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Original article

Metabolomics

Short-term NO_2 exposure is associated with long-chain fatty acids in prospective cohorts from Augsburg, Germany: results from an analysis of 138 metabolites and three exposures

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Abstract

Background: Short-term exposure to air pollution is associated with morbidity and mortality. Metabolites are intermediaries in biochemical processes, and associations between air pollution and metabolites can yield unique mechanistic insights.

Methods: We used independent cross-sectional samples with targeted metabolomics (138 metabolites across five metabolite classes) from three cohort studies, each a part of the Cooperative Health Research in the Region of Augsburg (KORA). The KORA cohorts are numbered (1 to 4) according to which survey they belong to, and lettered S or F according to whether the survey was a baseline or follow-up survey. KORA F4 ($N = 3044$) served as our discovery cohort, with KORA S4 ($N = 485$) serving as the primary replication cohort. KORA F4 and KORA S4 were primarily fasting cohorts. We used the non-fasting KORA F3 ($N = 377$) cohort to evaluate replicated associations in non-fasting individuals, and we performed a random effects meta-analysis of all three cohorts. Associations between the 0–4-day lags and the 5-day average of particulate matter $(PM)_{2.5}$, NO_2 and ozone were modelled via generalized additive models. All air pollution exposures were scaled to the interquartile range, and effect estimates presented as percent changes relative to the geometric mean of the metabolite concentration (ΔGM).

Results: There were 10 discovery cohort associations, of which seven were lysophosphatidylcholines (LPCs); NO_2 was the most ubiquitous exposure (5/10). The 5-day average NO_2-LPC(28:0) association was associated at a Bonferroni corrected *P*-value threshold ($P < 1.2x10^{-4}$) in KORA F4 [$\Delta GM = 11.5\%$; 95% confidence interval (CI) = 6.60, 16.3], and replicated ($P < 0.05$) in KORA S4 ($\Delta GM = 21.0\%$; CI = 4.56, 37.5). This association was not observed in the non-fasting KORA F3 cohort ($\Delta GM = -5.96\%$; CI = −26.3, 14.3), but remained in the random effects meta-analysis ($\Delta GM = 10.6\%$; CI = 0.16, 21).

Conclusions: LPCs are associated with short-term exposure to air pollutants, in particular NO_2. Further research is needed to understand the effect of nutritional/fasting status on these associations and the causal mechanisms linking air pollution exposure and metabolite profiles.

Key words: short-term air pollution, metabolomics, long-chain fatty acids, NO2

Key Messages

- Short-term air pollution is associated with serum metabolite profiles that may provide clues to the biological mechanisms linking air pollution and health.
- Short-term exposure to NO_2 is positively associated with multiple lysophosphatidylcholines, a type of fatty acid, and 5-day average NO_2 exposure is associated with a principal components analysis-derived lysophosphatidylcholine profile indicating a broad association between NO_2 and lysophosphatidylcholines.
- Large-scale metabolomics studies can provide unprecedented insights into the associations between environmental exposures and biochemical mediators of health.

Introduction

Short-term air pollution exposure is associated with inflammatory markers,[1–3] lung function,[4,5] ischaemic heart disease and stroke,[6–9] myocardial infarction,[7,10–12] and death.[8,13,14] Potential mechanisms linking air pollution exposure and health are disruption of the autonomic nervous system, increased oxidative stress and reactive oxygen species, and direct damage to the vasculature by particulate matter.[15] Metabolomic profiling of large cohorts offers researchers the opportunity to gain insights into the mechanisms linking air pollution and disease.

Metabolomics is the study of intermediate and end-products of biochemical processes within cells.[16,17] As such it gives a snapshot of the biochemical state of cells—a product of underlying genetics,[18,19] nutrition[20–22] and environmental exposures.[23,24] The importance of metabolomics in understanding complex diseases has been highlighted recently with metabolomics profiles being associated cardiovascular disease (CVD),[25–28] cancer,[29,30] obesity[31,32] and diabetes.[33–35]

Current publications considering air pollution exposures and blood chemistries have focused on traditional clinical parameters such as: low-density lipoprotein cholesterols,[36–38] cytokines[39,40] and C-reactive protein.[41–43] In the single broad survey of metabolomics and air pollution to date, Menni *et al.* examined 280 metabolites and showed that peripheral blood metabolites are associated with both long-term exposure to air pollution [particulate matter $(PM)_{2.5}$ and PM_{10}] and lung function, possibly via inflammatory processes.[44] This manuscript will explore the role of short-term (0–4-day) variation in air pollution exposure and its association with a broad spectrum of metabolites in population-based cohorts from Augsburg, Germany.

Methods

Subjects

Participants were taken from the KORA F3, KORA S4 and KORA F4 surveys, conducted in Augsburg, Germany. The KORA cohorts are numbered (1–4) according to which survey they belong to, and lettered S or F according to whether the survey was a baseline or follow-up survey, respectively. The KORA F3 cohort is a follow-up survey taken from the KORA S3, and was conducted from 2004 to 2005.[45,46] KORA S4 is a general population survey that began in 1999 and ended in 2001.[47] KORA F4 a follow-up survey of KORA S4, with participants examined from 2006 to 2008.[48] Detailed clinical and demographic information was collected from all participants, including blood samples for later analysis. All three studies were approved by the ethics committee of the Bavarian Medical Association in Munich, Germany. KORA F4 participants with metabolomics data after applying quality control (QC) procedures comprised our discovery cohort ($N = 3044$). Our replication cohort consisted of KORA S4 participants who had metabolomics data, passed QC procedures and did not participate in KORA F4 ($N = 485$). Similarly, we restricted KORA F3 to participants passing all QC procedures and who did not participate in KORA F4 or KORA S4 ($N = 377$).

Metabolite measurement

A total of 188 serum metabolites were assayed using the Biocrates Absolute*IDQ*™ P180 kit. Metabolites were measured in serum taken from each participant on their examination date. This examination date was then linked with fixed site monitoring data from the city of Augsburg for the relevant lags before the date of examination. Identical QC procedures were used for KORA S4, KORA F4 and KORA F3 and have been published in detail.[49,50] The QC procedure was a two-step procedure applied separately to KORA S3 and KORA F4. In the first step, a coefficient of variation was calculated for each metabolite, using a reference sample measured five times across 10 plates. Metabolites with a coefficient of variation greater than 25% or with more than 5% of values missing were removed. The second step controlled for outliers by removing metabolite measurements five standard deviations beyond the mean concentration of the metabolite for that individual. Individuals with more than three 'independent outliers', outliers with a correlation < 70% with all other outliers, were removed. Missing values were imputed via a linear regression approach implemented in the R package 'mice'.[51] Multiple imputation ($n = 5$) was used and the imputed datasets averaged to get a single imputed value for each metabolite. At total of 138 metabolites passed QC in KORA S4 and KORA F4, and were available for analysis. These 138 metabolites belonged to five general classes: amino acids, phosphatidylcholines, lysophosphatidlycholines (LPCs), sphingomyelins and fatty acids.

Air pollution assessment

Air pollution was assessed via fixed monitoring sites (one for $PM_{2.5}$ and ozone, three for NO_2) within Augsburg, Germany. For the KORA F4 cohort, $PM_{2.5}$ was assessed via Tapered Element Oscillating MicroBalance (TEOM) with Filter Dynamics Measurement System (FDMS), and for the KORA S4 cohort, $PM_{2.5}$ measurements were assessed with TEOM without FDMS. NO_2 was assessed as the mean of three monitors within the study area (two urban and one background), and ozone was assessed as the daily maximal 8-h running mean. For KORA F3, we imputed missing daily monitoring data using a modified APHEA (Air Pollution and Health: A European Approach) approach.[52,53] KORA F4 and KORA S4 participants did not have any missing monitor data. Exposures are reported in $\mu g/m^3$ for all assessment methods.

Statistical methods

A total of six exposure periods were considered for each pollutant: 0-, 1, 2-, 3- and 4-day lags and the 5-day average (arithmetic mean of the 0–4-day lags). All meteorological variables were taken so as to correspond to the lag being assessed. Before analysis, all air pollution exposures were scaled to the interquartile range.

Generalized additive models implemented via the mgcv package[54] in R v3.1.3[55] were used to assess the linear association between interquartile range transformed air pollution exposure and natural-log transformed metabolite concentrations. Our primary model adjusted for season (a four-level factor variable; December–February, March–May, June–August and September–November), time trend (count of days from study start to examination), temperature, relative humidity, day of the week, age, sex, body mass index and smoking (never vs former/current). This a priori selected model matches previous approaches to the analysis of short-term air pollution exposures, including the use of both season and time trend variables to account for seasonality.[5,56] We used regression splines to account for non-linearity in the time trend, temperature and relative humidity variables. Four degrees of freedom were used for the temperature and relative humidity regression splines and four degrees of freedom per year were used for the time trend regression spline, resulting in 6, 8 and 6 degrees of freedom being used for KORA F4, KORA S4 and KORA F3 respectively. As the metabolites for KORA F4 were assessed in separate batches, a categorical 'batch' variable was included in all KORA F4 analysis to remove potential confounding from variation in the technician performing the metabolite assessment or other laboratory-related factors. Metabolites for KORA S4 and KORA F3 were assessed in a single batch. All effect estimates were divided by the geometric mean of the metabolites so that each effect estimate represents the estimated effect relative to the geometric mean of the metabolite.

Given the three air pollution exposures and 138 metabolites, we set a *P*-value cutoff of $P < 1.2\text{x}10^{-4}$, 0.05/(138*3), for discovery associations; this corresponds to a Bonferroni correction.[57] Replicated metabolites were those with a consistent direction of association and $P < 0.05$ in the KORA S4 cohort. Metabolites which replicated in KORA S4 were then checked for replication ($P < 0.05$ and same direction of association) in the KORA F3. We additionally performed a random effects meta-analysis of all three cohorts for all metabolite-exposure combinations with $P < 1.2\text{x } 10^{-4}$ in KORA F4. We used a random effects meta-analysis model, due to potential for heterogeneity across studies. We report the I^2 [58] as an estimate of the heterogeneity across studies. All meta-analyses were conducted via the metafor R package.[59]

Associations from the KORA F4 primary model which passed our *P*-value cutoff were tested for association in a clinical model which included all primary model terms

plus: influenza days (assessed on the day of examination), socioeconomic status (SES) assessed via the Helmert method,[60] alcohol consumption (g/day) and diabetes status (presence of type 2 diabetes or no). We additionally examined discovery associations for interactions between the short-term air pollution exposures and sex and diabetes, as well as smoking status. The effect of fasting status was evaluated by analysing the clinical model in fasting KORA F4 participants ($N = 3028$). We also re-analysed the KORA F4 associations using a fixed 5-day average lag for all of the meteorological variables. We performed this sensitivity analysis to evaluate the impact of our choice to pair the lag for the meteorological variables with that of the air pollution exposures, which may represent a strong assumption on the link between meteorology and air pollution exposure.

To evaluate associations between short-term air pollution exposure and metabolite profiles, we used principal components analysis (PCA) via the 'stats' package in R[55] to construct principal components composed of metabolites, which we call metabolite profiles, and associate these with our air pollution exposures using the primary model. We constructed the metabolite profiles in two ways. First, by performing PCA on all metabolites, and second, by performing PCA in a metabolite class-specific manner based on those metabolite classes with replicated associations. Since PCA decomposes the total variance of a dataset, by focusing on those classes of metabolites shown to be associated in KORA F4 and replicated in KORA S4 we may enrich for associations. We set the significance level for each set of PCA metabolite profiles at 0.05/(number PCA components tested). For the profiles based on all metabolites, we used the Scree plot to determine which metabolite profiles accounted for an outsized proportion of the variance as compared with all PCA components. For the metabolite class phosphatidylcholines (PCs), we test all PCs from that metabolite class. All metabolite profiles were constructed in KORA F4 and replicated in KORA S4. We replicated the metabolite profiles from KORA F4 in KORA S4 by directly applying the calculated KORA F4 loadings for each metabolite to KORA S4, as opposed to re-doing the PCA in KORA S4 and obtaining new metabolite loadings.

Results

Clinical characteristics for all three cohorts used for this analysis are given in Table 1. All results are given as percent change relative to the geometric mean of the metabolite concentration (ΔGM) per interquartile range increase in air pollution. All confidence intervals (CI) given are 95% CIs.

Table 1. Clinical and meteorological covariates for the KORA F4, KORA S4 and KORA F3 cohorts

	KORA F4	KORA S4	KORA F3
N	3044	485	377
Sex (% males)	48.2	51.5	52.3
Age (years)	56.1 (13.2)	65.8 (5.31)	65.9 (7.37)
BMI (kg/m^2)	27.6 (4.82)	29.0 (4.72)	28.5 (3.93)
Smoking (% never smokers)	55.8	55.6	53.0
SES (Helmert)	14 (5.08)	11.8 (5.13)	
SES (years of education)			11 (2.39)
Alcohol consumption (g/day)	14.3 (19.5)	15.8 (20.8)	16.1 (21.7)
Type 2 diabetes (% yes)*	27	41.4	13.5
Temperature (°C)	8.98 (6.78)	10.7 (7.25)	6.42 (8.26)
Relative humidity	77.7 (12.5)	76.2 (12.9)	72.5 (13)
Number of influenza days/year	109	55.8	
$PM_{2.5}$ (24 h, μg/m^3)	14.8 (10.8)	16.0 (6.59)	15.6 (16.1)
Ozone (8 h max, μg/m^3)	62.2 (31.2)	65.9 (35.3)	67.9 (34.2)
NO_2 (24 h, μg/m^3)	34.5 (10.8)	41.8 (10.7)	48.6 (15.2)

Mean (SD) given for continuous variables.

*Diabetes case status for KORA F3 does not differentiate between type 1 and type 2 diabetes.

Associations in KORA F4 and replication in KORA S4 and KORA F3

Ten associations representing seven metabolites passed our discovery *P*-value cutoff ($P < 1.2 \times 10^{-4}$) in KORA F4 using our primary model (Supplementary Table 1, available as Supplementary data at *IJE* online). Five-day average NO_2 exposure was associated with four metabolites: lysophosphatidylcholine 28:0 [LPC(28:0)] (ΔGM = 11.5%; CI = 6.60, 16.3), lysophosphatidylcholine 26:1 [LPC(26:1)] (ΔGM = 0.66%; CI = 0.38, 0.94), C6:1 (ΔGM = 224%; CI = 126, 323); and lysophosphatidylcholine 28:1[LPC(28:1)] (ΔGM = 7.55%; CI = 4.21, 10.9). Three-day lag NO_2 was associated with LPC(26:1) (ΔGM = 0.54%; CI = 0.29, 0.78) and 2-day lag NO_2 was associated with phosphatidylcholine 40:1 [PC(40:1)] (ΔGM = 4.61%; CI = 2.29, 6.93). $PM_{2.5}$ exposure was associated with LPC(26:1) (0-day lag, ΔGM = 0.47%; CI = 0.24, 0.69) and LPC(28:0) (1-day lag, ΔGM = 7.78%; CI = 3.90, 11.7). Finally, we observed two associations with ozone: 3-day lag ozone with phosphatidylcholine (O-38:1) [PC(O-38:1)] (ΔGM = −9.88%; CI = −14.8, -4.91) and 5-day average ozone with lysophosphatidylcholine 24:0 [LPC(24:0)] (ΔGM = 21.2%; CI = 10.4, 31.9). The 5-day average NO_2-LPC(28:0) replicated in KORA S4 (Figure 1, Table 2); however, this association did not replicate in KORA

F3 (Table 2). Of the 10 KORA F4 associations in the discovery cohort, six were associated with exposures in a random effects meta-analysis, all of which were LPCs with the exception of the 3-day lag NO_2-C6:1 association (Figure 2, Table 3).

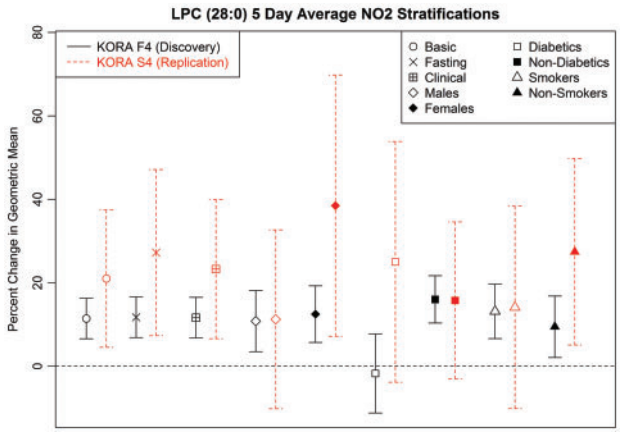

Figure 1. Associations for the primary model (Basic) clinical model (Clinical), primary model when restricted to fasting individuals (Fasting), and the stratified analyses by sex, diabetes and smoking for both the KORA F4 (solid) and KORA S4 (dashed) cohorts. The interaction between diabetes status and 5-day average NO_2 had a $P < 0.05$ in KORA F4. Associations for the Basic, Full and Fasting models were nearly identical.

Sensitivity analyses of KORA F4 associations

All 10 associations observed in KORA F4 primary model analysis remained in the clinical model and when restricted to fasting participants (Supplementary Table 1). We stratified the KORA F4 cohort on sex, diabetes and smoking status to determine if there were any clinical state-specific associations or potential interactions. As the LPC(28:0)-5 day average NO_2 association was the only replicated association, we focused our stratified and interaction analysis on this metabolite-lag-exposure. There were no strong differences according to sex in KORA F4; however, KORA S4 associations indicated that females had a weaker association than males. Individuals with type 2 diabetes had a stronger association in KORA F4. The interaction between air pollution and type 2 diabetes was the only interaction with $P < 0.05$ ($P = 0.02$; Figure 2, Table 2). As an additional sensitivity analysis, we evaluated the effect of our choice to match the meteorological variables to the air pollution lag being considered. When using a fixed 5-day average of the meteorological variables, all 10 KORA F4

Table 2. Regression estimates for the clinical and fasting models and diabetes, smoking and sex stratifications of the primary model for KORA F4 and KORA S4 for the LPC (28:0) - 5-day average NO_2 exposure. Interactions between the exposure and diabetes, sex and smoking were assessed in KORA F4. Though stratifications on clinical variables were examined in both KORA F4 and KORA S4, interactions with sex, diabetes status and smoking were only directly tested for in KORA F4 as described in Methods. For reference, the primary model associations are also given. The regression estimate scaled to a percent change in geometric mean (ΔGM) and 95% confidence interval (CI) are given. The 'Fasting Individuals Model' was the Clinical Model restricted to participants fasting at the time of sample collection

KORA F4		ΔGM (%)	CI	*P*-value	Interaction *P*-value
	Primary Model	11.5	6.6, 16.3	3.91×10^{-6}	
	Clinical Model	11.6	6.78, 16.5	2.77×10^{-6}	
	Fasting Individuals Model	11.8	6.89, 16.6	2.32×10^{-6}	
	Males	10.8	3.51, 18.1	0.004	0.81
	Females	12.5	5.70, 19.3	3.19×10^{-4}	
	Type 2 diabetes (no)	16.0	10.3, 21.7	3.54×10^{-8}	0.016
	Type 2 diabetes (yes)	−1.73	−11.2, 7.74	0.72	
	Never smokers	9.46	2.10, 16.8	0.01	0.16
	Smokers	13.2	6.65, 19.7	7.83×10^{-5}	
KORA S4					
	Primary Model	21.0	4.56, 37.5	0.013	
	Clinical Model	23.3	6.60, 40.0	0.007	
	Fasting Individuals Model	27.2	7.33, 47.1	0.008	
	Males	11.3	−10.2, 32.7	0.30	Interaction not assessed
	Females	38.0	7.18, 69.8	0.017	
	Type 2 diabetes (no)	15.8	−3.02, 34.6	0.10	Interaction not assessed
	Type 2 diabetes (yes)	25.0	−3.83, 53.9	0.091	
	Never smokers	27.4	5.10, 49.8	0.017	Interaction not assessed
	Smokers	14.1	−10.1, 38.4	0.26	

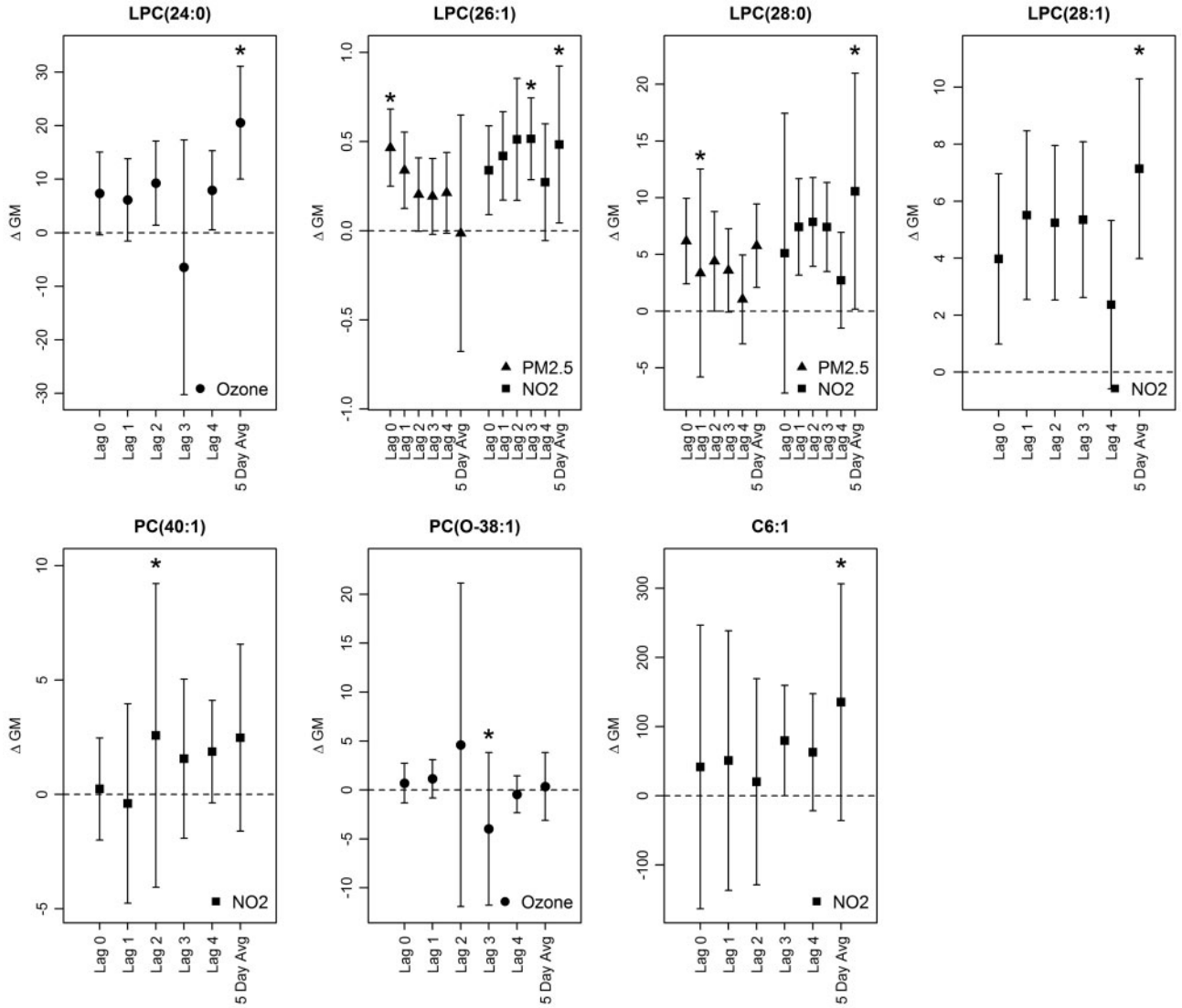

Figure 2. Random effects meta-analysis results for those metabolite-air pollution pairs with at least one association with $P < 1.2 \times 10^{-4}$ in KORA F4. The six lags are shown across the x-axis. On the y-axis is the percent change relative to the geometric mean (ΔGM). * = associations with $P < 1.2 \times 10^{-4}$ in KORA F4.

Table 3. Meta-analysis results for discovery metabolite associations with $P < 1.2 \times 10^{-4}$. Random effects meta-analysis of all three cohorts

Association	ΔGM (%)	CI	*P*	I^2
C6:1-NO_2 5-day average	135	−35.9, 307	0.12	40.4
PC(40:1)-NO_2 2-day lag	2.58	−4.05, 9.21	0.45	71.2
PC(O-38:1)-ozone 3-day lag	−3.97	−11.8, 3.84	0.32	80.9
LPC(24:0)-ozone 5-day average	20.6	10.0, 31.1	1.30×10^{-4}	0.0
LPC(26:1)-$PM_{2.5}$ 0-day lag	0.47	0.25, 0.68	2.6×10^{-5}	0.0
LPC(26:1)-NO_2 3-day lag	0.52	0.29, 0.75	1.1×10^{-5}	0.0
LPC(26:1)-NO_2 5-day average	0.48	0.04, 0.92	0.03	24.4
LPC(28:0)-$PM_{2.5}$ 1-day lag	3.36	−5.81, 12.5	0.47	41.9
LPC(28:0)-NO_2 5-day average*	10.6	0.16, 21.0	0.05	48.1
LPC(28:1)-NO_2 5-day average	7.14	3.99, 10.3	9.1×10^{-6}	0.0

I^2, I^2 statistic for heterogeneity.

*$P < 1.2 \times 10^{-4}$ in discovery (KORA F4) and $P < 0.05$ in replication (KORA S4) analyses of primary model.

associations with $P < 1.2x10^{-4}$ in the initial analysis remained associated, though some of the effect sizes were attenuated (Supplementary Table 2, available as Supplementary data at *IJE* online).

As the Bonferroni cutoff used for the discovery *P*-value threshold can be conservative, we also report all associations with $P < 0.05$ when using the Benjamini-Hochberg false discovery rate (FDR) correction.[61] Here there were 26 associations in KORA F4 with an FDR $P < 0.05$. As seen when using a Bonferroni *P*-value-based cutoff, the results with an FDR $P < 0.05$ were dominated by LPCs (14/26) and NO_2 was the most common exposure (18/26) (Supplementary Table 3, available as Supplementary data at *IJE* online).

Analyses of PCA constructed metabolite profiles

Based on the Scree plot from the PCA of all metabolites, only the first principal component, i.e. metabolite profile, contained a substantially greater proportion of variance than the other metabolite profiles (Supplementary Figure 2, available as Supplementary data at *IJE* online). We number the metabolite profiles in order of their percent variance explained. For both KORA S4 and KORA F4, this metabolite profile was composed of long-chain phosphatidylcholines but it was not associated with any air pollution exposure (data not shown). Given the multiple discovery associations and replicated association between LPCs and 5-day average NO_2 exposure, we investigated associations between LPC metabolite profiles and 5-day average NO_2. There was substantial correlation (Pearson's R) between KORA S4 and KORA F4 for the top LPC profiles (Supplementary Table 4, available as Supplementary data at *IJE* online). There were 13 total LPCs shared between KORA S4 and KORA F4, and PCA yielded 13 LPC profiles. Thus, associated metabolite profiles were determined to be those with $P < 0.0038$. For KORA F4 there were two LPC metabolite profiles associated with 5-day average NO_2 exposure: LPC profile 2 ($P = 9.9x10^{-6}$) and LPC profile 10 ($P = 0.001$). The 5-day average NO_2-LPC profile 2 association replicated in KORA S4 ($P = 0.047$). LPC profile 2 explained 7.7% of the LPC variation in KORA F4. KORA F4 loadings for LPC profile 2 are given in Supplementary Table 5 (available as Supplementary data at *IJE* online).

Discussion

We analysed the short-term effects of air pollution exposures on serum metabolites and metabolite profiles. Each of these air pollution exposures is known to have adverse health effects,[15,62–65] and many of the health outcomes associated with these exposures have themselves been associated with metabolites.[66–69] To overcome the potential for an excess of false-positives due to the number of tests performed, we used a Bonferroni correction for the number of metabolites and exposures assessed. This correction controls the family-wide error rate by imposing a *P*-value cutoff on associations, here $P < 1.2x10^{-4}$. This is an effective but restrictive method to limit false-positive associations. To give a broader examination of associations, we have included all those associations with an FDR $P < 0.05$ in Supplementary Table 3. The 5-day average NO_2 exposure had the greatest number of associations in our discovery cohort (Supplementary Table 1). Our primary model adjusted for age, sex, obesity(BMI) and smoking – all known to associate with metabolite concentrations.[50,70–72] We additionally controlled for season using both season indicator and linear terms. We feel this seasonal adjustment is warranted given the known association between metabolites and season.[73,74] Using the primary model we observed 10 associations in KORA F4, and we replicated the association between 5-day average NO_2 exposure and LPC(28:0). Six of the 10 discovery associations were associated in the meta-analysis, and four of the six meta-analysis associations were with NO_2 exposure. NO_2 is primarily associated with traffic-related air pollution,[75–77] and is associated with a variety of adverse health outcomes.[64,65,78,79]

LPCs and air pollution

In the discovery, replication and meta-analysis associations, the LPCs were the most consistently represented metabolite class. LPCs are generated via enzymatic reactions catalyzed by phospholipase A1 and phospholipase A2. In a previous study of pulmonary artery endothelial cells, short-term (24- and 48-h) exposure to NO_2 increased the activity of phospholipase A1 relative to cells receiving a control exposure.[80] Phospholipase A1 and phospholipase A2 generate specific isomers of LPCs via SN1 and SN2 reactions, respectively. Our LPC measures represent the sum of these two isomers, and further analysis is necessary to associate enzyme-specific isomers with NO_2 exposure.

Though only the NO_2 exposure replicated, both $PM_{2.5}$ and ozone additionally had associations with metabolites in the KORA F4 cohorts. The meta-analysis also revealed associations between ozone and $PM_{2.5}$ and LPCs particularly when examining the 5-day average (Figure 2, Supplementary Table 2). Thus, whereas NO_2 is the exposure most strongly and broadly associated with LPCs in our analysis, it is likely that multiple exposures are associated with specific LPCs. In an analysis of LPC metabolite profiles created via PCA, the 5-day average NO_2 exposure was

associated with two LPC metabolite profiles in KORA F4, one of which replicated in KORA S4.

Very-long chain fatty acids and health

All of the LPCs with $P < 1.2x10^{-4}$ in KORA F4 and $P < 0.05$ in the meta-analysis associations belong to a class of fatty acids referred to as very-long chain fatty acids (VLCFAs). The accumulation of VLCFAs has been linked to oxidative stress.[81] Additionally, X-linked adrenoleukodystrophy (X-ALD), a peroxisomal disorder, has been linked to the accumulation of VLCFAs.[82] Individuals with X-ALD have also been found to have increased reactive oxygen species in their fibroblasts,[83] suggesting that oxidative stress may be a contributing factor or by-product of X-ALD and the accumulation of VLCFAs. Thus, oxidative stress may link short-term air pollution exposure and LPCs given the known association between air pollution and oxidative stress.[84]

Strengths and limitations

The main strength of this study is the use of multiple cohorts to perform independent cross-sectional analyses to establish a relationship between short-term variation in air pollution and serum metabolites. We used three independent cohorts with a combined sample size of 3906 to discover and replicate our associations and perform a meta-analysis of replicated associations. In the meta-analysis, the majority of the associations showed little heterogeneity; however, for the majority of the associations the effect sizes and *P*-values were attenuated in the random effects meta-analysis as compared with a fixed effects meta-analysis (Table 3), and there was substantial inter-cohort variability in the effect estimates (Supplementary Figure 1, available as Supplementary data at *IJE* online), prompting us to focus our investigations on the random effects meta-analysis. The lack of reported heterogeneity despite the observed inter-cohort variability and fixed effects results attenuation could indicate that the heterogeneity is being underestimated as can occur in meta-analyses with low numbers of studies.[58] Reasons for the inter-cohort variability (especially with respect to KORA F3) include: variations in sample size; differences in fasting status; and the imputation procedure used to replace missing values for NO_2, $PM_{2.5}$ and ozone in KORA F3 – which was not necessary for KORA F4 or S4.

Another strength of our study is that air pollution and metabolite assessment were done independently and the large selection of metabolites assessed. We assessed 138 metabolites belonging to a variety of different classes. This provides a broad look at metabolite associations across several metabolite classes. Also, we were able to create class-specific metabolite profiles and demonstrate that an LPC metabolite profile was associated with 5-day average NO_2 exposure.

A limitation of our study is the fact that all cohorts were sampled from the same region in Southern Germany. This geographically restricted sampling may limit the generalizability of our associations. Further work to establish these associations in multi-ethnic cohorts should be undertaken. Also, although we adjusted for both clinical and meteorological factors in our primary model, and verified that associations remain in a more fully adjusted clinical model, the possibility persists that unobserved confounders may alter the observed associations. The most prominent of these would likely be dietary factors which were not assessed in the KORA cohorts. The effects from diet could be long-lasting and persist even if samples are taken in a fasting state. Future analyses should seek to collect detailed dietary information to establish the independence of these associations from dietary confounders. A final limitation is the use of a fixed monitoring site which could cause exposure misclassification. We expect that this error would be of the Berkson type, i.e. independent of true exposure, and therefore only impact on the standard error and should be offset by our large sample size for the discovery cohort and meta-analysis.

Conclusion

In conclusion, we observe multiple associations between short-term air pollution exposure and serum metabolites. Several LPCs were associated with short-term air pollution exposure, with NO_2 responsible for five of the 10 discovery cohort associations and the only replicated association [5-day average NO_2–LPC(28:0)]. In a meta-analysis, the strongest LPC associations by *P*-value were with NO_2, and NO_2 was the exposure most often associated with the LPCs. However, additional LPC associations were observed for $PM_{2.5}$ - LPC(28:0) and -LPC(26:1) and ozone [LPC(24:0)]. Finally, we observed an association between 5-day average NO_2 and a metabolite profile based on the LPCs, indicating that the LPCs may be broadly associated with short-term exposure to NO_2.

Supplementary Data

Supplementary data are available at *IJE* online.

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