Associations among plasma metabolite levels and short-term exposure to $PM_{2.5}$ and ozone in a cardiac catheterization cohort

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Abstract

Rationale: Exposure to ambient particulate matter (PM) and ozone has been associated with cardiovascular disease (CVD). However, the mechanisms linking PM and ozone exposure to CVD remain poorly understood.

Objective: This study explored associations between short-term exposures to PM with a diameter < 2.5 µm ($PM_{2.5}$) and ozone with plasma metabolite concentrations.

Methods and Results: We used cross-sectional data from a cardiac catheterization cohort at Duke University, North Carolina (NC), USA, accumulated between 2001 and 2007. Amino acids, acylcarnitines, ketones and total non-esterified fatty acids plasma concentrations were determined in fasting samples. Daily concentrations of $PM_{2.5}$ and ozone were obtained from a Bayesian space-time hierarchical model, matched to each patient's residential address. Ten metabolites were selected for the analysis based on quality criteria and cluster analysis. Associations between metabolites and $PM_{2.5}$ or ozone were analyzed using linear regression models adjusting for long-term trend and seasonality, calendar effects, meteorological parameters, and participant characteristics.

We found delayed associations between $PM_{2.5}$ or ozone and changes in metabolite levels of the glycine-ornithine-arginine metabolic axis and incomplete fatty acid oxidation associated with mitochondrial dysfunction. The strongest association was seen for an increase of 8.1 µg/m^3 in $PM_{2.5}$ with a lag of one day and decreased mean glycine concentrations (-2.5% [95% confidence interval: -3.8%; -1.2%]).

Conclusions: Short-term exposure to ambient $PM_{2.5}$ and ozone is associated with changes in plasma concentrations of metabolites in a cohort of cardiac catheterization patients. Our findings might help to understand the link between air pollution and cardiovascular disease.

Abstract: 247 words

Keywords: particulate matter, ozone, metabolomics, cardiovascular disease

Non-standard Abbreviations and Acronyms

AOD	Aerosol optical depth
AOD + GM	Combination of satellite-based aerosol optical depth (AOD) retrievals and $PM_{2.5}$ concentrations from ground monitors
CAD	Coronary artery disease
CATHGEN	**CATH**eterization **GEN**etics cohort
CMAQ	Models-3/Community Multiscale Air Quality
DDCD	Duke Databank for Cardiovascular Disease
IQR	Interquartile range
NARR	North American Regional Reanalysis
NEFA	Total non-esterified fatty acids
NO	Nitric oxide
PM	Particulate matter
$PM_{2.5}$	PM with an aerodynamic diameter less than 2.5 μm

INTRODUCTION

Exposure to ambient air pollution affects a range of cardiovascular events (Brook et al. 2010; Rückerl et al. 2011). Acute (day-to-day) exposure to particulate matter (PM) with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) is associated with increased risk of cardiovascular mortality, myocardial infarction, heart failure exacerbation, stroke (Atkinson et al. 2014; Mustafic et al. 2012; Shah et al. 2013; Shah et al. 2015) and induction of a variety of adverse cardiovascular outcomes (Brook et al. 2010; McGuinn et al. 2015). Epidemiological and controlled-exposure studies also suggest that exposure to ambient ozone may increase cardiovascular morbidity (Arjomandi et al. 2015; Devlin et al. 2012; Green et al. 2016; Hampel et al. 2012; Lanzinger et al. 2014). The elderly and those with underlying diseases, for example, cardiovascular diseases or diabetes, are particularly susceptible to the health effects of $PM_{2.5}$ (Lanzinger et al. 2014; Rückerl et al. 2011; Shumake et al. 2013; Stafoggia et al. 2010); however, current evidence for the risks of ozone are inconclusive (Goodman et al. 2014).

The physiological mechanisms linking $PM_{2.5}$ exposure to cardiovascular disease have yet to be fully elucidated. Biological pathways thought to be important include: systemic inflammation; changes in the autonomic balance; local inflammatory response; and oxidative stress due to translocation of particles or particle constituents (Brook et al. 2010; Peters et al. 2011). Further, inhalation of ozone may cause systemic inflammation and autonomic dysfunction (Brook et al. 2010; Devlin et al. 2012; Hampel et al. 2012). However, exploring the possibility that $PM_{2.5}$- or ozone-induced changes in metabolic pathways may contribute to or mediate cardiometabolic outcomes is becoming increasing important for understanding potential mechanisms of these effects.

Metabolomics, or metabolomic profiling, refers to the comprehensive analysis of metabolites - low molecular weight chemicals including sugars, acylcarnitines, amino acids, and lipids - present in biological specimens (Rhee and Gerszten 2012). Metabolomics has the potential for identifying novel biomarkers contributing to the onset or progression of cardiovascular disease (Shah et al. 2012a). Specific metabolomic profiles are associated with coronary artery disease (CAD) and atherosclerosis, and with major adverse cardiovascular events, including myocardial infarction, stroke, heart failure and death (Kordalewska and Markuszewski 2015; Shah et al. 2012a; Würtz et al. 2015).

Current literature on short-term exposures to air pollution and blood chemistries has focused on traditional clinical parameters such as C-reactive protein or cytokines (e.g. Chuang et al. 2007; Rückerl et al. 2007; Tsai et al. 2012). However, evaluating associations between air pollution and metabolite levels could provide further evidence of air pollution-related physiologic changes and offer further insights into the pathophysiologic mechanisms by which short-term exposures to air pollution may increase the risk of acute cardiovascular events. So far, there has been only one epidemiological study exploring the association between air

pollution and changes in metabolite levels (Menni et al. 2015). In this study using a subset of the TwinsUK cohort, long-term exposures to PM_{10} and $PM_{2.5}$ were linked with metabolites related to reduced lung function. Only a small number of animal or toxicological studies have reported associations between inhaled toxicants and metabolite levels (Miller et al. 2016; Miller et al. 2015; Wang et al. 2012; Wang et al. 2015; Wei et al. 2013).

This study aimed to explore the influence of short-term exposures to $PM_{2.5}$ and ozone on selected metabolites in a cohort of individuals undergoing cardiac catheterization for suspected coronary artery disease. Moreover, we evaluated whether these associations were modified by participant or lifestyle characteristics. Since the study population was at high risk for cardiovascular disease, our findings may help to uncover and clarify air pollution-metabolomics associations in a population particularly susceptible to the health effects of air pollution.

METHODS

Study population

This study was conducted using data from the **CATH**eterization **GEN**etics (CATHGEN) cohort, a large cohort of patients undergoing cardiac catheterization for suspected cardiovascular disease between 2001 – 2010 at the Duke University Cardiac Catheterization Clinic (Durham, NC)(Kraus et al. 2015).

For each of these patients, home addresses were extracted from medical records. Addresses were geocoded within the Children's Environmental Health Initiative (http://cehi.snre.umich.edu/), adding latitude and longitude information to each record (McGuinn et al. 2015; Ward-Caviness et al. 2015). Out of the entire cohort of 9,334 individuals, 8,071 (86%) addresses were successfully geocoded; 7,118 (76.3%) resided in North Carolina (Supplemental Material, Figure 1). For participants whose addresses changed over time, we used the most recent address entered into their records at catheterization. The average time at an address prior to the catheterization procedure was 587 days (Ward-Caviness et al. 2015).

Subjects fasted for a minimum of six hours before blood collection. Blood was drawn from the femoral artery at the time of arterial access for catheterization, immediately processed to separate plasma, and frozen at -80°C(Shah et al. 2010). Clinical data and patient characteristics were provided by the Duke Databank for Cardiovascular Disease (DDCD), a database of patients undergoing catheterization at Duke University since 1969.

The CATHGEN study was approved by the Duke University Institutional Review Board; written informed consent was obtained from all subjects prior to participation.

Metabolite data

Metabolomic profiling was available for 3,873 individuals in the interval 2001 to 2007. The plasma concentrations of 45 acylcarnitines and 15 amino acids were quantitatively determined using a

targeted mass spectrometry–based approach (Kraus et al. 2015). Proteins were first removed by precipitation with methanol; aliquoted supernatants were dried and esterified with hot, acidic methanol (acylcarnitines) or n-butanol (amino acids). For analysis, tandem mass spectrometry with a Quattro Micro instrument (Waters Corp, Milford, MA) was used. Adding mixtures of known quantities of stable-isotope internal standards facilitated quantification of "targeted" intermediary metabolites. Assay ranges are 0.05 to 50 µmol (acylcarnitines) and 5 to 1000 µmol (amino acids). Two acylcarnitines (C6 and C7DC) did not meet the quality standards and were, therefore, excluded for further analyses.

Quantitative determination of total ketones, β-hydroxybutyrate, and total non-esterified fatty acids (NEFA) was performed. Ketones (total and β-hydroxybutyrate) and NEFA were measured on a Beckman Coulter DxC600 clinical chemistry analyzer, using reagents from Wako (Richmond, VA) (Kraus et al. 2015). Methodology and measures of intra-individual variability have been previously reported (Shah et al. 2010). A complete list of all 61 metabolites can be found in Supplemental Material, Table 1.

Exposure data

Daily predictive surfaces of particulate matter with an aerodynamic diameter < 2.5µm ($PM_{2.5}$) (daily average in $\mu g/m^3$) and ozone (daily 8-h maximum in ppb) were provided by the U.S. Environmental Protection Agency (U.S. EPA) for the years 2001 to 2008 (www.epa.gov/esd/land-sci/lcb/lcb_faqsd.html). A Bayesian space-time "downscaler" fusion modeling approach was used to develop these predictive surfaces (Berrocal et al. 2010a; b; 2012). The approach uses input data from two sources: air quality monitoring data from the EPA Air Quality System (AQS) repository database and numerical output from the Models-3/Community Multiscale Air Quality (CMAQ; http://www.epa.gov/asmdnerl/CMAQ) model run at a 12 km spatial resolution. The fused model combines the two data sources attempting to adjust for the existing bias in the CMAQ model and produces predictions for census tract centroids across the entire state of North Carolina (Gray et al. 2013). Further details and descriptions of the modeling technique and predictive performance are available (Berrocal et al. 2012). Geocoded residential addresses of the study participants were assigned the exposure as estimated at the closest census tract centroid based on spatial location and date.

We obtained daily $PM_{2.5}$ concentrations from a second source to better compare the metabolic effects of $PM_{2.5}$ exposure with previously published cardiovascular effects in the CATHGEN cohort (McGuinn et al. 2015). Based on a combination of satellite-based aerosol optical depth (AOD) retrievals and $PM_{2.5}$ concentrations from ground monitors (McGuinn et al. 2015), $PM_{2.5}$ concentration levels ($\mu g/m^3$) were predicted at a 10 x 10 km spatial resolution for the state of North Carolina for 2002-2009 using recently developed statistical prediction models. Geocoded addresses were matched to the centroid of the nearest 10 x 10 km grid location based on spatial location and date.

Daily mean air temperature and relative humidity were obtained from the North American Regional Reanalysis (NARR) project (Mesinger et al. 2006). Geocoded addresses were matched to the meteorological data based on spatial location and date.

Statistical analysis

We restricted our analysis population to those residing in North Carolina and participants with complete information on exposure, covariates and metabolomics markers. The final analysis population consisted of 2,869 individuals. We selected only metabolites with less than 10% of values below the limit of detection and with a high measurement accuracy based on repeated profiling ($R^2 \geq 0.85$) reported in previous analyses (Shah et al. 2009). This reduced the large number of (correlated) metabolites to 23 (see Supplemental Table 1 for a complete list of metabolites). With these 23 remaining metabolites, we performed a hierarchical cluster analysis using Euclidian distances and the Ward method (Murtagh and Legendre 2014). The number of sufficient clusters was chosen based on various indices (e.g. Calinski and Harabasz index, Duda index, C-index) provided by the R package NbClust (Charrad et al. 2014). In general, all indices measure the inter- and intra-cluster variability. While the variability of observations within a cluster should be low, the between-cluster variance should be high. Of each of the resulting clusters, we chose the metabolite with the highest measurement accuracy (=highest R^2) and the lowest number of values below the detection limit as the "main" metabolite for the cluster. Metabolites within the same cluster which showed low correlation with the main metabolite ($|r|<0.4$) were also considered as metabolites of interest. At first, the metabolite showing the lowest correlation with the main metabolite was chosen. If a further metabolite also exhibited a low correlation with the main metabolite, it was only selected if it also showed low correlation with the metabolite selected in the previous step. This approach greatly reduced the number of analyzed metabolites and therefore reduced the multiple comparisons in the statistical analysis. Further, our approach allows the discussion of the study results to be streamlined by allowing each biologically relevant cluster to be represented by (a) single outcome(s). Metabolite levels were natural-log transformed prior to analysis.

To evaluate the associations of metabolite levels with air pollution concentrations, we used additive regression models in an *a priori* defined adjustment model. Penalized splines based on B-spline bases were used to allow for non-linear confounding effects (Eilers and Marx 1996). To control for systematic variation over time, we introduced a time trend term (using date order) as well as dummy variables for season and day of the week. We further included a fixed intercept at the county level to account for unmeasured variation due to population-level characteristics. As other potential confounders, we considered air temperature and relative humidity, and the subject-related variables age, body mass index (BMI), gender, race (European-Americans, African-American, and other race/ethnicity) and smoking status (current vs. never/former smoker). Time trend was modeled using

penalized splines with four degrees of freedom per year. Adjustment for air temperature was done by modeling high and low temperatures separately (Stafoggia et al. 2013). Specifically, to control for heat effects, we calculated the average temperature on the current and previous day (lag 0-1) and fit a natural spline with three degrees of freedom only for days on which the temperature was higher than the median annual temperature. Similarly, only for days on which temperature was below the median annual value, we adjusted for low temperatures by fitting a natural spline with two degrees of freedom for the average temperature on the previous four days (lag 1–4). Relative humidity was modeled using a 5-day average (lag 0-4) assuming that three degrees of freedom should suffice.

In the last step of the analysis, air pollutants were added separately to the model and associations estimated linearly. We analyzed single-day lags from 0 to 4 days and the average of lags 0–4 (5-day average). Effect estimates from our models and their 95% confidence intervals (95% CI) were transformed into percent changes of geometric mean outcome levels and reported per interquartile range (IQR) increase of pollutants.

Interaction terms for age (≤ 60 vs. > 60 years), gender (male vs. female), race (European-Americans vs. African-Americans vs. other race/ethnicity), history of hypertension (yes vs. no) and diabetes (yes vs. no), and smoking status (current vs. never/former smoker) were used to investigate effect modification of the association between the air pollution and metabolite levels.

Sensitivity analyses

We performed a number of sensitivity analyses to assess the robustness of the main findings. We adjusted the degrees of freedom for the trend spline to control for seasonal effects; we also varied the lag pattern and the degrees of freedom for air temperature and relative humidity. We estimated models without adjusting for counties, season or subject-related covariates. Two-pollutant models examined the independent effects of $PM_{2.5}$ and ozone. Finally, we checked the exposure–response functions for deviations from linearity by replacing the linear term of the particle metrics with a fixed 4-degrees of freedom regression spline. We used a likelihood ratio test with three degrees of freedom comparing the original main model with the smoothed model and visual inspection to assess whether the smoothed exposure–response curve resembled a straight line.

All the analyses were performed with R project for statistical computing (V.2.14.2; http://www.r-project.org/) using the 'mcgv' package.

RESULTS

Participant characteristics

Table 1 describes the study population. On average, participants were 59 years old with a mean BMI of 30 kg/m². About 58% of the participants were men; approximately half were current smokers. The

prevalence of CAD and hypertension was 50.4% and 67.9%, respectively; this reflects a population with increased risk for CAD.

Cluster analysis

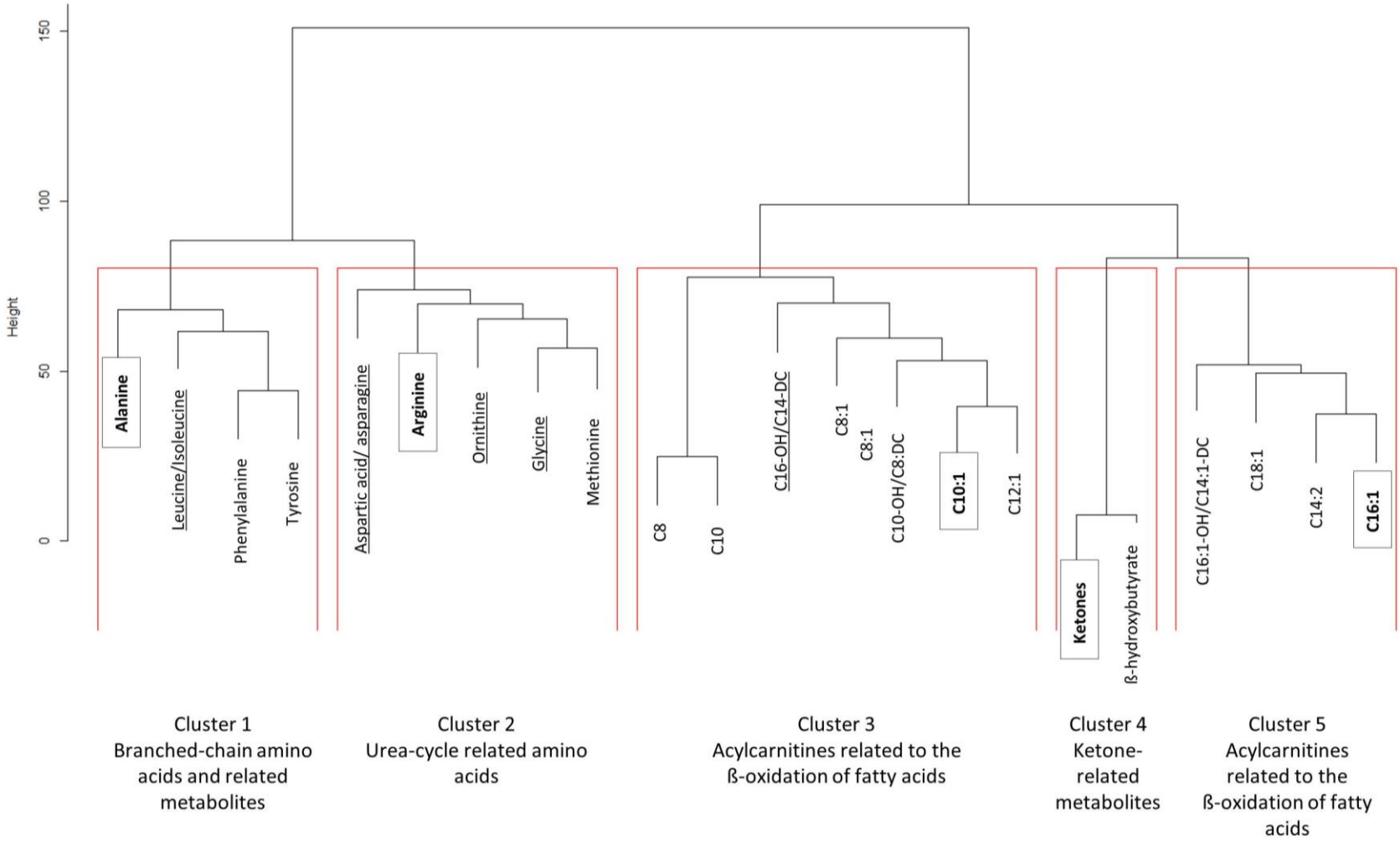
Twenty-three of the 61 metabolites met all the inclusion criteria. Results of the cluster analysis are shown in Figure 1. Most of the indices used to determine the relevant number of clusters identified five clusters as optimal to group the metabolites. These five clusters represent long neutral amino

Table 1. Descriptive statistics of the study population (n=2,869).

		Mean (SD)
Age (years)		59.4 (12.1)
BMI (kg/m^2)		30.3 (7.4)
SBP (mmHg)		149.9 (25.1)
DBP (mmHg)		79.8 (14.7)
		N(%)
Gender	Male	1,671 (58.2)
	Female	1,198 (41.8)
Race	European-American	1,991 (69.4)
	African-American	639 (22.3)
	Other	239 (8.3)
Smoking	Current	1,464 (51.0)
	Never/former	1,405 (49.0)
History of	Coronary artery disease	1,445 (50.4)
	Myocardial infarction	854 (29.8)
	Diabetes	854 (29.8)
	Hypertension	1,948 (67.9)
Family history of	Coronary disease	1,160 (40.4)

SD: standard deviation; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure

Figure 1


Height
150
100
50
0
Alanine
Leucine/Isoleucine
Phenylalanine
Tyrosine
Aspartic acid/ asparagine
Arginine
Ornithine
Glycine
Methionine
C8
C10
C16-OH/C14-DC
C8:1
C8:1
C10-OH/C8:DC
C10:1
C12:1
Ketones
ß-hydroxybutyrate
C16:1-OH/C14:1-DC
C18:1
C14:2
C16:1
Cluster 1
Branched-chain amino acids and related metabolites
Cluster 2
Urea-cycle related amino acids
Cluster 3
Acylcarnitines related to the ß-oxidation of fatty acids
Cluster 4
Ketone-related metabolites
Cluster 5
Acylcarnitines related to the ß-oxidation of fatty acids

acids, their metabolites and alanine (Cluster 1), urea cycle- related amino acids and glycine (Cluster 2), acylcarnitines-adducts of ß-oxidation of fatty acid metabolism (Clusters 3 and 5) and ketone metabolites (Cluster 4). Based on our described selection approach, we chose to represent the clusters: alanine and leucine/isoleucine (Cluster 1); arginine, aspartic acid/asparagine, ornithine and glycine (Cluster 2); decenoyl carnitine (C10:1) and 3-hydroxy-hexadecanoyl carnitine/tetradecanedioyl carnitine (C16-OH:C14-DC) (Cluster 3); total ketones (Cluster 4); and palmitoleyl carnitine (C16:1), (Cluster 5) as our outcomes of interest.

Metabolites and air pollution

Descriptive statistics of metabolites, modeled air pollutants and meteorology are presented in Table 2. The daily mean values of $PM_{2.5}$ and ozone derived from the downscaler fusion model were 13.3 $\mu g/m^3$ and 43.3 ppb, respectively. The daily mean value of $PM_{2.5}$ based on a combination of satellite-based aerosol optical depth (AOD) retrievals and $PM_{2.5}$ concentrations from ground monitors (AOD + GM) was 12.6 $\mu g/m^3$. Correlations between the metabolites can be found in Supplemental Material, Table 2. There was little or no correlation among $PM_{2.5}$, ozone and the meteorological parameters (Supplemental Material, Table 3). As expected, the $PM_{2.5}$ values predicted by the two different models were highly correlated (Spearman correlation coefficient = 0.857).

Table 2. Summary statistics of metabolite concentrations, air pollution concentrations and meteorological variables for the period 2001-2007. Metabolites selected first are marked in bold.

Metabolites	**N**	**Mean**	**SD**	**Min**	**25%**	**Med**	**75%**	**Max**
Cluster 1								
Alanine (µM)	2,869	316.7	94.4	104.4	250.6	302.9	369.2	944.4
Leucine/Isoleucine (µM)	2,869	67.0	14.2	21.6	57.8	65.4	73.8	217.7
Cluster 2								
Arginine (µM)	2,869	65.5	19.6	12.7	52.3	64.2	77.0	178.3
Aspartic acid/asparagine (µM)	2,869	87.3	21.1	13.8	73.5	84.0	98.4	223.0
Ornithine (µM)	2,869	76.6	21.9	24.4	61.5	74.0	88.1	230.8
Glycine (µM)	2,869	309.0	83.0	115.2	251.1	302.0	354.6	739.2
Cluster 3								
C10:1 (µM)	2,864	0.15	0.08	0.02	0.10	0.13	0.18	0.67
C16-OH:C14-DC (µM)	2,796	0.0047	0.0045	0.0001	0.0026	0.0039	0.0057	0.1007
Cluster 4								
Total Ketones (µM)	2,868	303.3	291.4	9.5	101.2	202.6	415.0	3220.9
Cluster 5								
C16:1 (µM)	2,856	0.027	0.016	0.005	0.018	0.024	0.033	0.332

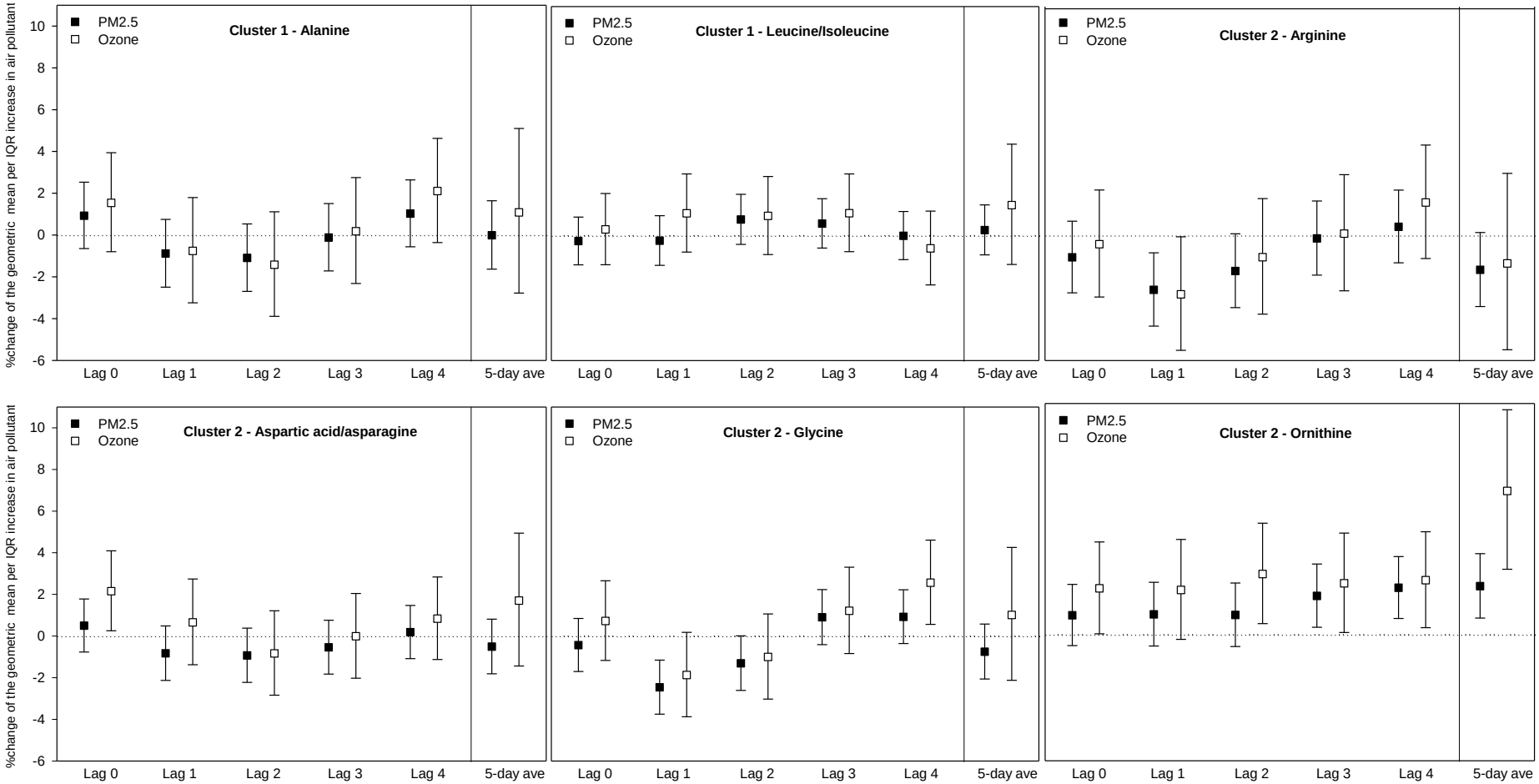
Meteorology and air pollution								
Air temperature (°C)	2,869	16.1	8.6	-8.1	9.2	16.8	23.5	33.0
Relative humidity (%)	2,869	73.8	10.8	38.5	66.5	75.3	82.2	95.3
$PM_{2.5}$ (daily mean; $\mu g/m^3$) - BDFM	2,869	13.3	6.1	0.6	8.7	12.3	16.8	49.4
Ozone (8-hour max; ppb) – BDFM	2,869	43.3	15.9	3.8	31.2	41.2	53.9	99.7
$PM_{2.5}$ (daily mean; $\mu g/m^3$) – AOD + GM	2,587	12.6	5.9	2.0	8.1	11.7	15.6	52.1

SD: standard deviation; Min: minimum; 25%: 25th percentile; Med: median; 75%: 75th percentile; Max: maximum, $PM_{2.5}$: particulate matter with an aerodynamic diameter<2.5µm; BDFM: Bayesian space-time "downscaler" fusion modeling approach; AOD + GM: combination of satellite-based aerosol optical depth retrievals and ground monitoring data.

Figure 2 and Supplemental Material, Table 4 show the associations between air pollutants and the selected amino acids. For alanine and leucine/isoleucine (Cluster 1), no associations with air pollution were found; whereas arginine (Cluster 2) was negatively associated with $PM_{2.5}$ and ozone. The strongest effects were found for lag 1 exposures with a -2.6% decrease (95% CI: -4.4%; -0.8%) per IQR increase (8.1 $\mu g/m^3$) in $PM_{2.5}$ and -2.8% decrease (95% CI: -5.5%; -0.1%) per IQR increase (22.7 ppb) in ozone. An IQR increase in $PM_{2.5}$ also resulted in decreased glycine levels with a lag of one day. Lag 1 ozone exposure showed an effect in the same direction; however, the association was not significant (on a significance level of 0.05). Both pollutants were consistently associated with increases in ornithine levels across several lags. For example, ornithine (Cluster 2) levels increased by 2.3% (95% CI: 0.8%; 3.9%) and 6.8% (95% CI: 3.1%; 10.7%) per IQR increase in 5-day average $PM_{2.5}$ and ozone, respectively.

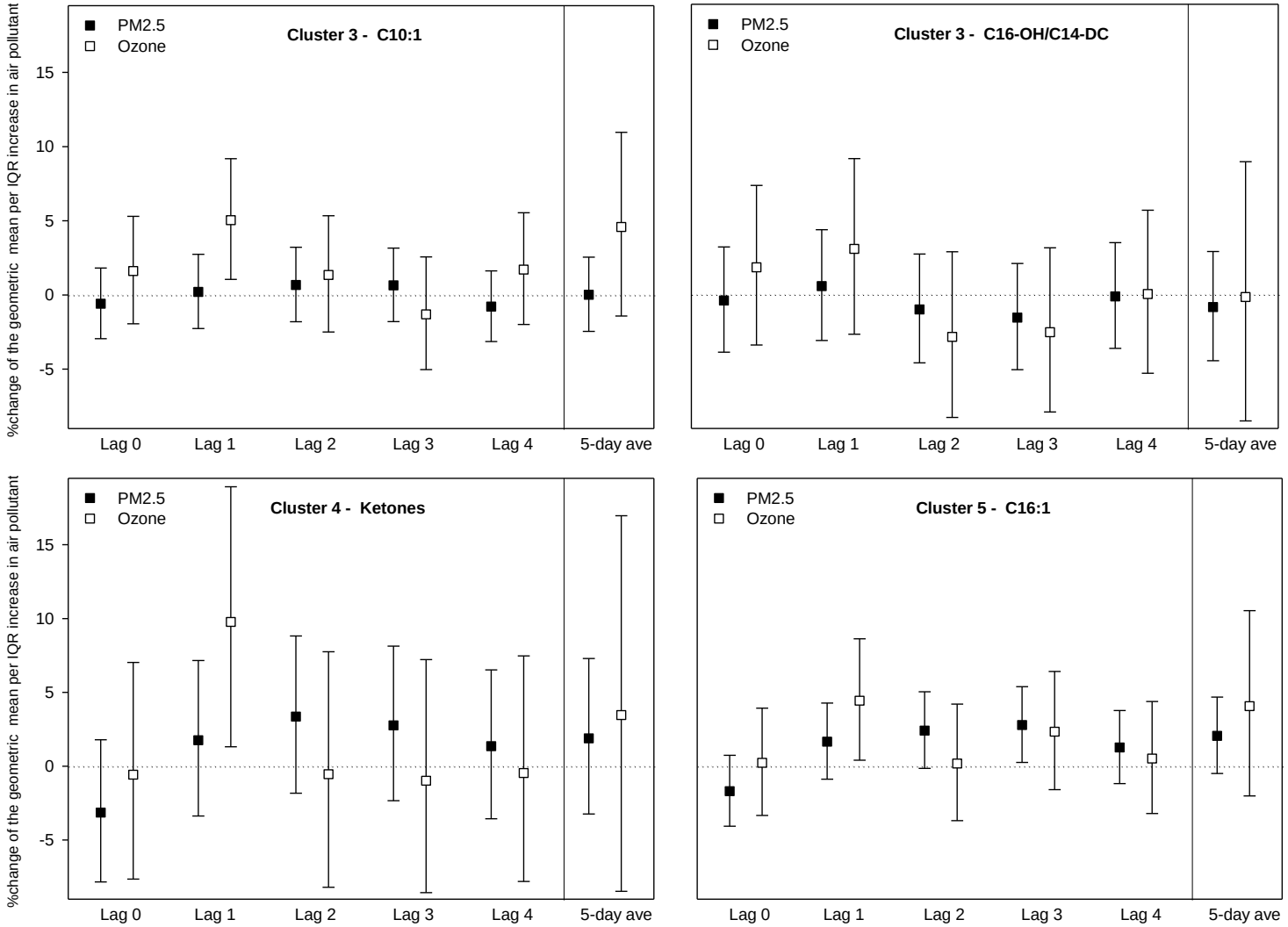
Results further suggest an association between ozone at lag 1 and C10:1 (Cluster 3), total ketones (Cluster 4) and C16:1 (Cluster 5), (Figure 3). Moreover, increases in $PM_{2.5}$ were associated with delayed increases in C16:1 levels; the strongest effect was a 3-day lagged 2.8% increase (95% CI: 0.3%; 5.4%).

Figure 2. Associations between $PM_{2.5}$, ozone (based on the Bayesian space-time "downscaler" fusion modeling approach) and amino acid levels per interquartile range increase of air pollutants[a].



[a] Models were adjusted for time trend, air temperature, relative humidity, age, gender, body mass index, race and smoking status.

Figure 3. Associations between $PM_{2.5}$, ozone (based on the Bayesian space-time "downscaler" fusion modeling approach) and acylcarnitine and total ketone levels per interquartile range increase of air pollutants[a].

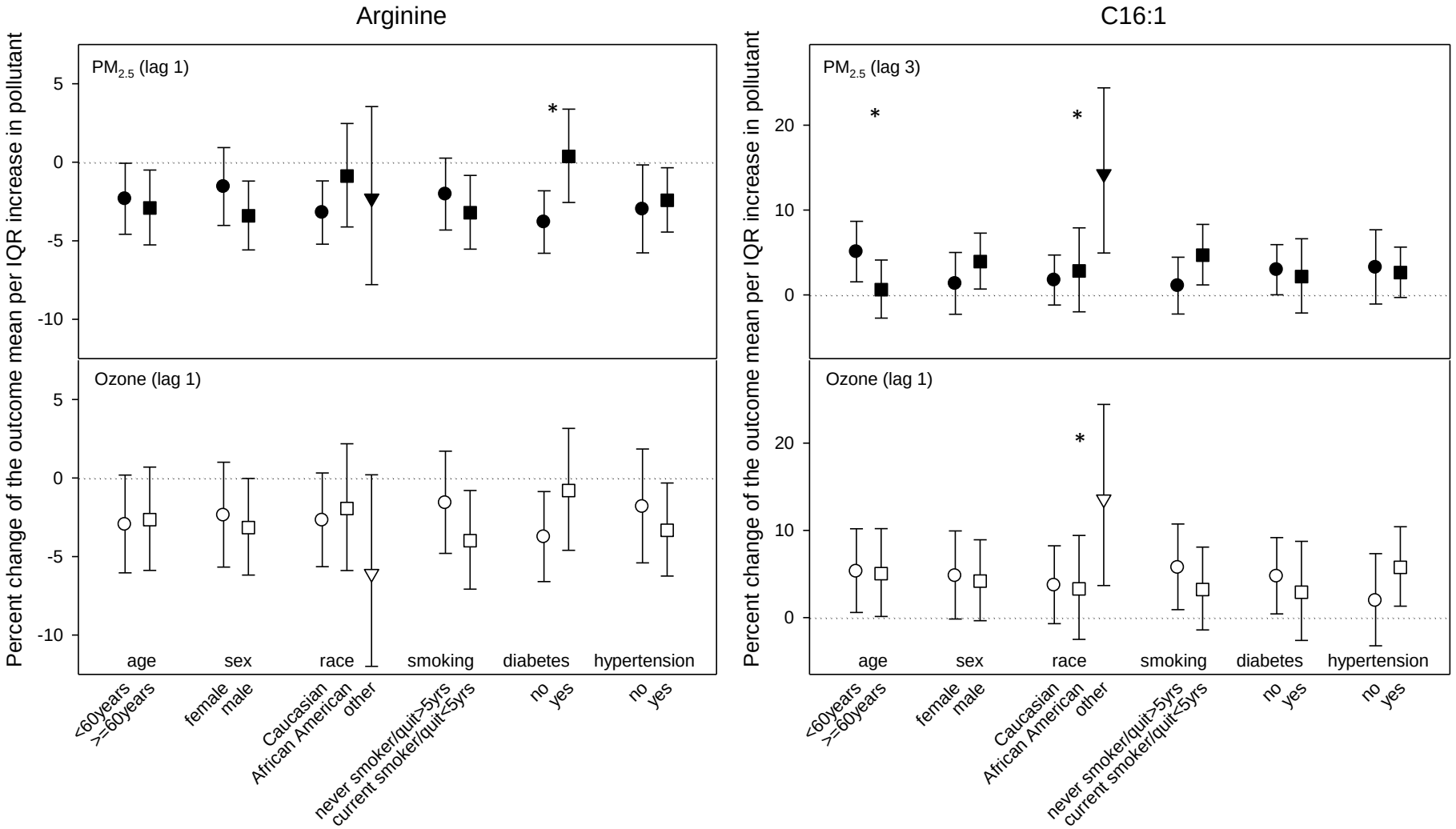


[a] Models were adjusted for time trend, air temperature, relative humidity, age, gender, body mass index, race and smoking status.

Statistically significant effect modifications were only observed for arginine and C16:1. Modifications of both $PM_{2.5}$ and ozone effects on arginine levels were observed for diabetes status; stronger associations were with those without diabetes (Figure 4). As further shown in Figure 4, both $PM_{2.5}$ and ozone effects on C16:1 were modified by race; the strongest increases were for those in the Other race/ethnicity category (for each IQR increase in $PM_{2.5}$, lag 3 or ozone, lag 1 C16:1 levels increased by 14.3% [95% CI: 4.9%; 24.4%] or 13.6% [95% CI: 3.7%; 24.4%], respectively). This category is composed mainly by self-declared Native Americans living in Southeastern North Carolina, with some minor composition from Asian, Hispanic, and unknown/undeclared individuals. Results also suggest that $PM_{2.5}$ and ozone effects on ornithine were more pronounced in African-Americans and individuals in the Other race/ethnicity category (Supplemental Material, Figure 2); moreover, effects of $PM_{2.5}$ on C16:1 were only observed in individuals younger than 60 years. Sex, smoking status, and history of hypertension did not have any modifying effects on the association between air pollution and metabolite levels (data not shown).

Using $PM_{2.5}$ data based on a combination of satellite-based aerosol optical depth retrievals and ground monitoring data (AOD + GM) gave similar results for the three amino acids arginine, glycine and ornithine compared with data from the Bayesian space-time "downscaler" fusion modeling approach (Table 3).

Figure 4. Air pollution and Arginine (left panel) or C16:1 (right panel) - effect modification.



* p-value of interaction < 0.05

Table 3. Percent change (95% confidence intervals) of the geometric mean of Cluster 2 amino acid levels per interquartile range increase in $PM_{2.5}$ based on the Bayesian space-time "downscaler" fusion modeling approach (BDFM) and based on a combination of satellite-based aerosol optical depth retrievals and ground monitoring data (AOD + GM).

Amino acids		$PM_{2.5}$ (BDFM)		$PM_{2.5}$ (AOD + GM)	
	Lag	% change	(95% CI)	% change	(95% CI)
Arginine	0	-1.05	(-2.76;0.68)	-1.36	(-3.11;0.42)
	1	-2.61	(-4.35;-0.84)**	-3.19	(-4.92;-1.43)†
	2	-1.71	(-3.46;0.08)	-2.10	(-3.92;-0.25)*
	3	-0.14	(-1.90;1.65)	-1.13	(-2.94;0.71)
	4	0.41	(-1.31;2.17)	0.94	(-0.86;2.78)
	5-day	-1.65	(-3.41;0.14)	-1.36	(-2.98;0.27)
Glycine	0	-0.44	(-1.70;0.84)	-0.64	(-1.93;0.67)
	1	-2.46	(-3.75;-1.16)†	-1.63	(-2.91;-0.33)*
	2	-1.31	(-2.61;0.01)	-0.87	(-2.22;0.49)
	3	0.90	(-0.41;2.23)	0.17	(-1.17;1.53)
	4	0.92	(-0.36;2.22)	0.42	(-0.89;1.75)
	5-day	-0.75	(-2.07;0.58)	-0.94	(-2.13;0.26)
Ornithine	0	0.92	(-0.52;2.39)	0.78	(-0.69;2.27)
	1	0.96	(-0.54;2.49)	1.20	(-0.28;2.70)
	2	0.93	(-0.57;2.46)	1.17	(-0.37;2.74)
	3	1.84	(0.35;3.36)*	1.31	(-0.21;2.86)
	4	2.23	(0.77;3.72)**	1.64	(0.16;3.15)*
	5-day	2.31	(0.79;3.85)**	2.10	(0.55;3.67)**

% change: Percent change of geometric mean 95% CI: 95% confidence interval; 5-day: 5-day average concentration;

Interquartile ranges for $PM_{2.5}$ (BDFM): Lags 0-4 8.1 $\mu g/m^3$, 5-day average 5.1 $\mu g/m^3$; Interquartile ranges for $PM_{2.5}$ (AOD + GM): Lags 0-4 7.5 $\mu g/m^3$, 5-day average 4.6 $\mu g/m^3$

* p-value < 0.05

** p-value < 0.01

† p-value < 0.001

Sensitivity analyses

As mentioned, we performed several sensitivity analyses: among them we tested increasing the degrees of freedom for the trend spline or excluding some of the confounders. None of the sensitivity analyses changed the significant associations between air pollution and metabolites (Supplemental Material, Table 5).

$PM_{2.5}$ showed independent effects on arginine and glycine, whereas ozone effects were attenuated in the two-pollutant models (Supplemental Material, Figure 3 and Table 6). Both pollutants exhibited slightly weaker associations with ornithine while ozone showed stronger effects on acylcarnitine C10:1 in the two-pollutant model. For all other metabolites except C16:1, the two-pollutant models did not change the effect estimates (Supplemental Material, Table 6). For C16:1, ozone effects exhibited stronger effects in the two-pollutant model.

Finally, we checked the exposure–response functions of metabolites and $PM_{2.5}$ or ozone for selected lags; as shown for arginine, glycine or ornithine, there was no indication for a deviation from linearity (Supplemental Material, Figures 4-6).

DISCUSSION

Summary

Prior day (1-day lag) increases in $PM_{2.5}$ were associated with decreases in the concentrations of the amino acids arginine and glycine; $PM_{2.5}$ was also associated with delayed increases in ornithine and C16:1. Increases in short-term exposures to ozone resulted in immediate and delayed increases of the amino acids aspartic acid/asparagine and ornithine; delayed increases were found for the acylcarnitines C10:1 and C16:1 as well as for total ketones. Results also suggested that there was effect modification by race on the associations of $PM_{2.5}$ and ozone with C16:1, C10:1 and ornithine.

Air pollution and metabolites

To our knowledge, there has been only one epidemiological study exploring the association between air pollution and small molecular blood-borne metabolites levels (Menni et al. 2015). Using a subset of the TwinsUK cohort, long-term exposure to PM_{10} and $PM_{2.5}$ were associated with metabolites related to reduced lung function. Eight metabolites were significantly negatively associated with PM, including asparagine and glycine. We also observed significant negative associations between glycine levels and prior day (1-day lag) increases in $PM_{2.5}$; however we did not observe significant relations between $PM_{2.5}$ and aspartic acid/asparagine. A small number of animal or toxicological studies have investigated the associations between welding fumes or ozone and metabolite levels (Miller et al. 2016; Miller et al. 2015; Wang et al. 2012; Wang et al. 2015; Wei et al. 2013). However, results of these studies are not directly comparable to our study because of differences in the study design, pollutants (e.g., welding fumes), time points of collection and fluid sampled.

Metabolites and cardiovascular disease

Metabolic profiling has the potential to identify novel biological mediators of cardiovascular disease (Shah et al. 2012a). Metabolic profiles are associated with CAD, atherosclerosis and with major adverse cardiovascular events including: myocardial infarction, stroke, heart failure and death (Kordalewska and Markuszewski 2015; Shah et al. 2012a; Würtz et al. 2015). Tang et al. (2009)(Tang et al. 2009) reported a strong association of arginine - and its downstream metabolites ornithine and citrulline - with CAD and incident major adverse cardiovascular events: death, myocardial infarction, and stroke. Individuals with CAD have significantly lower arginine, but greater ornithine and citrulline concentrations compared to CAD free individuals; this may be an indication of lower arginine bioavailability (Tang et al. 2009). Arginine is necessary for production of nitric oxide (NO); and NO is important for maintaining vascular health and homeostasis. Ornithine is produced by the cleavage of urea from arginine; this results in less arginine bioavailability. Low arginine bioavailability ratios (arginine:ornithine) are inversely associated with markers of endothelial function (Sourij et al. 2011).

In a recent study, plasma glycine was inversely associated with risk of subsequent myocardial infarction in individuals with suspected stable angina pectoris (Ding et al. 2016). Several metabolomic investigations have recorded an association of decreased glycine concentrations with diabetes (Ferrannini et al. 2013; Floegel et al. 2013) and obesity (Newgard et al. 2009). Further, lower glycine concentrations are a predictor of individuals who develop glucose intolerance and diabetes (Wang-Sattler et al. 2012). The mechanisms linking blood plasma glycine and diabetes are related but remain unexplained. However, it has been speculated that insulin resistance might result in increased expression of δ-aminolevulinic acid synthase 1 (ALAS1) and production of 5-aminolevulinic acid from glycine; alternatively, oxidative stress associated with diabetes leads to increased demand for glutathione and depletion of circulating glycine (Roberts et al. 2014). Further, glycine is the end product of a series of reactions from choline, through sarcosine whereby single carbon units are donated to the one-carbon folate pool — important for defense against oxidative stress. Finally, glycine, ornithine and arginine are involved in the condensation reaction producing creatine. The intriguing inverse associations among glycine and arginine with ornithine and air pollutants in this study should be noted. This observation may provide an important clue to the involvement of the creatine condensation reaction as a mediator of short-term air quality effects on cardiometabolic diseases.

Medium-chain acylcarnitines and long-chain dicarboxylacylcarnitines were positively associated with an increased risk for all-cause mortality in participants from the CATHGEN cohort (Shah et al. 2012b). Moreover, a metabolic factor related to medium- and long-chain acylcarnitines was associated with an increased risk for cardiovascular events in elderly individuals (Rizza et al. 2014). Higher levels of acylcarnitines indicate inefficient ß-oxidation and mitochondrial dysfunction; and

medium- and long-chained acylcarnitines are assumed to be an indicator of the defect in mitochondrial oxidative capacity associated with insulin resistance (Schooneman et al. 2013). Incomplete fatty acid oxidation in bodily tissues would be expected to yield a higher plasma ketone concentration (total ketone and beta-hydroxybuterate)—as observed in our study.

The observed short-term associations between air pollution and metabolite levels in our study are currently of unknown clinical significance; however, they provide evidence of air pollution-related physiologic changes and offer further insights into the pathophysiologic mechanisms by which air pollution may increase the risk of acute cardiovascular events.

Air pollution effects in potentially susceptible subgroups

Infants, the elderly, the obese, and those with underlying disease - particularly cardiovascular disease or type-2 diabetes - are particularly susceptible to the health effects of $PM_{2.5}$ and ozone (Lanzinger et al. 2014; Rückerl et al. 2011; Shumake et al. 2013; Stafoggia et al. 2010). Effect modifications were mostly non-significant in our study and confined to single metabolites. We observed stronger $PM_{2.5}$ effects on C16:1 levels in individuals younger than 60 years. Interestingly, air pollution effects on arginine were stronger in those without diabetes. This is contrary to many other studies that found individuals with diabetes to be more susceptible to the effects from air pollution (Dubowsky et al. 2006; O'Neill et al. 2007; Schneider et al. 2010); we are at a loss to explain this inconsistency. We observed stronger associations between short-term $PM_{2.5}$ or ozone exposure and C16:1, C10:1 and ornithine in the Other race/ethnicity category; a substantial number of these participants reside in Robeson County, where many people of Native American descent reside. Although these data suggest this population might be more susceptible to air pollution, additional research to better identify Native American participants in the CATHGEN cohort will be needed before definitive conclusions can be reached.

There is additional evidence for environmental effects on some of our selected metabolites. In a previous genetic study including 100 individuals in 10 families with early onset cardiovascular disease, we examined the heritability of metabolites as indicated by the correlation structures observed among parent-offspring, siblings and spouses (Shah et al. 2009). Spouses are generally genetically unrelated and thus, highly correlated metabolites between spouses could be attributed to the environments they share. We re-examined the correlations and found moderate to high spouse correlations ($r>0.5$) for arginine, glycine, ornithine, and C18:1. The latter showed an environmental influence on C16:1 ($r=0.73$).

Strengths and limitations

A strength of CATHGEN is the availability of detailed information on demographics and cardiometabolic risk factors; this enables appropriate adjustment for potential confounders (Kraus et

al. 2015). Moreover, all variables were assessed prospectively prior to catheterization in a fasting state.

A further strength is the confirmation of observations using alternative methods for determining air quality exposures. We obtained daily $PM_{2.5}$ concentrations from two different sources; using data based on a combination of satellite-based aerosol optical depth retrievals and ground monitoring data (AOD + GM) led to similar effects compared to results obtained from the Bayesian space-time "downscaler" fusion modeling approach.

One potential weakness is the risk for false discovery; in an exploratory analytic approach, we performed a large number of analyses in ten metabolites. We sought to minimize the risk of false discovery by reducing the number of metabolites through cluster analysis. We selected for analysis only metabolites having high measurement accuracy, a low percentage of values below the detection limit, and/or which were uncorrelated with other metabolites within the same cluster. By conducting appropriate sensitivity analyses we are confident of our findings. Irrespective of the risk of multiple comparisons, and given the limited knowledge concerning the effects of air pollution on metabolite exposures, these exploratory analyses hold substantial value; they may be useful for generating hypotheses regarding the biological mechanisms of cardiovascular disease.

No repeated measurements of metabolite levels for each participant are available; therefore, potential variation of metabolite levels within one individual could not be taken into account. In contrast, a large number of individuals were included in the analyses; this made possible the investigation of potential air pollution effect modifications by intrinsic individual characteristics. We were not able to adjust for medication, as this information was only available for a few hundred participants; however, medication use has not influenced previous studies in this population (Shah et al. 2010; Shah et al. 2012a; Shah et al. 2012b). A more detailed adjustment for smoking was not possible: only current smoking status was obtained in the study. Finally, one should be cautious in generalizing our observations to a community sample; only patients undergoing cardiac catheterization and with a high risk of CAD were included. Nevertheless, these studies may provide useful mechanistic clues to the metabolic underpinnings of cardiovascular disease.

Conclusions

Short-term ambient $PM_{2.5}$ and ozone exposures were associated with plasma concentrations of metabolites in a cohort of cardiac catheterization patients. Our findings suggest that environmental stressors — such as air pollution — are important factors to consider when examining the metabolic mechanisms of cardiovascular disease. The glycine-ornithine-arginine metabolic axis and incomplete fatty acid oxidation associated with mitochondrial dysfunction as mediators of cardiometabolic risk are of particular interest for further investigation.

Disclaimer

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