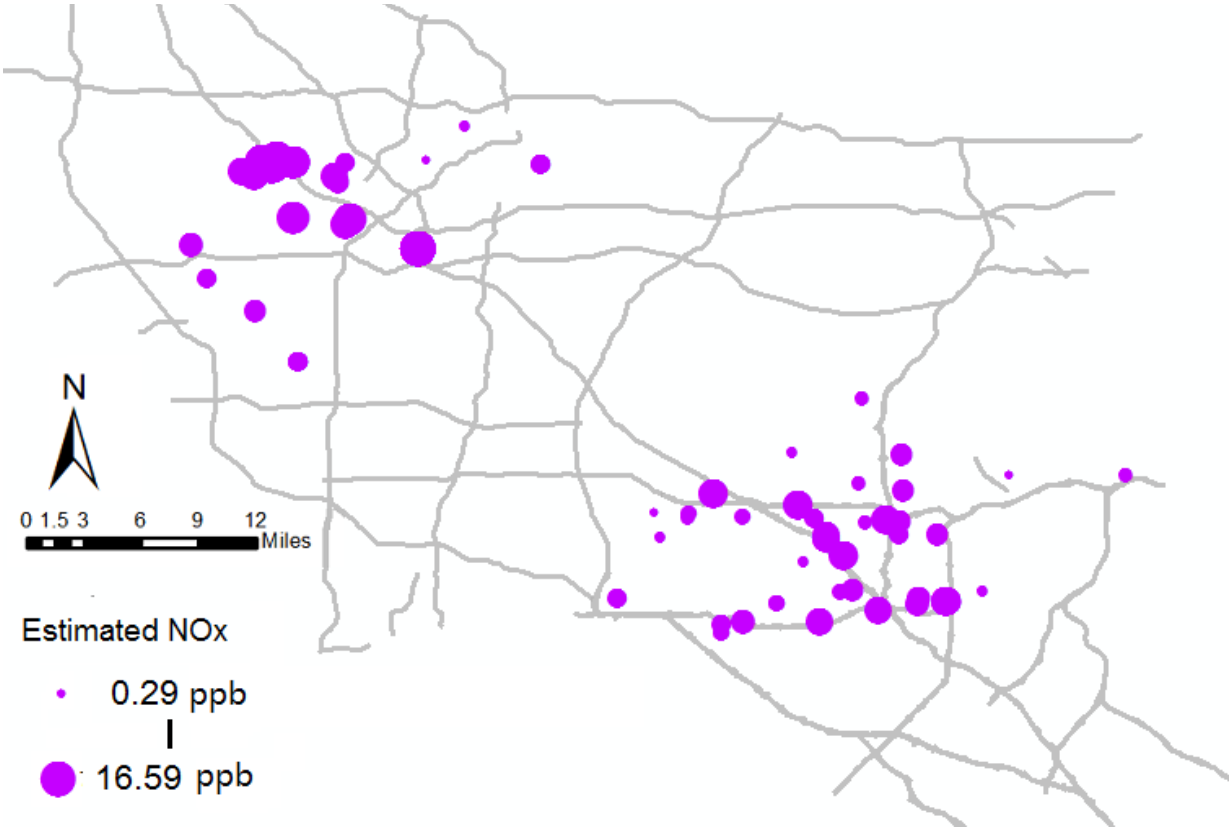


**Figure 5.1. $NO_x$ levels at subject's residential addresses: estimated by CALINE4.**
The size of circles indicates the estimated $NO_x$ level.

### 5.3.2. Regression analysis of effect modification

Inverse associations of RHI with daily traffic-related ambient pollutants (BC, CO, and $NO_x$) were stronger among subjects living at residences with higher CALINE4-modeled $NO_x$ exposure than subjects with lower CALINE4-modeled $NO_x$ exposure, and the effect modification was statistically significant for acute exposures (1-day average) (Figure 5.2 and Table 5.2). For example, among subjects with higher CALINE4-modeled $NO_x$, the estimated RHI was -0.074 (95% CI: -0.126, 0.021) with per interquartile range increase in ambient $NO_x$ at 1-day average, while the estimated RHI was -0.003 (95% CI: -0.056, 0.051) among subjects living at residences with lower dispersion modeled $NO_x$. The *p*-value for the interaction term was 0.071. Decreased RHI also has stronger associations with some of the ambient 5-day components and oxidative potential markers among subjects living at residences with higher CALINE4-modeled $NO_x$ (Figure 5.2 and Table 5.2). These include total $PM_{0.18}$ mass, hopanes in $PM_{0.18}$, OC in $PM_{0.18}$, total metals in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$, total ROS in $PM_{0.18}$ and $PM_{0.18\text{-}2.5}$ and DTT in $PM_{0.18}$. However, the effect modifications were non-significant except for total metal in $PM_{0.18\text{-}2.5}$ ($p$=0.095), where we observed a point estimate of -0.048 (95% CI: -0.088, -0.008) in RHI with per interquartile range increase in total metal in $PM_{0.18\text{-}2.5}$ among subjects living at residence with higher CALINE4-modeled NOx exposure while the point estimate was -0.007 (95% CI: -0.058, 0.044) among subjects living at residences with lower CALINE4-modeled NOx exposure.

For EBC MDA, all averaging times for daily traffic-related air pollutants (BC, CO, and $NO_x$) generated stronger positive associations among subjects living in areas with

higher CALINE4-modeled $NO_x$ although the effect modifications were not statistically significant (Figure 5.3 and Table 5.3). Consistent with daily exposure, many of 5-day components show stronger effects among subjects living at residence with higher CALINE4-modeled $NO_x$ exposure, although no product terms reached $p<0.1$. The modifying effects were most prominent for PAHs and hopanes.

Similar to RHI, the associations of FeNO with daily ambient BC, CO, and $NO_x$ at acute exposure (1-day and/or 3-day) were stronger among subjects living at residences with greater than median CALINE4-modeled $NO_x$ (Figure 5.4 and Table 5.4). The modifying effect for 5-day air pollutant components were less clear. We found unexpected significant effect modification with hopanes in $PM_{0.18\text{-}2.5,}$ where the stronger positive association was observed in subjects living in residences with lower CALINE4-modeled $NO_x$. EC in $PM_{0.18}$ showed an expected effect modification with stronger positive association among subjects living in residences with higher CALINE4-modeled $NO_X$. The effect modification by CALINE4-modeled NOx was observed between FeNO and GPx-1 in $PM_{0.18\text{-}2.5}$ (p=0.009), with inverse association among subjects living at residences with higher CALINE4-modeled NOx (estimate: -0.015, 95% CI: -0.027, -0.002) while association was non-significant among subjects living at residences with lower CALINE4-modeled NOx.

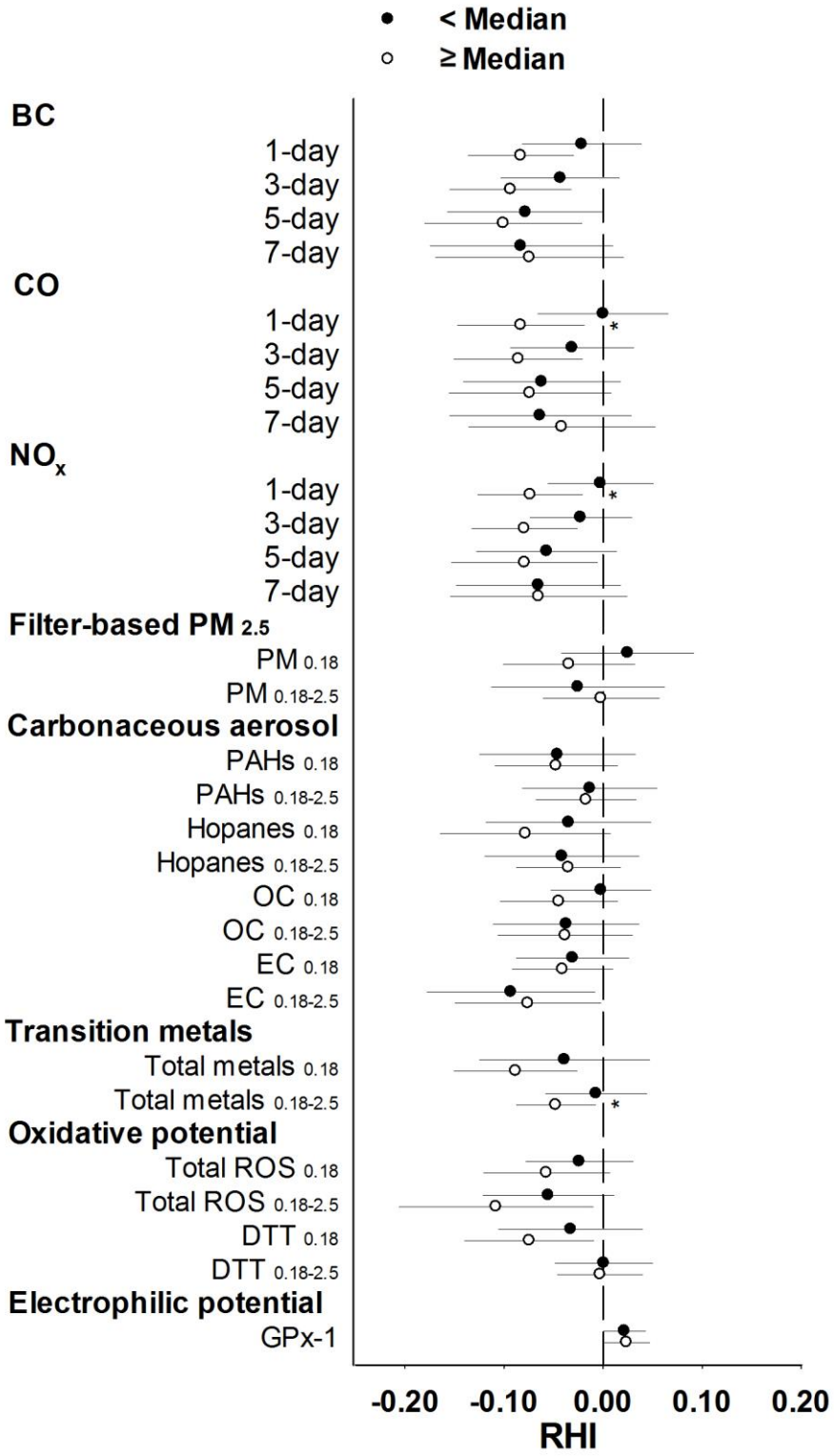


Figure 5.2. Associations of RHI with ambient daily air pollutants and 5-day air pollutant components. Effect modification by CALINE4-modeled $NO_x$ above and below median levels. * $p < 0.1$

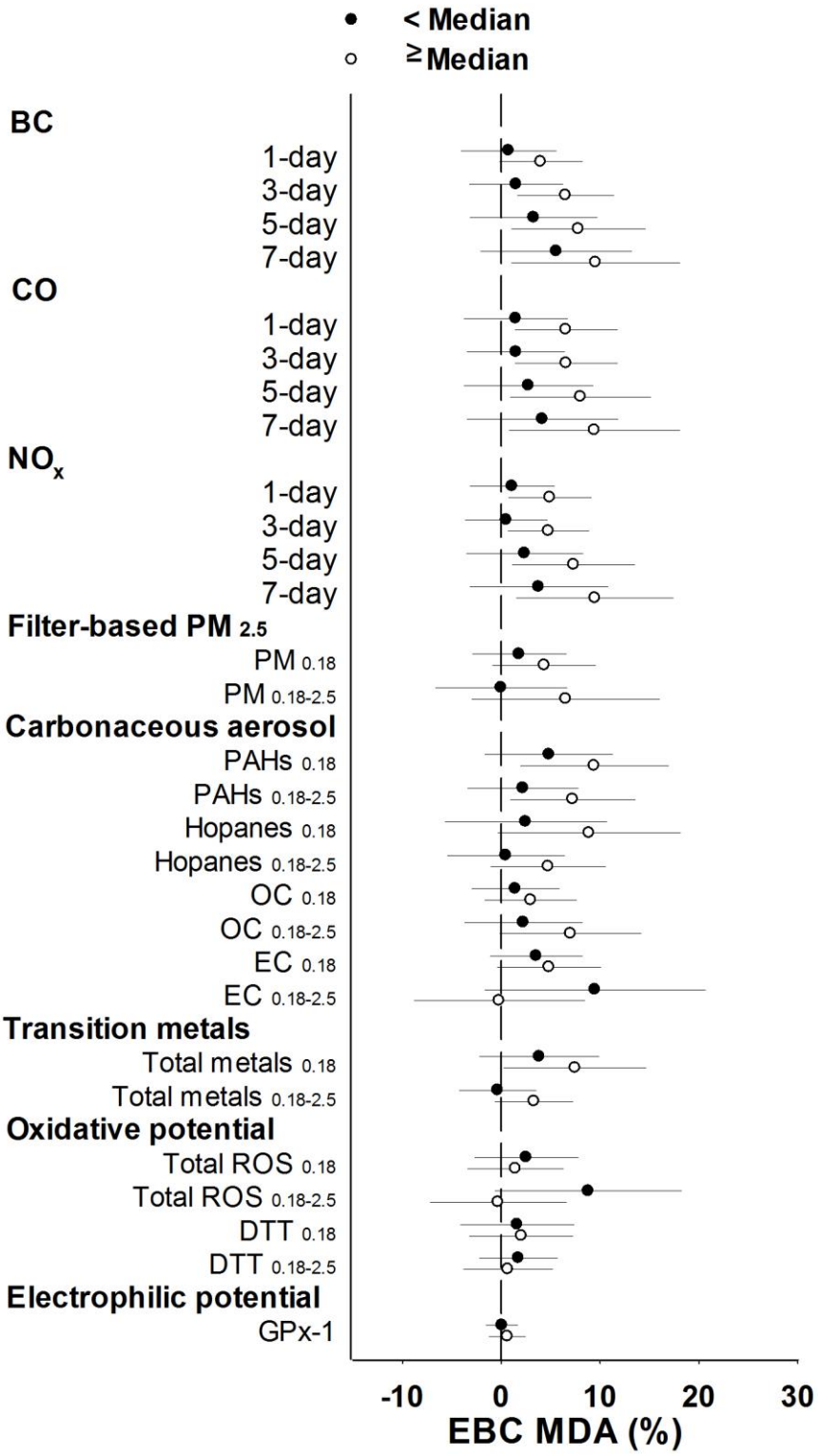


Figure 5.3. Associations of EBC with ambient daily air pollutants and 5-day air pollutant components. Effect modification by CALINE4-modeled $NO_x$ above and below median levels.

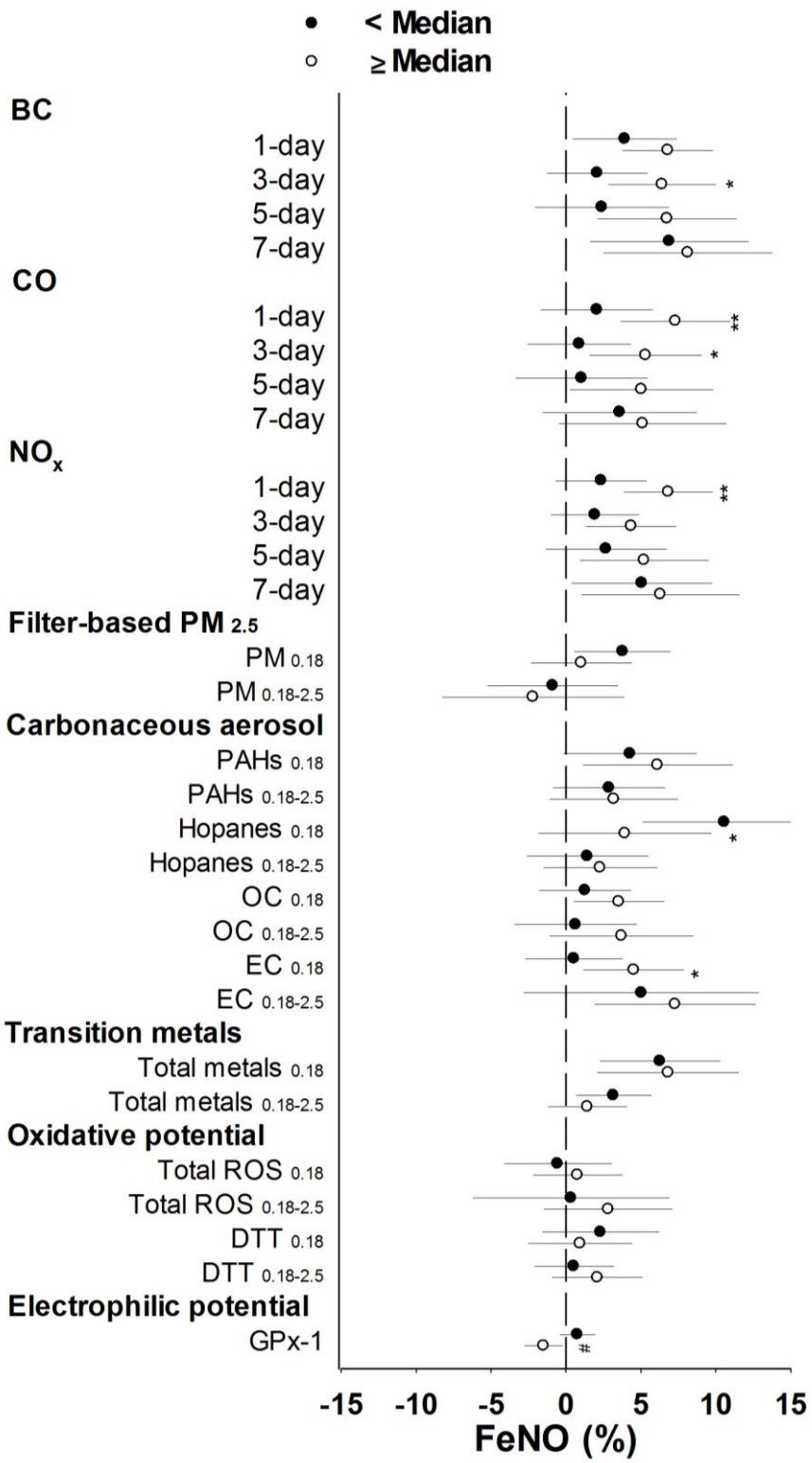


Figure 5.4. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components. Effect modification by CALINE4-modeled $NO_x$ above and below median levels. * $p < 0.1$, ** $p < 0.05$, # $p < 0.01$.

### 5.3.3. Secondary analyses

After stratifying by season, we found that, for all outcomes, the effect modification by CALINE4-modeled NOx observed above were only observed in the cool season, not in the warm season. Figure 5.5, Figure 5.6, and Figure 5.7 represents the results for RHI, EBC MDA, and FeNO, respectively.

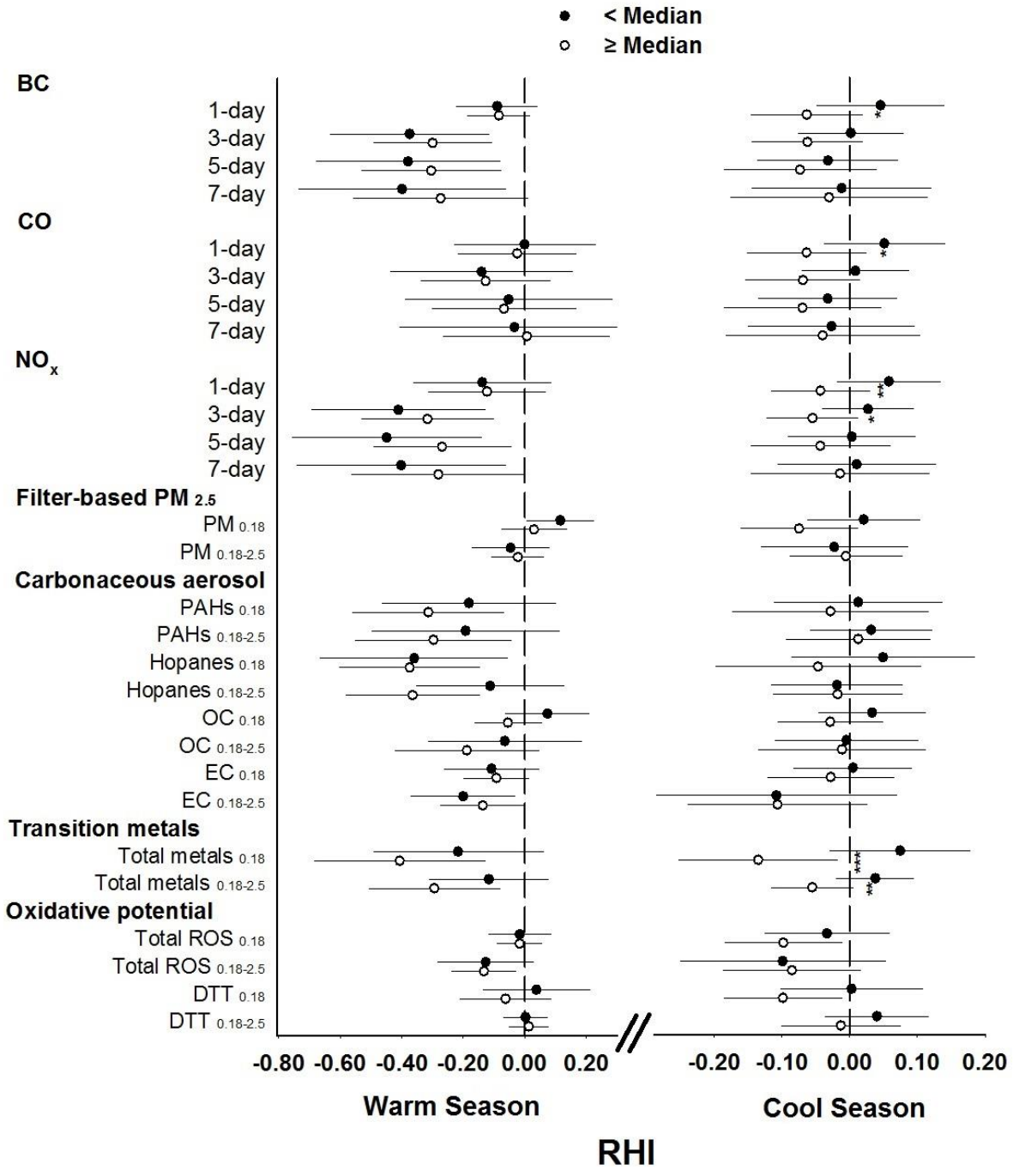


Figure 5.5. Associations of RHI with ambient daily air pollutants and 5-day air pollutant components, modifying by dispersion-modeled traffic-related air pollution above and below median levels: stratified by season. * $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$.

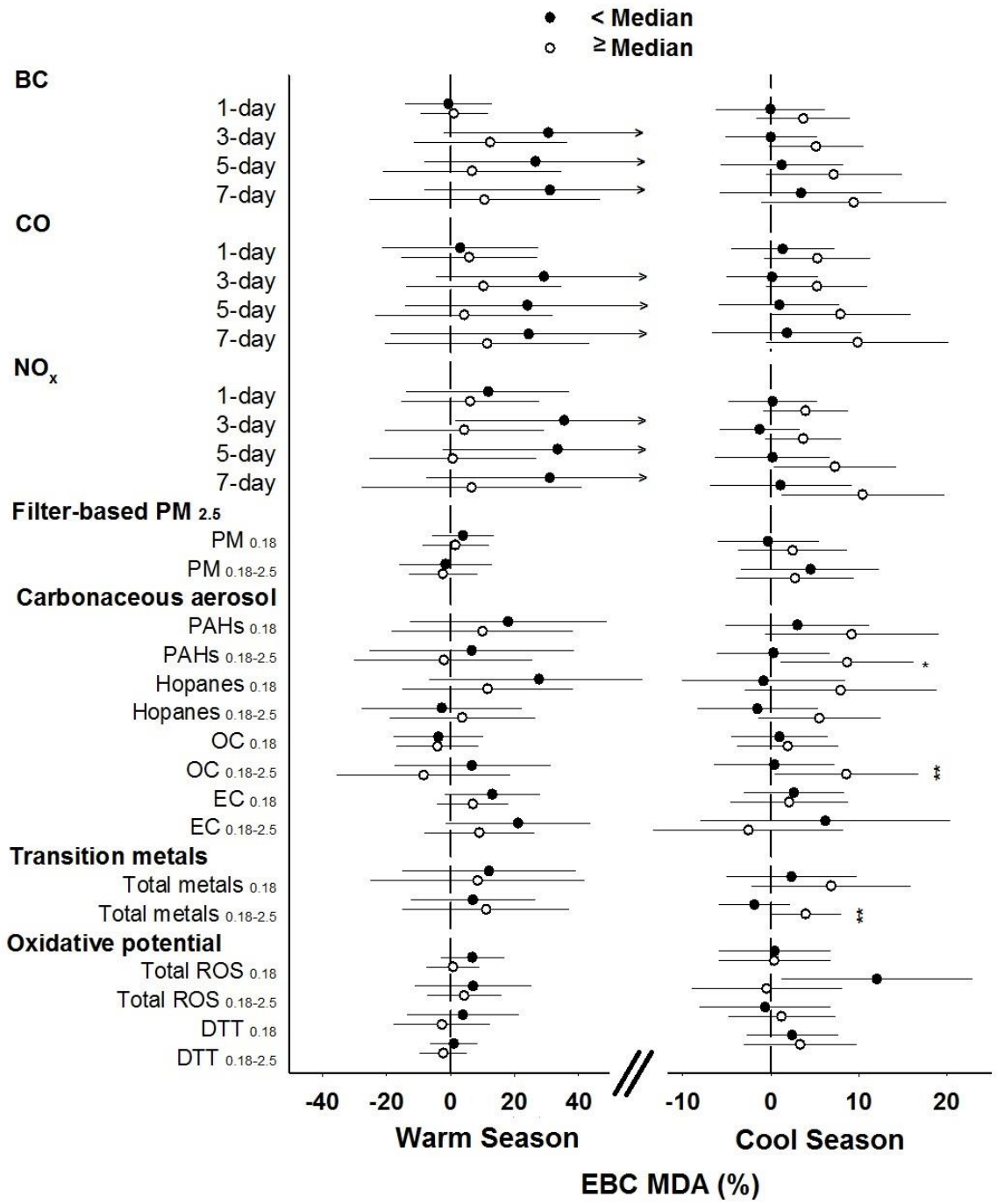


Figure 5.6. Associations of EBC MDA with ambient daily air pollutants and 5-day air pollutant components, modifying by dispersion-modeled traffic-related air pollution above and below median levels: stratified by season. * $p < 0.1$, ** $p < 0.05$.

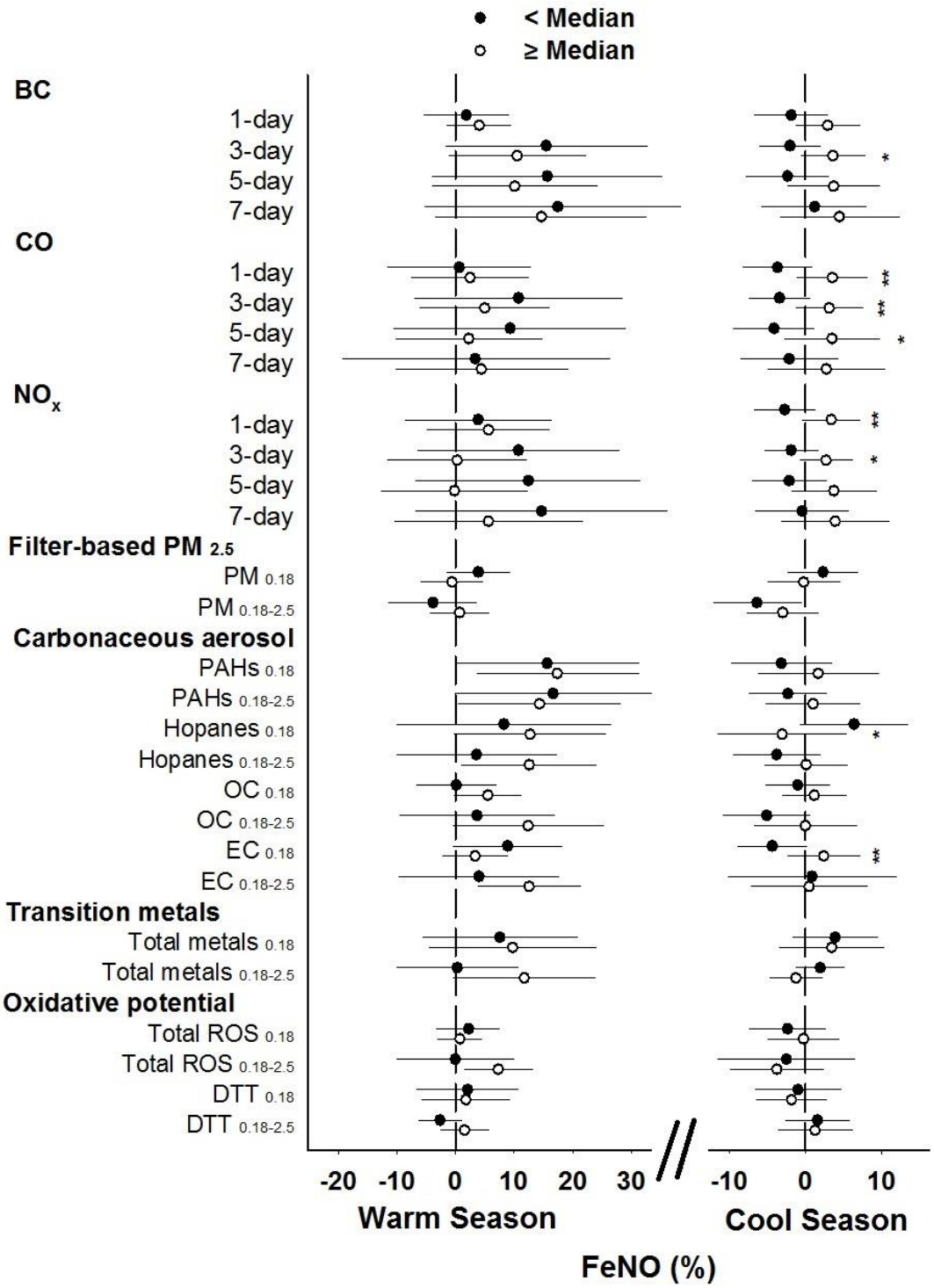


Figure 5.7. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components, modifying by dispersion-modeled traffic-related air pollution above and below median levels: stratified by season. * $p < 0.1$, ** $p < 0.05$.

As expected, adding these time-invariant subject characteristics (age, sex, study region, race, and SES) as adjustment covariates did not significantly change the estimations (Table 5.5, Table 5.6, and Table 5.7). As discussed before, these time-invariant subject characteristics were likely to be as effect modifiers rather than confounders. In our previous analyses, we explored the modifying effects of some of these covariates to explore the susceptibility. We did not observe significant effect modification by age, sex, or study region between RHI and short-term exposure to air pollutants (Chapter 3.3.4). We found some evidence of potential effect modification on EBC MDA by age and by study region. For example, subjects who were older (age > 75 years old) have stronger positive associations than younger subjects (≤ 75 years old). Regional differences were also observed (although not statistically significant) on the associations between EBC MDA and daily traffic-related air pollutants, where positive associations were stronger in Anaheim than in Los Angeles (Chapter 4.3.4 and Table 5.9). Of note, Anaheim had a lower estimated CALINE4-modeled NOx exposure than Los Angeles (Table 5.1). In this chapter, we additionally explored the modifying effects by SES and race/ethnicity. We found stronger inverse associations between RHI and short-term exposure to carbonaceous aerosol (e.g. PAHs, hopanes etc.) in Non-Hispanic Whites than others (Table 5.11), while EBC MDA has stronger positive associations with ambient daily pollutants in other race/ethnicities than in Non-Hispanic Whites (Table 5.12). There was not consistent effect modification by Non-Hispanic Whites and non-Whites on FeNO (Table 5.13). The associations between two airway biomarkers (EBM MDA and FeNO) and air pollutants were stronger in the lower SES

group than in the high SES group for the majority of the air pollutants, although the differences between the two level were mostly non-significant (Figure 5.5 and Figure 5.6). On the contrary, stronger inverse associations between RHI and air pollutants (especially with exposure to ambient daily traffic-related air pollutants) were observed in subjects with higher SES than lower SES (Figure 5.7).

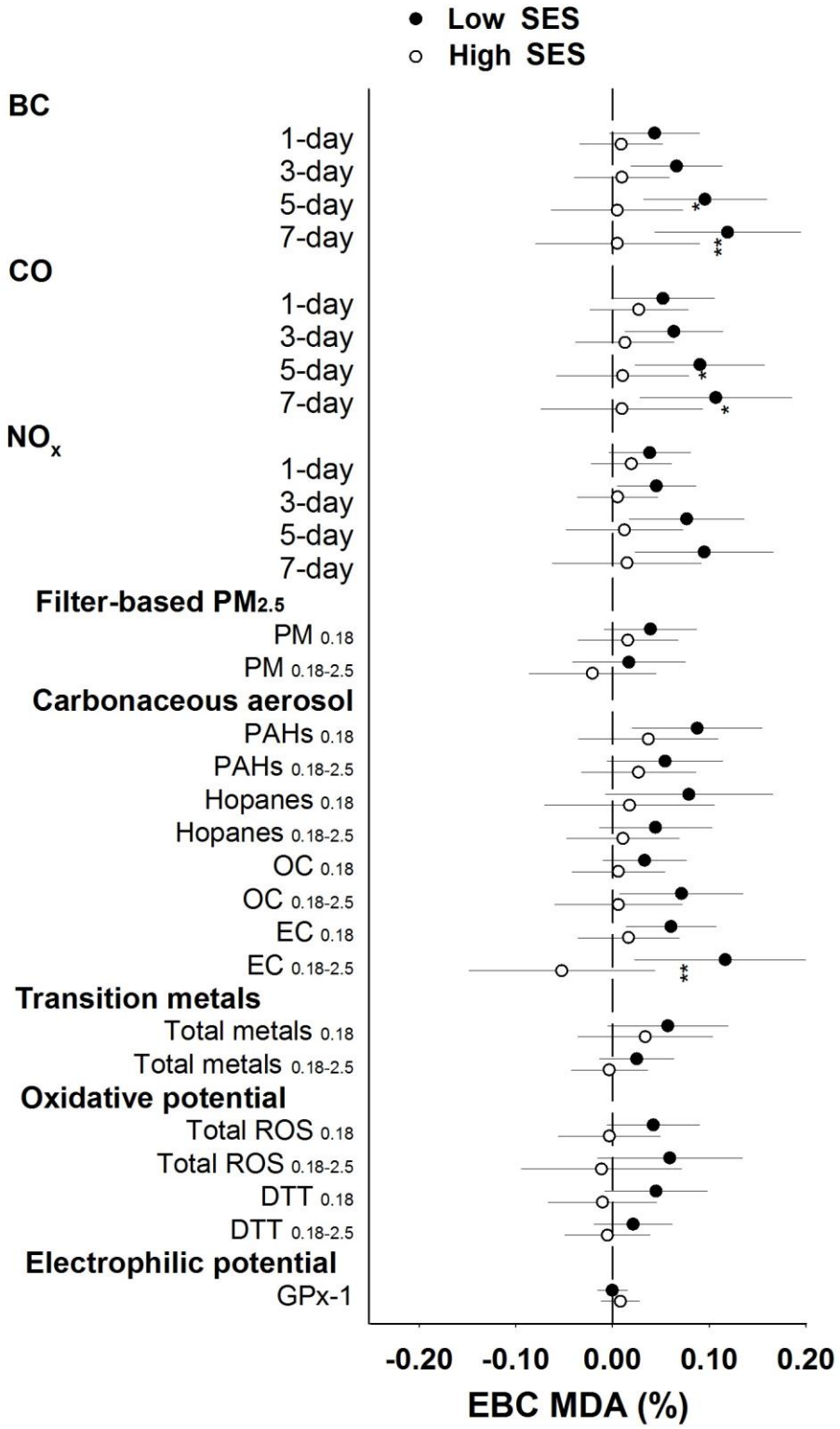


Figure 5.5 .Associations of EBC MDA with ambient daily air pollutants and 5-day air pollutant components. Effect modification by socioeconomic status. * $p < 0.1$, ** $p < 0.05$

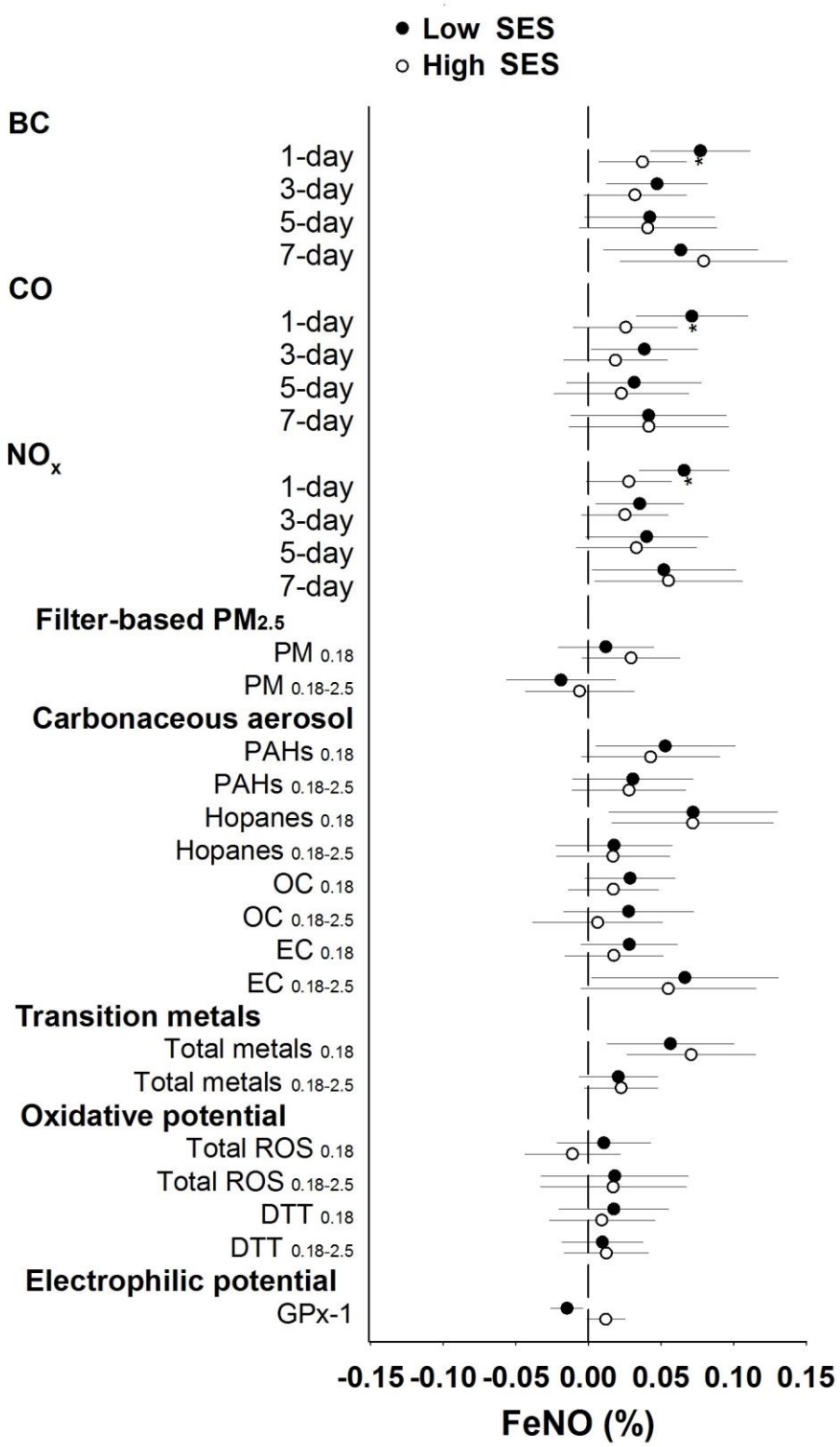


Figure 5.6. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components. Effect modification by socioeconomic status. * $p < 0.1$

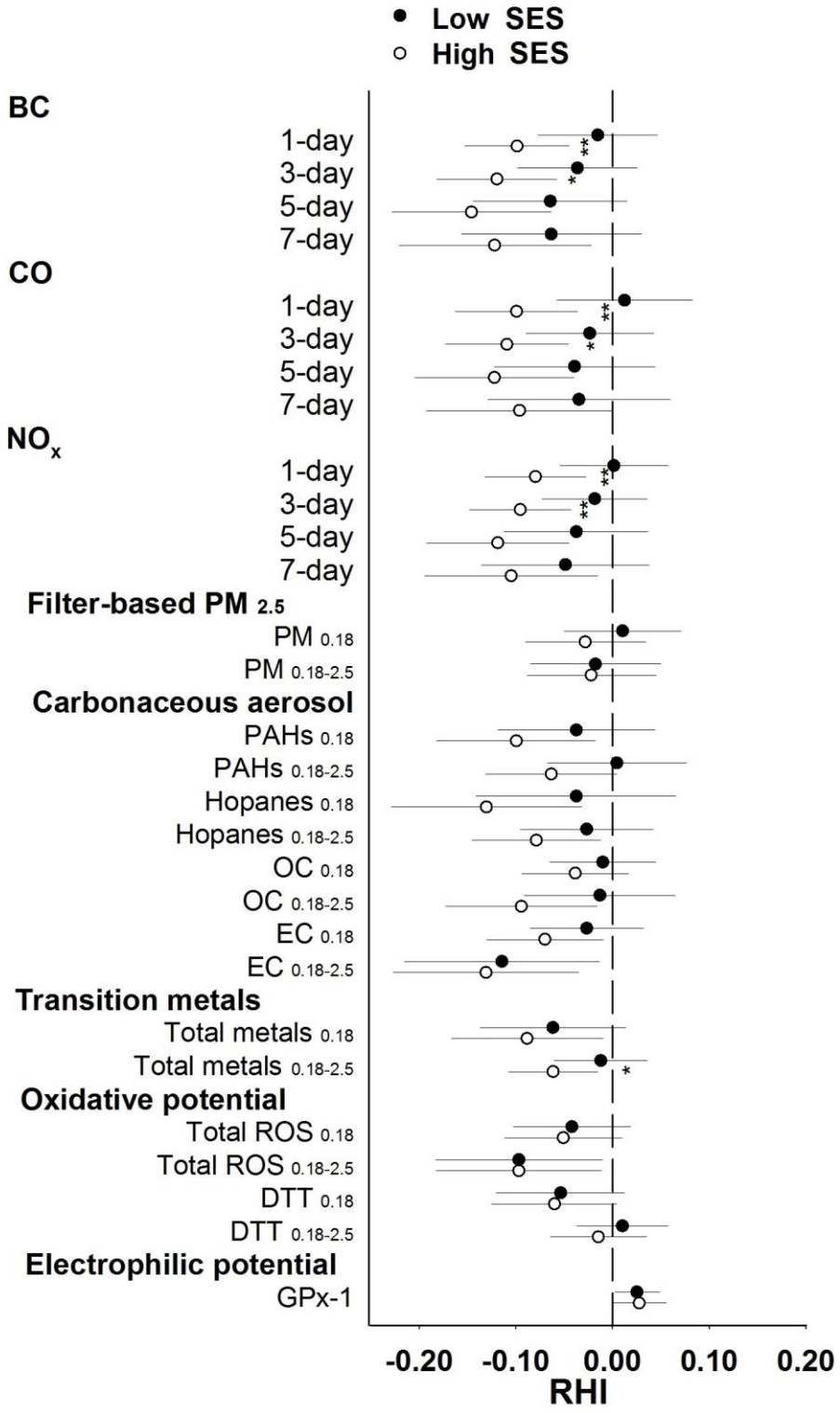


Figure 5.7. Associations of RHI with ambient daily air pollutants and 5-day air pollutant components. Effect modification by socioeconomic status. * $p < 0.1$, ** $p < 0.05$

By using models with 3-way interaction terms to explore the differential effect modification of CALINE4-model NOx level by these time-invariant characteristics, we found that the effect modifications in the associations between RHI and daily ambient exposures with BC, CO, and NOx at 1-day average or 3-day average differed by sex ($p$<0.1), with stronger effect modifications by CALINE4-modeled NOx observed in men than in women (Table 5.14). For FeNO, the effect modifications by CALINE4-modeled NOx were significant in younger subjects (age ≤ 75 years old) at 1-day average ambient BC, CO, and $NO_{x,}$ while no significant effect modifications were found in elder subjects (age >75) (Table 5.6). We found consistent evidence showing that the effect modifications in the associations between FeNO and many of the ambient air pollutants (21 out of 29 pollutant species) differed by study region ($p$<0.1), with stronger effect modifications observed in Anaheim than in Los Angeles (Table 5.7). There was also evidence suggesting effect modifications of associations of FeNO with ambient air pollution by CALINE4-modeled NOx were different in lower SES vs. higher SES groups. The effect modifications were observed in subjects in lower SES rather than those with higher SES (Table 5.8). These differences by age, study region, and SES that were observed only in FeNO were not found for RHI or EBC MDA (Data not shown). There was no consistent evidence that effect modification of associations by CALINE4-modeled NOx differed between Non-Hispanic Whites as compared to the combination of other race/ethnicities (Data not shown).

Sensitivity analysis by excluding subjects from three communities, where subjects were clustered (n=24), were similar with the results using all subjects, but

differences in association above and below the median dispersion-modeled strata were smaller and confidence intervals were wider (data not shown).

## 5.4. Discussion

In this large panel cohort study of elder subjects living in the Los Angeles area between 2012 and 2014, we found suggestions of effect modification by residential concentrations of NOx estimated by dispersion-modeling in the associations between cardiorespiratory outcomes and short-term exposure to traffic-related air pollution. Subjects with higher CALINE4-modeled $NO_x$ exposure had a stronger adverse cardiorespiratory effects (i.e., lower RHI sore, or greater increases in EBC MDA or FeNO) than subjects living at residences with lower CALINE4-modeled $NO_x$. Effect modification was observed between FeNO and GPx-1 activity by CALINE4-modeled NOx. Among subjects with higher CALINE4-modeled NOx exposure, a lower GPx-1 activity (inactivated by electrophilic potential) was associated with increased FeNO while the association was non-significant among subjects living at lower CALINE4-modeled NOx exposure. This indicated that chronic exposure to residential traffic-related air pollutants strengthened the inverse association between airway inflammation and GPx-1 activity and suggested that the null associations between airway biomarkers and GPx-1 (Chapter 4) may in part due to exposure misclassification resulted spatial variation (discussed below). The modifying effects were more evident in the cool season than in the warm season. These results are generally consistent with the only previous studies with a similar aim, which showed that positive associations of asthma-related hospital morbidity (admissions and emergency department visits) with daily ambient air

pollution were stronger among subjects living at residences with higher CALINE4-modeled predicted levels of air pollution from traffic sources than those with lower residential exposures (Delfino et al. 2014).

As discussed in the previous chapters, ambient exposure data obtained from central monitoring stations can lead to exposure error, especially for ultrafine particles, which have a great spatial variation (Saffari et al. 2015). Those high pro-oxidative particles are found in high concentration on and near major roadways and levels decrease exponentially away from the road (Zhu et al. 2002). Therefore, living in higher traffic density areas can have a greater impact on the association between adverse health effects and exposure to ambient air pollutants. Additionally, other studies have shown that subjects who lived closer to major roadways may have greater levels of oxidative stress and inflammation as a result of their chronic exposure to traffic-related air pollutants (Brook et al. 2010).

We observed the modifying effects in cool season rather than warm season. This is expected because the meteorological conditions that contribute to the amplification of traffic-related air pollution with increased ambient air pollution is more like to happen in the cool season (Zhu et al. 2004). The greater spatial variation of traffic-related air pollutants during the cool season can therefore lead to a stronger effect modification by residential traffic level.

Our secondary analyses by including time-invariant subject characteristics as adjustment variables in the main models were robust. To explore whether the observed effect modification by CALINE4- modeled NOx level was driven by the covariates that

varied with traffic, we further explored the effect modification by these characteristics (sex, race, age, study region, and SES). We observed evidence of effect modification by some of these characteristics. However, the observed effect modifications were not consistent in all three outcomes and the direction of modifying effects were specific to each outcome. For example, evidence suggests that the adverse effects on EBC MDA and FeNO of short-term exposure to air pollutants were stronger among subjects with low SES while the adverse effects on RHI were stronger among subjects with high SES. The underlying mechanism that driving the observed effect modifications was unclear. However, it unlikely that CALINE4-modeled NOx level was acting as surrogate for these characteristics because the different effect modifying patterns. It is also important to note that our race/ethnicity groups were only differentiated Non-Hispanic Whites from other race/ethnicities due to the small sample size. In additional, SES itself combines many different elements that may contribute to differential health responses to air pollution, which are not necessarily captured by total annual income or education levels that are measured in the present study.

In our 3-way interactions terms, we found evidence showing that, the modifying effects on the association of RHI with short-term exposure to traffic-related air pollution by CALINE4-modeled traffic exposure were stronger among men than women. This result need further investigation, in particular to better understand whether the differential effect modification by sex could be explained by gender specific differences in micro-environmental exposure and/or biological differences (e.g. thickness of vasculature). However, it is of note that the sample size within the strata for men was small and may not be representative. We found evidence that effect modifications of

associations of FeNO with ambient air pollutants by CALINE4-modeled NOx differed by SES and by study region. An indication of the greatest increase in FeNO in association with short-term exposure to traffic-related air pollutants was observed among subjects with higher CALINE4-modeled NOx exposure while having low SES. This finding is consistent with a study conducted in Paris investigating all-cause mortality, which found that subjects chronically exposed to higher dispersion modeled $NO_2$ level had a higher risk of all-cause mortality from exposure to short-term air pollution among subjects living in deprived census blocks (Deguen et al. 2015). Greater modifying effects between associations of FeNO to short-term air pollution exposure by CALINE4-modeled NOx were found among younger subjects than older subjects, and among subjects living in Anaheim than subjects living in Los Angeles. It is possible that older subjects were more like to have other chronic airway conditions (such as COPD). Therefore, the modifying effects by long-term traffic exposure may be obscured by their existing conditions. The modifying effects by CALINE4-modeled NOx between FeNO and ambient traffic-related pollutant were stronger in Anaheim than in Los Angeles. This may due to the fact that many subjects in Los Angeles were clustered in several communities. The shared micro-environment and lifestyle factors (e.g., diet) may confound the results and limit the power to detect the effect modification by CALINE4-modeled NOx among subjects living in Los Angeles. It is also important to note that these differences by demographic factors were not observed in the other two outcomes, suggesting that the interaction effect between these demographic factors and CALINE4-modeled NOx exposure on the associations between FeNO and traffic-related pollutants may be due to unknown mechanisms specifically related to FeNO. We observed no consistent differences by

race/ethnicity in effect modifications. However, we combined Asian, Hispanic, African American and other race/ethnicities into one group because of the limited number of subjects, which may obscure the potential effect modification.

To our knowledge, this is the first study to show that the associations of both cardiovascular and respiratory outcomes with short-term exposure to ambient air pollution are stronger among subjects living at residences with higher predicted levels of air pollution from local traffic sources.

One limitation is that our long-term exposure to local traffic emissions was based on the current residential addresses and the study period. It does not represent long-term exposure over a lifetime. However, our primary objective was to investigate the interaction effect between short-term ambient exposures and spatial variability in local traffic emissions on cardiorespiratory outcomes. The average CALINE4-modeled exposure over the study period reliably estimated spatial variability of local-traffic generated pollutants. Another limitation is that some subjects in Los Angeles were living in the same communities and they may share similar micro-environmental and life-style factors that affected the associations. However, our sensitivity analysis excluding these subjects found similar results. Finally, demographic characteristics that were correlated with residential traffic may confound the observed effect modification. Our secondary analyses did not support that CALINE4-modeled exposure level is acting as surrogates of these characteristics. However, residential air pollution level is closely related to many of the factors. To fully tease apart these factors is a difficult challenge in the present study. More research is needed to further investigate the whether the effect

modification is from residential traffic emissions or the correlated demographical factors, including other unmeasured factors.

In summary, this study suggested that the adverse cardiorespiratory effects of short-term exposure to traffic-related air pollution were modified by residential dispersion-modeled local traffic emissions, particularly during the cold season. Our results point to the potential underestimation of the cardiorespiratory effects with short-term ambient exposure using central air monitoring station data, especially where there are many subjects living in high traffic areas. This study adds to evidence that present regulations of regional air quality might need to consider the local variation of traffic sources. Further study is needed to investigate whether the observed modifying effects by residential traffic sources were confounded by demographic differences that vary with traffic.

Table 5.1. Characteristics of 97 subjects by estimated residential exposure to local traffic emissions

| Characteristic | Mean ± SD or N (%) | | |
|---|---|---|---|
| | **Low residential traffic level (N=48)** | **High residential traffic level (N=49)** | ***P* value** |
| Age (years) | 73.44 ± 7.69 | 76.16 ± 7.14 | 0.07 |
| BMI (kg/m$^2$) | | | 0.73 |
| Overweight (25-29.9) | 18 (37.5) | 16 (32.65) | |
| Obesity (≥30) | 14 (29.17) | 18 (36.73) | |
| Male (%) | 5 (10.42) | 20 (40.82) | <0.01 |
| Annual income ($) (%) | | | 0.32 |
| <14,000 | 5 (19.23) | 13 (36.11) | |
| 15,000-35,000 | 8 (30.77) | 10 (27.78) | |
| 35,000-50,000 | 7 (26.92) | 7 (19.44) | |
| 50,000-100,000 | 4 (15.38) | 6 (16.67) | |
| >100,000 | 2 (7.69) | 0 (0.00) | |
| missing | 22 | 13 | |
| Education (%) | | | 0.11 |
| Up to 8$^{th}$ grade | 4 (8.89) | 1 (2.08) | |
| High school | 30 (66.67) | 27 (56.25) | |
| 4 year college | 7 (15.56) | 8 (16.67) | |
| >4 year college | 4 (8.89) | 12 (25.00) | |
| Missing | 3 | 1 | |
| Former smoker (%) | 17 (35.42) | 25 (51.02) | 0.12 |
| Cardiovascular Disease (%) | 8 (16.67) | 8 (16.33) | 0.96 |
| Hypertension (%) | 28 (58.33) | 35 (71.43) | 0.47 |
| Hypercholesterolemia (by history) (%) | 25 (52.08) | 28 (57.14) | 0.62 |
| Lipid Profile | | | |
| Total cholesterol | | | 0.62 |
| > 200 mg/dL(%) | 20 (41.67) | 18 (36.73) | |
| LDL | | | 0.95 |
| > 130 mg/dL(%) | 15 (31.25) | 15 (30.61) | |
| HDL | | | 0.43 |
| Female <50 mg/dL, male <40 mg/dL(%) | 14 (29.17) | 18 (36.73) | |
| Adult-onset diabetes mellitus | 7 (14.58) | 15 (30.61) | 0.06 |
| COPD (%) | 3 (6.25) | 5 (10.2) | 0.48 |
| Asthma (%) | 6 (12.5) | 6 (12.24) | 0.97 |
| Anti-hypertensive medication (%) | 25 (52.08) | 28 (57.14) | 0.18 |
| Race/ethnicity (%) | | | 0.02 |
| Non-Hispanic White | 24 (50) | 40 (81.63) | |
| Hispanic | 5 (10.42) | 4 (8.16) | |
| African American | 8 (16.67) | 2 (4.08) | |
| Asian | 7 (14.58) | 2 (4.08) | |
| Other | 4 (8.33) | 1 (2.04) | |
| Region (%) | | | <0.01 |
| Los Angeles | 13 (27.08) | 35 (71.43) | |
| Anaheim | 35 (72.92) | 14 (28.57) | |
| Cardiorespiratory outcomes | | | |
| RHI | 1.98 ± 0.38 | 1.89 ± 0.36 | 0.22 |
| EBC MDA (nmol) | 7.86 ± 4.79 | 8.93 ± 5.38 | 0.32 |
| FeNO (ppb) | 28.10 ± 15.95 | 25.04 ± 10.06 | 0.26 |

Abbreviations: BMI, body mass index; COPD: chronic obstructive pulmonary disease; EBC: exhaled breath condensate; FeNO: exhaled nitric oxide; MDA: malondialdehyde; RHI: reactive hyperemia index.

Table 5.2. Associations of RHI with ambient daily air pollutants and 5-day air pollutant components: effect modification by dispersion-modeled local traffic emissions above and below median levels.

| | Estimated change in RHI (95% CI) | | *p* for interaction [a] | |
|---|---|---|---|---|
| | < Median NOx | ≥ Median NOx | | |
| **BC** | | | | |
| 1-day | -0.021 (-0.082, 0.039) | -0.083 (-0.137, -0.030) | 0.133 | |
| 3-day | -0.043 (-0.103, 0.017) | -0.094 (-0.155, -0.032) | 0.245 | |
| 5-day | -0.078 (-0.158, 0.001) | -0.101 (-0.180, -0.021) | 0.687 | |
| 7-day | -0.083 (-0.175, 0.009) | -0.074 (-0.170, 0.021) | 0.890 | |
| **CO** | | | | |
| 1-day | 0.000 (-0.066, 0.066) | -0.083 (-0.148, -0.018) | 0.079 | * |
| 3-day | -0.031 (-0.093, 0.031) | -0.085 (-0.151, -0.020) | 0.233 | |
| 5-day | -0.062 (-0.142, 0.018) | -0.074 (-0.156, 0.008) | 0.830 | |
| 7-day | -0.064 (-0.155, 0.028) | -0.042 (-0.136, 0.053) | 0.730 | |
| **NOx** | | | | |
| 1-day | -0.003 (-0.056, 0.051) | -0.074 (-0.126, -0.021) | 0.064 | * |
| 3-day | -0.023 (-0.074, 0.029) | -0.079 (-0.133, -0.026) | 0.128 | |
| 5-day | -0.057 (-0.128, 0.014) | -0.079 (-0.153, -0.005) | 0.650 | |
| 7-day | -0.065 (-0.149, 0.018) | -0.065 (-0.154, 0.024) | 0.996 | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | |
| $PM_{0.18}$ | 0.025 (-0.042, 0.092) | -0.034 (-0.101, 0.032) | 0.204 | |
| $PM_{0.18\text{-}2.5}$ | -0.025 (-0.113, 0.062) | -0.002 (-0.061, 0.057) | 0.679 | |
| **Carbonaceous aerosol components, 5-day** | | | | |
| $PAHs_{0.18}$ | -0.046 (-0.125, 0.033) | -0.047 (-0.110, 0.015) | 0.711 | |
| $PAHs_{0.18\text{-}2.5}$ | -0.013 (-0.081, 0.055) | -0.017 (-0.068, 0.034) | 0.782 | |
| $Hopanes_{0.18}$ | -0.035 (-0.118, 0.049) | -0.078 (-0.165, 0.008) | 0.406 | |
| $Hopanes_{0.18\text{-}2.5}$ | -0.041 (-0.120, 0.037) | -0.035 (-0.088, 0.018) | 0.885 | |
| $OC_{0.18}$ | -0.002 (-0.053, 0.048) | -0.045 (-0.104, 0.015) | 0.321 | |
| $OC_{0.18\text{-}2.5}$ | -0.037 (-0.111, 0.036) | -0.038 (-0.107, 0.030) | 0.849 | |
| $EC_{0.18}$ | -0.031 (-0.088, 0.026) | -0.041 (-0.092, 0.010) | 0.690 | |
| $EC_{0.18\text{-}2.5}$ | -0.093 (-0.178, -0.008) | -0.076 (-0.149, -0.002) | 0.613 | |
| **Oxidative potential, 5-day** | | | | |
| Total $ROS_{0.18}$ | -0.024 (-0.078, 0.030) | -0.057 (-0.121, 0.007) | 0.632 | |
| Total $ROS_{0.18\text{-}2.5}$ | -0.055 (-0.122, 0.011) | -0.108 (-0.206, -0.010) | 0.886 | |
| $DTT_{0.18}$ | -0.033 (-0.105, 0.040) | -0.074 (-0.140, -0.009) | 0.379 | |
| $DTT_{0.18\text{-}2.5}$ | 0.001 (-0.049, 0.050) | -0.003 (-0.046, 0.040) | 0.905 | |
| **Transition metals, 5-day** | | | | |
| Total $metals_{0.18}$ | -0.039 (-0.125, 0.047) | -0.088 (-0.151, -0.026) | 0.095 | * |
| Total $metals_{0.18\text{-}2.5}$ | -0.007 (-0.058, 0.044) | -0.048 (-0.088, -0.008) | 0.095 | * |
| **Electrophilic potential, 5-day** | | | | |
| GPx-$1_{0.18}$ | 0.029 (0.000, 0.058) | 0.031 (0.000, 0.062) | 0.888 | |

[a] *$p < 0.1$*

Table 5.3. Associations of EBC MDA with ambient daily air pollutants and 5-day air pollutant components: effect modification by dispersion-modeled local traffic emissions above and below median levels.

| | Estimated change in EBC MDA (95% CI) | | *p* for interaction |
|---|---|---|---|
| | < Median NOx | ≥ Median NOx | |
| **BC** | | | |
| 1-day | 0.008 (-0.041, 0.056) | 0.040 (-0.002, 0.082) | 0.319 |
| 3-day | 0.015 (-0.033, 0.063) | 0.065 (0.016, 0.114) | 0.150 |
| 5-day | 0.033 (-0.032, 0.098) | 0.078 (0.010, 0.146) | 0.331 |
| 7-day | 0.056 (-0.021, 0.132) | 0.096 (0.011, 0.181) | 0.469 |
| **CO** | | | |
| 1-day | 0.015 (-0.038, 0.067) | 0.066 (0.014, 0.118) | 0.178 |
| 3-day | 0.015 (-0.035, 0.065) | 0.066 (0.014, 0.118) | 0.163 |
| 5-day | 0.028 (-0.038, 0.093) | 0.080 (0.009, 0.151) | 0.268 |
| 7-day | 0.042 (-0.035, 0.119) | 0.094 (0.008, 0.181) | 0.345 |
| **NOx** | | | |
| 1-day | 0.011 (-0.031, 0.054) | 0.049 (0.008, 0.091) | 0.209 |
| 3-day | 0.005 (-0.037, 0.047) | 0.048 (0.007, 0.089) | 0.150 |
| 5-day | 0.024 (-0.035, 0.083) | 0.073 (0.011, 0.136) | 0.232 |
| 7-day | 0.038 (-0.032, 0.108) | 0.095 (0.015, 0.175) | 0.254 |
| **Filter-based $PM_{2.5}$, 5-day** | | | |
| $PM_{0.18}$ | 0.018 (-0.030, 0.066) | 0.044 (-0.008, 0.096) | 0.465 |
| $PM_{0.18\text{-}2.5}$ | 0.014 (-0.055, 0.082) | -0.006 (-0.063, 0.051) | 0.662 |
| **Carbonaceous aerosol components, 5-day** | | | |
| $PAHs_{0.18}$ | 0.048 (-0.017, 0.113) | 0.094 (0.019, 0.170) | 0.305 |
| $PAHs_{0.18\text{-}2.5}$ | 0.022 (-0.034, 0.078) | 0.072 (0.009, 0.136) | 0.166 |
| $Hopanes_{0.18}$ | 0.025 (-0.057, 0.107) | 0.089 (-0.004, 0.182) | 0.254 |
| $Hopanes_{0.18\text{-}2.5}$ | 0.005 (-0.054, 0.064) | 0.048 (-0.011, 0.106) | 0.249 |
| $OC_{0.18}$ | 0.014 (-0.030, 0.059) | 0.030 (-0.017, 0.077) | 0.622 |
| $OC_{0.18\text{-}2.5}$ | 0.022 (-0.037, 0.082) | 0.070 (-0.002, 0.142) | 0.276 |
| $EC_{0.18}$ | 0.036 (-0.012, 0.083) | 0.048 (-0.004, 0.101) | 0.717 |
| $EC_{0.18\text{-}2.5}$ | 0.095 (-0.017, 0.207) | -0.002 (-0.089, 0.085) | 0.179 |
| **Oxidative potential, 5-day** | | | |
| Total $ROS_{0.18}$ | 0.025 (-0.027, 0.078) | 0.015 (-0.034, 0.063) | 0.767 |
| Total $ROS_{0.18\text{-}2.5}$ | 0.088 (-0.006, 0.183) | -0.003 (-0.072, 0.066) | 0.128 |
| $DTT_{0.18}$ | 0.016 (-0.041, 0.074) | 0.020 (-0.032, 0.073) | 0.912 |
| $DTT_{0.18\text{-}2.5}$ | 0.017 (-0.022, 0.057) | 0.007 (-0.039, 0.053) | 0.725 |
| **Transition metals, 5-day** | | | |
| Total metals $_{0.18}$ | 0.039 (-0.022, 0.100) | 0.075 (0.003, 0.147) | 0.400 |
| Total metals $_{0.18\text{-}2.5}$ | -0.003 (-0.042, 0.035) | 0.033 (-0.006, 0.073) | 0.153 |
| **Electrophilic potential, 5-day** | | | |
| GPx-1 $_{0.18}$ | 0.001 (-0.016, 0.017) | 0.006 (-0.012, 0.025) | 0.660 |

Table 5.4. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components: effect modification by dispersion-modeled local traffic emissions above and below median levels.

| ` | Estimated change in FeNO (95% CI) < Median NOx | ≥ Median NOx | *p* for interaction [a] | |
|---|---|---|---|---|
| **BC** | | | | |
| 1-day | 0.039 (0.004, 0.074) | 0.068 (0.038, 0.098) | 0.211 | |
| 3-day | 0.021 (-0.013, 0.055) | 0.064 (0.029, 0.100) | 0.084 | * |
| 5-day | 0.024 (-0.021, 0.069) | 0.068 (0.021, 0.114) | 0.181 | |
| 7-day | 0.069 (0.016, 0.122) | 0.082 (0.025, 0.138) | 0.745 | |
| **CO** | | | | |
| 1-day | 0.021 (-0.017, 0.058) | 0.073 (0.037, 0.110) | 0.046 | ** |
| 3-day | 0.009 (-0.026, 0.043) | 0.053 (0.016, 0.091) | 0.088 | * |
| 5-day | 0.010 (-0.034, 0.055) | 0.050 (0.003, 0.098) | 0.223 | |
| 7-day | 0.036 (-0.015, 0.087) | 0.051 (-0.005, 0.107) | 0.684 | |
| **NOx** | | | | |
| 1-day | 0.023 (-0.007, 0.054) | 0.068 (0.039, 0.098) | 0.036 | ** |
| 3-day | 0.019 (-0.010, 0.049) | 0.044 (0.014, 0.074) | 0.255 | |
| 5-day | 0.027 (-0.013, 0.067) | 0.052 (0.009, 0.095) | 0.386 | |
| 7-day | 0.051 (0.004, 0.098) | 0.063 (0.010, 0.116) | 0.719 | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | |
| $PM_{0.18}$ | 0.038 (0.006, 0.070) | 0.010 (-0.023, 0.044) | 0.238 | |
| $PM_{0.18-2.5}$ | -0.028 (-0.071, 0.015) | -0.001 (-0.034, 0.032) | 0.327 | |
| **Carbonaceous aerosol components, 5-day** | | | | |
| $PAHs_{0.18}$ | 0.043 (-0.002, 0.087) | 0.061 (0.011, 0.111) | 0.561 | |
| $PAHs_{0.18-2.5}$ | 0.029 (-0.008, 0.066) | 0.032 (-0.010, 0.075) | 0.902 | |
| $Hopanes_{0.18}$ | 0.106 (0.051, 0.160) | 0.039 (-0.018, 0.097) | 0.078 | * |
| $Hopanes_{0.18-2.5}$ | 0.014 (-0.026, 0.055) | 0.023 (-0.015, 0.061) | 0.743 | |
| $OC_{0.18}$ | 0.013 (-0.018, 0.044) | 0.035 (0.005, 0.066) | 0.298 | |
| $OC_{0.18-2.5}$ | 0.007 (-0.035, 0.048) | 0.037 (-0.011, 0.085) | 0.319 | |
| $EC_{0.18}$ | 0.005 (-0.027, 0.038) | 0.045 (0.012, 0.079) | 0.094 | |
| $EC_{0.18-2.5}$ | 0.050 (-0.028, 0.129) | 0.073 (0.019, 0.127) | 0.640 | |
| **Oxidative potential, 5-day** | | | | |
| Total $ROS_{0.18}$ | -0.006 (-0.041, 0.030) | 0.008 (-0.022, 0.038) | 0.577 | |
| Total $ROS_{0.18-2.5}$ | 0.003 (-0.062, 0.069) | 0.028 (-0.015, 0.071) | 0.534 | |
| $DTT_{0.18}$ | 0.023 (-0.016, 0.062) | 0.010 (-0.025, 0.045) | 0.601 | |
| $DTT_{0.18-2.5}$ | 0.005 (-0.021, 0.032) | 0.021 (-0.009, 0.052) | 0.434 | |
| **Transition metals, 5-day** | | | | |
| Total metals $_{0.18}$ | 0.063 (0.022, 0.103) | 0.068 (0.021, 0.116) | 0.850 | |
| Total metals $_{0.18-2.5}$ | 0.032 (0.007, 0.057) | 0.014 (-0.012, 0.041) | 0.321 | |
| **Electrophilic potential, 5-day** | | | | |
| GPx-1 $_{0.18}$ | 0.008 (-0.004, 0.019) | -0.015 (-0.027, -0.002) | 0.009 | # |

[a] **p < 0.1, **p < 0.05, #p < 0.01*

Table 5.5. Associations of RHI with ambient daily air pollutants and 5-day air pollutant components: effect modification by dispersion-modeled local traffic emissions above and below median levels. Models were adjusted for age, sex, race, study region, and socioeconomic status.

| | Estimated change in RHI (95% CI) | | *p* for interaction [a] |
|---|---|---|---|
| | < Median NOx | ≥ Median NOx | |
| **BC** | | | |
| 1-day | -0.011 (-0.072, 0.050) | -0.077 (-0.131, -0.024) | 0.107 |
| 3-day | -0.034 (-0.094, 0.027) | -0.086 (-0.148, -0.024) | 0.228 |
| 5-day | -0.061 (-0.141, 0.020) | -0.084 (-0.165, -0.003) | 0.676 |
| 7-day | -0.063 (-0.157, 0.031) | -0.054 (-0.151, 0.042) | 0.891 |
| **CO** | | | |
| 1-day | 0.010 (-0.057, 0.076) | -0.079 (-0.144, -0.014) | 0.062 * |
| 3-day | -0.023 (-0.085, 0.040) | -0.080 (-0.145, -0.014) | 0.212 |
| 5-day | -0.047 (-0.127, 0.034) | -0.061 (-0.143, 0.022) | 0.804 |
| 7-day | -0.045 (-0.138, 0.048) | -0.025 (-0.120, 0.071) | 0.748 |
| **NOx** | | | |
| 1-day | 0.003 (-0.051, 0.057) | -0.072 (-0.125, -0.019) | 0.050 * |
| 3-day | -0.016 (-0.068, 0.036) | -0.076 (-0.129, -0.022) | 0.112 |
| 5-day | -0.043 (-0.114, 0.029) | -0.066 (-0.141, 0.008) | 0.633 |
| 7-day | -0.050 (-0.134, 0.034) | -0.050 (-0.140, 0.040) | 0.997 |
| **Filter-based $PM_{2.5}$, 5-day** | | | |
| $PM_{0.18}$ | 0.016 (-0.043, 0.076) | -0.038 (-0.100, 0.024) | 0.203 |
| $PM_{0.18\text{-}2.5}$ | -0.003 (-0.083, 0.077) | 0.015 (-0.046, 0.075) | 0.715 |
| **Carbonaceous aerosol components, 5-day** | | | |
| $PAHs_{0.18}$ | -0.026 (-0.104, 0.052) | -0.041 (-0.127, 0.044) | 0.755 |
| $PAHs_{0.18\text{-}2.5}$ | 0.008 (-0.058, 0.073) | -0.004 (-0.075, 0.068) | 0.784 |
| $Hopanes_{0.18}$ | -0.008 (-0.108, 0.092) | -0.057 (-0.161, 0.046) | 0.437 |
| $Hopanes_{0.18\text{-}2.5}$ | -0.009 (-0.080, 0.062) | -0.019 (-0.084, 0.046) | 0.817 |
| $OC_{0.18}$ | -0.010 (-0.066, 0.046) | -0.051 (-0.105, 0.002) | 0.276 |
| $OC_{0.18\text{-}2.5}$ | -0.023 (-0.096, 0.050) | -0.030 (-0.113, 0.054) | 0.899 |
| $EC_{0.18}$ | -0.025 (-0.084, 0.033) | -0.039 (-0.100, 0.021) | 0.746 |
| $EC_{0.18\text{-}2.5}$ | -0.080 (-0.211, 0.050) | -0.054 (-0.147, 0.039) | 0.736 |
| **Oxidative potential, 5-day** | | | |
| Total $ROS_{0.18}$ | -0.027 (-0.093, 0.039) | -0.047 (-0.104, 0.009) | 0.641 |
| Total $ROS_{0.18\text{-}2.5}$ | -0.056 (-0.175, 0.062) | -0.051 (-0.129, 0.027) | 0.936 |
| $DTT_{0.18}$ | -0.033 (-0.101, 0.035) | -0.070 (-0.131, -0.008) | 0.410 |
| $DTT_{0.18\text{-}2.5}$ | -0.004 (-0.052, 0.043) | -0.007 (-0.056, 0.043) | 0.943 |
| **Transition metals, 5-day** | | | |
| Total metals $_{0.18}$ | -0.037 (-0.108, 0.034) | -0.120 (-0.201, -0.040) | 0.089 * |
| Total metals $_{0.18\text{-}2.5}$ | -0.005 (-0.050, 0.040) | -0.053 (-0.100, -0.005) | 0.113 |
| **Electrophilic potential, 5-day** | | | |
| GPx-1 $_{0.18}$ | 0.021 (-0.001, 0.042) | 0.024 (0.000, 0.047) | 0.851 |

[a] **p < 0.1, **p < 0.05, #p < 0.01*

Table 5.6. Associations of EBC MDA with ambient daily air pollutants and 5-day air pollutant components: effect modification by dispersion-modeled local traffic emissions above and below median levels. Models were adjusted for age, sex, race, study region, and socioeconomic status.

| | Estimated change in EBC MDA (95% CI) | | *p* for interaction |
|---|---|---|---|
| | < Median NOx | ≥ Median NOx | |
| **BC** | | | |
| 1-day | 0.002 (-0.047, 0.050) | 0.038 (-0.004, 0.080) | 0.268 |
| 3-day | 0.010 (-0.038, 0.058) | 0.063 (0.014, 0.112) | 0.130 |
| 5-day | 0.024 (-0.041, 0.089) | 0.072 (0.004, 0.140) | 0.300 |
| 7-day | 0.046 (-0.031, 0.123) | 0.088 (0.003, 0.173) | 0.447 |
| **CO** | | | |
| 1-day | 0.009 (-0.043, 0.062) | 0.064 (0.012, 0.116) | 0.150 |
| 3-day | 0.011 (-0.039, 0.060) | 0.064 (0.011, 0.116) | 0.145 |
| 5-day | 0.020 (-0.046, 0.085) | 0.075 (0.004, 0.146) | 0.246 |
| 7-day | 0.032 (-0.045, 0.110) | 0.087 (-0.000, 0.173) | 0.332 |
| **NOx** | | | |
| 1-day | 0.008 (-0.034, 0.050) | 0.049 (0.007, 0.090) | 0.180 |
| 3-day | 0.002 (-0.040, 0.044) | 0.047 (0.006, 0.088) | 0.131 |
| 5-day | 0.016 (-0.043, 0.076) | 0.069 (0.006, 0.131) | 0.208 |
| 7-day | 0.030 (-0.040, 0.101) | 0.089 (0.009, 0.169) | 0.241 |
| **Filter-based $PM_{2.5}$, 5-day** | | | |
| $PM_{0.18}$ | 0.020 (-0.028, 0.067) | 0.048 (-0.004, 0.100) | 0.419 |
| $PM_{0.18\text{-}2.5}$ | 0.002 (-0.067, 0.071) | -0.014 (-0.071, 0.043) | 0.726 |
| **Carbonaceous aerosol components, 5-day** | | | |
| $PAHs_{0.18}$ | 0.040 (-0.025, 0.106) | 0.088 (0.012, 0.163) | 0.289 |
| $PAHs_{0.18\text{-}2.5}$ | 0.014 (-0.041, 0.070) | 0.067 (0.004, 0.130) | 0.149 |
| $Hopanes_{0.18}$ | 0.009 (-0.073, 0.092) | 0.075 (-0.019, 0.168) | 0.247 |
| $Hopanes_{0.18\text{-}2.5}$ | -0.007 (-0.066, 0.053) | 0.041 (-0.017, 0.100) | 0.198 |
| $OC_{0.18}$ | 0.018 (-0.027, 0.062) | 0.036 (-0.011, 0.082) | 0.573 |
| $OC_{0.18\text{-}2.5}$ | 0.017 (-0.042, 0.077) | 0.068 (-0.004, 0.139) | 0.253 |
| $EC_{0.18}$ | 0.032 (-0.015, 0.079) | 0.046 (-0.007, 0.098) | 0.706 |
| $EC_{0.18\text{-}2.5}$ | 0.054 (-0.061, 0.169) | -0.023 (-0.111, 0.065) | 0.288 |
| **Oxidative potential, 5-day** | | | |
| Total $ROS_{0.18}$ | 0.023 (-0.030, 0.075) | 0.014 (-0.034, 0.062) | 0.809 |
| Total $ROS_{0.18\text{-}2.5}$ | 0.061 (-0.035, 0.157) | -0.018 (-0.088, 0.052) | 0.187 |
| $DTT_{0.18}$ | 0.015 (-0.042, 0.073) | 0.020 (-0.032, 0.073) | 0.896 |
| $DTT_{0.18\text{-}2.5}$ | 0.020 (-0.019, 0.060) | 0.010 (-0.036, 0.056) | 0.732 |
| **Transition metals, 5-day** | | | |
| Total $metals_{0.18}$ | 0.041 (-0.020, 0.102) | 0.080 (0.008, 0.152) | 0.361 |
| Total $metals_{0.18\text{-}2.5}$ | -0.005 (-0.044, 0.034) | 0.033 (-0.007, 0.072) | 0.138 |
| **Electrophilic potential, 5-day** | | | |
| $GPx\text{-}1_{0.18}$ | 0.001 (-0.015, 0.018) | 0.008 (-0.011, 0.026) | 0.600 |

Table 5.7. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components: effect modification by dispersion-modeled local traffic emissions above and below median levels. Models were adjusted for age, sex, race, study region, and socioeconomic status.

| | Estimated change in FeNO (95% CI) | | *p* for interaction [a] |
|---|---|---|---|
| | < Median NOx | ≥ Median NOx | |
| **BC** | | | |
| 1-day | 0.039 (0.004, 0.074) | 0.067 (0.037, 0.097) | 0.226 |
| 3-day | 0.021 (-0.013, 0.054) | 0.063 (0.028, 0.099) | 0.088 * |
| 5-day | 0.024 (-0.020, 0.069) | 0.066 (0.020, 0.113) | 0.196 |
| 7-day | 0.069 (0.016, 0.122) | 0.080 (0.024, 0.137) | 0.775 |
| **CO** | | | |
| 1-day | 0.020 (-0.017, 0.058) | 0.072 (0.035, 0.109) | 0.049 ** |
| 3-day | 0.008 (-0.026, 0.043) | 0.052 (0.015, 0.089) | 0.093 * |
| 5-day | 0.010 (-0.034, 0.054) | 0.049 (0.001, 0.096) | 0.244 |
| 7-day | 0.036 (-0.016, 0.087) | 0.049 (-0.007, 0.105) | 0.727 |
| **NOx** | | | |
| 1-day | 0.023 (-0.008, 0.053) | 0.068 (0.038, 0.097) | 0.036 ** |
| 3-day | 0.019 (-0.011, 0.048) | 0.043 (0.013, 0.073) | 0.256 |
| 5-day | 0.027 (-0.013, 0.067) | 0.051 (0.008, 0.094) | 0.405 |
| 7-day | 0.050 (0.003, 0.098) | 0.062 (0.009, 0.115) | 0.743 |
| **Filter-based $PM_{2.5}$, 5-day** | | | |
| $PM_{0.18}$ | 0.038 (0.005, 0.070) | 0.012 (-0.022, 0.046) | 0.272 |
| $PM_{0.18\text{-}2.5}$ | -0.027 (-0.071, 0.016) | -0.001 (-0.035, 0.032) | 0.351 |
| **Carbonaceous aerosol components, 5-day** | | | |
| $PAHs_{0.18}$ | 0.043 (-0.002, 0.087) | 0.060 (0.010, 0.110) | 0.574 |
| $PAHs_{0.18\text{-}2.5}$ | 0.028 (-0.009, 0.066) | 0.031 (-0.012, 0.073) | 0.925 |
| $Hopanes_{0.18}$ | 0.107 (0.052, 0.161) | 0.037 (-0.021, 0.095) | 0.067 * |
| $Hopanes_{0.18\text{-}2.5}$ | 0.014 (-0.027, 0.055) | 0.022 (-0.016, 0.060) | 0.762 |
| $OC_{0.18}$ | 0.012 (-0.019, 0.043) | 0.037 (0.006, 0.067) | 0.262 |
| $OC_{0.18\text{-}2.5}$ | 0.006 (-0.035, 0.047) | 0.036 (-0.011, 0.084) | 0.320 |
| $EC_{0.18}$ | 0.005 (-0.027, 0.038) | 0.045 (0.012, 0.078) | 0.096 * |
| $EC_{0.18\text{-}2.5}$ | 0.058 (-0.022, 0.139) | 0.070 (0.016, 0.125) | 0.800 |
| **Oxidative potential, 5-day** | | | |
| Total $ROS_{0.18}$ | -0.006 (-0.042, 0.030) | 0.008 (-0.022, 0.038) | 0.565 |
| Total $ROS_{0.18\text{-}2.5}$ | 0.008 (-0.058, 0.075) | 0.029 (-0.015, 0.072) | 0.613 |
| $DTT_{0.18}$ | 0.023 (-0.016, 0.062) | 0.010 (-0.025, 0.044) | 0.600 |
| $DTT_{0.18\text{-}2.5}$ | 0.005 (-0.022, 0.032) | 0.021 (-0.009, 0.052) | 0.421 |
| **Transition metals, 5-day** | | | |
| Total $metals_{0.18}$ | 0.063 (0.022, 0.103) | 0.070 (0.023, 0.118) | 0.794 |
| Total $metals_{0.18\text{-}2.5}$ | 0.032 (0.007, 0.057) | 0.015 (-0.012, 0.041) | 0.330 |
| **Electrophilic potential, 5-day** | | | |
| $GPx\text{-}1_{0.18}$ | 0.007 (-0.004, 0.019) | -0.014 (-0.027, -0.002) | 0.011 ** |

[a] **p < 0.1, **p < 0.05*

Table 5.8. Associations of RHI with ambient daily air pollutants and 5-day air pollutant components. Effect modification by region.

| | Estimated change in RHI (95% CI) | | *p* for interaction [a] |
|---|---|---|---|
| | Los Angeles | Anaheim | |
| **BC** | | | |
| 1-day | -0.054 (-0.107, -0.002) | -0.035 (-0.102, 0.031) | 0.661 |
| 3-day | -0.072 (-0.146, 0.001) | -0.050 (-0.104, 0.004) | 0.629 |
| 5-day | -0.068 (-0.165, 0.030) | -0.074 (-0.146, -0.002) | 0.912 |
| 7-day | -0.008 (-0.128, 0.111) | -0.079 (-0.161, 0.003) | 0.304 |
| **CO** | | | |
| 1-day | -0.053 (-0.120, 0.014) | -0.016 (-0.081, 0.049) | 0.439 |
| 3-day | -0.067 (-0.140, 0.006) | -0.037 (-0.095, 0.021) | 0.521 |
| 5-day | -0.050 (-0.143, 0.044) | -0.053 (-0.128, 0.021) | 0.949 |
| 7-day | 0.008 (-0.098, 0.115) | -0.060 (-0.146, 0.027) | 0.306 |
| **NOx** | | | |
| 1-day | -0.043 (-0.100, 0.014) | -0.027 (-0.078, 0.024) | 0.674 |
| 3-day | -0.052 (-0.109, 0.006) | -0.039 (-0.088, 0.010) | 0.733 |
| 5-day | -0.039 (-0.122, 0.044) | -0.062 (-0.128, 0.005) | 0.658 |
| 7-day | -0.001 (-0.107, 0.104) | -0.071 (-0.147, 0.005) | 0.254 |
| **Filter-based $PM_{2.5}$, 5-day** | | | |
| $PM_{0.18}$ | -0.027 (-0.097, 0.043) | -0.001 (-0.055, 0.054) | 0.547 |
| $PM_{0.18-2.5}$ | -0.001 (-0.058, 0.056) | 0.035 (-0.061, 0.131) | 0.519 |
| **Carbonaceous aerosol components, 5-day** | | | |
| $PAHs_{0.18}$ | -0.026 (-0.121, 0.070) | -0.042 (-0.115, 0.030) | 0.758 |
| $PAHs_{0.18-2.5}$ | 0.024 (-0.047, 0.095) | -0.023 (-0.090, 0.044) | 0.263 |
| $Hopanes_{0.18}$ | -0.037 (-0.141, 0.068) | -0.033 (-0.133, 0.066) | 0.958 |
| $Hopanes_{0.18-2.5}$ | -0.004 (-0.068, 0.060) | -0.046 (-0.121, 0.029) | 0.336 |
| $OC_{0.18}$ | -0.039 (-0.099, 0.021) | -0.029 (-0.080, 0.023) | 0.785 |
| $OC_{0.18-2.5}$ | -0.010 (-0.107, 0.088) | -0.037 (-0.103, 0.029) | 0.617 |
| $EC_{0.18}$ | -0.038 (-0.104, 0.028) | -0.028 (-0.083, 0.027) | 0.823 |
| $EC_{0.18-2.5}$ | -0.055 (-0.144, 0.033) | -0.113 (-0.343, 0.117) | 0.658 |
| **Oxidative potential, 5-day** | | | |
| Total $ROS_{0.18}$ | -0.044 (-0.100, 0.012) | -0.028 (-0.095, 0.039) | 0.715 |
| Total $ROS_{0.18-2.5}$ | -0.059 (-0.131, 0.013) | -0.020 (-0.247, 0.206) | 0.755 |
| $DTT_{0.18}$ | -0.059 (-0.122, 0.003) | -0.045 (-0.113, 0.023) | 0.753 |
| $DTT_{0.18-2.5}$ | 0.004 (-0.053, 0.061) | -0.011 (-0.054, 0.032) | 0.673 |
| **Transition metals, 5-day** | | | |
| Total metals $_{0.18}$ | -0.107 (-0.205, -0.008) | -0.065 (-0.130, -0.001) | 0.437 |
| Total metals $_{0.18-2.5}$ | -0.031 (-0.078, 0.016) | -0.025 (-0.071, 0.021) | 0.844 |
| **Electrophilic potential, 5-day** | | | |
| GPx-1 $_{0.18}$ | 0.008 (0.002, 0.014) | 0.002 (-0.000, 0.004) | 0.075 * |

[a] *$p < 0.1$*

Table 5.9. Associations of EBC MDA with ambient daily air pollutants and 5-day air pollutant components. Effect modification by region.

| ` | | Estimated change in EBC MDA (95% CI) | | *p* for interaction [a] | |
|---|---|---|---|---|---|
| | | Los Angeles | Anaheim | | |
| **BC** | | | | | |
| | 1-day | 0.001 (-0.039, 0.041) | 0.065 (0.009, 0.120) | 0.074 | * |
| | 3-day | 0.002 (-0.054, 0.058) | 0.056 (0.013, 0.100) | 0.130 | |
| | 5-day | 0.002 (-0.080, 0.084) | 0.068 (0.010, 0.125) | 0.186 | |
| | 7-day | 0.031 (-0.074, 0.136) | 0.078 (0.009, 0.146) | 0.440 | |
| **CO** | | | | | |
| | 1-day | 0.019 (-0.032, 0.070) | 0.059 (0.004, 0.113) | 0.293 | |
| | 3-day | 0.004 (-0.052, 0.060) | 0.058 (0.011, 0.105) | 0.140 | |
| | 5-day | 0.003 (-0.078, 0.083) | 0.068 (0.007, 0.129) | 0.191 | |
| | 7-day | 0.021 (-0.075, 0.117) | 0.076 (0.003, 0.148) | 0.349 | |
| **NOx** | | | | | |
| | 1-day | 0.007 (-0.035, 0.049) | 0.052 (0.009, 0.095) | 0.144 | |
| | 3-day | -0.010 (-0.053, 0.033) | 0.054 (0.014, 0.094) | 0.031 | ** |
| | 5-day | -0.007 (-0.077, 0.063) | 0.069 (0.015, 0.124) | 0.076 | * |
| | 7-day | 0.013 (-0.079, 0.104) | 0.075 (0.010, 0.139) | 0.242 | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | | |
| | $PM_{0.18}$ | 0.018 (-0.041, 0.077) | 0.040 (-0.004, 0.084) | 0.075 | * |
| | $PM_{0.18\text{-}2.5}$ | -0.021 (-0.074, 0.032) | 0.025 (-0.056, 0.105) | 0.550 | |
| **Carbonaceous aerosol components, 5-day** | | | | | |
| | $PAHs_{0.18}$ | 0.035 (-0.050, 0.120) | 0.071 (0.010, 0.132) | 0.023 | ** |
| | $PAHs_{0.18\text{-}2.5}$ | 0.001 (-0.063, 0.066) | 0.061 (0.005, 0.118) | 0.034 | ** |
| | $Hopanes_{0.18}$ | 0.029 (-0.062, 0.119) | 0.046 (-0.039, 0.131) | 0.286 | |
| | $Hopanes_{0.18\text{-}2.5}$ | -0.010 (-0.068, 0.047) | 0.052 (-0.010, 0.114) | 0.098 | * |
| | $OC_{0.18}$ | 0.013 (-0.038, 0.065) | 0.034 (-0.007, 0.076) | 0.103 | |
| | $OC_{0.18\text{-}2.5}$ | 0.006 (-0.079, 0.090) | 0.049 (-0.007, 0.104) | 0.084 | * |
| | $EC_{0.18}$ | 0.024 (-0.031, 0.079) | 0.048 (0.002, 0.094) | 0.039 | ** |
| | $EC_{0.18\text{-}2.5}$ | -0.007 (-0.086, 0.072) | 0.072 (-0.126, 0.271) | 0.476 | |
| **Oxidative potential, 5-day** | | | | | |
| | Total $ROS_{0.18}$ | 0.018 (-0.031, 0.067) | 0.018 (-0.035, 0.071) | 0.500 | |
| | Total $ROS_{0.18\text{-}2.5}$ | -0.007 (-0.068, 0.054) | 0.149 (-0.037, 0.336) | 0.117 | |
| | $DTT_{0.18}$ | -0.005 (-0.057, 0.048) | 0.046 (-0.012, 0.104) | 0.119 | |
| | $DTT_{0.18\text{-}2.5}$ | -0.012 (-0.065, 0.040) | 0.028 (-0.008, 0.065) | 0.128 | |
| **Transition metals, 5-day** | | | | | |
| | Total metals $_{0.18}$ | 0.019 (-0.066, 0.104) | 0.070 (0.014, 0.126) | 0.015 | ** |
| | Total metals $_{0.18\text{-}2.5}$ | -0.008 (-0.047, 0.030) | 0.038 (-0.002, 0.078) | 0.063 | * |
| **Electrophilic potential, 5-day** | | | | | |
| | GPx-1 $_{0.18}$ | -0.012 (-0.053, 0.029) | 0.005 (-0.008, 0.018) | 0.415 | |

[a] **p < 0.1, **p < 0.05*

Table 5.10. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components. Effect modification by region.

| | Estimated change in FeNO (95% CI) | | *p* for interaction [a] | |
|---|---|---|---|---|
| | Los Angles | Anaheim | | |
| **BC** | | | | |
| 1-day | 0.051 (0.023, 0.080) | 0.066 (0.026, 0.105) | 0.562 | |
| 3-day | 0.060 (0.019, 0.100) | 0.030 (-0.000, 0.061) | 0.262 | |
| 5-day | 0.065 (0.011, 0.120) | 0.033 (-0.007, 0.073) | 0.349 | |
| 7-day | 0.065 (-0.001, 0.132) | 0.080 (0.032, 0.127) | 0.717 | |
| **CO** | | | | |
| 1-day | 0.060 (0.023, 0.098) | 0.034 (-0.003, 0.071) | 0.321 | |
| 3-day | 0.046 (0.005, 0.086) | 0.018 (-0.015, 0.051) | 0.302 | |
| 5-day | 0.052 (-0.002, 0.105) | 0.014 (-0.027, 0.055) | 0.271 | |
| 7-day | 0.043 (-0.018, 0.105) | 0.043 (-0.006, 0.091) | 0.984 | |
| **NOx** | | | | |
| 1-day | 0.051 (0.021, 0.082) | 0.042 (0.012, 0.072) | 0.658 | |
| 3-day | 0.029 (-0.002, 0.061) | 0.033 (0.005, 0.062) | 0.842 | |
| 5-day | 0.042 (-0.005, 0.089) | 0.036 (-0.001, 0.074) | 0.850 | |
| 7-day | 0.048 (-0.011, 0.107) | 0.061 (0.017, 0.105) | 0.720 | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | |
| $PM_{0.18}$ | 0.009 (-0.028, 0.047) | 0.035 (0.005, 0.065) | 0.282 | |
| $PM_{0.18\text{-}2.5}$ | -0.016 (-0.047, 0.016) | -0.001 (-0.052, 0.050) | 0.636 | |
| **Carbonaceous aerosol components, 5-day** | | | | |
| $PAHs_{0.18}$ | 0.049 (-0.007, 0.105) | 0.052 (0.010, 0.093) | 0.937 | |
| $PAHs_{0.18\text{-}2.5}$ | 0.021 (-0.022, 0.063) | 0.038 (0.001, 0.076) | 0.507 | |
| $Hopanes_{0.18}$ | 0.047 (-0.010, 0.104) | 0.103 (0.048, 0.159) | 0.132 | |
| $Hopanes_{0.18\text{-}2.5}$ | 0.014 (-0.023, 0.052) | 0.025 (-0.017, 0.068) | 0.683 | |
| $OC_{0.18}$ | 0.034 (0.001, 0.067) | 0.017 (-0.011, 0.046) | 0.450 | |
| $OC_{0.18\text{-}2.5}$ | 0.005 (-0.050, 0.061) | 0.026 (-0.011, 0.064) | 0.516 | |
| $EC_{0.18}$ | 0.043 (0.007, 0.079) | 0.011 (-0.021, 0.042) | 0.181 | |
| $EC_{0.18\text{-}2.5}$ | 0.054 (0.004, 0.104) | 0.166 (0.033, 0.299) | 0.130 | |
| **Oxidative potential, 5-day** | | | | |
| Total $ROS_{0.18}$ | 0.005 (-0.025, 0.035) | -0.003 (-0.039, 0.033) | 0.734 | |
| Total $ROS_{0.18\text{-}2.5}$ | 0.010 (-0.029, 0.049) | 0.114 (-0.010, 0.238) | 0.121 | |
| $DTT_{0.18}$ | -0.008 (-0.042, 0.027) | 0.050 (0.010, 0.090) | 0.029 | ** |
| $DTT_{0.18\text{-}2.5}$ | 0.021 (-0.016, 0.057) | 0.009 (-0.015, 0.033) | 0.574 | |
| **Transition metals, 5-day** | | | | |
| Total $metals_{0.18}$ | 0.057 (0.004, 0.111) | 0.069 (0.032, 0.107) | 0.703 | |
| Total $metals_{0.18\text{-}2.5}$ | 0.002 (-0.024, 0.027) | 0.049 (0.023, 0.075) | 0.007 | # |
| **Electrophilic potential, 5-day** | | | | |
| GPx-$1_{0.18}$ | -0.031 (-0.056, -0.005) | 0.000 (-0.009, 0.009) | 0.026 | ** |

[a] **p < 0.1, **p < 0.05, #p < 0.01*

Table 5.11. Associations of RHI with ambient daily air pollutants and 5-day air pollutant components. Effect modification by race/ethnicity.

| | Estimated change in RHI (95% CI) | | *p* for interaction [a] |
|---|---|---|---|
| | Non-Hispanic Whites | Others | |
| **BC** | | | |
| 1-day | -0.085 (-0.133, -0.036) | 0.003 (-0.067, 0.074) | 0.930 |
| 3-day | -0.089 (-0.142, -0.036) | -0.029 (-0.101, 0.043) | 0.428 |
| 5-day | -0.118 (-0.188, -0.048) | -0.039 (-0.134, 0.055) | 0.412 |
| 7-day | -0.110 (-0.194, -0.026) | -0.029 (-0.137, 0.079) | 0.599 |
| **CO** | | | |
| 1-day | -0.078 (-0.135, -0.021) | 0.024 (-0.055, 0.102) | 0.554 |
| 3-day | -0.080 (-0.136, -0.024) | -0.017 (-0.091, 0.057) | 0.644 |
| 5-day | -0.099 (-0.171, -0.027) | -0.015 (-0.109, 0.080) | 0.761 |
| 7-day | -0.083 (-0.168, 0.001) | -0.004 (-0.111, 0.102) | 0.937 |
| **NOx** | | | |
| 1-day | -0.064 (-0.110, -0.018) | 0.009 (-0.055, 0.073) | 0.772 |
| 3-day | -0.068 (-0.113, -0.022) | -0.018 (-0.080, 0.045) | 0.574 |
| 5-day | -0.097 (-0.161, -0.032) | -0.018 (-0.102, 0.065) | 0.666 |
| 7-day | -0.098 (-0.177, -0.019) | -0.015 (-0.111, 0.082) | 0.767 |
| **Filter-based $PM_{2.5}$, 5-day** | | | |
| $PM_{0.18}$ | -0.032 (-0.087, 0.023) | 0.044 (-0.025, 0.113) | 0.082 * |
| $PM_{0.18\text{-}2.5}$ | 0.007 (-0.048, 0.062) | -0.067 (-0.158, 0.025) | 0.171 |
| **Carbonaceous aerosol components, 5-day** | | | |
| $PAHs_{0.18}$ | -0.097 (-0.172, -0.021) | 0.004 (-0.083, 0.091) | 0.049 ** |
| $PAHs_{0.18\text{-}2.5}$ | -0.048 (-0.112, 0.016) | 0.023 (-0.050, 0.097) | 0.089 * |
| $Hopanes_{0.18}$ | -0.105 (-0.197, -0.013) | -0.012 (-0.121, 0.096) | 0.140 |
| $Hopanes_{0.18\text{-}2.5}$ | -0.066 (-0.126, -0.007) | 0.009 (-0.070, 0.087) | 0.091 * |
| $OC_{0.18}$ | -0.047 (-0.095, 0.001) | 0.030 (-0.034, 0.094) | 0.050 * |
| $OC_{0.18\text{-}2.5}$ | -0.069 (-0.142, 0.003) | -0.005 (-0.087, 0.078) | 0.208 |
| $EC_{0.18}$ | -0.071 (-0.124, -0.018) | 0.007 (-0.059, 0.074) | 0.069 * |
| $EC_{0.18\text{-}2.5}$ | -0.110 (-0.192, -0.027) | -0.115 (-0.248, 0.017) | 0.942 * |
| **Oxidative potential, 5-day** | | | |
| Total $ROS_{0.18}$ | -0.053 (-0.104, -0.002) | -0.018 (-0.096, 0.060) | 0.471 |
| Total $ROS_{0.18\text{-}2.5}$ | -0.097 (-0.167, -0.028) | -0.066 (-0.193, 0.062) | 0.668 |
| $DTT_{0.18}$ | -0.075 (-0.131, -0.019) | -0.008 (-0.087, 0.072) | 0.158 |
| $DTT_{0.18\text{-}2.5}$ | 0.003 (-0.040, 0.047) | -0.007 (-0.061, 0.048) | 0.767 |
| **Transition metals, 5-day** | | | |
| Total metals $_{0.18}$ | -0.112 (-0.182, -0.041) | -0.002 (-0.084, 0.081) | 0.027 ** |
| Total metals $_{0.18\text{-}2.5}$ | -0.048 (-0.090, -0.006) | -0.001 (-0.053, 0.051) | 0.844 |
| **Electrophilic potential, 5-day** | | | |
| GPx-1 $_{0.18}$ | 0.003 (0.000, 0.005) | 0.003 (-0.000, 0.006) | 0.876 |

[a] **p < 0.1, **p < 0.05*

Table 5.12. Associations of EBC MDA with ambient daily air pollutants and 5-day air pollutant components. Effect modification by race/ethnicity.

| | Estimated change in EBC (95% CI) | | *p* for interaction [a] | |
|---|---|---|---|---|
| | Non-Hispanic Whites | Others | | |
| **BC** | | | | |
| 1-day | 0.010 (-0.027, 0.047) | 0.069 (0.009, 0.130) | 0.102 | |
| 3-day | 0.024 (-0.018, 0.065) | 0.074 (0.014, 0.134) | 0.174 | |
| 5-day | 0.035 (-0.023, 0.093) | 0.095 (0.015, 0.175) | 0.222 | |
| 7-day | 0.055 (-0.016, 0.127) | 0.110 (0.015, 0.204) | 0.342 | |
| **CO** | | | | |
| 1-day | 0.018 (-0.026, 0.063) | 0.087 (0.022, 0.153) | 0.088 | * |
| 3-day | 0.021 (-0.023, 0.065) | 0.074 (0.012, 0.136) | 0.171 | |
| 5-day | 0.031 (-0.029, 0.091) | 0.090 (0.010, 0.171) | 0.235 | |
| 7-day | 0.046 (-0.027, 0.119) | 0.099 (0.005, 0.193) | 0.357 | |
| **NOx** | | | | |
| 1-day | 0.010 (-0.025, 0.046) | 0.075 (0.022, 0.128) | 0.048 | ** |
| 3-day | 0.011 (-0.024, 0.046) | 0.065 (0.011, 0.118) | 0.094 | * |
| 5-day | 0.026 (-0.028, 0.079) | 0.091 (0.018, 0.163) | 0.139 | |
| 7-day | 0.039 (-0.029, 0.106) | 0.103 (0.018, 0.188) | 0.211 | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | |
| $PM_{0.18}$ | 0.009 (-0.035, 0.053) | 0.064 (0.006, 0.122) | 0.130 | |
| $PM_{0.18\text{-}2.5}$ | -0.010 (-0.062, 0.042) | 0.035 (-0.045, 0.115) | 0.351 | |
| **Carbonaceous aerosol components, 5-day** | | | | |
| $PAHs_{0.18}$ | 0.047 (-0.019, 0.112) | 0.097 (0.022, 0.173) | 0.267 | |
| $PAHs_{0.18\text{-}2.5}$ | 0.026 (-0.030, 0.082) | 0.069 (0.004, 0.135) | 0.258 | |
| $Hopanes_{0.18}$ | 0.049 (-0.032, 0.131) | 0.058 (-0.037, 0.154) | 0.873 | |
| $Hopanes_{0.18\text{-}2.5}$ | 0.012 (-0.041, 0.064) | 0.062 (-0.009, 0.133) | 0.218 | |
| $OC_{0.18}$ | 0.008 (-0.033, 0.049) | 0.044 (-0.009, 0.098) | 0.270 | |
| $OC_{0.18\text{-}2.5}$ | 0.020 (-0.041, 0.081) | 0.074 (0.002, 0.145) | 0.228 | |
| $EC_{0.18}$ | 0.028 (-0.016, 0.073) | 0.063 (0.007, 0.119) | 0.334 | |
| $EC_{0.18\text{-}2.5}$ | 0.034 (-0.045, 0.113) | 0.050 (-0.089, 0.189) | 0.842 | |
| **Oxidative potential, 5-day** | | | | |
| Total $ROS_{0.18}$ | 0.012 (-0.030, 0.054) | 0.039 (-0.027, 0.105) | 0.509 | |
| Total $ROS_{0.18\text{-}2.5}$ | 0.021 (-0.042, 0.084) | 0.069 (-0.052, 0.190) | 0.489 | |
| $DTT_{0.18}$ | -0.000 (-0.048, 0.047) | 0.061 (-0.008, 0.129) | 0.138 | |
| $DTT_{0.18\text{-}2.5}$ | -0.011 (-0.049, 0.027) | 0.050 (0.003, 0.096) | 0.042 | ** |
| **Transition metals, 5-day** | | | | |
| Total metals $_{0.18}$ | 0.033 (-0.028, 0.095) | 0.081 (0.009, 0.153) | 0.278 | |
| Total metals $_{0.18\text{-}2.5}$ | 0.007 (-0.028, 0.042) | 0.029 (-0.017, 0.076) | 0.421 | |
| **Electrophilic potential, 5-day** | | | | |
| GPx-1 $_{0.18}$ | -0.004 (-0.020, 0.011) | 0.016 (-0.004, 0.036) | 0.109 | |

[a] **p < 0.1, **p < 0.05*

Table 5.13. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components. Effect modification by race/ethnicity.

| | Estimated change in FeNO (95% CI) | | *p* for interaction [a] | |
|---|---|---|---|---|
| | Non-Hispanic Whites | Others | | |
| **BC** | | | | |
| 1-day | 0.056 (0.028, 0.083) | 0.055 (0.014, 0.097) | 0.991 | |
| 3-day | 0.046 (0.016, 0.076) | 0.031 (-0.011, 0.074) | 0.590 | |
| 5-day | 0.049 (0.009, 0.089) | 0.033 (-0.022, 0.089) | 0.646 | |
| 7-day | 0.067 (0.019, 0.115) | 0.086 (0.022, 0.151) | 0.634 | |
| **CO** ` | | | | |
| 1-day | 0.061 (0.028, 0.093) | 0.020 (-0.025, 0.065) | 0.150 | |
| 3-day | 0.041 (0.010, 0.073) | 0.006 (-0.037, 0.049) | 0.198 | |
| 5-day | 0.042 (0.001, 0.082) | 0.003 (-0.051, 0.057) | 0.258 | |
| 7-day | 0.048 (0.000, 0.096) | 0.031 (-0.032, 0.094) | 0.665 | |
| **NOx** | | | | |
| 1-day | 0.054 (0.028, 0.080) | 0.030 (-0.007, 0.068) | 0.310 | |
| 3-day | 0.036 (0.010, 0.061) | 0.021 (-0.016, 0.058) | 0.511 | |
| 5-day | 0.044 (0.008, 0.081) | 0.026 (-0.024, 0.075) | 0.550 | |
| 7-day | 0.058 (0.013, 0.102) | 0.051 (-0.007, 0.108) | 0.841 | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | |
| $PM_{0.18}$ | 0.026 (-0.003, 0.056) | 0.022 (-0.017, 0.060) | 0.840 | |
| $PM_{0.18\text{-}2.5}$ | -0.007 (-0.038, 0.024) | -0.027 (-0.078, 0.024) | 0.508 | |
| **Carbonaceous aerosol components, 5-day** | | | | |
| $PAHs_{0.18}$ | 0.046 (0.003, 0.090) | 0.057 (0.005, 0.109) | 0.741 | |
| $PAHs_{0.18\text{-}2.5}$ | 0.024 (-0.013, 0.061) | 0.040 (-0.004, 0.083) | 0.548 | |
| $Hopanes_{0.18}$ | 0.046 (-0.005, 0.097) | 0.119 (0.056, 0.182) | 0.054 | * |
| $Hopanes_{0.18\text{-}2.5}$ | 0.012 (-0.023, 0.046) | 0.033 (-0.015, 0.081) | 0.445 | |
| $OC_{0.18}$ | 0.030 (0.003, 0.057) | 0.015 (-0.022, 0.052) | 0.501 | |
| $OC_{0.18\text{-}2.5}$ | 0.022 (-0.018, 0.063) | 0.014 (-0.035, 0.063) | 0.798 | |
| $EC_{0.18}$ | 0.038 (0.009, 0.067) | -0.000 (-0.039, 0.039) | 0.122 | |
| $EC_{0.18\text{-}2.5}$ | 0.070 (0.019, 0.121) | 0.033 (-0.056, 0.123) | 0.480 | |
| **Oxidative potential, 5-day** | | | | |
| Total $ROS_{0.18}$ | 0.011 (-0.016, 0.038) | -0.021 (-0.065, 0.022) | 0.213 | |
| Total $ROS_{0.18\text{-}2.5}$ | 0.022 (-0.019, 0.062) | 0.004 (-0.073, 0.082) | 0.699 | |
| $DTT_{0.18}$ | 0.016 (-0.015, 0.048) | 0.014 (-0.031, 0.060) | 0.944 | |
| $DTT_{0.18\text{-}2.5}$ | 0.014 (-0.012, 0.040) | 0.010 (-0.022, 0.042) | 0.837 | |
| **Transition metals, 5-day** | | | | |
| Total metals $_{0.18}$ | 0.068 (0.027, 0.108) | 0.062 (0.014, 0.110) | 0.845 | |
| Total metals $_{0.18\text{-}2.5}$ | 0.021 (-0.002, 0.044) | 0.028 (-0.002, 0.058) | 0.710 | |
| **Electrophilic potential, 5-day** | | | | |
| GPx-1 $_{0.18}$ | -0.004 (-0.015, 0.006) | -0.000 (-0.014, 0.014) | 0.624 | |

[a] **p < 0.1*

Table 5.14. Associations of RHI with ambient daily air pollutants and 5-day air pollutant components, modifying by dispersion-modeled local traffic emissions above and below median levels: stratified by sex.

| | Female | | Male | | *p* for interaction [a] | |
|---|---|---|---|---|---|---|
| | Estimates (95% CI) < median NOx (n=43) | Estimates (95% CI) ≥ median NOx (n=29) | Estimates (95% CI) < median NOx (n=5) | Estimates (95% CI) ≥ median NOx (n=20) | | |
| **BC** | | | | | | |
| 1-day | -0.048 (-0.115, 0.019) | -0.057 (-0.125, 0.011) | 0.042 (-0.096, 0.180) | -0.048 (-0.115, 0.019) | 0.025 | ** |
| 3-day | -0.064 (-0.133, 0.005) | -0.073 (-0.151, 0.005) | -0.014 (-0.138, 0.111) | -0.064 (-0.133, 0.005) | 0.058 | * |
| 5-day | -0.086 (-0.174, 0.002) | -0.068 (-0.166, 0.029) | -0.025 (-0.187, 0.137) | -0.086 (-0.174, 0.002) | 0.158 | |
| 7-day | -0.084 (-0.184, 0.016) | -0.036 (-0.148, 0.077) | -0.007 (-0.191, 0.177) | -0.084 (-0.184, 0.016) | 0.202 | |
| **CO** | | | | | | |
| 1-day | -0.021 (-0.095, 0.052) | -0.056 (-0.137, 0.026) | 0.037 (-0.101, 0.176) | -0.021 (-0.095, 0.052) | 0.039 | ** |
| 3-day | -0.043 (-0.112, 0.026) | -0.057 (-0.139, 0.025) | -0.011 (-0.142, 0.120) | -0.043 (-0.112, 0.026) | 0.087 | * |
| 5-day | -0.061 (-0.147, 0.025) | -0.038 (-0.137, 0.062) | -0.027 (-0.189, 0.136) | -0.061 (-0.147, 0.025) | 0.226 | |
| 7-day | -0.058 (-0.155, 0.039) | 0.000 (-0.112, 0.112) | -0.016 (-0.201, 0.170) | -0.058 (-0.155, 0.039) | 0.252 | |
| **NOx** | | | | | | |
| 1-day | -0.027 (-0.089, 0.035) | -0.053 (-0.121, 0.015) | 0.030 (-0.084, 0.144) | -0.027 (-0.089, 0.035) | 0.045 | ** |
| 3-day | -0.043 (-0.103, 0.018) | -0.065 (-0.135, 0.005) | -0.009 (-0.120, 0.103) | -0.043 (-0.103, 0.018) | 0.105 | |
| 5-day | -0.058 (-0.137, 0.021) | -0.047 (-0.138, 0.044) | -0.024 (-0.172, 0.124) | -0.058 (-0.137, 0.021) | 0.251 | |
| 7-day | -0.060 (-0.150, 0.029) | -0.024 (-0.130, 0.082) | -0.012 (-0.183, 0.159) | -0.060 (-0.150, 0.029) | 0.283 | |
| **Filter-based $PM_{2.5}$, 5-day** | | | | | | |
| $PM_{0.18}$ | 0.018 (-0.046, 0.081) | -0.001 (-0.076, 0.074) | 0.028 (-0.089, 0.146) | 0.018 (-0.046, 0.081) | 0.212 | |
| $PM_{0.18\text{-}2.5}$ | -0.032 (-0.112, 0.048) | 0.001 (-0.076, 0.079) | 0.057 (-0.135, 0.248) | -0.032 (-0.112, 0.048) | 0.283 | |
| **Carbonaceous aerosol components, 5-day** | | | | | | |
| $PAHs_{0.18}$ | -0.048 (-0.130, 0.034) | -0.027 (-0.128, 0.074) | -0.014 (-0.176, 0.148) | -0.048 (-0.130, 0.034) | 0.293 | |
| $PAHs_{0.18\text{-}2.5}$ | -0.012 (-0.080, 0.056) | 0.012 (-0.074, 0.099) | 0.014 (-0.128, 0.156) | -0.012 (-0.080, 0.056) | 0.297 | |
| $Hopanes_{0.18}$ | -0.045 (-0.146, 0.055) | -0.049 (-0.175, 0.077) | 0.030 (-0.211, 0.271) | -0.045 (-0.146, 0.055) | 0.267 | |
| $Hopanes_{0.18\text{-}2.5}$ | -0.047 (-0.121, 0.026) | -0.016 (-0.095, 0.064) | 0.055 (-0.098, 0.207) | -0.047 (-0.121, 0.026) | 0.118 | |
| $OC_{0.18}$ | -0.000 (-0.059, 0.058) | -0.018 (-0.083, 0.047) | -0.003 (-0.105, 0.099) | -0.000 (-0.059, 0.058) | 0.366 | |
| $OC_{0.18\text{-}2.5}$ | -0.048 (-0.125, 0.029) | -0.003 (-0.101, 0.095) | 0.019 (-0.128, 0.167) | -0.048 (-0.125, 0.029) | 0.072 | * |
| $EC_{0.18}$ | -0.032 (-0.095, 0.030) | -0.021 (-0.096, 0.054) | -0.025 (-0.159, 0.108) | -0.032 (-0.095, 0.030) | 0.373 | |
| $EC_{0.18\text{-}2.5}$ | -0.141 (-0.264, -0.017) | -0.035 (-0.147, 0.077) | -0.014 (-0.358, 0.330) | -0.141 (-0.264, -0.017) | 0.183 | |
| **Oxidative potential, 5-day** | | | | | | |
| Total $ROS_{0.18}$ | -0.027 (-0.098, 0.043) | -0.027 (-0.099, 0.045) | -0.053 (-0.209, 0.102) | -0.027 (-0.098, 0.043) | 0.714 | |
| Total $ROS_{0.18\text{-}2.5}$ | -0.101 (-0.213, 0.012) | -0.060 (-0.158, 0.038) | -0.028 (-0.389, 0.333) | -0.101 (-0.213, 0.012) | 0.559 | |
| $DTT_{0.18}$ | -0.033 (-0.105, 0.039) | -0.048 (-0.124, 0.029) | -0.033 (-0.185, 0.119) | -0.033 (-0.105, 0.039) | 0.544 | |
| $DTT_{0.18\text{-}2.5}$ | -0.006 (-0.055, 0.044) | 0.013 (-0.048, 0.074) | 0.035 (-0.060, 0.129) | -0.006 (-0.055, 0.044) | 0.113 | |
| **Transition metals, 5-day** | | | | | | |
| Total metals $_{0.18}$ | -0.035 (-0.111, 0.041) | -0.077 (-0.173, 0.019) | -0.023 (-0.176, 0.129) | -0.035 (-0.111, 0.041) | 0.342 | |
| Total metals $_{0.18\text{-}2.5}$ | -0.012 (-0.060, 0.035) | -0.021 (-0.081, 0.039) | 0.024 (-0.079, 0.127) | -0.012 (-0.060, 0.035) | 0.082 | * |
| **Electrophilic potential, 5-day** | | | | | | |
| $GPx\text{-}1_{0.18}$ | 0.016 (-0.009, 0.041) | 0.029 (0.002, 0.056) | 0.033 (-0.003, 0.070) | 0.016 (-0.009, 0.041) | 0.367 | |

[a] *p < 0.1, **p < 0.05, the p-value for interaction is testing the null hypothesis that effect modification by CALINE4 classification is the same across demographic strata.

Table 5.15. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components, modifying by dispersion-modeled local traffic emissions above and below median levels: stratified by age

| | Age ≤ 75 years | | Age >75 years | | *p* for interaction [a] |
|---|---|---|---|---|---|
| | Estimates (95% CI) < median NOx (n=34) | Estimates (95% CI) ≥ median NOx (n=23) | Estimates (95% CI) < median NOx (n=14) | Estimates (95% CI) ≥ median NOx (n=26) | |
| **BC** | | | | | |
| 1-day | 0.023 (-0.020, 0.067) | 0.104 (0.055, 0.154) | 0.044 (-0.012, 0.101) | 0.023 (-0.020, 0.067) | 0.050 * |
| 3-day | 0.010 (-0.032, 0.052) | 0.073 (0.018, 0.127) | 0.015 (-0.042, 0.071) | 0.010 (-0.032, 0.052) | 0.387 |
| 5-day | 0.010 (-0.043, 0.064) | 0.069 (0.004, 0.134) | 0.022 (-0.053, 0.097) | 0.010 (-0.043, 0.064) | 0.564 |
| 7-day | 0.054 (-0.008, 0.117) | 0.076 (0.002, 0.151) | 0.066 (-0.024, 0.155) | 0.054 (-0.008, 0.117) | 0.825 |
| **CO** | | | | | |
| 1-day | 0.006 (-0.040, 0.051) | 0.106 (0.052, 0.160) | 0.027 (-0.034, 0.088) | 0.006 (-0.040, 0.051) | 0.049 ** |
| 3-day | 0.001 (-0.041, 0.043) | 0.064 (0.008, 0.119) | -0.002 (-0.060, 0.056) | 0.001 (-0.041, 0.043) | 0.363 |
| 5-day | 0.001 (-0.051, 0.054) | 0.060 (-0.006, 0.126) | 0.004 (-0.070, 0.078) | 0.001 (-0.051, 0.054) | 0.391 |
| 7-day | 0.031 (-0.028, 0.090) | 0.066 (-0.007, 0.139) | 0.022 (-0.065, 0.109) | 0.031 (-0.028, 0.090) | 0.537 |
| **NOx** | | | | | |
| 1-day | 0.006 (-0.033, 0.044) | 0.098 (0.053, 0.143) | 0.036 (-0.014, 0.086) | 0.006 (-0.033, 0.044) | 0.021 ** |
| 3-day | 0.005 (-0.032, 0.043) | 0.049 (0.002, 0.096) | 0.017 (-0.033, 0.066) | 0.005 (-0.032, 0.043) | 0.297 |
| 5-day | 0.006 (-0.043, 0.055) | 0.060 (-0.004, 0.124) | 0.033 (-0.034, 0.099) | 0.006 (-0.043, 0.055) | 0.248 |
| 7-day | 0.033 (-0.023, 0.089) | 0.077 (0.005, 0.150) | 0.051 (-0.028, 0.130) | 0.033 (-0.023, 0.089) | 0.327 |
| **Filter-based $PM_{2.5}$, 5-day** | | | | | |
| $PM_{0.18}$ | 0.043 (0.006, 0.081) | 0.005 (-0.042, 0.051) | 0.026 (-0.029, 0.081) | 0.043 (0.006, 0.081) | 0.479 |
| $PM_{0.18\text{-}2.5}$ | -0.019 (-0.066, 0.028) | 0.020 (-0.023, 0.062) | -0.056 (-0.144, 0.032) | -0.019 (-0.066, 0.028) | 0.869 |
| **Carbonaceous aerosol components, 5-day** | | | | | |
| $PAHs_{0.18}$ | 0.037 (-0.015, 0.089) | 0.083 (0.013, 0.154) | 0.053 (-0.021, 0.127) | 0.037 (-0.015, 0.089) | 0.366 |
| $PAHs_{0.18\text{-}2.5}$ | 0.023 (-0.021, 0.068) | 0.057 (-0.008, 0.121) | 0.037 (-0.023, 0.097) | 0.023 (-0.021, 0.068) | 0.305 |
| $Hopanes_{0.18}$ | 0.116 (0.053, 0.179) | 0.028 (-0.054, 0.110) | 0.083 (-0.010, 0.176) | 0.116 (0.053, 0.179) | 0.487 |
| $Hopanes_{0.18\text{-}2.5}$ | 0.008 (-0.041, 0.056) | 0.036 (-0.018, 0.091) | 0.026 (-0.040, 0.093) | 0.008 (-0.041, 0.056) | 0.428 |
| $OC_{0.18}$ | 0.009 (-0.027, 0.045) | 0.039 (-0.003, 0.081) | 0.019 (-0.031, 0.070) | 0.009 (-0.027, 0.045) | 0.662 |
| $OC_{0.18\text{-}2.5}$ | 0.005 (-0.044, 0.053) | 0.060 (-0.009, 0.128) | 0.009 (-0.059, 0.078) | 0.005 (-0.044, 0.053) | 0.440 |
| $EC_{0.18}$ | 0.005 (-0.033, 0.043) | 0.046 (0.002, 0.091) | 0.006 (-0.054, 0.066) | 0.005 (-0.033, 0.043) | 0.968 |
| $EC_{0.18\text{-}2.5}$ | 0.082 (-0.010, 0.173) | 0.085 (0.008, 0.162) | -0.026 (-0.169, 0.118) | 0.082 (-0.010, 0.173) | 0.385 |
| **Oxidative potential, 5-day** | | | | | |
| Total $ROS_{0.18}$ | -0.005 (-0.048, 0.037) | 0.007 (-0.034, 0.047) | -0.005 (-0.071, 0.061) | -0.005 (-0.048, 0.037) | 0.954 |
| Total $ROS_{0.18\text{-}2.5}$ | 0.005 (-0.065, 0.076) | 0.031 (-0.024, 0.086) | 0.014 (-0.145, 0.172) | 0.005 (-0.065, 0.076) | 0.942 |
| $DTT_{0.18}$ | 0.021 (-0.025, 0.067) | 0.017 (-0.034, 0.068) | 0.027 (-0.039, 0.094) | 0.021 (-0.025, 0.067) | 0.698 |
| $DTT_{0.18\text{-}2.5}$ | 0.004 (-0.027, 0.034) | 0.027 (-0.015, 0.068) | 0.009 (-0.036, 0.054) | 0.004 (-0.027, 0.034) | 0.611 |
| **Transition metals, 5-day** | | | | | |
| Total metals $_{0.18}$ | 0.057 (0.010, 0.104) | 0.074 (0.011, 0.138) | 0.075 (0.006, 0.144) | 0.057 (0.010, 0.104) | 0.627 |
| Total metals $_{0.18\text{-}2.5}$ | 0.029 (-0.001, 0.059) | 0.019 (-0.019, 0.057) | 0.038 (-0.004, 0.079) | 0.029 (-0.001, 0.059) | 0.631 |
| **Electrophilic potential, 5-day** | | | | | |
| GPx-$1_{0.18}$ | 0.012 (-0.003, 0.027) | -0.016 (-0.033, 0.001) | 0.001 (-0.017, 0.019) | 0.012 (-0.003, 0.027) | 0.408 |

[a] *p < 0.1, **p < 0.05, the p-value for interaction is testing the null hypothesis that effect modification by CALINE4 classification is the same across demographic strata.

Table 5.16. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components, modifying by dispersion-modeled local traffic emissions above and below median levels: stratified by region.

| | Los Angeles | | Anaheim | | |
|---|---|---|---|---|---|
| | Estimates (95% CI) | Estimates (95% CI) | Estimates (95% CI) | Estimates (95% CI) | *p* for interaction [a] |
| | < median NOx (n=13) | ≥median NOx (n=35) | < median NOx (n=35) | ≥ median NOx (n=14) | |
| **BC** | | | | | |
| 1-day | 0.041 (-0.008, 0.090) | 0.045 (0.013, 0.077) | 0.021 (-0.027, 0.069) | 0.041 (-0.008, 0.090) | 0.031 ** |
| 3-day | 0.058 (-0.015, 0.132) | 0.034 (-0.016, 0.085) | -0.001 (-0.039, 0.037) | 0.058 (-0.015, 0.132) | 0.057 * |
| 5-day | 0.087 (-0.011, 0.186) | 0.035 (-0.028, 0.098) | -0.004 (-0.053, 0.045) | 0.087 (-0.011, 0.186) | 0.035 ** |
| 7-day | 0.109 (-0.007, 0.224) | 0.039 (-0.034, 0.112) | 0.046 (-0.011, 0.103) | 0.109 (-0.007, 0.224) | 0.068 * |
| **CO** | | | | | |
| 1-day | 0.053 (-0.011, 0.117) | 0.048 (0.005, 0.090) | -0.005 (-0.049, 0.039) | 0.053 (-0.011, 0.117) | 0.027 ** |
| 3-day | 0.043 (-0.029, 0.115) | 0.018 (-0.032, 0.067) | -0.012 (-0.051, 0.027) | 0.043 (-0.029, 0.115) | 0.032 ** |
| 5-day | 0.069 (-0.024, 0.161) | 0.017 (-0.044, 0.079) | -0.015 (-0.064, 0.033) | 0.069 (-0.024, 0.161) | 0.027 ** |
| 7-day | 0.068 (-0.034, 0.170) | 0.021 (-0.046, 0.087) | 0.017 (-0.039, 0.073) | 0.068 (-0.034, 0.170) | 0.089 * |
| **NOx** | | | | | |
| 1-day | 0.036 (-0.018, 0.090) | 0.044 (0.007, 0.080) | 0.007 (-0.030, 0.044) | 0.036 (-0.018, 0.090) | 0.075 * |
| 3-day | 0.024 (-0.033, 0.081) | 0.001 (-0.041, 0.043) | 0.002 (-0.033, 0.037) | 0.024 (-0.033, 0.081) | 0.027 ** |
| 5-day | 0.063 (-0.018, 0.143) | 0.002 (-0.055, 0.059) | -0.001 (-0.046, 0.044) | 0.063 (-0.018, 0.143) | 0.009 # |
| 7-day | 0.082 (-0.016, 0.179) | 0.015 (-0.052, 0.081) | 0.028 (-0.024, 0.080) | 0.082 (-0.016, 0.179) | 0.036 ** |
| **Filter-based $PM_{2.5}$, 5-day** | | | | | |
| $PM_{0.18}$ | 0.061 (-0.008, 0.130) | -0.008 (-0.050, 0.034) | 0.033 (-0.002, 0.068) | 0.061 (-0.008, 0.130) | 0.103 |
| $PM_{0.18\text{-}2.5}$ | -0.046 (-0.107, 0.014) | -0.006 (-0.041, 0.029) | -0.012 (-0.071, 0.046) | -0.046 (-0.107, 0.014) | 0.985 |
| **Carbonaceous aerosol components, 5-day** | | | | | |
| $PAHs_{0.18}$ | 0.093 (0.001, 0.186) | 0.031 (-0.032, 0.094) | 0.028 (-0.020, 0.076) | 0.093 (0.001, 0.186) | 0.028 ** |
| $PAHs_{0.18\text{-}2.5}$ | 0.047 (-0.017, 0.112) | 0.006 (-0.043, 0.056) | 0.018 (-0.025, 0.061) | 0.047 (-0.017, 0.112) | 0.029 ** |
| $Hopanes_{0.18}$ | 0.121 (0.021, 0.220) | 0.018 (-0.046, 0.083) | 0.101 (0.040, 0.163) | 0.121 (0.021, 0.220) | 0.161 |
| $Hopanes_{0.18\text{-}2.5}$ | 0.038 (-0.025, 0.101) | 0.004 (-0.039, 0.046) | -0.003 (-0.053, 0.047) | 0.038 (-0.025, 0.101) | 0.023 ** |
| $OC_{0.18}$ | 0.055 (-0.008, 0.118) | 0.027 (-0.009, 0.064) | 0.003 (-0.030, 0.037) | 0.055 (-0.008, 0.118) | 0.074 * |
| $OC_{0.18\text{-}2.5}$ | 0.038 (-0.053, 0.129) | -0.009 (-0.072, 0.054) | -0.003 (-0.047, 0.041) | 0.038 (-0.053, 0.129) | 0.021 ** |
| $EC_{0.18}$ | 0.082 (0.009, 0.155) | 0.032 (-0.008, 0.072) | -0.013 (-0.048, 0.023) | 0.082 (0.009, 0.155) | 0.008 *** |
| $EC_{0.18\text{-}2.5}$ | 0.031 (-0.062, 0.125) | 0.060 (0.005, 0.116) | 0.098 (-0.056, 0.252) | 0.031 (-0.062, 0.125) | 0.158 |
| **Oxidative potential, 5-day** | | | | | |
| Total $ROS_{0.18}$ | 0.024 (-0.037, 0.086) | 0.000 (-0.034, 0.034) | -0.021 (-0.064, 0.023) | 0.024 (-0.037, 0.086) | 0.123 |
| Total $ROS_{0.18\text{-}2.5}$ | -0.021 (-0.096, 0.055) | 0.020 (-0.024, 0.064) | 0.077 (-0.065, 0.219) | -0.021 (-0.096, 0.055) | 0.433 |
| $DTT_{0.18}$ | 0.017 (-0.046, 0.079) | -0.016 (-0.055, 0.023) | 0.027 (-0.020, 0.074) | 0.017 (-0.046, 0.079) | 0.042 ** |
| $DTT_{0.18\text{-}2.5}$ | 0.028 (-0.040, 0.096) | 0.018 (-0.022, 0.059) | 0.003 (-0.025, 0.030) | 0.028 (-0.040, 0.096) | 0.454 |
| **Transition metals, 5-day** | | | | | |
| Total $metals_{0.18}$ | 0.100 (0.006, 0.194) | 0.041 (-0.019, 0.102) | 0.055 (0.012, 0.098) | 0.100 (0.006, 0.194) | 0.087 * |
| Total $metals_{0.18\text{-}2.5}$ | 0.016 (-0.025, 0.057) | -0.005 (-0.035, 0.024) | 0.039 (0.010, 0.069) | 0.016 (-0.025, 0.057) | 0.095 * |
| **Electrophilic potential, 5-day** | | | | | |
| $GPx\text{-}1_{0.18}$ | -0.006 (-0.051, 0.040) | -0.039 (-0.067, -0.010) | 0.008 (-0.004, 0.020) | -0.006 (-0.051, 0.040) | 0.570 |

[a]* $p$ < 0.1, ** $p$ < 0.05, #$p$ < 0.01, the p-value for interaction is testing the null hypothesis that effect modification by CALINE4 classification is the same across demographic strata.

Table 5.17. Associations of FeNO with ambient daily air pollutants and 5-day air pollutant components, modifying by dispersion-modeled traffic-related air pollution above and below median levels: stratified by socioeconomic status (SES).

| | Low SES | | High SES | | *p* for interaction [a] |
|---|---|---|---|---|---|
| | Estimate (95% CI) < median NOx (n=28) | Estimate (95% CI) ≥ median NOx (n=22) | Estimate (95% CI) < median NOx (n=19) | Estimate (95% CI) ≥ median NOx (n=26) | |
| **BC** | | | | | |
| 1-day | 0.029 (-0.018, 0.076) | 0.115 (0.066, 0.164) | 0.035 (-0.016, 0.086) | 0.029 (-0.018, 0.076) | 0.040 ** |
| 3-day | 0.011 (-0.034, 0.056) | 0.082 (0.026, 0.137) | 0.018 (-0.034, 0.070) | 0.011 (-0.034, 0.056) | 0.173 |
| 5-day | 0.001 (-0.057, 0.060) | 0.082 (0.015, 0.150) | 0.038 (-0.030, 0.105) | 0.001 (-0.057, 0.060) | 0.114 |
| 7-day | 0.024 (-0.043, 0.091) | 0.108 (0.029, 0.186) | 0.112 (0.033, 0.191) | 0.024 (-0.043, 0.091) | 0.018 ** |
| **CO** | | | | | |
| 1-day | 0.018 (-0.032, 0.068) | 0.126 (0.069, 0.182) | 0.012 (-0.043, 0.066) | 0.018 (-0.032, 0.068) | 0.046 ** |
| 3-day | 0.001 (-0.046, 0.047) | 0.074 (0.017, 0.132) | 0.005 (-0.046, 0.056) | 0.001 (-0.046, 0.047) | 0.126 |
| 5-day | -0.007 (-0.065, 0.052) | 0.069 (-0.001, 0.138) | 0.022 (-0.042, 0.086) | -0.007 (-0.065, 0.052) | 0.089 * |
| 7-day | 0.006 (-0.061, 0.073) | 0.080 (0.002, 0.157) | 0.066 (-0.007, 0.140) | 0.006 (-0.061, 0.073) | 0.030 ** |
| **NOx** | | | | | |
| 1-day | 0.021 (-0.021, 0.062) | 0.110 (0.063, 0.156) | 0.015 (-0.030, 0.061) | 0.021 (-0.021, 0.062) | 0.064 * |
| 3-day | 0.005 (-0.035, 0.046) | 0.055 (0.007, 0.103) | 0.018 (-0.027, 0.063) | 0.005 (-0.035, 0.046) | 0.155 |
| 5-day | -0.000 (-0.054, 0.054) | 0.078 (0.012, 0.144) | 0.041 (-0.018, 0.100) | -0.000 (-0.054, 0.054) | 0.033 ** |
| 7-day | 0.008 (-0.054, 0.070) | 0.102 (0.027, 0.178) | 0.085 (0.017, 0.153) | 0.008 (-0.054, 0.070) | 0.006 *** |
| **Filter-based $PM_{2.5}$, 5-day** | | | | | |
| $PM_{0.18}$ | 0.014 (-0.028, 0.056) | 0.010 (-0.040, 0.059) | 0.065 (0.018, 0.112) | 0.014 (-0.028, 0.056) | 0.147 |
| $PM_{0.18\text{-}2.5}$ | -0.048 (-0.105, 0.010) | 0.001 (-0.046, 0.048) | -0.004 (-0.066, 0.058) | -0.048 (-0.105, 0.010) | 0.290 |
| **Carbonaceous aerosol components, 5-day** | | | | | |
| $PAHs_{0.18}$ | 0.019 (-0.040, 0.077) | 0.111 (0.038, 0.184) | 0.074 (0.011, 0.137) | 0.019 (-0.040, 0.077) | 0.012 ** |
| $PAHs_{0.18\text{-}2.5}$ | 0.008 (-0.042, 0.058) | 0.072 (0.007, 0.138) | 0.057 (0.004, 0.109) | 0.008 (-0.042, 0.058) | 0.024 ** |
| $Hopanes_{0.18}$ | 0.083 (0.008, 0.157) | 0.063 (-0.024, 0.150) | 0.136 (0.061, 0.211) | 0.083 (0.008, 0.157) | 0.175 |
| $Hopanes_{0.18\text{-}2.5}$ | 0.000 (-0.053, 0.054) | 0.040 (-0.017, 0.097) | 0.037 (-0.021, 0.095) | 0.000 (-0.053, 0.054) | 0.173 |
| $OC_{0.18}$ | 0.009 (-0.031, 0.048) | 0.054 (0.010, 0.098) | 0.019 (-0.026, 0.063) | 0.009 (-0.031, 0.048) | 0.176 |
| $OC_{0.18\text{-}2.5}$ | -0.008 (-0.063, 0.046) | 0.093 (0.023, 0.163) | 0.030 (-0.029, 0.090) | -0.008 (-0.063, 0.046) | 0.009 # |
| $EC_{0.18}$ | -0.005 (-0.049, 0.040) | 0.068 (0.020, 0.115) | 0.020 (-0.028, 0.069) | -0.005 (-0.049, 0.040) | 0.085 * |
| $EC_{0.18\text{-}2.5}$ | 0.024 (-0.079, 0.127) | 0.097 (0.017, 0.177) | 0.093 (-0.027, 0.212) | 0.024 (-0.079, 0.127) | 0.206 |
| **Oxidative potential, 5-day** | | | | | |
| Total $ROS_{0.18}$ | -0.001 (-0.049, 0.047) | 0.021 (-0.022, 0.065) | -0.015 (-0.069, 0.040) | -0.001 (-0.049, 0.047) | 0.724 |
| Total $ROS_{0.18\text{-}2.5}$ | -0.028 (-0.112, 0.056) | 0.046 (-0.016, 0.108) | 0.046 (-0.056, 0.149) | -0.028 (-0.112, 0.056) | 0.14 |
| $DTT_{0.18}$ | 0.006 (-0.045, 0.057) | 0.031 (-0.022, 0.085) | 0.044 (-0.013, 0.101) | 0.006 (-0.045, 0.057) | 0.103 |
| $DTT_{0.18\text{-}2.5}$ | -0.001 (-0.036, 0.034) | 0.026 (-0.016, 0.069) | 0.016 (-0.022, 0.053) | -0.001 (-0.036, 0.034) | 0.288 |
| **Transition metals, 5-day** | | | | | |
| Total metals $_{0.18}$ | 0.034 (-0.019, 0.087) | 0.093 (0.026, 0.160) | 0.095 (0.038, 0.153) | 0.034 (-0.019, 0.087) | 0.043 ** |
| Total metals $_{0.18\text{-}2.5}$ | 0.007 (-0.027, 0.041) | 0.040 (-0.000, 0.080) | 0.057 (0.022, 0.092) | 0.007 (-0.027, 0.041) | 0.004 # |
| **Electrophilic potential, 5-day** | | | | | |
| GPx-$1_{0.18}$ | -0.004 (-0.018, 0.010) | -0.031 (-0.049, -0.014) | 0.027 (0.008, 0.046) | -0.004 (-0.018, 0.010) | 0.976 |

[a]* $p < 0.1$, ** $p < 0.05$, #$p < 0.01$the p-value for interaction is testing the null hypothesis that effect modification by CALINE4 classification is the same across demographic strata.

# Chapter 6. Discussion of overall project

## 6.1. Summary of the main results

Overall, the current study investigated the sources and components of air pollutants in relation to cardiorespiratory outcomes, and to explore potentially susceptible subgroups and risk factors. The summary of the main results is outlined in the section 6.1.1 – 6.1.3.

### 6.1.1. Cardiorespiratory outcomes and daily ambient air pollutants

We found that exposure to ambient traffic-related air pollutants (BC, CO, and $NO_x$) was related to impaired microvascular endothelial function (represented by RHI score), elevated airway oxidative stress and inflammation (represented by EBC MDA and FeNO, respectively), and acute increase in systemic inflammation (represented by IL-6). Our findings with daily ambient pollutants suggested some plausible biological mechanisms that explain the adverse health effects from air pollution. Air pollutants, especially from mobile sources, are capable of generating ROS and provoke alveolar oxidative stress and inflammation, and inducing the release of inflammatory mediators to circulating system. Once circulating, these mediator would have direct effect on alterations in autonomic tone and vascular function (Brook et al. 2010).

By assessing daily exposure, we found the associations for microvascular function and airway biomarkers were generally persisted throughout 1-day to 7-day

averages, with the strongest associations for 5-day or 7-day averages, indicating delayed effects of pollutants. Meanwhile, the significant associations for systemic inflammation biomarker were only observed for 1-day averages and became non-significant with longer averaging times. A similar trend was observed for the systemic oxidative stress biomarker, oxLDL, even though the associations were largely nonsignificant. As Miller et.al. reviewed, vascular endothelial effects can happen as soon as 2 hours after the exposure, while the local and systemic inflammation and oxidative stress increase gradually and reach a peak at 1 day after the exposure (Miller et al. 2012). However, this review is based largely on controlled exposures to concentrated ambient particles and cohort studies of subjects living in Beijing. Under both conditions, the PM concentration and composition can be quite different from the present study. Because our clinic outcomes were measured at the same time, we could not further explore the temporal relationships among these outcomes.

In exploring susceptible subgroups, we found differential effects of pollutants on microvascular function by smoking status and BMI. The observed point estimates were stronger in former smokers than never smokers and slightly stronger associations were found in subjects who had BMI ≥ 30 $kg/m^2$ compared with those who had BMI < 30 $kg/m^2$. These differential effects were not observed on biomarkers in the airway or circulation.

Traffic-related air pollutants and ultrafine particles were also associated with stronger increases of EBC MDA (markers of airway oxidative stress) in subjects above a cardiovascular risk score of 0.2, compared with those below a cardiovascular risk score of 0.2. Similar effects were observed in subjects above 75 years of age compared

with subjects below 75 years of age, and total cholesterol/HDL ratio above 3.5 compared with and total cholesterol/HDL ratio below 3.5. Even though the effect modifications by demographic characteristics were different across outcomes, it all suggested that subjects with poorer health condition were more susceptible.

The effect modification of microvascular function and airway inflammation biomarkers with traffic-related air pollutants by CALINE4-modeled $NO_x$ was observed for acute exposure averages (1-day or 3-day averages), particularly during the cool season (Chapter 5). It is likely that the spatial variation of traffic-related air pollutants is greater for shorter averaging times than in longer averaging times (Pope et al. 2014), which leads to greater exposure misclassification. It is also possible that the chronic changes in oxidative stress and inflammation resulted from long-term residential exposure to traffic-related air pollutants rendering subjects more sensitive to the acute exposures.

### 6.1.2. Cardiorespiratory outcomes and 7-day personal exposure

There were no significant associations found between any cardiorespiratory outcomes and 7-day personal $NO_x$, while significant associations were found for both microvascular function and airway biomarkers with exposure to ambient 7-day $NO_x$ (section 3.3.2 and section 4.3). The null results for the associations of systemic biomarkers with 7-day personal may be because the effect of personal exposure to traffic-related pollutants on systemic endpoints is rather transient (section 4.3.3). However, the discrepancy of personal and ambient $NO_x$ on microvascular function and

airway biomarkers was unexpected because exposure measured by central monitor data could be subject to greater exposure misclassification than exposure measured by a personal sampler. The evidence of effect modification of ambient air pollutants by CALINE4-modeled $NO_x$ found in our analysis (Chapter 5) may indicate the potential exposure misclassification from using central monitoring data. However, several limitations of our personal exposure measurement were discussed in sections 3.4. and 4.4.7. Briefly, subject non-compliance, face velocity effects on the passive $NO_x$ badge from variations in airflow, and unadjusted indoor sources of $NO_x$ were the main limitations of our personal $NO_x$ measurement. Additionally, in order to assess the transient changes potentially occurring in the circulation, a more sensitive personal monitoring device that could measure high resolution of exposure (i.e., daily or perhaps hourly) is needed to better understand the time course of the relationships.

### 6.1.3. Cardiorespiratory outcomes and 5-day PM components

In recent years, an increasing literature has focused on measuring size-fractionated PM and/or its chemical constituents in epidemiological studies to investigate air pollution effects on cardiorespiratory outcomes (Delfino et al. 2008; Delfino et al. 2009; Delfino et al. 2010a; Chen et al. 2015; Wu et al. 2015; Lin et al. 2016). Particles that have smaller aerodynamic diameter have been demonstrated to have a greater adverse effects on health because they can penetrate deeper in the lung and they have a greater surface area to carry toxic components than larger size particles (Elder et al. 2006). In addition to PM size, the chemical composition of air

pollution is another important determinant of health effects (Kelly et al. 2012). The present study not only measured chemically-characterized PM in different size-fractions, but also added novel exposure assessments using markers of oxidative potential and electrophilic potential to characterize PM.

As discussed in the section 6.1.1, the associations between systemic biomarkers and traffic-related pollutants were transient (within 1-day average). Therefore, we did not observe consistent associations between systemic biomarkers and 5-day PM components.

We found stronger adverse associations between airway biomarkers and total PM mass in the ultrafine mode ($PM_{0.18}$) than in larger size-fractions ($PM_{0.18-2.5}$ and $PM_{2.5-10}$), while this was not observed for the associations between microvascular function and total PM mass. This may indicate that size alone was insufficient to explain the adverse effect of PM on microvascular function, rather PM sources or composition was an important determinant (for example, mobile-source PAH).

For carbonaceous aerosol components, we found adverse effects on microvascular function and airway biomarkers with exposure to PAHs and hopanes, and the associations were stronger in $PM_{0.18}$ than in $PM_{0.18-2.5}$. Results also suggested adverse effects on microvascular function and airway with exposure to EC or OC, however, with no consistent indications of stronger associations in ultrafine particles than larger particles. The equally strong or even stronger associations in $PM_{2.5-10}$ than in smaller fractions may be because the distribution of primary OC and secondary OC in

different PM size-fractions was different or EC and OC in $PM_{2.5-10}$ served as surrogate for other toxic substances (such as transition metals).

Consistent evidence showed that impaired microvascular function, increased airway oxidative stress and inflammation were related to the increase of the transition metals Cr, Mn, Cu, and Fe in all three size-fractions. Even though the effects were generally stronger in $PM_{0.18}$ than in $PM_{0.18-2.5}$, the effects in $PM_{2.5-10}$ were strong as well. Metals in $PM_{2.5-10}$ are mostly a product of non-exhaust emissions, such as re-suspension of road dust and vehicular abrasion, while metals in $PM_{2.5}$ are the production of exhaust emissions, which is dominated by combustion processes (Pant et al. 2013). Our results of adverse effects on cardiorespiratory outcomes by transition metals in $PM_{2.5-10}$ suggested that the traffic-source from non-exhaust emissions may have importance to adverse health effects as well. Regulations should be considered for non-exhaust emissions as current regulations have focused almost exclusively on exhaust emissions. In other exposure research from CHAPS investigators, data shows that over the years in southern California non-exhaust emissions have not decreased as much as exhaust emissions (Shirmohammadi et al. 2016).

We found suggestive evidence that PM oxidative potential (measured by both *in vitro* macrophage ROS and DTT) was associated with microvascular function and airway oxidative stress and inflammation. In regard to the associations with PM oxidative potential in different size fractions, ROS and DTT showed different trends and had no evidence suggesting a stronger association in smaller particles than in larger particles as observed for markers of fossil fuel combustion sources. This indicated that the adverse health effects from transition metal exposures and PM oxidative potential

does not solely depend on particle size, but is likely a result of composition. Our results also suggested that future studies should take both macrophage ROS and DTT measurements into consideration to assess PM oxidative potential because they measured intrinsically different aspects of oxidative activity of particles (discussed in the section 2.5.4).

The results showed positive association between microvascular function and GPx-1 activity in $PM_{0.18}$. This is expected because low GPx-1 activity indicates an increase in electrophilic potential (higher toxicity), which could have an adverse effect on vascular function. This result indirectly suggests that the observed effects of traffic-related air pollutants on microvascular function may be at least in part attributable to the potential of traffic-related air pollutants to generate oxidative stress by increasing electrophilic potential. For airway and systemic biomarkers, no significant associations were observed except for the positive association (unexpected) between oxLDL and GPx-1 activity in $PM_{0.18}$.

The effect modification by CALINE4-modeled NOx between microvascular function and biomarkers of airway and 5-day PM components were mostly non-signification. However, for all outcomes, there was evidence of stronger adverse effects among subjects with higher CALINE4-modeled NOx. Along with similar finding with daily ambient air pollutants, these results indicated potential underestimation of adverse health effects using exposure data from central monitoring stations.

## 6.2. Strengthens and limitations

There are strengths and limitations to each Aim of this thesis and they were discussed in respective chapters. The key strengthen in this study is the chemically-characterized exposure assessment. In addition to measurements of source-specific markers (e.g., PAHs, hopanes, and BC) of PM in different size-fractions, we implemented several novel markers to evaluate PM oxidative potential (macrophage ROS and DTT) and electrophilic potential (GPx-1 activity). In addition, the cohort panel study design with repeated-measures can effectively control for the confounding by the time-invariant covariates. Lastly, we simultaneous measured microvascular function and multiple airway and systemic biomarkers, some of which (e.g., EndoPAT, EBC MDA) are novel and have seldom been used in previous air pollution studies.

The limitations to individual study were discussed in respective chapter. However, serval shared limitations are important to consider. First, this study included only elderly subjects selected in specific geographic regions. Generalization of the study results to other populations is somewhat limited. Second, the measurement of ambient air pollutants from central air monitoring stations may be associated with exposure error. This was further evidenced by the analysis of effect modification by residential CALINE4-modeled $NO_x$. However, the exposure misclassification in this study was likely to be non-differential and to have resulted in underestimation of associations. Third, the multiplicity of pollutants and studied outcomes over several averages could lead to multiple testing bias. Multiple testing is common in air pollution-related health effects studies and that potentially increase the likelihood of type I error. However, this study was undertaken with a clear rationale and plausible biological mechanisms (discussed

in Chapter 1). The acute cardiovascular outcome and biomarkers investigated in this study were selected to represent specific biological responses and different biological pathways linking gaseous and particulate air pollutants to cardiovascular disease. The statistical tests were thus driven by plausible hypothesis and were not exploratory or confirmatory in nature. Therefore, statistical adjustment for multiple testing is unnecessary in such conditions (Rothman 1990). However, we acknowledge that at least some of the observed significant results may have occurred by chance.

## 6.3. Overall significance

This study shed light on the potential mechanisms linking cardiovascular diseases with air pollution. Aside from our previous studies (Delfino et al. 2010a; Delfino et al. 2010b), very few epidemiological studies (Huang et al. 2012; Roy et al. 2014; Altemose et al. 2016) have systemically evaluated the health effects of air pollution on both the respiratory and circulatory systems. Huang et al. observed improvements in biomarkers of pulmonary inflammation and biomarkers of respiratory and systemic oxidative stress during the Beijing Olympic air pollution control period in young healthy subjects (Huang et al. 2012). This group further investigated the temporal pattern of cardiorespiratory effects of ambient air pollution using a comparative hierarchical pathway analysis and concluded that among this healthy young adult population, the airway inflammation and oxidative stress pathways are the first to respond to ambient air pollution exposure (within 24 hours). The initial airway response may sequentially contribute to the more gradual systemic changes that likely ultimately involve the

cardiovascular system (Roy et al. 2014). These results were, in general, consistent with our current findings. However, in our study, we further characterized chemical components of PM in a great detail. Overall, our results support and add new evidence to the previous hypothesized mechanism that air pollutants in ultrafine and fine PM that incorporate reactive organic and transition metal components could enter the lung and cause local oxidative stress and inflammation and then induce inflammation in the systemic circulation. Systemic effects could include the release of pro-inflammatory cytokines (e.g., IL-6) and endothelial dysfunction (Pope et al. 2004; Franklin et al. 2015). This is important because the acute changes in the vasculature could play vital roles in the genesis of chronic cardiovascular diseases (Brook et al. 2010). The enhanced associations of cardiorespiratory outcomes with short-term exposure to air pollutants among subjects living in areas with high traffic-related air pollution suggests the underestimate effects of ambient air pollutants on cardiorespiratory outcomes in elderly population. This could be as a result of acutely increased vulnerability (spatial variation of traffic-related air pollution) or chronically increased susceptibility (comorbidities).

This study provides recommendations for future work in investigating the associations between air pollution and cardiovascular diseases.

1. More research is needed on how to minimize exposure measurement error, including implementing more sensitive and effective personal exposure assessment.
2. Further research is need to confirm which individuals are the most sensitive to the health effects of air pollution in order to facilitate intervention strategies.

3. Further studies are needed to investigate whether airway and/or systemic oxidative stress and inflammation serve as the operating mechanisms to explain the link between air pollution and adverse cardiovascular morbidity and mortality.

## 6.4. Conclusion

The results of this study provide evidence linking airway oxidative stress and inflammation, systemic inflammation, and impaired microvascular function to short-term elevations in traffic-related air pollutants, transition metals (including Cr, Mn, Cu, and Fe) and ultrafine PM, all of which have high oxidative potential. Possible increased susceptibility to the observed associations among specific demographic subpopulations were observed. Although this effect modification was varied across different outcomes, overall, it pointed to unhealthy subjects as being more susceptible than healthier subjects. The results also provided evidence that chronic exposure to residential $NO_x$ from local traffic emissions and acute exposure to ambient pollutants with high oxidative potential positively interacted to adversely affect health outcomes. These findings may have important public health implications in air pollution regulations and prevention of air pollution-related health effects.

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