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Full length article

Longitudinal associations between metabolites and long-term exposure to ambient air pollution: Results from the KORA cohort study

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ABSTRACT

Background: Long-term exposure to air pollution has been associated with cardiopulmonary diseases, while the underlying mechanisms remain unclear.
Objectives: To investigate changes in serum metabolites associated with long-term exposure to air pollution and explore the susceptibility characteristics.
Methods: We used data from the German population-based Cooperative Health Research in the Region of Augsburg (KORA) S4 survey (1999–2001) and two follow-up examinations (F4: 2006–08 and FF4: 2013–14). Mass-spectrometry-based targeted metabolomics was used to quantify metabolites among serum samples. Only participants with repeated metabolites measurements were included in the current analysis. Land-use regression (LUR) models were used to estimate annual average concentrations of ultrafine particles, particulate matter (PM) with an aerodynamic diameter less than 10 μm (PM_{10}), coarse particles (PM_{coarse}), fine particles, $PM_{2.5}$ absorbance (a proxy of elemental carbon related to traffic exhaust, $PM_{2.5abs}$), nitrogen oxides (NO_2, NO_x), and ozone at individuals' residences. We applied confounder-adjusted mixed-effects regression models to examine the associations between long-term exposure to air pollution and metabolites.
Results: Among 9,620 observations from 4,261 KORA participants, we included 5,772 (60.0%) observations from 2,583 (60.6%) participants in this analysis. Out of 108 metabolites that passed stringent quality control across three study points in time, we identified nine significant negative associations between phosphatidylcholines (PCs) and ambient pollutants at a Benjamini-Hochberg false discovery rate (FDR) corrected p-value < 0.05. The strongest association was seen for an increase of 0.27 μg/m^3 (interquartile range) in $PM_{2.5abs}$ and decreased phosphatidylcholine acyl-alkyl C36:3 (PC ae C36:3) concentrations [percent change in the geometric mean: −2.5% (95% confidence interval: −3.6%, −1.5%)].
Conclusions: Our study suggested that long-term exposure to air pollution is associated with metabolic alterations, particularly in PCs with unsaturated long-chain fatty acids. These findings might provide new insights into potential mechanisms for air pollution-related adverse outcomes.

1. Introduction

Epidemiological studies have shown associations between chronic exposure to ambient air pollution and pulmonary, cardio-metabolic, and neurological disease, and even mortality (Bae et al. 2021; Cao et al. 2020; Hales et al. 2021; Kasdagli et al. 2022; Liu et al. 2021; Mortamais et al. 2021; Park et al. 2021; Wolf et al. 2021). However, the underlying biological mechanisms are not yet fully elucidated. Hypothesized pathways linking air pollution exposure and health include the direct translocation of ambient particles with a smaller aerodynamic diameter (e.g., ultrafine particles) and gaseous air pollutants (e.g., nitrogen dioxide and ozone) from the lung into the blood leading to alternations of

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blood parameters (Nemmar et al. 2002). Another possible pathway is the induction of local inflammatory responses in the lung by larger inhaled ambient particles leading to autonomic cardiac, systemic inflammatory, and haemostatic activities (Brook et al. 2010).

The blood metabolome is a collection of biologically active chemicals in the human blood, derived from endogenous processes and exogenous exposure to food, medicines, and pollutants (Rappaport et al. 2014). Metabolomics has become a well-developed tool to investigate small molecular metabolites presented in the biological systems and corresponding cellular responses perturbed by endogenous or exogenous stimuli (Holmes et al. 2008). Recent epidemiological studies have provided evidence of adverse air pollution-induced effects on metabolomic biomarkers (Chen et al. 2019a; Gaskins et al. 2021; Hood et al. 2022; Li et al. 2017; Li et al. 2021; Liang et al. 2018; Ritz et al. 2022; van Veldhoven et al. 2019; Vlaanderen et al. 2017; Ward-Caviness et al. 2016). However, these studies mainly focused on short-term and intermediate exposures (day-to-day changes) to air pollution. Only a few studies have examined the metabolomics signatures in response to long-term air pollution exposures (e.g., annual averages) within cohort studies (Jeong et al. 2018; Nassan et al. 2021a; Nassan et al. 2021b; Walker et al. 2019). These studies were either limited to small sample sizes or focused on specific individuals, for example, older men or participants with adult-onset asthma or cardio-cerebrovascular diseases.

Given the limited evidence, especially within a general population cohort study, we aimed to determine the associations between long-term ambient air pollution and targeted metabolomics within the population-based Cooperative Health Research in the Region of Augsburg (KORA) cohort, conducted in the area of Augsburg, Germany. Additionally, we explored the role of potential individual characteristics in modifying the effects of air pollution effects, including body mass index (BMI), lifestyle (e.g., smoking status, alcohol consumption, physical activity, and dietary patterns), pre-existing diseases (e.g., hypertension and diabetes), and medication intakes (e.g., anti-hypertensive, anti-diabetic, and lipid-lowering medication). We hypothesized that long-term exposure to air pollution is associated with the perturbation of serum metabolite concentrations involved in some metabolic pathways related to adverse health effects from ambient air pollution and that individuals' characteristics can modify these health effects.

2. Methods

2.1. Study design and participants

In this longitudinal study, we used data from the KORA cohort. The fourth cross-sectional health survey of the KORA cohort (KORA S4) was conducted from October 1999 to April 2001. It involved 4,261 participants aged 25–74 years with German citizenship in the city of Augsburg, Germany, and two adjacent counties. Two follow-up examinations were carried out: within the first follow-up (KORA F4), 3,080 participants were examined between Oct 2006 and May 2008, whereas the second follow-up (KORA FF4) consisted of 2,279 participants with examinations between June 2013 and Sept 2014.

A computer-assisted personal interview, a self-administered questionnaire, and physical examinations were performed at each visit by trained investigators at the study centre. Physical activity was categorized based on the time spent on physical exercise into low (no or almost no physical exercise), medium (regular or irregular approx. one hour per week), and high (more than two hours per week) levels. Alcohol consumption was categorized into no (0 g/day), moderate (men 0.1–39.9 g/day and women 0.1–19.9 g/day), and high (men $\geq$ 40 g/day and women $\geq$ 20 g/day) consumption. Smoking status was categorized into current (regular or irregular smokers), former (ex-smokers), and never (never-smokers) smokers. A diet questionnaire with a qualitative food frequency list was performed to collect the dietary intake; a continuous dietary score and categorical dietary patterns were defined based on participants' answers. Briefly, the individuals' dietary intake was collected using a food-frequency questionnaire investigating 24 food groups. An index was built rating the frequency with which each food was consumed by assigning either 0, 1, or 2 points based on recommendations of the German Nutrition Society (DGE). Higher scores reflect better compliance with DGE recommendations. A sum dietary score ranging from 0 to 27 was calculated according to DGE guidelines and subsequently grouped into three categories: adverse ($\leq$13 points), ordinary (14 $\sim$ 15 points), and favourable ($\geq$16 points) dietary patterns. This approach was established in earlier KORA studies and was validated against a weighed 7-day dietary protocol (Rabel et al. 2018; Winkler and Döring 1998).

Only participants who attended at least two visits across the entire study period were included in this longitudinal analysis. Additionally, we excluded participants with missing data on covariates used in our main analysis (**Fig. S1**). Written informed content was obtained from all participants. The KORA study was approved by the ethics committee of the Bavarian Chamber of Physicians (Munich, Germany).

2.2. Biomarker measurements

Blood samples were drawn into serum gel tubes between 8:00 am and 10:30 am after at least 8 h of overnight fasting. The blood samples were kept at 4 °C up to six hours after blood withdrawal for further procedure. Serum was collected and filled into synthetic straws, and stored in liquid nitrogen (−80 °C) until the further analyses were conducted.

2.3. High-sensitivity C-reactive protein (hs-CRP)

The high-sensitivity C-reactive protein (hs-CRP) assay was performed shortly after the blood withdrawal for each study wave (KORA S4 (September-December 2001), KORA F4 (July-October 2008), and for KORA FF4 (December 2015-March 2016)). hs-CRP was measured in serum by a BN nephelometer (Siemens Healthcare Diagnostics Product GmbH, Marburg, Germany) in the collaborating Biomarker Laboratory at the University of Ulm, Germany.

2.4. Targeted metabolomics

The metabolite profiling in serum samples was done with the AbsoluteIDQ^{TM} p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) for KORA S4 (March–April 2011) and FF4 (February–October 2019), allowing for the simultaneous quantification of 188 metabolites. KORA F4 samples were measured with the AbsoluteIDQ^{TM} p150 kit to detect 163 metabolites in August 2008–March 2009. The assay procedures have been described previously in detail (Römisch-Margl et al. 2012).

Identical quality control (QC) procedures were used in each of the three study points in time. Each metabolite should meet the following three criteria: (1) The average value of the coefficient of variance (CV) in the five/six reference samples or three quality control samples should be less than 25%; (2) 50% of all measured sample concentrations for the metabolite should be above the limit of detection (LOD), which was defined as three times the median of zero samples; (3) The rate of missing value of metabolite should be less than 5%. The non-detectable values of each metabolite were randomly imputed by values ranging from 75% to 125% of half of the lowest measured value of the corresponding metabolite in each plate. In order to minimize the plate effects in each visit, plate normalization factors were calculated by dividing the mean of reference sample values (QC samples in KORA F4) in each plate by the mean of all reference sample values in all plates, and then used to normalize each metabolite (Han et al. 2022; Huang et al. 2020).

Additionally, to control for the effects of the different kits between KORA F4 and KORA S4/FF4, up to eight participants' samples were randomly selected from each of the 36 kit plates in KORA F4 and re-measured using the same AbsoluteIDQt p180 kit used in KORA S4/FF4

in September–October 2019 (Han et al. 2022). The difference in each metabolite between the corresponding participants in KORA F4 and re-measured KORA F4, and a further mean difference of each metabolite were calculated. The kit normalization factor was calculated by dividing the mean of each metabolite in KORA F4 by the mean of each metabolite in KORA F4 minus the mean difference between KORA F4 and re-measured KORA F4, and used to correct KORA F4 metabolite data. Extreme outliers of each metabolite were defined as a value beyond the range of mean ± 5 × standard deviations and imputed by the K-nearest neighbors algorithm (KNN).

In total, 135 metabolites in KORA S4, 114 in KORA F4, and 145 in KORA FF4 passed the quality control. Out of these, 108 metabolites were overlapped among KORA S4, F4, and FF4 and were used in the subsequent analysis. Metabolites covered the following compound classes: 12 amino acids, 12 acylcarnitines, 72 glycerophospholipids (including 32 phosphatidylcholines with acyl-acyl (diacyl) side chains, 33 phosphatidylcholines with acyl-alkyl side chains, and seven lysophosphatidylcholines), 11 sphingomyelins (SM) and a sum of hexoses (including glucose). The complete list of metabolites is presented in the supplementary material (**Table S1**).

2.5. Exposure assessment

Residential annual mean exposure to air pollution including ultrafine particles (particulate matter (PM) ≤ 100 nm in aerodynamic diameter, represented by particle number concentration (PNC)), PM with an aerodynamic diameter less than 10 μm (PM_{10}), between 2.5 and 10 μm (PM_{coarse}), and less than 2.5 μm ($PM_{2.5}$), $PM_{2.5}$ absorbance (a proxy of elemental carbon related to traffic exhaust, $PM_{2.5abs}$), nitrogen oxides (NO_2, NO_x), and ozone (O_3) was estimated using land-use regression (LUR) models. The performance of LUR models was evaluated by leave-one-out cross-validation (LOOCV) (Wolf et al. 2017). Briefly, three bi-weekly measurements at 20 locations within the KORA study area were carried out between March 2014 and April 2015 to cover the warm, cold, and intermediate seasons. Simultaneously, measurements were obtained at a reference site throughout the whole period to adjust for temporal variation. Annual average air pollutant concentrations were then calculated at those sites. The LUR model was built by regressing the measured annual average concentrations in 2014–15 against geographic information system-based spatial predictors including local land use (e.g. residential land, industrial, commercial and transport units, urban green, and water bodies), building density, population density, household density, topography, coordinates, and traffic variables (e.g. total traffic load of all (major) roads in a buffer, traffic intensity on nearest (major) road, and heavy-duty traffic intensity on nearest (major) road) (Wolf et al. 2017). Participants' home addresses were applied to the fitted models to determine residential exposure levels. The adjusted model-explained variance (R^2) of the LUR models ranged from 68% (PM_{coarse}) to 94% (NO_2), and the adjusted LOOCV R^2 was between 55% (PM_{coarse}) and 89% (NO_2), which indicated a good model fit. The process has been described in detail elsewhere (Wolf et al. 2017). For participants who moved during the study period, the updated residential addresses were used for exposure assignment; otherwise, the same exposure levels were assigned across different visits.

3. Statistical analyses

3.1. Statistical methods

Basic descriptive analyses were performed for participant characteristics, air pollutants, and meteorological parameters. Kruskal-Wallis test (one-way ANOVA) and Pearson's Chi-squared test were applied for continuous and categorical variables, respectively. Spearman's rank correlation coefficient was used to calculate correlations between air pollutants.

We applied linear mixed-effects models with random participant-specific intercepts to examine the associations between repeatedly measured metabolite levels and air pollutants. In addition, linear mixed-effects models were also performed between hs-CRP and air pollutants to investigate the systemic inflammatory response. All outcomes (metabolites and hs-CRP) were natural-log transformed to increase the conformity to normal distributions of residuals. Covariates included in the models were selected a priori based on previous studies and the Bayesian Information Criterion (BIC) (Holmes et al. 2008; Lacruz et al. 2016; Nassan et al. 2021a; Sun et al. 2020b; Ward-Caviness et al. 2016). Minimum models adjusted for age, sex, body-mass index (BMI), an indicator of each visit (KORA S4, KORA F4, or KORA FF4), and season of blood withdrawal (winter: December–February, spring: March–May, summer: June–August, and autumn: September–November). Main models additionally included smoking status (never/former/current), alcohol consumption (g/day), physical activity (low/medium/high), educational attainment (primary school/high school/college), fasting status (overnight fasting of 8 h or not) and diet score (continuous). Extended models further added hypertension, diabetes, medication intake (anti-hypertensive, anti-diabetic, and lipid-lowering medication), high-density lipoproteins (HDL), and total cholesterol. Effect estimates are presented as percent changes in the geometric mean (together with 95% confidence intervals [95% CI]) of the repeatedly assessed outcomes per interquartile range (IQR) increase in air pollutant concentrations.

Single – Pollutant models

$$\log\left(Y_{ij}\right) = \beta_0 + \mu_i + \beta_1 \times AP_{ij} + \beta_{2-n} \times Covariates_{ij} + e_{ij}$$

In the formula, Y_{ij} is the metabolite concentration of participant i at visit j. β_0 denotes the fixed intercept, and μ_i represents the random intercept for subject i. β_1 is the estimate of each air pollutant and AP_{ij} indicates the annual averages of the air pollutants (PM_{10}, PM_{coarse}, $PM_{2.5}$, $PM_{2.5abs}$, PNC, NO_2, NO_x, and O_3) for participant i at visit j. $\beta_{2\text{-}n}$ is estimate for each covariate, and $Covariates_{ij}$ represents the measurement of covariates for participant i at visit j. e_{ij} is the residual normal error.

Effect modification was investigated by including an interaction term between each air pollutant and the potential effect modifier assessed at each visit. The examined modifiers included age (<65 years *vs* ≥ 65 years; the age 65 years is the current official retirement age in Germany)), sex (male *vs* female), obesity (BMI < 30 kg/m^2 *vs* ≥ 30 kg/m^2), smoking status (current *vs* never/former smoker), alcohol consumption (low *vs* medium *vs* high), education (low *vs* high (high school/college)), physical activity (low *vs* medium *vs* high), dietary pattern (adverse *vs* ordinary *vs* favourable), hypertension (no *vs* yes), diabetes (no *vs* yes), and medication intakes (no *vs* yes). The effect modification analyses were only conducted for those metabolites significantly associated with air pollutants.

We performed several sensitivity analyses in this study: 1) We included all participants with data on air pollution, phenotypes, and metabolites in the analysis. 2) We restricted our analyses to participants who did fasting eight hours before the blood withdrawal throughout the entire study period. 3) Additionally, we restricted our main analysis to participants who did not move within the study period. 4) To control for selection bias introduced by selecting participants with more than one measurement, we estimated weights for those included using the inverse probability weighting (IPW) method (Weuve et al. 2012). Briefly, the probability of being included in our main analysis among all study participants in KORA S4 was calculated using logistic regression. We used individual characteristics of our main analysis as possible predictors. Then, we applied the inverse of the predicted probability determined from the logistic regression as the weight in our main model. 5) Given the temporal variation of each air pollutant exposure, we used back-extrapolated annual average air pollutant concentrations from the respective years of KORAS4, F4 and FF4 instead of using annual average air pollutant concentration estimated by the LUR models in 2014–2015 **(Text S1)**. Briefly, the absolute differences between the LUR model and the air pollutants data from monitors in the period of each visit were

calculated. They were then used to correct each visit's air pollutant concentrations, respectively. 6) To examine the influence of air pollution-associated systemic inflammation, we further included high-sensitivity C-reactive protein (hs-CRP) in our main models. 7) We performed two-pollutant models by including two air pollutants simultaneously if their Spearman correlation was smaller than 0.7. 8) We also performed a mixed-effects quantile regression to assess the association between air pollution exposure and metabolites at deciles of the metabolites. 9) To investigate the co-effects between long-term and short-term air pollution exposure, we simultaneously included short-term exposures (at the day of blood withdrawal, one day, two days, three days, four days, as well as two-day, five-day and two-week moving averages before the blood withdrawal) to each air pollutant in the corresponding long-term exposure model. The short-term exposure included $PM_{2.5}$, PM_{10}, PM_{coarse}, NO_2, NO_x and O_3, was measured consecutively by local monitors and the daily average exposure concentration of each air pollutant was assigned to each participant based on the date of blood withdrawal in each visit (**Text S1**). 10) To assess the effect of the storage time (Haid et al. 2018), we performed an additional sensitivity analysis, including the storage year in our main models. Briefly, we calculated the storage time between the collection date of the blood sample for each participant and the detection time (middle date in the whole measurement period). Then, we included this storage year in our main models. We assumed a non-linear relationship between the change of metabolites concentrations and the storage time, so we used a spline for the storage years to account for non-linearity in these relationships. 11) In the main models, we assessed the exposure–response relationships between all metabolites and air pollutants for deviations from linearity using penalized splines with the degree of freedom selected by generalized cross-validation, and restricted our analyses to the linear section of the relationship.

All statistical analyses were done with R (version 3.6.2), and the *p*-value cut-off was set as 5.8×10^{-5} to account for multiple testing introduced by assessing eight air pollutants and 108 metabolites in this study (0.05/(108*8)). We also report all associations with *p*-values<0.05 after Benjamini-Hochberg false discovery rate (FDR) correction since the Bonferroni method for adjusting *p*-values is more conservative.

3.2. Pathway analysis for metabolites

For metabolites showing significant associations with air pollutants after correcting for multiple testing, we performed pathway analysis using the "Pathway Analysis" module in MetaboAnalyst 5.0, a web-based software for metabolomics data analysis (Pang et al. 2021). This module supports pathway analysis by integrating two parts, enrichment analysis and topology analysis, based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, which is a collection of manually drawn pathway maps representing the knowledge of molecular interaction, reaction, and relation networks. In the enrichment analysis, the *p*-value is calculated by the one-tailed Fisher's exact test, which represents the probability of observing at least *k* metabolites in a pathway, if there is no association with air pollution:

$$p(X \geq k) = 1 - \sum_{i=0}^{k-1} \frac{\binom{M}{i}\binom{N-M}{n-i}}{\binom{N}{n}}$$

where *N* represents the number of the metabolites detected by the platform, *M* indicates the metabolites in the pathway of interest (i^{th}), *n* is the metabolites significantly associated with air pollution, and *k* means the number of metabolites overlapped between *M* and *n* mapping to the i^{th} pathway (Wieder et al. 2021). The pathway topology analysis uses two well-established node centrality measures to estimate node importance. Furthermore, to take into account the comparison among different pathways, the node importance values calculated from centrality measures are further normalized by the sum of the importance of the pathway. Therefore, the total/maximum importance of each pathway is one. The importance measure of each metabolite node reflects the percentage with regard to the total pathway importance, and the pathway impact value is the cumulative percentage from the matched metabolite nodes. Pathways with a *p*-value ≤ 0.1, or with an impact value > 0.5 while *p*-value ≤ 0.3 were considered the most relevant pathways.

4. Results

4.1. Characteristics of study participants

Participant characteristics are summarized in Table 1. Only participants attending at least two visits during the entire study period with no missing information in the main confounders were included in our main analyses. Therefore, among 9,620 observations from 4,261 study participants in the KORA cohort, we included 5,772 (60.0%) observations from 2,583 (60.6%) participants in this analysis. Specifically, 1,977 (76.5%) out of the 2,583 participants attended two examinations, and 606 (23.5%) attended all three examinations (Table 1).

Due to the fasting status restriction in KORA S4, only 1,601 from overall 4,261 participants had data on metabolite levels, mainly elderly individuals. Therefore, on average, KORA S4 participants were older than those of KORA F4 and KORA FF4 (Table 1). Meanwhile, the average educational attainment, the percentages of 8 h overnight fasting before blood withdrawal, current smoker, unhealthy dietary pattern, and medium and high levels of physical activity of KORA S4 were lower (*p*-value < 0.01). In contrast, the mean BMI, alcohol consumption, cholesterol, HDL, and hs-CRP and the percentage of hypertension were higher in KORA S4 (*p*-value < 0.01).

4.2. Characteristics of air pollutants

Annual average concentrations of $PM_{2.5}$, PM_{10} and NO_2 at participant's residences were below the EU air quality standard values of 25 $\mu g/m^3$ for $PM_{2.5}$, and 40 $\mu g/m^3$ for PM_{10} and NO_2, respectively. While they were all higher than the WHO air quality guideline values of 5 $\mu g/m^3$, 10 $\mu g/m^3$ and 10 $\mu g/m^3$ for $PM_{2.5}$, PM_{10} and NO_2, respectively. The maximum annual O_3 concentration (45.9 $\mu g/m^3$) was also below the WHO air quality guideline values calculated from peak season (60 $\mu g/m^3$) (Table 2). All pollutants showed a strong positive relationship, except for O_3, which showed weak or negative correlations with other air pollutants (Table 2).

4.3. Association between metabolites and long-term air pollution

In our main models, several metabolites from the phosphatidylcholines group showed significant negative associations with PM_{coarse}, $PM_{2.5abs}$ and NO_2, respectively (Fig. 1). Specifically, PC ae C34:2 and PC ae C36:3 were negatively associated with PM_{coarse} and $PM_{2.5abs}$ (at a *p*-value $< 5.8 \times 10^{-5}$). Additionally, at an FDR-corrected *p*-value < 0.05, we observed decreases in PC ae C34:2 and PC ae C36:3 in association with NO_2. Moreover, PC ae C36:4 showed negative associations with $PM_{2.5abs}$ and PM_{coarse}, and PC ae C34:3 with $PM_{2.5abs}$, respectively. These results were robust in our minimum and extended models (Fig. 2). In addition, we observed positive associations between hs-CRP and PM_{coarse}, PM_{10}, PNC and NO_x (uncorrected *p*-value < 0.05) (**Fig. S2**). While the four identified metabolites showed moderate to high correlations with each other, hs-CRP was not associated with them at all (**Fig. S3**).

4.4. Pathway analysis

In the pathway analysis, we uploaded the four metabolites significantly associated with at least one of the long-term exposures to $PM_{2.5abs}$, PM_{coarse}, or NO_2. We identified four metabolic pathways,

Table 1
Descriptive statistics of participant characteristics for KORA S4, F4 and FF4 (N = 5,772).

Variable	S4 (N = 1,129) Mean ± SD / N (%)	F4 (N = 2,556) Mean ± SD / N (%)	FF4 (N = 2,087) Mean ± SD / N (%)	*p*–value
Age (years)	63.3 ± 5.4	57.5 ± 13.3	60.7 ± 12.3	< 0.001
Sex (male)	570 (50.6)	1,240 (48.5)	1,012 (48.5)	0.46
Education				< 0.001
Primary school	753 (66.7)	1,357 (53.1)	1034 (49.5)	
High school	221 (19.6)	621 (24.3)	530 (25.4)	
College	155 (13.7)	578 (22.6)	523 (25.1)	
BMI (kg/m^2)	28.4 ± 4.2	27.7 ± 4.7	27.8 ± 4.9	< 0.001
Alcohol consumption (g/day)	16.2 ± 20.9	14.4 ± 19.5	14.9 ± 20.1	0.025
Dietary score	16.2 ± 3.6	15.3 ± 3.6	15.1 ± 3.6	< 0.001
Dietary patterns				< 0.001
Adverse	271 (24.0)	817 (31.9)	715 (34.3)	
Ordinary	212 (18.8)	541 (21.2)	451 (21.6)	
Favorable	646 (57.2)	1,198 (46.9)	921 (44.1)	
Fasting (8 h) (% yes)	1,016 (90.0)	2,543 (99.5)	2,074 (99.4)	< 0.001
Smoking status				0.002
Current smoker	137 (12.1)	384 (15.0)	307 (14.7)	
Former smoker	437 (38.7)	1,066 (41.7)	902 (43.2)	
Never smoker	555 (49.2)	1,106 (43.3)	878 (42.1)	
Physical activity				< 0.001
Low	444 (39.3)	818 (32.0)	589 (28.2)	
Medium	479 (42.4)	1,115 (43.6)	952 (45.6)	
High	206 (18.3)	623 (24.4)	546 (26.2)	
Hypertension (% yes)	609 (53.9)	1,016 (39.8)	825 (39.5)	< 0.001
Diabetes (% yes)	92 (8.2)	224 (8.8)	215 (10.3)	0.11
Medication intake (% yes)				
Anti–hypertension medication	397 (35.2)	861 (33.7)	782 (37.5)	0.03
Anti–diabetes medication	53 (4.7)	154 (6.0)	174 (8.3)	< 0.001
Lipid lowering medication	128 (11.3)	351 (13.7)	342 (16.4)	< 0.001
Cholesterol (mg/dL) *	243.6 ± 40.8	216.1 ± 38.8	216.7 ± 39.5	< 0.001
HDL (mg/dL)*	58.1 ± 16.5	56.1 ± 14.4	65.9 ± 18.8	< 0.001
hs-CRP (mg/L)*	3.1 ± 4.9	2.4 ± 4.8	2.5 ± 4.6	< 0.001

KORA = Cooperative Health Research in the Region of Augsburg; S4 = fourth cross-sectional health survey of the KORA cohort; F4 = first follow-up examination of KORA S4; FF4 = second follow-up examination of KORA S4; BMI = body mass index; HDL = high density lipoprotein; hs-CRP = high sensitivity C-reactive protein; S4 participants were selected based on whether they did fasting or not. Dietary patterns was classified by the dietary score basing on the assessment of individual's dietary intake (questionnaire): Adverse = ≤13 points, Ordinary = 14 ~ 15 points, Favourable = ≥16 points. Physical activity was defined according to the exercise time per week: Low = almost or no sporting activity, Medium = regular/ irregular approx. 1 h per week, High = regularly>2 h in the week. *Cholesterol was missing for one (0.09%) participant in KORA S4, and one (0.05%) in KORA FF4; HDL was missing for one (0.09%) participant in KORA S4, one (0.04%) in KORA F4, and one (0.05%) in KORA FF4; hs-CRP was missing for 12 (1.06%) participants in KORA S4, five (0.22%) in KORA F4, and two (0.10%) in KORA FF4. 1,977 participants attended two examinations, and 606 attended three examinations. *p*-value was based on the Kruskal-Wallis test for continuous variables, and Pearson's Chi-squared test for categorical variables.

including the arachidonic acid (p-value = 0.003, impact value = 0), linoleic acid (p-value = 0.008, impact value = 0), alpha-linolenic acid (p-value = 0.02, impact value = 0), and glycerophospholipid (p-value = 0.02, impact value = 0.1) metabolisms that were related to long-term exposure to $PM_{2.5abs}$, PM_{coarse}, and NO_2 exposure, where p-value was from enrichment analysis and pathway impact value was from the topology analysis. However, they were insignificant after using the FDR method to correct the raw p-value (Fig. 3 and **Table S2**).

4.5. Effect modification

Effect modification analyses were conducted for the four metabolites significantly associated with long-term exposure to $PM_{2.5abs}$, PM_{coarse} and NO_2. Results are presented in Fig. 4 showing that the associations between PC ae C34:3 and $PM_{2.5abs}$, PM_{coarse} and NO_2 were significantly modified by physical activity (Bonferroni-corrected $p < 0.004$). Participants with low physical activity showed the strongest effects. A similar pattern was seen for PC ae 34:2, PC ae 36:3 and $PM_{2.5abs}$ and NO_2 (uncorrected $p < 0.05$). Moreover, results indicated a consistent modification of the air pollutant effects on PC ae 34:2 by education. Participants with a lower education showed stronger effects compared to those with a higher education. Results also suggested a consistent modification of the air pollutant effects on PC ae 36:4 by obesity - obese individuals showed stronger associations between metabolites and air pollutants. We did not find consistent differences between smokers and participants who never smoked and participants with medium or high alcohol consumption versus those without alcohol intake. Additionally, results suggested effect modification by disease status (hypertension and diabetes) and medication intake, while the differences were not statistically significant (**Fig. S4**, **Fig. S5**). This might be due to the large difference in the sample sizes of the different groups since much fewer participants had diabetes or intake of anti-hypertension, anti-diabetes, or lowering-lipid medicines. We also did not find significant differences between males and females in most metabolites except that a few PCs decreased more in females than males when exposed to O_3 (**Fig. S6**).

4.6. Sensitivity analyses

The associations between air pollution and the four metabolites were generally robust in different sensitivity analyses. Results remained stable when restricting the participants to fasting individuals or those who did not move their residences during the whole study period (Fig. 5). Additionally, including all participants, using predicted inverse probabilities, or using back-extrapolated air pollutant exposures to adjust for measurement error did not change the results. The results were still robust after further including hs-CPR in the main and extended models (**Fig. S7**).

The associations between metabolites and particulate air pollutants (PM_{10}, PM_{coarse}, $PM_{2.5abs}$, and PNC) were robust after additionally adjusting for $PM_{2.5}$ except for PNC where associations were attenuated (**Fig. S8**). The associations between metabolites and particle metrics ($PM_{2.5}$, PM_{10}, PM_{coarse}, $PM_{2.5abs}$, and PNC) and NO_2 were also stable after adjusting for O_3 (**Fig. S9**). After additionally adjusted by the storage year of blood samples into the main model, the effect estimates keep stable (**Fig. S10**). The additional adjustment of short-term air pollution exposure slightly strengthened the effect estimates of long-

Table 2
Descriptive statistics and Spearman correlation coefficients of air pollution concentrations in long-term analysis (N = 2,583).

Pollutant	Mean ± SD	Range	IQR	Spearman correlation coefficients							
				$PM_{2.5}$	PM_{10}	$PM_{2.5abs}$	PM_{Coarse}	PNC	O_3	NO_2	NO_x
$PM_{2.5}$ (μg/m^3)	11.8 ± 1.0	8.2–14.3	1.4	1							
PM_{10} (μg/m^3)	16.6 ± 1.5	12.3–22.3	2.1	0.52	1						
$PM_{2.5abs}$ (10^{-5}/m)	1.2 ± 0.18	0.8–1.8	0.3	0.61	0.78	1					
PM_{coarse} (μg/m^3)	4.9 ± 1.0	2.6–8.7	1.4	0.57	0.78	0.81	1				
PNC (10^3/cm^3)	7.3 ± 1.8	3.2–15.0	2.0	0.65	0.80	0.78	0.76	1			
O_3 (μg/m^3)	39.1 ± 2.4	31.3–45.9	3.4	–0.18	0.05	–0.10	0.14	–0.03	1		
NO_2 (μg/m^3)	14.1 ± 4.4	6.9–27.5	6.9	0.72	0.72	0.86	0.83	0.78	–0.16	1	
NO_x (μg/m^3)	21.8 ± 7.4	4.0–50.5	8.8	0.75	0.73	0.72	0.75	0.90	–0.06	0.83	1

*Exposure levels were estimated at participants' residences in KORA S4. In total, 2153 participants didn't move since S4, and 430 participants moved between S4 and F4, or F4 to FF4. For participants who changed residence among S4, F4 and FF4, the updated residential addresses were used for exposure assignment to the respective study. Otherwise, the same exposure levels from KORA S4 were assigned across different visits. $PM_{2.5}$ = particulate matter with an aerodynamic diameter less than or equal to 2.5 μm; PM_{coarse} = particulate matter with an aerodynamic diameter of 2.5–10 μm; PM_{10} = particulate matter with an aerodynamic diameter less than or equal to 10 μm; $PM_{2.5abs}$ = $PM_{2.5}$ absorbance; PNC = particle number concentration; NO_2 = nitrogen dioxide; NO_x = nitrogen oxide; O_3 = ozone.

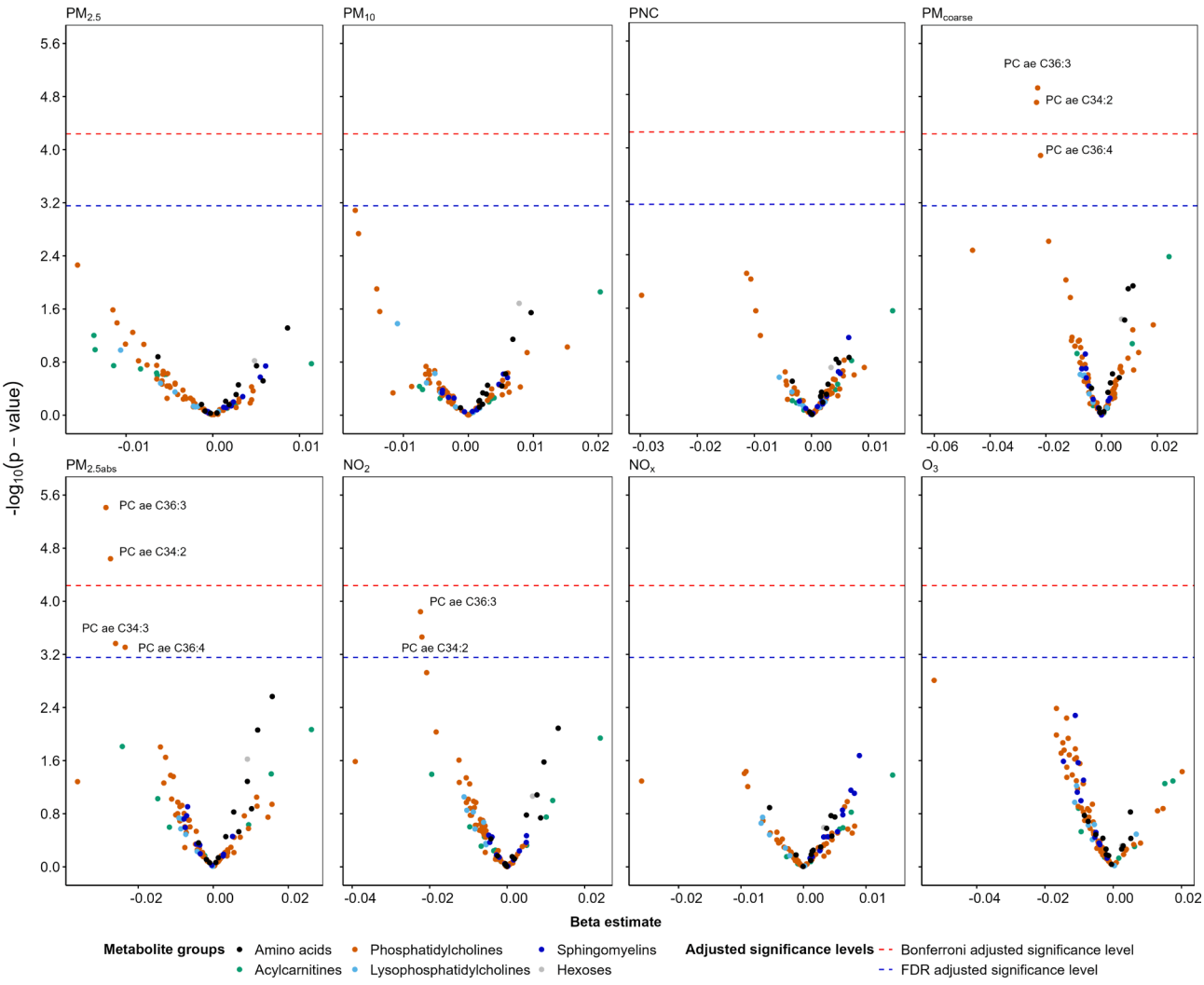

Fig. 1. Volcano plots presenting the associations between long-term air pollutant exposure and metabolites. The results were derived from the main models adjusted for age, sex, body-mass index (BMI), an indicator for each visit(KORA S4, KORA F4, or KORA FF4), season of blood withdrawal, smoking status, alcohol consumption, physical activity, educational attainment, fasting status, and dietary score. The Y axis shows the negative logarithm of the *p*-value (logarithmic base of 10). The X axis indicates the association between air pollutants and metabolites. The red and blue dashed lines represent adjusted statistical significance levels according to Bonferroni and FDR methods, respectively. The points with six different colors represent six metabolite groups involved in this study including amino acids (black), acylcarnitines (green), phosphatidylcholines (orange), lysophosphatidylcholines (light blue), sphingomyelins (blue), and hexoses (grey). PC ae: acyl-alkyl phosphatidylcholine. $PM_{2.5}$ = particulate matter with an aerodynamic diameter less than or equal to 2.5 μm; PM_{coarse} = particulate matter with an aerodynamic diameter of 2.5–10 μm; PM_{10} = particulate matter with an aerodynamic diameter less than or equal to 10 μm; $PM_{2.5abs}$ = $PM_{2.5}$ absorbance; PNC = particle number concentration; NO_2 = nitrogen dioxide; NO_x = nitrogen oxide; O_3 = ozone.

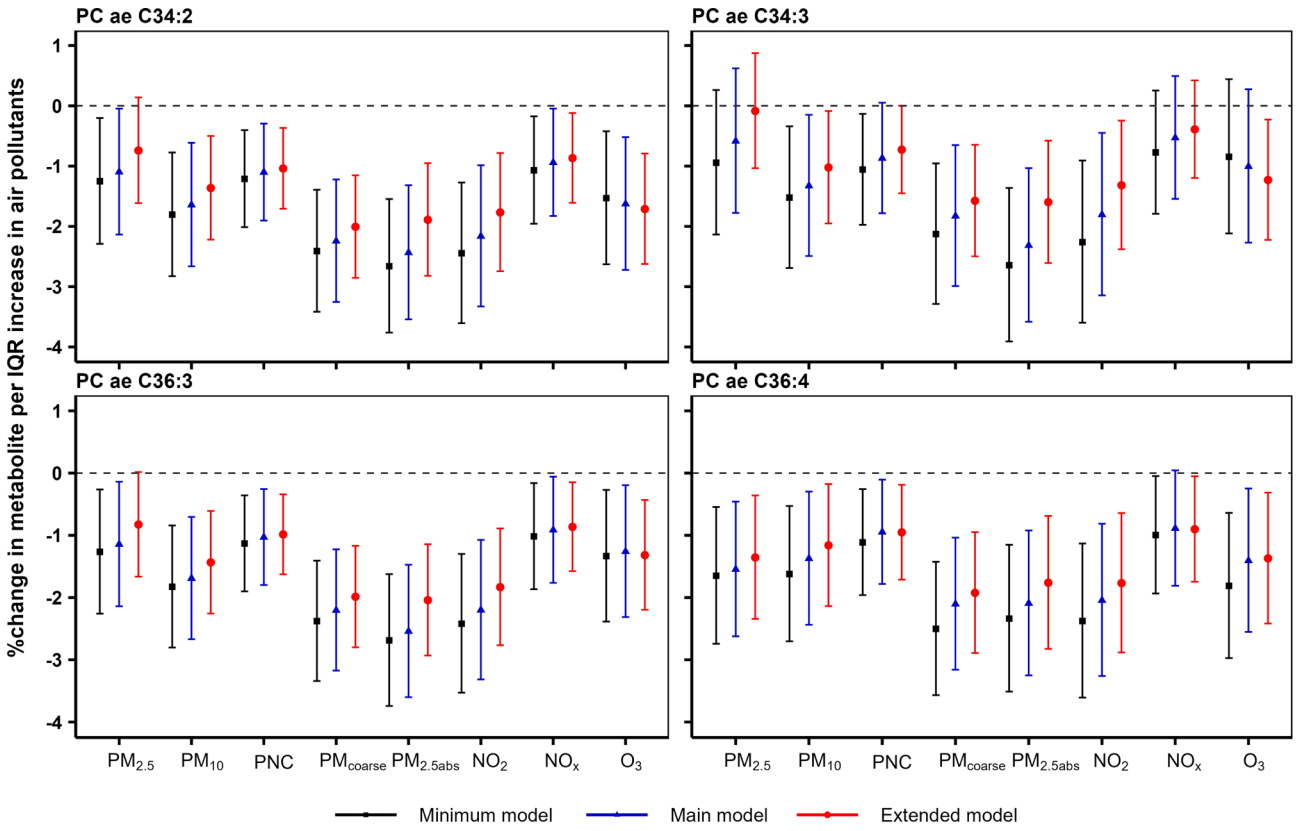

Fig. 2. Comparisons of percent changes (95% CIs) of metabolites per IQR increase in air pollutant concentrations between the results from minimum, main and extended models. Minimum model: minimum models were adjusted for age, sex, BMI, season, an indicator of each visit (KORA S4, KORA F4, or KORA FF4); Main model: further adjusted for educational attainment, smoking status, fasting status, alcohol consumption, physical activity, and dietary score; Extended model: additionally included hypertension, diabetes, and medication intake (anti-hypertension, anti-diabetes, and lipid lowering medications), HDL, and total cholesterol into the main models. An IQR increase was 1.40 μg/m^3 for $PM_{2.5}$, 2.06 μg/m^3 for PM_{10}, 1.36 μg/m^3 for PM_{coarse}, 1.95×10^3/cm^3 for PNC, 0.27×10^{-5}/m for $PM_{2.5abs}$, 6.86 μg/m^3 for NO_2, 8.69 μg/m^3 for NO_x, and 3.45 μg/m^3 for O_3. PC ae: acyl-alkyl phosphatidylcholine. $PM_{2.5}$ = particulate matter with an aerodynamic diameter less than or equal to 2.5 μm; PM_{coarse} = particulate matter with an aerodynamic diameter of 2.5–10 μm; PM_{10} = particulate matter with an aerodynamic diameter less than or equal to 10 μm; $PM_{2.5abs}$ = $PM_{2.5}$ absorbance; PNC = particle number concentration; NO_2 = nitrogen dioxide; NO_x = nitrogen oxide; O_3 = ozone.

term exposure but showed consistent estimates across the different exposure windows (Fig. S11).

Mixed-effects quantile regression showed similar associations for $PM_{2.5abs}$ exposure across deciles (Fig. S12). In contrast, there were stronger associations between PM_{coarse} exposure and the four metabolites from the 10th percentile up to the fifth decile (Fig. S13). Similarly, stronger associations were seen between the four metabolites and NO_2 exposure from the second to the sixth decile (Fig. S14).

We also checked the exposure–response relationships of all metabolites and $PM_{2.5abs}$, PM_{coarse} and NO_2 exposure. There was no deviation from linearity for NO_2 with the four metabolites (Fig. S15), while slight deviations were observed for $PM_{2.5abs}$ and PM_{coarse} (Fig. S16, Fig. S17). We excluded the extreme values for $PM_{2.5abs}$ (>99% of total $PM_{2.5bs}$) and PM_{coarse} (<5% of total PM_{coarse} and > 95% of total PM_{coarse}) from the dataset to ensure a linear exposure–response relationship for $PM_{2.5abs}$, the results kept robust with our main analysis results (Fig. S18-S21).

5. Discussion

This longitudinal study identified nine associations between long-term exposure to air pollution and targeted serum metabolites, mainly from the phosphatidylcholine subgroup. In particular, we observed that participants exposed to higher $PM_{2.5abs}$, PM_{coarse} and NO_2 had lower levels of PC ae C34:2 and PC ae C36:3. In addition, PC ae C36:4 showed a negative association with $PM_{2.5abs}$ and PM_{coarse}, and PC ae C34:3 was negatively associated with $PM_{2.5abs}$. In the subsequent pathway analysis, they were identified as related to glycerophospholipid, linoleic acid and alpha-linolenic acid metabolism. Moreover, we found effect modifications for several individual characteristics: participants with older age, obesity, lower educational attainment, low physical activity levels, or adverse dietary patterns showed stronger associations than their counterparts. In addition, we could confirm positive associations between several air pollutants (PM_{coarse}, PM_{10}, PNC, and NO_x) and hs-CRP as previously reported cross-sectionally for FF4 (Pilz et al. 2018), where we saw positive but non-significant associations with PNC, PM_{10}, PM_{coarse}, $PM_{2.5abs}$, NO_2, and NO_x.

Metabolites are the intermediates or end products of metabolism, and could affect cellular physiology through modulation of other "omics" levels and represent changes induced by exposures (Rinschen et al. 2019). Alterations in the lipid metabolism due to the unbalance of anti- and pro-inflammatory biomarkers and oxidative stress levels could be one of the underlying mechanisms linking air pollution exposure to adverse health effects. Only a few studies explored the associations between long-term exposure to air pollution and metabolites in a cohort setting. A cross-sectional study based on the TwinsUK cohort reported eight inflammation and oxidative stress-related metabolites out of 280 untargeted metabolomics profiling. For example, α-tocopherol, glycine, and benzoate were associated with long-term $PM_{2.5}$. Moreover, CRP was negatively associated with seven of these eight metabolites (Menni et al. 2015). A study including cohorts from Italy and Switzerland reported that long-term exposure to air pollution on adult asthma and cardiovascular disease was related to unsaturated fatty acids e.g., linolenic acid metabolism (Jeong et al. 2018). Another cross-sectional cohort study based on 79 metabolites indicated that annual ultrafine particles

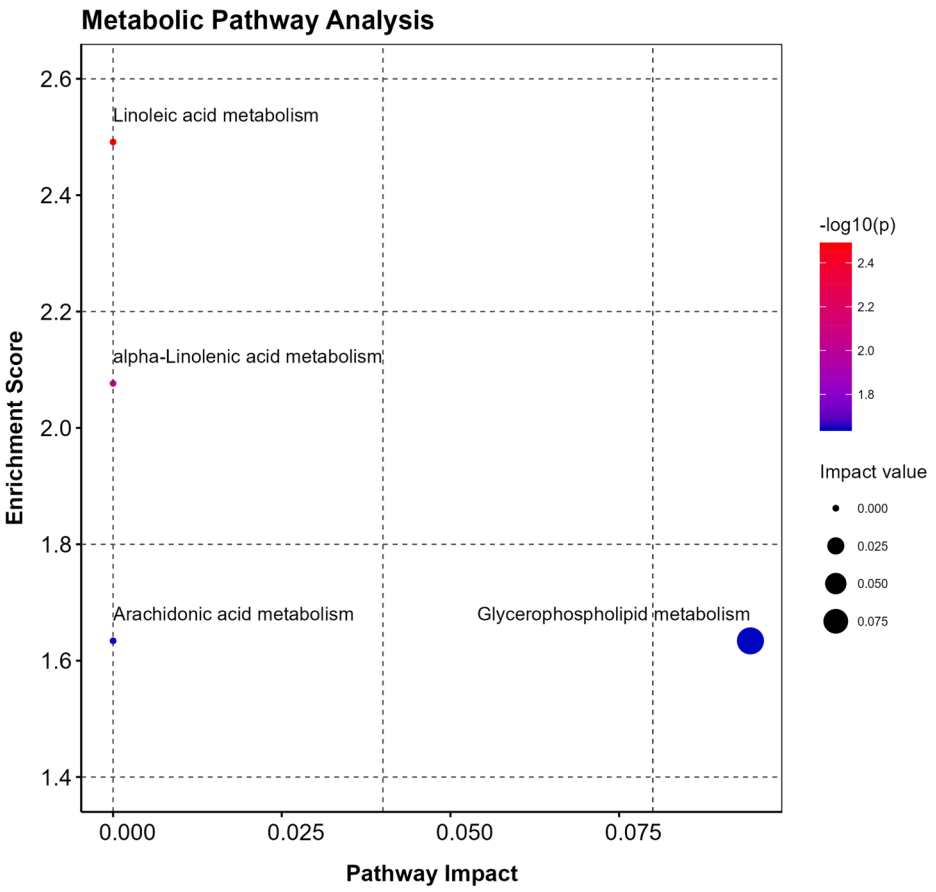

Fig. 3. Metabolic pathways identified for long-term exposure to $PM_{2.5abs}$, PM_{coarse} and NO_2. The plot is the same for $PM_{2.5abs}$, PM_{coarse} and NO_2, since the corresponding ID in the KEGG database for these four metabolites that were significantly associated with $PM_{2.5abs}$, PM_{coarse} or NO_2 exposure is identical. The pathway analysis is based on both enrichment analysis and pathway topology analysis. The Y-axis is the negative logarithm of the *p*-value (logarithmic base of 10) from the enrichment test. The X-axis indicates the structural impact of $PM_{2.5abs}$, PM_{coarse} or NO_2 related metabolites in the enriched pathways, which is based on the cumulative importance of all the significant metabolites within the pathway. The size of each bubble represents the impact value. The colour of each bubble represents the significance of the enrichment. PM_{coarse} = particulate matter with an aerodynamic diameter of 2.5–10 μm; $PM_{2.5abs}$ = $PM_{2.5}$ absorbance; NO_2 = nitrogen dioxide.

(UFP) exposure was associated with metabolites that might increase oxidative stress and affect inflammatory processes and endothelial function (Walker et al. 2019). Two studies within the Normative Aging Study (NAS, a closed cohort study) reported that long-term exposure to $PM_{2.5}$ species (e.g., UFP, black carbon), $PM_{2.5}$, and air temperature was associated with perturbed metabolic pathways, including glycerophospholipid, sphingolipid, and biosynthesis of unsaturated fatty acids etc. (Nassan et al. 2021a; Nassan et al. 2021b). They also reported that long-term NO_2 exposure was positively associated with four lipid metabolites, while these metabolites were not significantly associated with any metabolomics pathway.

Since untargeted metabolomics was used in all these studies, comparing our results with those from single metabolite levels is difficult. Nevertheless, they are mostly consistent in identifying metabolic pathways related to inflammation, unsaturated fatty acids, and glycerophospholipid associated with long-term exposure to air pollution. Potential differences in results compared with our findings might also be due to the small sample size (less than 1,000 participants) of some of these studies, differences in study designs (e.g., case-control study), and selected study population (e.g., older men).

Several studies reported the associations between metabolomics and short-term and intermediate exposure to air pollution. A longitudinal study on the effects of high-level $PM_{2.5}$ exposure on serum metabolomics reported that metabolites related to phospholipid metabolism (lysophosphatidic acid, phospholipid acid, and lysophosphatidylethanolamine) were decreased for a 10 $\mu g/m^3$ increase in $PM_{2.5}$ (Huan et al. 2021), which supports our findings to some extent where four phosphatidylcholine metabolites were decreased in association with $PM_{2.5abs}$, PM_{coarse} and NO_2. In a previous cross-sectional analysis based on KORA S4, F4 and the follow-up of survey 3 (KORA F3), Ward-Caviness et al. observed a significant positive association between one lysophosphatidylcholine (LPC) and short-term NO_2 exposures (Ward-Caviness et al. 2016). This longitudinal analysis did not find any associations between LPC and long-term exposures to NO_2 or other air pollutants. However, we observed decreased levels of four PCs in association with long-term $PM_{2.5abs}$, PM_{coarse} and NO_2 exposure. A perturbation between LPC and PC was also reported in two other studies investigating the associations between short-term exposure to air pollution and untargeted metabolomics profiling (Chen et al. 2019a; Yan et al. 2019). Chen et al. indicated that two fatty acids, five phospholipids (phosphatidylserine, PEs, phosphatidic acid), and one sphingosine in urine significantly decreased with a higher short-term $PM_{2.5}$ exposure; these metabolites were related to energy metabolism, oxidative stress and inflammation (Chen et al. 2019a). In the second study, Yan et al. investigated the associations between exposure to traffic-related air pollution (NO_x, $PM_{2.5}$) during the first trimester and serum metabolites measured in mid-pregnancy. They observed that higher exposure to air pollution was related to alterations in several oxidative stress and inflammatory pathways, including fatty acid, phospholipid, linoleate, and eicosanoid metabolism (Yan et al. 2019).

Phosphatidylcholine (PC) is the representative and important component of lipoproteins that belongs to glycerophospholipid. It has a polar phosphocholine head group, which is connected via a glycerol backbone to two fatty acid side chains of varying lengths and degrees of saturation. The fatty acids are bound to the sn1 and sn2 positions of the glycerol backbone, either via two esters (acyl) bonds (diacyl-PC, PC aa) or by one ester and one ether (alkyl) bond (acyl-alkyl-PC, PC ae). It is the most abundant phospholipid in all mammalian cell membranes and subcellular organelles and could be attacked by reactive oxygen species (ROS) and lead to lipid peroxidation, especially the polyunsaturated fatty acids (Ayala et al. 2014; Cole et al. 2012; van der Veen et al. 2017). PC and LPC serve as reservoirs and transporters of glycerophospholipid components: fatty acids, phosphate, glycerol, and choline, which could regulate homeostatic and inflammatory processes.

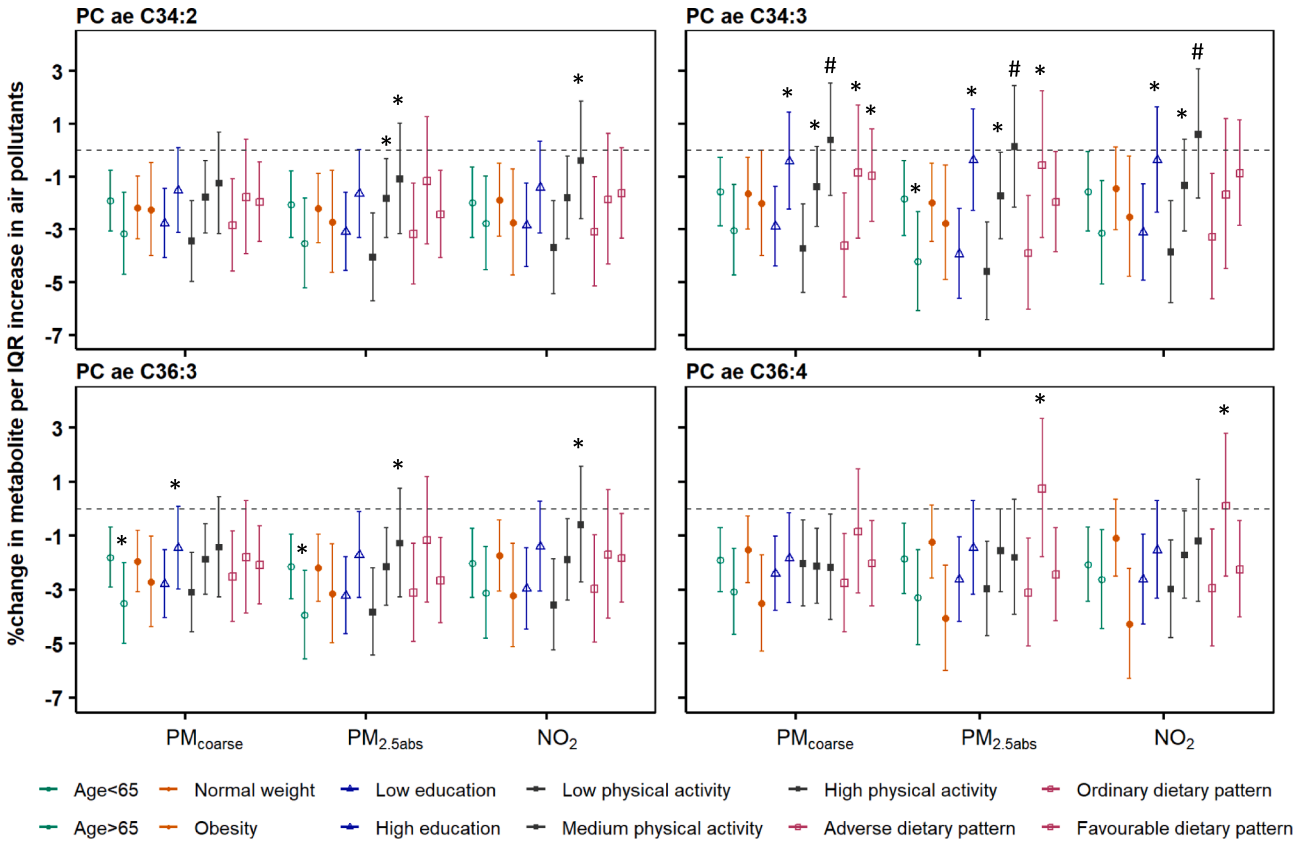

Fig. 4. Percent changes (95% CIs) of metabolites per IQR increase in air pollutant concentrations stratified by age, BMI, educational attainment, physical activity level and dietary patterns. Results were from our main models adjusted for covariates including age, sex, BMI, season, an indicator of each visit (KORA S4, KORA F4, or KORA FF4), educational attainment, smoking status, fasting status, alcohol consumption, physical activity, and dietary score, while the continuous variable will be replaced by each corresponding effect modifier. An IQR increase was 1.36 μg/m^3 for PM_{coarse}, 0.27 × 10^{-5}/m for $PM_{2.5abs}$, and 6.86 μg/m^3 for NO_2. PC ae: acyl-alkyl phosphatidylcholine. PM_{coarse} = particulate matter with an aerodynamic diameter of 2.5–10 μm; $PM_{2.5abs}$ = $PM_{2.5}$ absorbance; NO_2 = nitrogen dioxide. * $p < 0.05$ # $p < 0.004$ (0.05/(3*4)).

Lysophosphatidylcholine (LPC) could be hydrolyzed by PC via Phospholipase A2 (PLA2). The decreased concentrations of PCs, as well as increased PLA2, might indicate increased turnover of PCs for the synthesis of pro- and anti-inflammatory factors (Kertys et al. 2020). In an *in vivo* study, significant reductions in LPC and PC concentrations were observed after chronically exposing to ambient $PM_{2.5}$, which might result from repeated inflammation (Chen et al. 2014). This might explain the negative associations between PCs and long-term NO_2 exposure in this study compared to the study of Ward-Caviness and colleagues, which observed a positive association between LPCs and short-term NO_2 exposure. Furthermore, given the positive associations between CRP and long-term exposure to air pollution (PM_{coarse}, PM_{10}, PNC, and NO_x), these findings suggested a perturbation of anti-inflammation and pro-inflammation after long-term exposure to air pollution.

The higher abundance of PC in human tissues compared to other phospholipid classes has been shown to play an important role in health and diseases (van der Veen et al. 2017). The inhibition of hepatic PC synthesis and changes in hepatic phospholipid composition were related to fatty liver disease and impaired liver regeneration after surgery (van der Veen et al. 2017). The altered PC metabolism may also promote the development of Alzheimer's and cardiovascular diseases (CVD) (Tang et al. 2013; Whiley et al. 2014). In other metabolomics-related studies using the KORA cohort, a decrease in a few acyl-alkyl-PCs was associated with smoking (including an overlapped PC ae C34:3) and ageing (Chak et al. 2019; Xu et al. 2013). Plasmalogens are a subclass of alkyl-PCs with antioxidant properties (Engelmann 2004). A decreased serum concentration of acyl-alkyl-PCs and alkenyl-PC (plasmalogen) lipids were found in stable coronary artery disease (CAD) and acute myocardial infarction (MI) (Moxon et al. 2017; Sutter et al. 2016). Our findings, therefore, might indicate that acyl-alkyl-PCs could be the underlying biomarkers involved in the biological mechanisms of chronic air pollution exposure-associated diseases.

Apart from environmental impacts, pathological stimuli and normal physiological variations can also lead to differences in metabolic profiles (Lacruz et al. 2016; Soininen et al. 2015; Suhre et al. 2010). Lifestyle factors, including obesity, smoking, alcohol consumption, physical activity and dietary patterns, were considered risk factors for metabolism (Lacruz et al. 2016). Previous studies reported that male, older, obese, smoking, and unhealthy individuals are more susceptible to air pollution exposures (Chen et al. 2019b; Hou et al. 2020; Sun et al. 2020a; Yazdi et al. 2021; Zhang et al. 2021). We observed that older participants (>65 years old) showed a stronger association than the younger ones, which could be explained by a greater susceptibility in older adults to oxidative stress and also a higher prevalence of pre-existing diseases in the older group (Peters et al. 2021; Sacks et al. 2011). We did not find significant differences between males and females in most metabolites except that a few PCs decreased more in females than males when exposed to O_3. In contrast, males were more susceptible when exposed to the other air pollutants. The obese subgroup was more susceptible to long-term exposure to air pollution in this study, which could be hypothesized that altered PCs facilitate inflammation in obese participants. Physical activity has immediate beneficial effects, accumulating over time. In the long run, it reduces the risk of developing cardiovascular and respiratory diseases, type 2 diabetes, and certain types of cancers and reduces the risk of all-cause and cause-specific mortality (Tainio

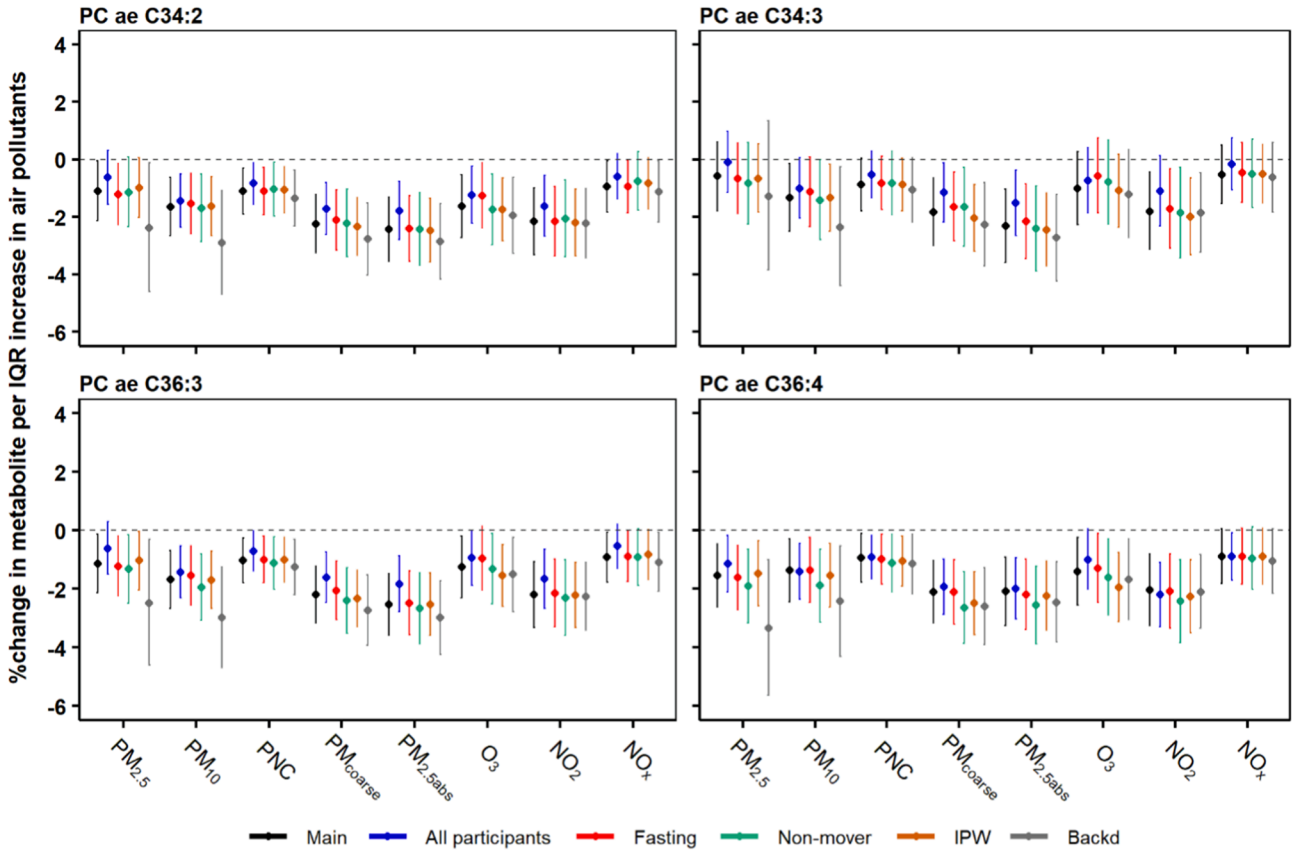

Fig. 5. Percent changes (95% CIs) of metabolites per IQR increase in air pollutant concentrations in different sensitivity analyses. The confounders used in different sensitivity analyses were the same as used in our main model including age, sex, BMI, season, indicator of each visit, educational attainment, smoking status, fasting status, alcohol consumption, physical activity, and dietary score. An IQR increase was 1.40 μg/m^3 for $PM_{2.5}$, 2.06 μg/m^3 for PM_{10}, 1.36 μg/m^3 for PM_{coarse}, 1.95 × 10^3/cm^3 for PNC, 0.27 × 10^{-5}/m for $PM_{2.5abs}$, 6.86 μg/m^3 for NO_2, 8.69 μg/m^3 for NO_x, and 3.45 μg/m^3 for O_3. Main: results from the main models (participants with repeated measurements); All participants: all participants with at least one visit in KORA S4, KORA F4 or KORA FF4; Fasting: participants who did overnight fasting; No-mover: participants who never change their residences during the entire study; IPW: further add predicted inverse probability of each participant into the main models; Backd: back-extrapolated annual average air pollutant concentrations were used rather the LUR estimated air pollutant concentrations. PC ae: acyl-alkyl phosphatidylcholine. $PM_{2.5}$ = particulate matter with an aerodynamic diameter less than or equal to 2.5 μm; PM_{coarse} = particulate matter with an aerodynamic diameter of 2.5–10 μm; PM_{10} = particulate matter with an aerodynamic diameter less than or equal to 10 μm; $PM_{2.5abs}$ = $PM_{2.5}$ absorbance; PNC = particle number concentration; NO_2 = nitrogen dioxide; NO_x = nitrogen oxide; O_3 = ozone.

et al. 2021). Our results suggested that higher physical activity attenuated the adverse effects of air pollution, which suggests that the long-term beneficial effects of physical activity might outweigh the harmful effects of air pollution, as previous studies reported (Fuertes et al. 2018; Sun et al. 2020b). Participants with low educational attainment were more vulnerable to air pollution exposure. Low education has been associated with increased susceptibility to adverse health effects of air pollution due to a higher prevalence of pre-existing diseases and limited access to medical care and fresh foods (Sacks et al. 2011). We observed that individuals with healthier dietary patterns showed weaker associations with air pollution, which might follow the findings that sufficient intakes of essential micronutrients (e.g., vitamins and long-chain polyunsaturated fatty acids) could modulate air pollution-induced harmful effects by reducing the oxidative stress and inflammatory response (Lim et al. 2019; Péter et al. 2015).

The targeted metabolomics approach used in our study has the strength to give an annotation of all metabolites compared to untargeted metabolomics analysis (unknown metabolites were also quantified), which might mislead false annotation for metabolites. To our knowledge, this is the first study using repeated measurements of targeted metabolomics to explore the health effects of long-term exposure to ambient air pollution within a population-representative cohort study of adults, and also with the largest number of study participants. We further assessed multiple air pollutants, including different particle matters and gaseous air pollutants. Moreover, the KORA cohort is a well-characterized study with standardized and comprehensive methods to collect individual information, enhancing our results' reliability. The longitudinal study design with repeated measurements of biomarkers strengthened statistical power and reduced potential residual confounding from unmeasured factors. It might also provide analytical improvement to previous cross-sectional analyses despite the lack of replication by other cohorts. Furthermore, the residential air pollutant concentrations were estimated using well-defined LUR models, which captured the spatial variation in exposure and enabled us to conclude consistent patterns across various air pollutants, reducing the risk of chance findings. This study also has the strength to assess the susceptibility from both external and intrinsic factors, especially dietary intake and lifestyle, which are known to affect the human metabolome. However, targeted metabolomics lowered the opportunity for new biomarkers discovery and could not fully represent the whole metabolome. Another limitation of our study is that the annual average concentrations of air pollutants were estimated using spatial models for 2014–15. We believe these exposure estimates are valid for the historical spatial contrasts because previous studies have shown that the spatial variation in exposure remained stable over time (de Hoogh et al. 2018; Eeftens et al. 2011; Gulliver et al. 2013; Wang et al. 2013). Using the air pollution concentrations obtained with a back-extrapolation approach, we investigated the potential effects of temporal variation. In addition,

we restricted our study to non-movers (participants who did not move within the study period) to reduce the exposure misclassification. The robust results from both analyses validated our exposure assessment approach. Nevertheless, we cannot rule out the potential impact of measurement error and note that exposure measurement error driven by spatial and/or temporal misalignments could lead to biases in either direction, as well as incorrect standard errors of health effect estimates.

6. Conclusions

Our study suggested that long-term air pollution exposure is associated with metabolic alterations, particularly in PCs with unsaturated long-chain fatty acids. These findings could provide new insights into potential mechanisms for air pollution-associated adverse outcomes in the general adult population.

CRediT authorship contribution statement

Yueli Yao: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Alexandra Schneider:** Conceptualization, Supervision, Writing - review & editing. **Kathrin Wolf:** Conceptualization, Writing - review & editing. **Siqi Zhang:** Conceptualization, Writing - review & editing. **Rui Wang-Sattler:** Conceptualization, Writing - review & editing. **Annette Peters:** Conceptualization, Data curation, Funding acquisition, Supervision, Writing - review & editing. **Susanne Breitner:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We thank all participants for their long-term commitment to the KORA study, the staff for data collection and research data management and the members of the KORA Study Group (https://www.helmholtz-munich.de/en/epi/cohort/kora) who are responsible for the design and conduct of the study.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2022.107632.

References

Ayala, A., Muñoz, M.F., Argüelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cellular Longevity 2014, 360438–360438. http://doi.org/10.1155/2014/360438.

Bae, H.R., Chandy, M., Aguilera, J., Smith, E.M., Nadeau, K.C., Wu, J.C., Paik, D.T., 2021. Adverse effects of air pollution-derived fine particulate matter on cardiovascular homeostasis and disease. Trends Cardiovasc. Med. https://doi.org/10.1016/j.tcm.2021.09.010.

Brook, R.D., Rajagopalan, S., Pope, C.A., Brook, J.R., Bhatnagar, A., Diez-Roux, A.V., Holguin, F., Hong, Y., Luepker, R.V., Mittleman, M.A., Peters, A., Siscovick, D., Smith, S.C., Whitsel, L., Kaufman, J.D., 2010. Particulate matter air pollution and cardiovascular disease: An update to the scientific statement from the American Heart Association. Circulation 121 (21), 2331–2378.

Cao, H., Li, B., Peng, W., Pan, L.i., Cui, Z.e., Zhao, W., Zhang, H., Tang, N., Niu, K., Sun, J., Han, X., Wang, Z., Liu, K., He, H., Cao, Y., Xu, Z., Shan, A., Meng, G.e., Sun, Y., Guo, C., Liu, X., Xie, Y., Wen, F., Shan, G., Zhang, L., 2020. Associations of long-term exposure to ambient air pollution with cardiac conduction abnormalities in Chinese adults: The CHCN-BTH cohort study. Environ. Int. 143, 105981.

Chak, C., Lacruz, M., Adam, J., Brandmaier, S., Covic, M., Huang, J., Meisinger, C., Tiller, D., Prehn, C., Adamski, J., Berger, U., Gieger, C., Peters, A., Kluttig, A., Wang-Sattler, R., 2019. Ageing Investigation Using Two-Time-Point Metabolomics Data from KORA and CARLA Studies. Metabolites 9 (3), 44.

Chen, C., Li, H., Niu, Y., Liu, C., Lin, Z., Cai, J., Li, W., Ge, W., Chen, R., Kan, H., 2019a. Impact of short-term exposure to fine particulate matter air pollution on urinary metabolome: A randomized, double-blind, crossover trial. Environ. Int. 130, 104878.

Chen, W., Lin, C., Yan, Y., Cheng, K.T., Cheng, T.-J., 2014. Alterations in rat pulmonary phosphatidylcholines after chronic exposure to ambient fine particulate matter. Mol. BioSyst. 10 (12), 3163–3169. https://doi.org/10.1039/C4MB00435C.

Chen, Z., Newgard, C.B., Kim, J.S., Ilkayeva, O., Alderete, T.L., Thomas, D.C., Berhane, K., Breton, C., Chatzi, L., Bastain, T.M., McConnell, R., Avol, E., Lurmann, F., Muehlbauer, M.J., Hauser, E.R., Gilliland, F.D., 2019b. Near-roadway air pollution exposure and altered fatty acid oxidation among adolescents and young adults – The interplay with obesity. Environ. Int. 130, 104935.

Cole, L.K., Vance, J.E., Vance, D.E., 2012. Phosphatidylcholine biosynthesis and lipoprotein metabolism. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of. Lipids 1821 (5), 754–761. https://doi.org/10.1016/j.bbalip.2011.09.009.

de Hoogh, K., Chen, J., Gulliver, J., Hoffmann, B., Hertel, O., Ketzel, M., Bauwelinck, M., van Donkelaar, A., Hvidtfeldt, U.A., Katsouyanni, K., Klompmaker, J., Martin, R.V., Samoli, E., Schwartz, P.E., Stafoggia, M., Bellander, T., Strak, M., Wolf, K., Vienneau, D., Brunekreef, B., Hoek, G., 2018. Spatial PM2.5, NO2, O3 and BC models for Western Europe – Evaluation of spatiotemporal stability. Environ. Int. 120, 81–92.

Eeftens, M., Beelen, R., Fischer, P., Brunekreef, B., Meliefste, K., Hoek, G., 2011. Stability of measured and modelled spatial contrasts in NO2 over time. Occup. Environ. Med. 68 (10), 765–770.

Engelmann, B., 2004. Plasmalogens: targets for oxidants and major lipophilic antioxidants. Biochem. Soc. Trans. 32 (1), 147–150. https://doi.org/10.1042/bst0320147.

Fuertes, E., Markevych, I., Jarvis, D., Vienneau, D., de Hoogh, K., Antó, J.M., Bowatte, G., Bono, R., Corsico, A.G., Emtner, M., Gislason, T., Gullón, J.A., Heinrich, J., Henderson, J., Holm, M., Johannessen, A., Leynaert, B., Marcon, A., Marchetti, P., Moratalla, J.M., Pascual, S., Probst-Hensch, N., Sánchez-Ramos, J.L., Siroux, V., Sommar, J., Weyler, J., Kuenzli, N., Jacquemin, B., Garcia-Aymerich, J., 2018. Residential air pollution does not modify the positive association between physical activity and lung function in current smokers in the ECRHS study. Environ. Int. 120, 364–372.

Gaskins, A.J., Tang, Z., Hood, R.B., Ford, J., Schwartz, J.D., Jones, D.P., Laden, F., Liang, D., 2021. Periconception air pollution, metabolomic biomarkers, and fertility among women undergoing assisted reproduction. Environ. Int. 155, 106666.

Gulliver, J., de Hoogh, K., Hansell, A., Vienneau, D., 2013. Development and back-extrapolation of NO2 land use regression models for historic exposure assessment in Great Britain. Environ. Sci. Technol. 47 (14), 7804–7811. https://doi.org/10.1021/es4008849.

Haid, M., Muschet, C., Wahl, S., Romisch-Margl, W., Prehn, C., et al., 2018. Long-Term Stability of Human Plasma Metabolites during Storage at -80 degrees C. J. Proteome Res. 17 (1), 203–211. https://doi.org/10.1021/acs.jproteome.7b00518.

Hales, S., Atkinson, J., Metcalfe, J., Kuschel, G., Woodward, A., 2021. Long term exposure to air pollution, mortality and morbidity in New Zealand: Cohort study. Sci. Total Environ. 801, 149660 https://doi.org/10.1016/j.scitotenv.2021.149660.

Han, S., Huang, J., Foppiano, F., Prehn, C., Adamski, J., et al., 2022. TIGER: technical variation elimination for metabolomics data using ensemble learning architecture. Brief Bioinform 23, 2. https://doi.org/10.1093/bib/bbab535.

Holmes, E., Wilson, I.D., Nicholson, J.K., 2008. Metabolic Phenotyping in Health and Disease. Cell 134 (5), 714–717. https://doi.org/10.1016/j.cell.2008.08.026.

Hood, R.B., Liang, D., Tang, Z., Kloog, I., Schwartz, J., et al., 2022. Length of PM2.5 exposure and alterations in the serum metabolome among women undergoing infertility treatment. Environ. Epidemiol, 6, 1, e191. https://doi.org/10.1097/EE9.0000000000000191.

Hou, J., Liu, X., Tu, R., Dong, X., Zhai, Z., Mao, Z., Huo, W., Chen, G., Xiang, H., Guo, Y., Li, S., Wang, C., 2020. Long-term exposure to ambient air pollution attenuated the association of physical activity with metabolic syndrome in rural Chinese adults: A cross-sectional study. Environ. Int. 136, 105459.

Huan, S., Jin, S., Liu, H., Xia, W., Liang, G., Xu, S., Fang, X., Li, C., Wang, Q., Sun, X., Li, Y., 2021. Fine particulate matter exposure and perturbation of serum metabolome: A longitudinal study in Baoding, China. Chemosphere 276, 130102.

Huang, J., Huth, C., Covic, M., Troll, M., Adam, J., Zukunft, S., Prehn, C., Wang, L.i., Nano, J., Scheerer, M.F., Neschen, S., Kastenmüller, G., Suhre, K., Laxy, M., Schliess, F., Gieger, C., Adamski, J., Hrabe de Angelis, M., Peters, A., Wang-Sattler, R., 2020. Machine Learning Approaches Reveal Metabolic Signatures of Incident Chronic Kidney Disease in Individuals With Prediabetes and Type 2 Diabetes. Diabetes 69 (12), 2756–2765.

Jeong, A., Fiorito, G., Keski-Rahkonen, P., Imboden, M., Kiss, A., Robinot, N., Gmuender, H., Vlaanderen, J., Vermeulen, R., Kyrtopoulos, S., Herceg, Z., Ghantous, A., Lovison, G., Galassi, C., Ranzi, A., Krogh, V., Grioni, S., Agnoli, C., Sacerdote, C., Mostafavi, N., Naccarati, A., Scalbert, A., Vineis, P., Probst-Hensch, N., 2018. Perturbation of metabolic pathways mediates the association of air pollutants with asthma and cardiovascular diseases. Environ. Int. 119, 334–345.

Kasdagli, M.-I., Katsouyanni, K., de Hoogh, K., Lagiou, P., Samoli, E., 2022. Investigating the association between long-term exposure to air pollution and greenness with mortality from neurological, cardio-metabolic and chronic obstructive pulmonary diseases in Greece. Environ. Pollut. 292, 118372 https://doi.org/10.1016/j.envpol.2021.118372.

Kertys, M., Grendar, M., Kosutova, P., Mokra, D., Mokry, J., 2020. Plasma based targeted metabolomic analysis reveals alterations of phosphatidylcholines and oxidative stress markers in guinea pig model of allergic asthma. Biochim. Biophys. Acta Mol. Basis Dis. 1866 (1), 165572 https://doi.org/10.1016/j.bbadis.2019.165572.

Lacruz, M.E., Kluttig, A., Tiller, D., Medenwald, D., Giegling, I., Rujescu, D., Prehn, C., Adamski, J., Frantz, S., Greiser, K.H., Emeny, R.T., Kastenmüller, G., Haerting, J., 2016. Cardiovascular Risk Factors Associated With Blood Metabolite Concentrations and Their Alterations During a 4-Year Period in a Population-Based Cohort. Circ. Cardiovasc. Genet. 9 (6), 487–494.

Li, H., Cai, J., Chen, R., Zhao, Z., Ying, Z., Wang, L., Chen, J., Hao, K.e., Kinney, P.L., Chen, H., Kan, H., 2017. Particulate Matter Exposure and Stress Hormone Levels: A Randomized, Double-Blind, Crossover Trial of Air Purification. Circulation 136 (7), 618–627.

Li, Z., Liang, D., Ye, D., Chang, H.H., Ziegler, T.R., Jones, D.P., Ebelt, S.T., 2021. Application of high-resolution metabolomics to identify biological pathways perturbed by traffic-related air pollution. Environ. Res. 193, 110506.

Liang, D., Moutinho, J.L., Golan, R., Yu, T., Ladva, C.N., Niedzwiecki, M., Walker, D.I., Sarnat, S.E., Chang, H.H., Greenwald, R., Jones, D.P., Russell, A.G., Sarnat, J.A., 2018. Use of high-resolution metabolomics for the identification of metabolic signals associated with traffic-related air pollution. Environ. Int. 120, 145–154.

Lim, C.C., Hayes, R.B., Ahn, J., Shao, Y., Silverman, D.T., Jones, R.R., Thurston, G.D., 2019. Mediterranean Diet and the Association Between Air Pollution and Cardiovascular Disease Mortality Risk. Circulation 139 (15), 1766–1775.

Liu, S., Jørgensen, J.T., Ljungman, P., Pershagen, G., Bellander, T., Leander, K., Magnusson, P.K.E., Rizzuto, D., Hvidtfeldt, U.A., Raaschou-Nielsen, O., Wolf, K., Hoffmann, B., Brunekreef, B., Strak, M., Chen, J., Mehta, A., Atkinson, R.W., Bauwelinck, M., Varraso, R., Boutron-Ruault, M.-C., Brandt, J., Cesaroni, G., Forastiere, F., Fecht, D., Gulliver, J., Hertel, O., de Hoogh, K., Janssen, N.A.H., Katsouyanni, K., Ketzel, M., Klompmaker, J.O., Nagel, G., Oftedal, B., Peters, A., Tjønneland, A., Rodopoulou, S.P., Samoli, E., Bekkevold, T., Sigsgaard, T., Stafoggia, M., Vienneau, D., Weinmayr, G., Hoek, G., Andersen, Z.J., 2021. Long-term exposure to low-level air pollution and incidence of chronic obstructive pulmonary disease: The ELAPSE project. Environ. Int. 146, 106267.

Menni, C., Metrustry, S.J., Mohney, R.P., Beevers, S., Barratt, B., Spector, T.D., Kelly, F. J., Valdes, A.M., 2015. Circulating levels of antioxidant vitamins correlate with better lung function and reduced exposure to ambient pollution. Am. J. Respir. Crit. Care Med. 191 (10), 1203–1207.

Mortamais, M., Gutierrez, L.-A., de Hoogh, K., Chen, J., Vienneau, D., Carrière, I., Letellier, N., Helmer, C., Gabelle, A., Mura, T., Sunyer, J., Benmarhnia, T., Jacquemin, B., Berr, C., 2021. Long-term exposure to ambient air pollution and risk of dementia: Results of the prospective Three-City Study. Environ. Int. 148, 106376.

Moxon, J.V., Jones, R.E., Wong, G., Weir, J.M., Mellett, N.A., Kingwell, B.A., Meikle, P.J., Golledge, J., 2017. Baseline serum phosphatidylcholine plasmalogen concentrations are inversely associated with incident myocardial infarction in patients with mixed peripheral artery disease presentations. Atherosclerosis 263, 301–308.

Nassan, F.L., Kelly, R.S., Kosheleva, A., Koutrakis, P., Vokonas, P.S., Lasky-Su, J.A., Schwartz, J.D., 2021a. Metabolomic signatures of the long-term exposure to air pollution and temperature. Environ. Health 20 (1). https://doi.org/10.1186/s12940-020-00683-x.

Nassan, F.L., Wang, C., Kelly, R.S., Lasky-Su, J.A., Vokonas, P.S., Koutrakis, P., Schwartz, J.D., 2021b. Ambient PM2.5 species and ultrafine particle exposure and their differential metabolomic signatures. Environ. Int. 151, 106447.

Nemmar, A., Hoet, P.H.M., Vanquickenborne, B., Dinsdale, D., Thomeer, M., Hoylaerts, M.F., Vanbilloen, H., Mortelmans, L., Nemery, B., 2002. Passage of inhaled particles into the blood circulation in humans. Circulation 105 (4), 411–414.

Pang, Z., Chong, J., Zhou, G., de Lima Morais, D.A., Chang, L., et al. 2021. MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. Nucl. Acids Res., 49, W1, W388–W396. https://doi.org/10.1093/nar/gkab382.

Park, J., Kim, H.-J., Lee, C.-H., Lee, C.H., Lee, H.W., 2021. Impact of long-term exposure to ambient air pollution on the incidence of chronic obstructive pulmonary disease: A systematic review and meta-analysis. Environ. Res. 194, 110703 https://doi.org/10.1016/j.envres.2020.110703.

Péter, S., Holguin, F., Wood, L., Clougherty, J., Raederstorff, D., Antal, M., Weber, P., Eggersdorfer, M., 2015. Nutritional Solutions to Reduce Risks of Negative Health Impacts of Air Pollution. Nutrients 7 (12), 10398–10416.

Peters, A., Nawrot, T.S., Baccarelli, A.A., 2021. Hallmarks of environmental insults. Cell 184 (6), 1455–1468. https://doi.org/10.1016/j.cell.2021.01.043.

Pilz, V., Wolf, K., Breitner, S., Rückerl, R., Koenig, W., Rathmann, W., Cyrys, J., Peters, A., Schneider, A., 2018. C-reactive protein (CRP) and long-term air pollution with a focus on ultrafine particles. Int. J. Hyg. Environ. Health 221 (3), 510–518.

Rabel, M., Laxy, M., Thorand, B., Peters, A., Schwettmann, L., et al., 2018. Clustering of Health-Related Behavior Patterns and Demographics. Results From the Population-Based KORA S4/F4 Cohort Study. Front. Public Health 6, 387. https://doi.org/10.3389/fpubh.2018.00387.

Rappaport, S.M., Barupal, D.K., Wishart, D., Vineis, P., Scalbert, A., 2014. The Blood Exposome and Its Role in Discovering Causes of Disease. Environ. Health Perspect. 122 (8), 769–774.

Rinschen, M.M., Ivanisevic, J., Giera, M., Siuzdak, G., 2019. Identification of bioactive metabolites using activity metabolomics. Nat. Rev. Mol. Cell Biol. 20 (6), 353–367. https://doi.org/10.1038/s41580-019-0108-4.

Ritz, B., Yan, Q.i., He, D.i., Wu, J., Walker, D.I., Uppal, K., Jones, D.P., Heck, J.E., 2022. Child serum metabolome and traffic-related air pollution exposure in pregnancy. Environ. Res. 203, 111907.

Römisch-Margl, W., Prehn, C., Bogumil, R., Röhring, C., Suhre, K., Adamski, J., 2012. Procedure for tissue sample preparation and metabolite extraction for high-throughput targeted metabolomics. Metabolomics 8 (1), 133–142.

Sacks, J.D., Stanek, L.W., Luben, T.J., Johns, D.O., Buckley, B.J., Brown, J.S., Ross, M., 2011. Particulate matter-induced health effects: who is susceptible? Environ. Health Perspect. 119 (4), 446–454.

Soininen, P., Kangas, A.J., Wurtz, P., Suna, T., Ala-Korpela, M., 2015. Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. Circ. Cardiovasc. Genet. 8 (1), 192–206. https://doi.org/10.1161/CIRCGENETICS.114.000216.

Suhre, K., Meisinger, C., Doring, A., Altmaier, E., Belcredi, P., et al. 2010. Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. PLoS One 5, 11, e13953. https://doi.org/10.1371/journal.pone.0013953.

Sun, S., Cao, W., Chan, K.-P., Ran, J., Ge, Y., Zhang, Y., Feng, Y., Zeng, Q., Lee, R.-Y., Wong, C.-M., Tian, L., Lei, Y., 2020a. Cigarette smoking increases deaths associated with air pollution in Hong Kong. Atmos. Environ. 223, 117266.

Sun, S., Cao, W., Qiu, H., Ran, J., Lin, H., et al. 2020b. Benefits of physical activity not affected by air pollution: a prospective cohort study. Int. J. Epidemiol., 49, 1, 142–152. https://doi.org/10.1093/ije/dyz184.

Sutter, I., Klingenberg, R., Othman, A., Rohrer, L., Landmesser, U., Heg, D., Rodondi, N., Mach, F., Windecker, S., Matter, C.M., Lüscher, T.F., von Eckardstein, A., Hornemann, T., 2016. Decreased phosphatidylcholine plasmalogens–A putative novel lipid signature in patients with stable coronary artery disease and acute myocardial infarction. Atherosclerosis 246, 130–140.

Tainio, M., Jovanovic Andersen, Z., Nieuwenhuijsen, M.J., Hu, L., de Nazelle, A., An, R., Garcia, L.M.T., Goenka, S., Zapata-Diomedi, B., Bull, F., Sá, T.H.d., 2021. Air pollution, physical activity and health: A mapping review of the evidence. Environ. Int. 147, 105954.

Tang, W.H.W., Wang, Z., Levison, B.S., Koeth, R.A., Britt, E.B., Fu, X., Wu, Y., Hazen, S.L., 2013. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N. Engl. J. Med. 368 (17), 1575–1584.

van der Veen, J.N., Kennelly, J.P., Wan, S., Vance, J.E., Vance, D.E., Jacobs, R.L., 2017. The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. Biochimica et Biophysica Acta (BBA) - Biomembranes 1859 (9), 1558–1572.

van Veldhoven, K., Kiss, A., Keski-Rahkonen, P., Robinot, N., Scalbert, A., Cullinan, P., Chung, K.F., Collins, P., Sinharay, R., Barratt, B.M., Nieuwenhuijsen, M., Rodoreda, A.A., Carrasco-Turigas, G., Vlaanderen, J., Vermeulen, R., Portengen, L., Kyrtopoulos, S.A., Ponzi, E., Chadeau-Hyam, M., Vineis, P., 2019. Impact of short-term traffic-related air pollution on the metabolome – Results from two metabolome-wide experimental studies. Environ. Int. 123, 124–131.

Vlaanderen, J.J., Janssen, N.A., Hoek, G., Keski-Rahkonen, P., Barupal, D.K., Cassee, F. R., Gosens, I., Strak, M., Steenhof, M., Lan, Q., Brunekreef, B., Scalbert, A., Vermeulen, R.C.H., 2017. The impact of ambient air pollution on the human blood metabolome. Environ. Res. 156, 341–348.

Walker, D.I., Lane, K.J., Liu, K., Uppal, K., Patton, A.P., Durant, J.L., Jones, D.P., Brugge, D., Pennell, K.D., 2019. Metabolomic assessment of exposure to near-highway ultrafine particles. J. Eposure Sci. Environ. Epidemiol. 29 (4), 469–483.

Wang, R., Henderson, S.B., Sbihi, H., Allen, R.W., Brauer, M., 2013. Temporal stability of land use regression models for traffic-related air pollution. Atmos. Environ. 64, 312–319. https://doi.org/10.1016/j.atmosenv.2012.09.056.

Ward-Caviness, C.K., Breitner, S., Wolf, K., Cyrys, J., Kastenmüller, G., Wang-Sattler, R., Schneider, A., Peters, A., 2016. Short-term NO2 exposure is associated with long-chain fatty acids in prospective cohorts from Augsburg, Germany: results from an analysis of 138 metabolites and three exposures. Int. J. Epidemiol. 45 (5), 1528–1538.

Weuve, J., Tchetgen Tchetgen, E.J., Glymour, M.M., Beck, T.L., Aggarwal, N.T., Wilson, R.S., Evans, D.A., Mendes de Leon, C.F., 2012. Accounting for bias due to selective attrition: the example of smoking and cognitive decline. Epidemiology 23 (1), 119–128.

Whiley, L., Sen, A., Heaton, J., Proitsi, P., García-Gómez, D., Leung, R., Smith, N., Thambisetty, M., Kloszewska, I., Mecocci, P., Soininen, H., Tsolaki, M., Vellas, B., Lovestone, S., Legido-Quigley, C., 2014. Evidence of altered phosphatidylcholine metabolism in Alzheimer's disease. Neurobiol. Aging 35 (2), 271–278.

Wieder, C., Frainay, C., Poupin, N., Rodríguez-Mier, P., Vinson, F., et al., 2021. Pathway analysis in metabolomics: Recommendations for the use of over-representation analysis. PLoS Comput. Biol. 17, 9, https://doi.org/e1009105. 10.1371/journal.pcbi.1009105.

Winkler, G., Döring, A., 1998. Validation of a short qualitative food frequency list used in several German large scale surveys. Zeitschrift für Ernährungswissenschaft 37 (3), 234–241. https://doi.org/10.1007/PL00007377.

Wolf, K., Cyrys, J., Harcinίková, T., Gu, J., Kusch, T., Hampel, R., Schneider, A., Peters, A., 2017. Land use regression modeling of ultrafine particles, ozone, nitrogen oxides and markers of particulate matter pollution in Augsburg, Germany. Sci. Total Environ. 579, 1531–1540.

Wolf, K., Hoffmann, B., Andersen, Z.J., Atkinson, R.W., Bauwelinck, M., Bellander, T., Brandt, J., Brunekreef, B., Cesaroni, G., Chen, J., de Faire, U., de Hoogh, K., Fecht, D., Forastiere, F., Gulliver, J., Hertel, O., Hvidtfeldt, U.A., Janssen, N.A.H., Jørgensen, J.T., Katsouyanni, K., Ketzel, M., Klompmaker, J.O., Lager, A., Liu, S., MacDonald, C.J., Magnusson, P.K.E., Mehta, A.J., Nagel, G., Oftedal, B., Pedersen, N. L., Pershagen, G., Raaschou-Nielsen, O., Renzi, M., Rizzuto, D., Rodopoulou, S., Samoli, E., van der Schouw, Y.T., Schramm, S., Schwarze, P., Sigsgaard, T., Sørensen, M., Stafoggia, M., Strak, M., Tjønneland, A., Verschuren, W.M.M., Vienneau, D., Weinmayr, G., Hoek, G., Peters, A., Ljungman, P.L.S., 2021. Long-term exposure to low-level ambient air pollution and incidence of stroke and coronary heart disease: a pooled analysis of six European cohorts within the ELAPSE project. The Lancet Planetary Health 5 (9) e620–e632.

Xu, T., Holzapfel, C., Dong, X., Bader, E., Yu, Z., Prehn, C., Perstorfer, K., Jaremek, M., Roemisch-Margl, W., Rathmann, W., Li, Y., Wichmann, H.-E., Wallaschofski, H., Ladwig, K.H., Theis, F., Suhre, K., Adamski, J., Illig, T., Peters, A., Wang-Sattler, R., 2013. Effects of smoking and smoking cessation on human serum metabolite profile: results from the KORA cohort study. BMC Med. 11 (1) https://doi.org/10.1186/1741-7015-11-60.

Yan, Q.i., Liew, Z., Uppal, K., Cui, X., Ling, C., Heck, J.E., von Ehrenstein, O.S., Wu, J., Walker, D.I., Jones, D.P., Ritz, B., 2019. Maternal serum metabolome and traffic-related air pollution exposure in pregnancy. Environ. Int. 130, 104872.

Yazdi, M.D., Wang, Y., Di, Q., Requia, W.J., Wei, Y., Shi, L., Sabath, M.B., Dominici, F., Coull, B., Evans, J.S., Koutrakis, P., Schwartz, J.D., 2021. Long-term effect of exposure to lower concentrations of air pollution on mortality among US Medicare participants and vulnerable subgroups: a doubly-robust approach. The Lancet Planetary Health 5 (10) e689–e697.

Zhang, S., Mwiberi, S., Pickford, R., Breitner, S., Huth, C., Koenig, W., Rathmann, W., Herder, C., Roden, M., Cyrys, J., Peters, A., Wolf, K., Schneider, A., 2021. Longitudinal associations between ambient air pollution and insulin sensitivity: results from the KORA cohort study. The Lancet Planetary Health 5 (1), e39–e49.