C-reactive protein (CRP) and long-term air pollution with a focus on ultrafine particles

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HIGHLIGHTS

- We analyzed associations of residential long-term air pollution and the inflammatory marker CRP measured by a high-sensitivity (hs) assay.
- Air pollutants including UFP (measured as PNC) were modeled by land-use regression.
- PNC and PM_{10} showed a positive association with hs-CRP in single-pollutant models.
- PNC and PM_{10} were significantly associated with hs-CRP after adjustment for $PM_{2.5}$.
- Never smokers, non-obese and non-diabetic participants indicated higher effect estimates.

ABSTRACT

BACKGROUND. Long-term exposure to ambient air pollution contributes to the global burden of disease by particularly affecting cardiovascular (CV) causes of death. We investigated the association between particle number concentration (PNC), a marker for ultrafine particles, and other air pollutants and high sensitivity C-reactive protein (hs-CRP) as a potential link between air pollution and CV disease.

METHODS. We cross-sectionally analysed data from the second follow up (2013 and 2014) of the German KORA baseline survey which was conducted in 1999 to 2001. Residential long-term exposure to PNC and various other size fractions of particulate matter (PM_{10} with size of < 10 µm in aerodynamic diameter, PM_{coarse} 2.5-10 µm or $PM_{2.5}$ < 2.5 µm, respectively), soot ($PM_{2.5}$abs: absorbance of $PM_{2.5}$), nitrogen oxides (nitrogen dioxide NO_2 or oxides NO_x, respectively) and ozone (O_3) were estimated by land-use regression models. Associations between annual air pollution concentrations and hs-CRP were modeled in 2,252 participants using linear regression models adjusted for several confounders. Potential effect-modifiers were examined by interaction terms and two-pollutant models were calculated for pollutants with Spearman inter-correlation <0.70.

RESULTS. Single pollutant models for PNC, PM_{10}, PM_{coarse}, $PM_{2.5}$abs, NO_2 and NO_x showed positive but non-significant associations with hs-CRP. For PNC, an interquartile range (2,000 particles/cm^3) increase was associated with a 3.6% (95% CI: -0.9%, 8.3%) increase in hs-CRP. A null association was found for $PM_{2.5}$. Effect estimates were higher for women, non-obese participants, for participants without diabetes and without a history of cardiovascular disease whereas ex-smokers showed lower estimates compared to smokers or non-smokers. For O_3, the dose-response function suggested a non-linear relationship. In two-pollutant models, adjustment for $PM_{2.5}$ strengthened the effect estimates for PNC and PM_{10} (6.3% increase per 2,000 particles/cm^3 [95% CI: 0.4%; 12.5%] and 7.3% per 16.5 µg/m^3 [95% CI: 0.4%; 14.8%], respectively).

CONCLUSION. This study adds to a scarce but growing body of literature showing associations between long-term exposure to ultrafine particles and hs-CRP, one of the most intensely studied blood biomarkers for cardiovascular health. Our results highlight the role of ultrafine particles within the complex mixture of ambient air pollution and their inflammatory potential.

KEYWORDS

Air pollution; Ultrafine particles; Particulate matter; Long-term exposure; C-reactive protein; Inflammation

INTRODUCTION

According to the WHO, ambient air pollution contributed to 3.7 million premature deaths in 2012 worldwide including 280,000 in high-income countries of Europe and is therefore the largest environmental contributor to the burden of disease (1). This risk factor particularly affects cardiovascular and cerebrovascular causes of death since these account for 80% of mortality attributable to ambient air pollution (2, 3). Previous studies provide consistent evidence that long-term exposure to particulate matter (PM) air pollution is associated with cardiovascular morbidity and mortality (4, 5). Although the exact mechanisms behind these effects are still unclear, an inflammatory response has been hypothesized to play an important role which is also supported by several animal studies (6, 7). C-reactive protein (CRP) is a reliable measure indicating systemic inflammation and high sensitivity (hs) CRP has also been reported to be a predictor for an increased risk of cardiovascular diseases (CVD) (8).

Several epidemiological studies have reported associations of long-term exposure to air pollution with an increased level of CRP (9-11) while others did not see an association (12, 13). A recent review on PM and hs-CRP did not find conclusive evidence yet (14). However, previous studies mainly focused on larger particles like $PM_{2.5}$ or PM_{10} (particulate matter with a size of < 2.5 µm in aerodynamic diameter or < 10 µm, respectively) whereas ultrafine particles (< 100 nm) came into focus only recently. They are hypothesized to be more harmful than other particles (15). Due to their small size they might even translocate from the lungs into the circulation which could directly lead to systemic inflammation (16). Furthermore, their large surface may provide a mechanism for delivering much more potentially toxic absorbed organic material (17). The California Teachers Study by Ostro et al. (18) was the first study which investigated long-term effects of ultrafine particles on health and it prospectively included more than 100,000 participants. They reported a significant association of long-term exposure to ultrafine particles with all-cause and cardiovascular mortality. So far, only two studies have examined the association between long-term exposure to particle number concentration (PNC), as a proxy for ultrafine particles, and hs-CRP. Viehmann et al. (19) examined a population based prospective cohort study incorporating approximately 4,800 participants finding a non-significant association whereas Lane et al. (20) investigated 409 participants with regard to long-term exposure of near-highway ultrafine particle exposure in a cross-sectional design. The study rom the U.S. (20) also found a positive but non-significant association among all participants which even reached significance when including only the white non-Hispanic population.

To date, reliable data of chronic exposure to ultrafine particles are still lacking which in turn leads to the fact that no air quality standards are established regarding this air pollution metric. In terms of filling this gap, we examined the association of long-term exposure to air pollution and the inflammatory marker hs-CRP. We focused on ultrafine particles, but also other PM metrics, soot, nitrogen oxides and

ozone were of interest. We hypothesized that long-term exposure to ambient air pollution would be positively associated with hs-CRP with ultrafine particles comprising higher toxicity.

METHODS

Study population

This cross-sectional analysis is part of the KORA (Cooperative Health Research in the Region of Augsburg) study, a population-based, prospective cohort study conducted in southern Germany. Study participants were selected randomly from population registries in the city of Augsburg and two adjacent counties. A total of 4,261 participants were examined at baseline (1999 to 2001) and 2,279 of these participants took part in the second follow-up conducted from 2013 to 2014. The latter served as the data base for the current analyses. The survey consisted of a computer-assisted personal interview and a self-administered questionnaire. All individuals were physically examined and blood samples were taken. The study design, sampling method and data collection have been described in detail elsewhere (21, 22). All participants gave written informed consent to the study, which was approved by the ethics committee of the Bavarian Medical Association.

Outcome definition

As a marker of inflammation hs-CRP (mg/L) was assayed in serum on a BN II nephelometer (Siemens Healthcare Diagnostics Product GmbH, Marburg, Germany) with intra-assay coefficient of variation of 2.13%. Samples were kept at 4°C after blood draw, until further processing within a maximum of six hours and aliquots were then stored at -80°C. Samples were measured by the collaborating Biomarker Laboratory at the University of Ulm, Germany. Measurements below the limit of detection of 0.175 mg/L were set to its half, 0.09 mg/L.

Air pollution exposure

Residential exposure to ambient air pollution was estimated within the framework of the ULTRA 3 project (Environmental Nanoparticles and Health: Exposure, Modeling and Epidemiology of Nanoparticles and their Composition) for all KORA participants. By the use of land-use regression (LUR) models, long-term exposure was assigned individually to the study participants' home addresses and estimated as mean annual concentration. In order to characterize spatial variation, LUR models were built on the basis of annual average measurements and predictors like traffic, land-use, population and building density (23). Measurements and modeling strategy were based on the standardized ESCAPE (European Study of Cohorts for Air Pollution Effects) approach (24, 25). Data on air pollutants were collected at 20 monitoring sites in the city and region of Augsburg and comprised traffic, urban background and rural background sites. The sampling time comprised three periods of two weeks in the cold, warm and intermediate (spring or autumn) season between March 2014 and April 2015 (23). In order to adjust for temporal variation, a reference site was operated continuously throughout the whole measurement period. The following air pollutants were modeled: PNC; PM10; PM2.5; coarse particulate matter (PMcoarse, 2.5-10 µm); absorbance of PM2.5 (PM2.5 abs); nitrogen dioxide (NO2); nitrogen oxide (NOx) and ozone (O3). By measuring the absorbance of PM2.5 the content of soot could be

determined. PNC was measured by four GRIMM ultrafine particle counters (model EDM 465 UFPC, GRIMM aerosol, Ainring, Germany) measuring total PNC with a cut-off at 7 nm and one NanoScan SMPS Nanoparticle Sizer (model 3910, TSI, Shoreview, MN, USA) measuring PNC in 13 size channels in the size range from 10 to 420 nm. A diffusion dryer was used in the sheath air loop of the Nanoscan-SPMS in order to minimize the influence of particle growth under conditions of high relative humidity. At the reference site, PNC was measured in the size range from 3 nm to 10 µm by use of a combination of custom-made Twin Differential Mobility Particle Spectrometry (TDMPS) based on Birmili et al (26)and an aerodynamic particle sizer (APS, Model 3321, TSI Inc., U.S.). Comparisons of all instruments were conducted every two weeks and correction factors were applied if necessary. The LUR model for PNC indicated a very good fit with an adjusted model explained variance (R^2) of 0.89 (cross-validation adjusted $R^2 = 0.81$) and included as predictors building footprints and traffic in the close vicinity (25m and 50m), seminatural and industrial areas in a 100m and 300m buffer, respectively, and green area within 500m. A more detailed description of the measurement techniques, predictors, model building, quality and validation can be found elsewhere (23).

Covariates

Potential confounding factors including demographic, socioeconomic, lifestyle and clinical characteristics as well as medical history and medication intake are summarized in Table 1. Socio-economic status (SES) was measured by the Helmert-Index (27) and neighborhood SES by area level household income (percentage of households with low income < 1.250 € in 5000 m² grid cell). Cumulative smoking exposure was assessed for current and ex-smokers as pack-years (packages per day*years of smoking). CVD was defined as history of myocardial infarction, angina pectoris or hypertension whereas the diagnosis of hypertension was defined by either blood pressure above 140/90 mmHg or treatment of known hypertension (28). Socio-demographic covariates and lifestyle characteristics were based on self-reported information. The diagnosis of diabetes mellitus (type 1 and 2) was validated either by an oral glucose tolerance test (OGTT) for participants with unknown diabetes, or by the physician's diagnosis or the current intake of glucose-lowering agents for previously diagnosed diabetes. Pre-diabetes was defined as impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). Current intake of CRP-lowering drugs like anti-inflammatory drugs (NSAID: non-steroidal anti-inflammatory drugs), or lipid-lowering (including statins) was classified according to the Anatomical Therapeutic Chemical Classification Index (29). Statins have been shown to reduce CRP levels independent of the reduction of LDL-cholesterol (referenz Arevalo-Lorido) and might therefore attenuate possible associations between air pollution and CRP. Clinical measurements were assayed by standard enzymatic procedures. Long-term road traffic noise was assessed by maximum annual A-weighted equivalent day-evening-night (Lden, 24-h) and night time (Lnight , 22.00-06.00 h) continuous sound pressure levels (dB(A)), estimated for the home address of each participant by the company ACCON GmbH (DIN ENISO14001:2009 certified), an environmental and engineering consultancy for

sound and vibration technology, air pollution control and environmental planning. For further details we refer to Pitchika et al. 2017 (30).

Statistical Analyses

Multiple linear regression models were conducted for each pollutant separately to examine the association between air pollutants and hs-CRP. Hs-CRP was log-transformed to approximate normal distribution of the residuals and to stabilize variance. We restricted our analysis to the study population with complete information on hs-CRP measurements, exposures and main covariates. We adjusted for several socio-economic and individual-level demographic characteristics as well as clinical variables. First, we defined the minimum model with a-priori chosen covariates: age, sex, body mass index (BMI), smoking status and month of blood draw. Based on minimizing the Schwarz Bayesian criterion, a supervised stepwise forward selection was then applied separately on three variable blocks in the given order: (I) socio-economic covariates (marital status, education, occupational status, income, SES, neighborhood SES), (II) lifestyle-related covariates (waist circumference, waist-hip ratio, physical activity, cumulative smoking exposure, alcohol consumption) and (III) clinical parameters (total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, triglycerides). As environmental tobacco smoke (ETS) exposure contained more than 10% of missing values (N = 433) this variable was not considered in the selection process. The final main model comprised the following covariates: age, sex, BMI, smoking status, month of blood draw, waist-hip ratio and HDL cholesterol. Effect estimates are presented as percent change of the geometric mean of hs-CRP and the corresponding 95% confidence interval (95% CI) for an interquartile range (IQR) increase in each air pollutant.

We examined possible effect modifiers by including interaction terms in the main model. We investigated age ($\geq$ 65 years vs. < 65 years), sex, obesity (BMI $\geq$ 30 kg/m^2 vs. < 30 kg/m^2), smoking status (current vs. ex- vs. non-smokers), diabetes (diabetes vs. pre-diabetes vs. no diabetes), having a history of CVD, the intake of CRP-lowering drugs and occupational status (employed vs. not employed). Statins have been shown to reduce CRP levels independent of the reduction of LDL-cholesterol (31) and might therefore attenuate possible associations between air pollution and CRP."

A number of sensitivity analyses were conducted to evaluate the robustness of our results. We investigated outliers by visual inspection of residual plots. The linearity of the dose-response function was examined by including the air pollutants as penalized splines in a generalized additive model. Smoothing parameters were estimated by optimizing the generalized cross-validation criterion. We examined independent effects of particle metrics by the use of two-pollutant models with the restriction that Spearman inter-correlation between pollutants was below 0.70. Furthermore, quantile regression was performed as an alternative method being less sensitive to outliers. In terms of evaluating the clinical

relevance of the results, we applied a logistic regression on the binary outcome hs-CRP > 3 mg/L vs. ≤ 3 mg/L as this cut-off point denotes an increased cardiovascular risk (32). We additionally adjusted for educational level and physical activity in an extended model to account for our strict variables selection process. Furthermore we additionally adjusted for ETS or traffic noise in separate regression models. Since an underlying systemic inflammation due to an acute infection might change CRP concentrations, we excluded measurements of hs-CRP ≥ 10 mg/L (32). Hs-CRP values smaller than the limit of detection were excluded in a further step. Moreover, potential exposure misclassification was analyzed by excluding the subgroup of participants who changed residence between first and second follow-up.

To assess statistical significance $p < 0.05$ was used. All statistical analyses were performed using R Statistical Software Version 3.3.2. (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Study population

Characteristics of the study population are presented in Table 1. In total, 2,279 subjects participated in the study of which 25 had to be excluded due to missing data in either the outcome (N = 19), the exposure (N = 1) or the covariates (N = 5) of the main model. Two outliers were excluded after the inspection of residual plots and thus 2,252 participants remained for our analyses. The study population available for analyses did not show systematic differences regarding outcome, exposure or covariates compared to the study population without exclusions (N=2,279; data not shown). Study participants were on average 60 years old and had a mean BMI of 27.8 kg/m^2. With 325 and 765 subjects having diabetes or pre-diabetes, respectively, only about 50% of the study population showed no history of diabetes. In total, 996 participants had a history of CVD of which the majority (879) was hypertensive. The geometric mean of hs-CRP concentration was 1.26 mg/L and hs-CRP concentrations exceeded 3 mg/L in 22% of participants.

Table 1. Descriptive statistics of the study population (N = 2,252).

	Mean ± SD or N (%)[a]	Missing N (%)
Personal characteristics		
Age (years)	60.3 ± 12.3	-
Sex (male)	1,091 (48.4)	-
Socio-economic covariates		
Marital status		-
Single	153 (6.8)	
Married or living with partner	1,655 (73.5)	
Divorced or separated	240 (10.7)	
Widowed	204 (9.0)	
Education (years)	11.9 ± 2.6	5 (0.1)
Occupational status		1 (0.1)
Employed, self-employed or in training	1,124 (49.9)	
Unemployed	31 (1.4)	
Homemaker	162 (7.2)	
Retired	934 (41.5)	
Income, per capita (Euro)	1,321.0 ± 659.6	117 (5.2)
SES (points, Helmert)	15.0 ± 5.1	8 (0.1)
Neighborhood SES (%)	27.7 ± 17.8	-
Lifestyle covariates		
BMI (kg/m^2)	27.8 ± 5.0	-
Waist circumference (cm)	96.9 ± 14.2	-
Waist–hip ratio	0.9 ± 0.1	-
Physical activity		-
Low (none)	640 (28.4)	
Medium (~1 h/week)	1,027(45.6)	
High (>2 h/week)	585 (26.0)	
Smoking status		-
Non-smoker	938 (41.6)	
Ex-smoker	965 (42.9)	
Smoker	349 (15.5)	
Cumulative smoking exposure (pack-years)	12.3 ± 19.7	55 (2.4)
Alcohol consumption (g/d)	14.7 ± 20.0	1 (0.1)
Clinical characteristics		
Hs-CRP (mg/L; arithmetic mean)	2.5 ± 4.1	-
Total cholesterol (mg/dL)	216.0 ± 39.4	-
HDL cholesterol (mg/dL)	65.6 ± 18.8	-
LDL cholesterol (mg/dL)	134.3 ± 35.2	-
Triglycerides (mg/dL)	121.5 ± 72.0	-
Medical history and medication		
Diabetes status		83 (3.7)
No diabetes	1,079 (49.7)	
Pre-diabetes	765 (35.3)	
Diabetes	325 (15.0)	
Cardiovascular disease[b]	996 (44.2)	-
Intake of medication affecting CRP [c]	944 (42.0)	4 (0.1)

BMI: body mass index; HDL: high density lipoprotein; hs-CRP: high sensitivity C-reactive protein; KORA: Cooperative Health Research in the Region of Augsburg; LDL: low density lipoprotein; N: total number; SD: standard deviation; SES: socio-economic status.

[a] Percentages are calculated based on observations with available information.

[b] History of myocardial infarction, angina pectoris or hypertension.

[c] Intake of non-steroidal anti-inflammatory drugs, lipid-lowering drugs (including statins).

Air pollutants

The descriptive statistics of annual average concentrations of air pollutants at participants' residences are presented in Table 2. Modelled long-term exposure to PNC was on average 7,200 particles/cm^3. The maximum concentrations of PM_{10}, $PM_{2.5}$ and NO_2 in the study area in 2014/2015 did not exceed the EU limits (Directive 2008/50/EC) but were higher than the WHO recommendations for $PM_{2.5}$ and PM_{10} (33). No annual EU limits or WHO guidelines are established for the other pollutants. Spearman correlations between air pollutants were strong or very strong. NO_x was very highly correlated with PNC (r_s = 0.90), as well as with NO_2 (r_s = 0.83). Only $PM_{2.5}$ and O_3 showed lower or no correlation with the other air pollutants.

Table 2. Descriptive statistics of residential air pollutants exposure as annual average concentrations (March 2014 - April 2015) and corresponding Spearman correlation coefficients (N = 2,252).

Pollutant (unit)	Mean± SD	Min	Q1	Median	Q3	Max	Spearman correlation coefficient						
							PNC	PM_{10}	PM_{coarse}	$PM_{2.5}$	$PM_{2.5}$abs	NO_2	NO_x
PNC (10^3/cm^3)	7.2 ± 1.8	3.2	6.1	7.2	8.1	15.0							
PM_{10} (µg/m^3)	16.5 ± 1.5	12.7	15.4	16.2	18.0	22.3	0.80						
PM_{coarse} (µg/m^3)	4.9 ± 1.0	2.4	4.2	4.9	5.6	8.6	0.75	0.79					
$PM_{2.5}$ (µg/m^3)	11.8 ± 1.0	8.3	11.1	11.8	12.5	14.2	0.65	0.52	0.56				
$PM_{2.5}$abs (10^{-5}/m)	1.2 ± 0.2	0.8	1.1	1.2	1.3	1.8	0.77	0.79	0.80	0.61			
NO_2 (µg/m^3)	14.0 ± 4.4	6.9	10.4	13.5	17.2	27.5	0.78	0.73	0.83	0.72	0.86		
NO_x (µg/m^3)	21.7 ± 7.3	4.0	17.2	22.5	25.9	50.5	0.90	0.73	0.74	0.76	0.72	0.83	
O_3 (µg/m^3)	39.1 ± 2.4	31.5	37.4	39.2	40.9	45.8	-0.03	0.06	0.17	-0.18	-0.08	-0.14	-0.07

Max: maximum; Min: minimum; N: total number; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; O_3: ozone; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration; Q1, Q3: first and third quartile, respectively; SD: standard deviation.

Regression Analyses

Effect estimates of the association between long-term residential exposure to air pollutants and hs-CRP adjusted with the minimum and main confounder model are reported in Table 3.Effect estimates for O_3 are not shown since the dose-response function suggested non-linearity and thus, a linear model seemed not to be adequate. For the other exposures, an IQR increase in air pollutant concentration was positively associated with an increase in hs-CRP but the associations were not significant. Our strongest finding was seen for PM_{10}. In comparison with the minimum adjusted model, the effect sizes decreased only marginally when applying the main adjustment model except for the $PM_{2.5}$ estimate which changed into a very weak negative association.

Table 3. Effect estimates and 95% CI of the association between long-term exposure to air pollution and hs-CRP, per IQR in air pollutant for the minimum and main confounder model.

Pollutant (unit)	IQR	Percent change per IQR (95% CI)	
		Minimum model[a]	Main model[b]
PNC (10^3/cm^3)	2.0	3.87 (-0.68, 8.62) (*)	3.63 (-0.86, 8.33)
PM_{10} (µg/m^3)	2.1	5.27 (-0.63, 11.52) (*)	5.15 (-0.69, 11.33) (*)
PM_{coarse} (µg/m^3)	1.4	4.32 (-1.53, 10.52)	4.45 (-1.36, 10.59)
$PM_{2.5}$ (µg/m^3)	1.4	0.16 (-5.38, 6.02)	-0.29 (-5.75, 5.49)
$PM_{2.5}$abs (10^{-5}/m)	0.3	3.10 (-3.08, 9.67)	2.80 (-3.31, 9.29)
NO_2 (µg/m^3)	6.8	1.37 (-4.92, 8.08)	1.19 (-5.03, 7.83)
NO_x (µg/m^3)	8.7	3.62 (-1.36, 8.85)	3.45 (-1.48, 8.61)

CI: confidence interval; hs-CRP: high sensitivity C-reactive protein; IQR: interquartile range; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.

[a] Adjusted for age, sex, body mass index (BMI), smoking status and month of blood draw.
[b] Adjusted for age, sex, BMI, smoking status, month of blood draw, waist–hip ratio and high density lipoprotein (HDL) cholesterol.

(*) Effect estimates with p-values < 0.1.

Effect Modification

Selected effect modifications of the associations between an IQR increase in exposure for each air pollutant and hs-CRP are shown in Figure 1. We observed significant interactions for sex (Supplemental Table A 1), obesity, smoking status, diabetes and CVD. As NO_x was highly correlated with PNC, the pattern was very similar with regard to directionality, magnitude and strength of association (data not shown). Effect estimates were higher across all air pollutants for non-obese participants, participants free of diabetes or CVD. Also, women showed larger effect sizes although confidence intervals were wide and the interactions significant (data not shown). With regard to PNC, PM_{10}, $PM_{2.5}$abs and NO_2, participants without diabetes showed significant effect estimates per IQR increase in air pollutant with the highest one for PM_{10} of 14.71% (per 16.5 µg/m^3; 95% CI: 5.47%; 24.77%). No clear pattern could be detected for participants with pre-diabetes or by smoking status though ex-smokers indicated lower estimates compared to smokers or non-smokers. Moreover, age, medication intake affecting CRP- levels or occupational status did not significantly modify the association. However, effect estimates for occupational status were found to be higher for persons who were not employed (Supplemental Table A 2)

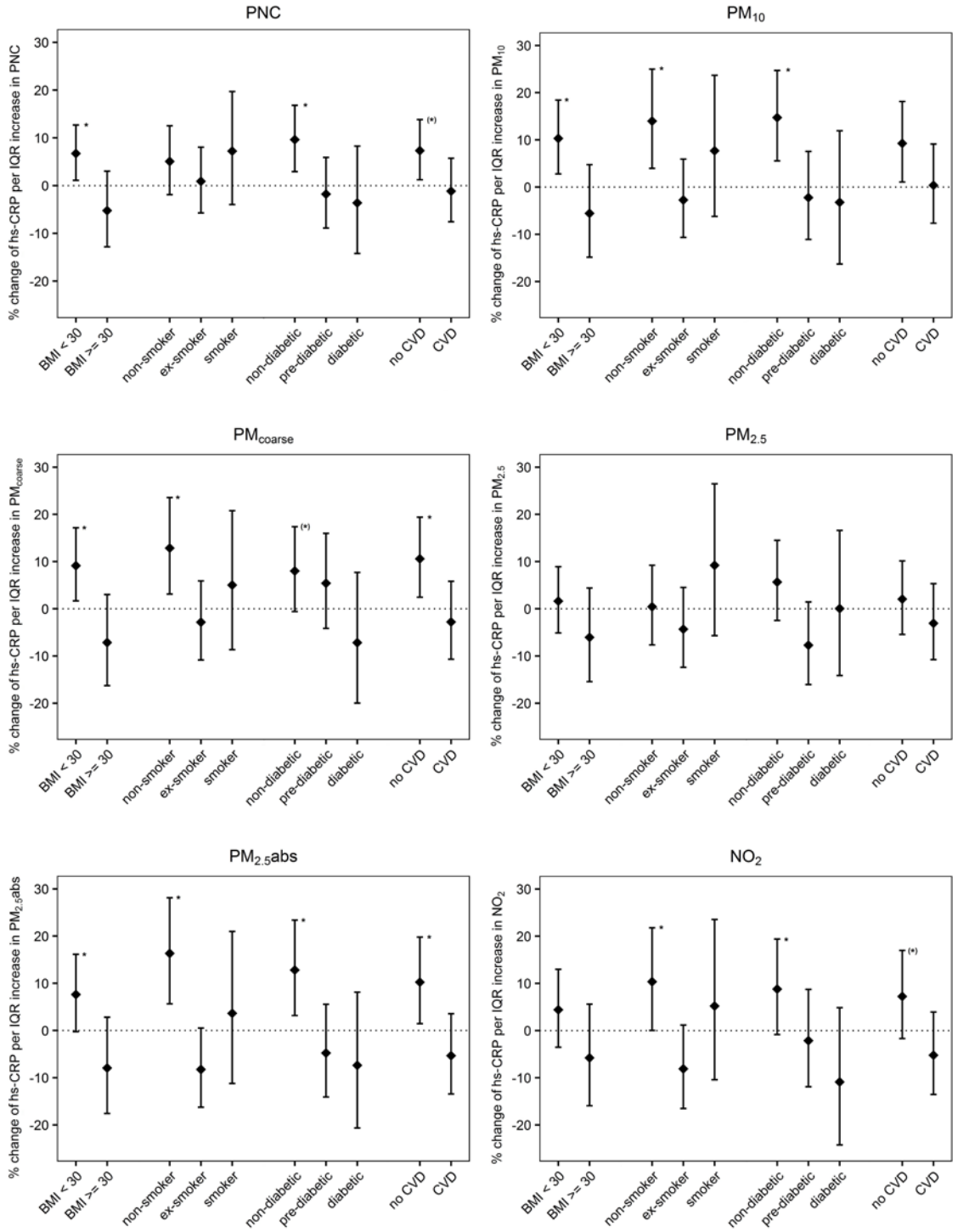

Figure 1. Effect modification by obesity, smoking status, diabetes and CVD of the association between air pollutants and high sensitivity C-reactive protein (hs-CRP) adjusting for main covariates.

BMI: body mass index in kg/m^2; CI: confidence interval; CVD: cardiovascular diseases; IQR: interquartile range; NO_2: nitrogen dioxide; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.

* p-value of interaction < 0.05.
(*) p-value of interaction < 0.10.

Sensitivity Analyses

Whereas most air pollutants showed a linear dose-response function, linearity could not be confirmed for O_3 (Figure 2). Therefore, this association could not be adequately estimated using a linear regression model.

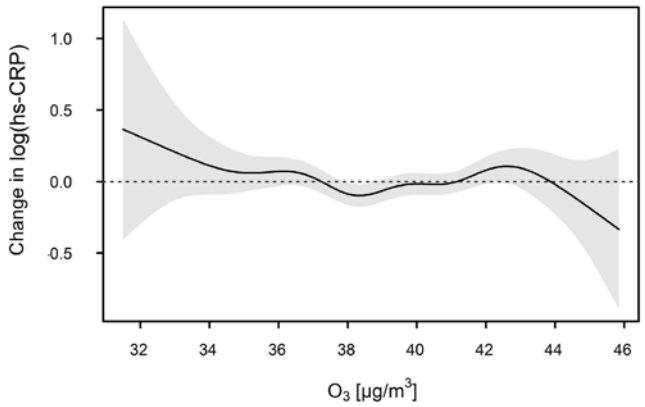

Figure 2. Smooth association between ozone (O_3) and high sensitivity C-reactive protein (hs-CRP) adjusted for main covariates. Grey shades indicate the 95% confidence interval. Adjusted for the main confounder model.

Two-pollutant models with O_3 incorporated as a smooth function showed comparable estimates for the other pollutants as in the single pollutant models. In contrast, adjustment for $PM_{2.5}$ strengthened the effect estimates for PNC, PM_{10}, PM_{coarse} and $PM_{2.5}$abs. Associations of PNC and PM_{10} even became significant with percent changes of 6.31% (per 2.0 $10^3/cm^3$; 95% CI: 0.42%; 12.53%) and 7.34% (per 2.1 µg/m^3; 95% CI: 0.40%; 14.77%), respectively (Figure 3).

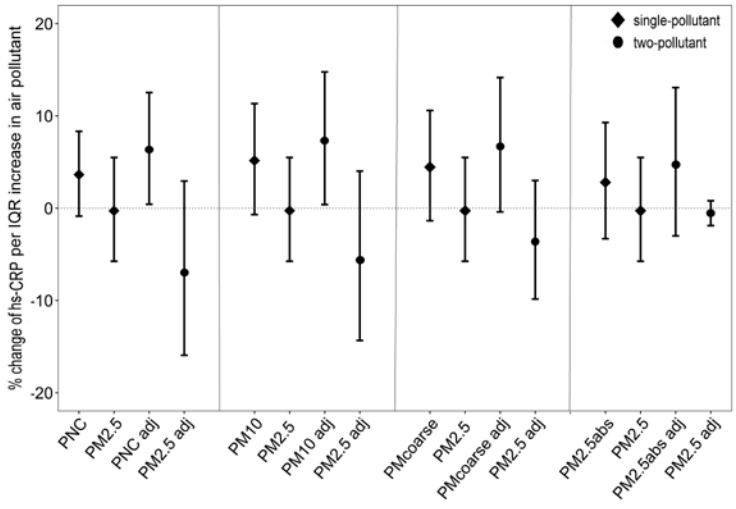

Figure 3. Single-pollutant and two-pollutant models of the association between air pollutants and high sensitivity C-reactive protein (hs-CRP) adjusting for main covariates.

adj: adjusted for the respective air pollutant, CI: confidence interval, IQR: interquartile range, PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and <2.5 µm respectively, $PM_{2.5}$abs: absorbance of $PM_{2.5}$, PNC: particle number concentration.

The quantile regression indicated heterogeneity of the association between long-term exposure to air pollution and hs-CRP across quantiles (Supplemental Figure A. 1). We observed stronger associations in the upper deciles of the hs-CRP distribution, though standard errors increased considerably. Among participants with hs-CRP below 0.37 mg/L (10th percentile), an IQR increase in PNC exposure was associated with a 0.01 mg/L (95% CI: -0.04, 0.04) increase in hs-CRP while among subjects with hs-CRP levels close to 5.83 mg/L (90th percentile), the same exposure was related to a 0.29 mg/L (95% CI: -0.28, 0.85) increase in hs-CRP. The logistic regression analysis examined the effect of long-term exposure to air pollutants on the probability of having an hs-CRP concentration greater than 3 mg/L (N = 491). In conformity to the linear regression, the logistic regression did not yield significant results (Supplemental Table A 3). Our results remained robust when additionally adjusted for educational level and physical activity, ETS or noise (Supplemental Table A. 4-6), and after the exclusion of participants having hs-CRP values greater than 10 mg/L (N = 82) or smaller than the limit of detection (N = 79) (data not shown). Associations were affected only marginally by the exclusion of participants who changed their residence between the two follow-up studies (N = 269, Supplemental Table A 7). Analyses for the study population without change of residence (n=1,866) were slightly smaller for PNC, PM_{10} and PM_{coarse} while the negative association for $PM_{2.5}$ disappeared. Overall, the CI stayed fairly similar.

DISCUSSION

Summary

This cross-sectional study conducted in the city and the region of Augsburg in Germany investigated the association between long-term exposure to ultrafine particles and further ambient air pollutants and the inflammatory marker hs-CRP. Although we did not find statistically significant associations the results showed a trend to a positive association for PNC, PM_{10}, PM_{coarse}, $PM_{2.5}$abs, NO_2 and NO_x. No association was seen for $PM_{2.5}$. The effect estimates were enhanced for females, non-obese participants, for participants without diabetes and without a history of CVD whereas smoking status showed no clear effect modifying pattern. For O_3, the dose-response function suggested a non-linear relationship twining around the null. In two-pollutant models, the adjustment for $PM_{2.5}$ strengthened the effects of the other air pollutants.

Biologic Mechanisms

Biological pathways linking the inhalation of air pollution to adverse cardiovascular effects are still not fully elucidated but inflammation has been suggested to play an important role (4). One possible mechanism is that particles depositing in the alveoli could activate an innate immune system response with macrophages to express toll-like receptors (TLRs) and evoke oxidative stress initiated by the formation of reactive oxygen species (4). A local and finally a systemic inflammation which is then no

more limited to the pulmonary system could be promoted by the release of proinflammatory mediators and vasculoactive molecules from lung-based cells. The systemic inflammation results in an increase in various inflammatory markers or acute phase reactants like CRP. Hs-CRP has been shown to be a reliable measure for systemic inflammation and a predictor for future myocardial infarction or for an increased risk of CVD (8). The cardiovascular risk may be increased as a consequence of endothelial dysfunction or an induced pro-coagulatory state leading to the progression and destabilization of atherosclerotic plaques and finally myocardial ischemia (4, 34).

However, toxicological studies reported that ultrafine particles might act through mechanisms that are not shared with larger particles (16). Ultrafine particles show a different deposition pattern than larger particles. Due to their small size they can penetrate deeper into the lungs and their large surface may provide a mechanism for delivering potentially toxic absorbed organic material (17). Moreover, ultrafine particles might have the ability to migrate from the lungs into the systemic circulation, which can have a direct effect on any organ and e.g. trigger acute cardiovascular events or an indirect effect via systemic inflammation processes (16, 34-36).

Comparison of Findings

Most of the studies linking CVD with air pollution have focused on particle mass but it is still not clear if PM is the best metric for quantifying adverse health outcomes. Although ultrafine particles are included in PM, they only represent a minor fraction of the mass but dominate particle numbers (5). So far, only two studies have examined the effect of long-term exposure to PNC and the inflammatory marker CRP (19, 20). Our findings are in line with a study from the U.S. (20), which also found a positive but non-significant association of annual exposure to PNC and hs-CRP among all participants which even reached significance when including only the white non-Hispanic population (32.7% per $10^4/cm^3$; 95% CI: 3.7%, 67.2%). Generally, this population is comparable to ours regarding e.g. age or BMI. However, the exposure assessments differed since Lane et al. (20) conducted PNC measurements by mobile monitoring and used a spatial-temporal model adjusted for individual time-activity patterns. Higher exposure values were found in this study compared to our exposures which might be the reason for our smaller effect estimate for PNC (19.5% per $10^4/cm^3$; 95% CI: -4.2%, 49.2%). The second study was conducted in the highly industrialized Ruhr district (Germany) and found a positive, non-significant association of 1.4% (95% CI: -0.2%, 3.0%) per $10^4/cm^3$ increase in annual exposure to PNC (19). Viehmann et al. (19) modeled exposures with a residential exposure assessment approach as we did, but used a chemistry transport and dispersion model which however could not be calibrated with measurements from the study region. As substantially higher median PNC exposure levels (75,000 particles/cm^3) compared to our study (7,220 particles/cm^3) were reported, it is questionable how well this model can be compared to our LUR-model approach.

We studied multiple air pollutants assessing the associations between an increase in hs-CRP and an IQR increase per pollutant to be able to directly compare the effect estimates. Our highest effect estimate was seen for PM_{10}, which was positive but only borderline significant (5.15% per 2.1 µg/m^3; 95% CI: -0.69%, 11.33%). This trend is in concordance with previous studies which reported a positive significant (10) or non-significant association (19, 37, 38) of long-term exposure to PM_{10} and CRP. PM_{10} and $PM_{2.5}$ are the most frequently investigated particulate size ranges in the context of inflammatory markers. We found a null association between $PM_{2.5}$ and hs-CRP but several previous studies reported differently. For $PM_{2.5}$, a significant positive association was found in a longitudinal cohort study from the Ruhr area, Germany (10) which observed an association of 4.5% (95% CI: 2.8%, 6.3%) for a 1 µg/m^3 increment. After re-examination of this cohort with a refined exposure grid, the findings remained mainly the same (19). This finding is strengthened by further long-term exposure studies with either significant (9) or non-significant results (37, 39). However, Hoffmann et al. (11) only found a positive association in men whereas females showed a null association and one study from the U.S. reported a small negative, non-significant association (12). One reason for our null findings for $PM_{2.5}$ might be the small spatial exposure contrast of $PM_{2.5}$ (23). In two-pollutant models, the $PM_{2.5}$ estimate even got negative whereas the positive effect estimates of PM_{coarse}, $PM_{2.5}$abs, PM_{10} and PNC strengthened with the latter two becoming significant. In specific, the effect estimates increased from 3.63 (-0.86, 8.33) to 6.31 (0.42, 12.53) for PNC and from 5.15 (-0.69, 11.33) to 7.34 (0.4, 14.77) for PM_{10} per IQR increase when adjusting for $PM_{2.5}$. In contrast, $PM_{2.5}$ estimates decreased and CI widened from 0.16 (-5.38, 6.02) to -6.97 (-15.93, 2.94) or -5.61 (-14.34, 4.02) when adjusting for PNC or PM_{10}, respectively. Thus, our results suggest non-independent effects of $PM_{2.5}$ and the mentioned air pollutants on hs-CRP and might strengthen the importance of PNC and PM_{10} as traffic related air pollutants given the exponential decay pattern for UFPs and greater heterogeneity of exposure levels across the study area for both pollutants than $PM_{2.5}$.

The association between hs-CRP and air pollutants has already been examined previously in our study population at baseline (1999 to 2001) (32) and at the first follow up (2006 to 2008) (37, 40). In both analyses, $PM_{2.5}$ showed the highest effect size. This discrepancy to our study might be explained by differences in exposure classification. The earlier two studies used an identical exposure model which was based on measurements in a much larger region (including the Augsburg and Munich area) conducted in 2008-2009 (24, 25). Our LUR model, is a refined version on the basis of new air pollution measurements in only the Augsburg region. The adjusted R^2s were similar or higher for the new models compared to the former ones with the exception of PM_{coarse}(23).

Next to ultrafine particles, NO_2, NO_X and $PM_{2.5}$abs are common constituents from motor vehicles combustion and are considered as indicators for near-road traffic-related emissions. We did not find significant associations for these air pollutants and our analysis did not reveal a certain pollutant to be more influential than the other. However, the clearest result was seen for NO_x. In the literature, NO_2 has

been intensively investigated with positive, but non-significant associations (37, 38, 41) or even no association (13, 39).

In addition to these primary pollutants, we also examined the secondary pollutant O_3 which is primarily formed by photochemical reactions of naturally occurring and man-made precursor pollutants such as volatile organic compounds and NO_x in the presence of solar ultraviolet radiation. As ozone can be reduced by nitric oxide in fresh motor vehicle exhaust, but can also be regenerated during transport, ozone concentrations are usually higher in suburban and rural areas downwind of the sources than in urban areas (42). In our analysis, the dose-response function of O_3 suggested a non-linear relationship. Previous studies examining long-term exposure to O_3 and CRP reported negative, non-significant effect estimates (38, 43). Both associations were modeled linearly but the authors did not report if they tested the linearity assumption of their dose-response function. If O_3 has been modeled linearly in our study, the effect estimate would have been negative as well (-1.84% per 3.5 µg/m^3; 95% CI -7.69%, 4.39%).

Effect Modification

Along with variations in the composition of air pollution and the intensity of exposures, susceptibility of a population also plays an important role in inflammatory responses. Participants with metabolic syndrome or the elderly were suggested to be especially susceptible to air pollution (44, 45). A systematic review on the effects of air pollution on CRP did not find consistent results among subgroups with chronic inflammatory conditions such as CVD, diabetes and obesity (14). However, with regard to long-term exposure to air pollution and CRP, several studies reported that participants with diabetes showed stronger associations (9, 11, 12). In a large, prospective cohort study in the U.S. (12), an effect estimate of 36.9% (95% CI: 0.1%, 87.2%) per 10 µg/m^3 increase in annual average exposure to $PM_{2.5}$ in participants with diabetes was reported. In the first follow-up of our cohort, the highest effect size was seen for pre-diabetic participants (40) which could, however, not be confirmed in our analysis since we found the highest associations for participants without diabetes (40). We do not have a straightforward explanation but possible reasons might be a differential loss to follow-up, the ageing of the study population or the refined exposure assessment. One further explanation could be that our subgroup of participants with diabetes showed the highest medication intake affecting hs-CRP and had the greatest proportion of participants with a history of CVD compared to participants with pre-diabetes or without diabetes (Supplemental Table A 8). Evidence for obese participants or participants with a history of CVD is less clear as only no effect modifications (9, 10, 19, 20) and also negative, but non-significant associations were reported (12). However, diagnosed diabetic or obese participants and subjects with CVD appeared less susceptible to air pollution exposure in our analyses. It has to be mentioned that a clear distinction between these subgroups is not possible as obese participants and participants with a history of CVD were predominantly found to be pre-diabetic or diabetic.

We found no clear effect modifying pattern of smoking status across all air pollutants. Positive associations were found for both, smokers and non-smokers depending on the examined air pollutant. However, generalization might be restricted with the fact that smokers tended to be younger and healthier (less histories of diabetes or CVD percentage-wise) compared to ex- or non-smokers (Supplemental Table A 9). Generally, smokers have higher levels of inflammatory markers due to tobacco smoke inhalation and they are thus hypothesized to show less effect to further air pollution exposure (41, 46). Previous studies of long-term exposure to air pollution and CRP strengthened this hypothesis (11-13).

Strengths and Limitations

A major strength of this study is the population-based design of a well-examined large population. Thus, a wide range of information on patient characteristics was available that allowed for a broad control for confounders and the investigation of potential effect modifying factors. Furthermore, our results were consistent in several sensitivity analyses which showed the robustness of our results. Our air pollutant exposure models were based on a considerable number of measurement periods and monitoring sites directly located in the study region.

Potential limitations of this study should be taken into account when interpreting the results. The cross-sectional design of our study is limiting the determination of causal evidence as the population is only captured and analyzed at a single time point. Furthermore, the study population is probably highly selected due to the 14 years between the baseline survey and the second follow-up leading to drop-outs in sick participants rather than in healthier ones. The long-term air pollution concentrations were measured up to two years after our study was conducted which could infer exposure misclassification. However, it has been shown that spatial contrasts of air pollution were stable for periods up to ten years and longer (47-49). The estimation of ambient air pollution exposure was based on a LUR model and although it is an established method the exposure allocation is associated with some degree of uncertainty and model performance variations among different air pollution components. Furthermore, our air pollution model provided relatively small exposure contrast for $PM_{2.5}$ limiting the power to detect effects with increased air pollution exposures (23). A further cause for a potential exposure misclassification might be the change of residence of some participants in the study period so that no clear exposure allocation can be ensured. Beyond residential movement patterns of the study participants their air pollution exposure at work could lead to exposure misclassification, however the respective data was unfortunately not available for this study. Our study results are possibly impacted by residual confounding because the analysis lacked individual factors like the personal exposure or environmental tobacco smoke that might influence the exposure to air pollution. We did not adjust for traffic noise exposure or short-term exposure to air pollution. However, neither traffic noise nor short-term exposure to air pollution seem to affect the estimates as shown by Hennig et al. (10) and Viehmann et al. (19), respectively.

CONCLUSION

Single-pollutant models showed a positive yet statistically non-significant association of long-term exposure to ultrafine particles and several other air pollutants with elevated levels of the inflammatory marker hs-CRP but no association for $PM_{2.5}$ or O_3. After adjustment for $PM_{2.5}$, effect estimates increased for PNC and PM_{10} and reached significance. Our results highlight the role of ultrafine particles within the complex mixture of ambient air pollution and their inflammatory potential and might help filling a research gap since studies on chronic exposure to ultrafine particles are still scarce.

DECLARATION OF INTEREST

Nothing to declare.

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SUPPLEMENTARY DATA

Supplemental Table A 1. Effect Modification by sex examined by interaction terms in the main confounder model: effect estimates and 95% CI for the association of air pollutants and hs-CRP, presented as percent change of hs-CRP scaled per IQR of air pollutant.

Pollutant (unit)	IQR	Percent change per IQR (95% CI)	
		Female	Male
PNC ($10^3/cm^3$)	2.0	7.38 (1.05, 14.11)	-0.45 (-6.68, 6.19)
PM_{10} ($\mu g/m^3$)	2.1	11.49 (3.1, 20.55)	-1.59 (-9.45, 6.94)
PM_{coarse} ($\mu g/m^3$)	1.4	10.79 (2.33, 19.95)	-1.84 (-9.52, 6.50)
$PM_{2.5}$ ($\mu g/m^3$)	1.4	4.77 (-3.08, 13.24)	-5.47 (-12.8, 2.49)
$PM_{2.5}$abs ($10^{-5}/m$)	0.3	11.26 (2.28, 21.03)	-5.8 (-13.76, 2.91)
NO_2 ($\mu g/m^3$)	6.8	9.34 (0.2, 19.32)	-7.06 (-15.18, 1.84)
NO_x ($\mu g/m^3$)	8.7	6.88 (-0.03, 14.27)	-0.28 (-7.09, 7.04)

CI: confidence interval; hs-CRP: high sensitivity C-reactive protein; IQR: interquartile range; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.

(*) Effect estimates with p-values < 0.1.

Supplemental Table A 2. Effect Modification by occupational status examined by interaction terms in the main confounder model: effect estimates and 95% CI for the association of air pollutants and hs-CRP, presented as percent change of hs-CRP scaled per IQR of air pollutant.

Pollutant (unit)	IQR	Percent change per IQR (95% CI)	
		Employed [a]	Not employed [a]
PNC ($103/cm^3$)	2.0	3.61 (-2.56, 10.17)	3.13 (-3.23, 9.9)
PM_{10} ($\mu g/m^3$)	2.1	3.51 (-4.65, 12.36)	6.25 (-1.82, 14.99)
PM_{coarse} ($\mu g/m^3$)	1.4	4.01 (-4.1, 12.81)	5.09 (-2.94, 13.79)
$PM_{2.5}$ ($\mu g/m^3$)	1.4	-5.18 (-12.36, 2.59)	4.19 (-3.78, 12.83)
$PM_{2.5abs}$ ($10^{-5}/m$)	0.3	1.36 (-7.08, 10.57)	4.09 (-4.47, 13.43)
NO_2 ($\mu g/m^3$)	6.8	-1.85 (-10.26, 7.36)	3.85 (-5.03, 13.56)
NO_x ($\mu g/m^3$)	8.7	1.77 (-4.89, 8.89)	4.45 (-2.57, 11.97)

CI: confidence interval; hs-CRP: high sensitivity C-reactive protein; IQR: interquartile range; NO2: nitrogen dioxide; NOx: nitrogen oxides; PM10, PMcoarse, PM2.5: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; PM2.5abs: absorbance of PM2.5; PNC: particle number concentration

[a] Adjusted for age, sex, BMI, smoking status, month of blood draw, waist–hip ratio and high density lipoprotein (HDL) cholesterol.

Supplemental Table A 3. Logistic regression results: Odds Ratio and 95% CI for hs-CRP > 3 mg/L (N = 487) versus hs-CRP ≤ 3 mg/L per IQR increase in air pollutants (N = 1,765).

Pollutant	IQR	Odds Ratio (95% CI)[a]
PNC	2.0	1.10 (0.98, 1.24)
PM_{10}	2.1	1.15 (0.99, 1.33) (*)
PM_{coarse}	1.4	1.06 (0.91, 1.23)
$PM_{2.5}$	1.4	1.02 (0.88, 1.19)
$PM_{2.5}$abs	0.3	1.02 (0.86, 1.20)
NO_2	6.8	1.03 (0.88, 1.22)
NO_x	8.7	1.13 (0.99, 1.29) (*)

CI: confidence interval; hs-CRP: high sensitivity C-reactive protein; IQR: interquartile range; N: total number; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.

[a] Adjusted for age, sex, body mass index (BMI), smoking status, month of blood draw, waist–hip ratio and high density lipoprotein (HDL) cholesterol.

(*) Effect estimates with p-values < 0.1.

Supplemental Table A 4. Sensitivity Analysis on an extended model including educational level and physical activity as further covariates to the main confounder model: effect estimates and 95% CI for the association of air pollutants and hs-CRP, presented as percent change of hs-CRP scaled per IQR of air pollutant.

Pollutant (unit)	IQR	Percent change per IQR (95% CI)	
		Main model[a]	Extended Model[b]
PNC ($10^3/cm^3$)	2.0	3.63 (-0.86, 8.33)	3.78 (-0.72, 8.49)
PM_{10} ($\mu g/m^3$)	2.1	5.15 (-0.69, 11.33) (*)	5.35 (-0.50, 11.55) (*)
PM_{coarse} ($\mu g/m^3$)	1.4	4.45 (-1.36, 10.59)	4.90 (-0.95, 11.09)
$PM_{2.5}$ ($\mu g/m^3$)	1.4	-0.29 (-5.75, 5.49)	-0.29 (-5.75, 5.49)
$PM_{2.5}$abs ($10^{-5}/m$)	0.3	2.80 (-3.31, 9.29)	2.96 (-3.15, 9.47)
NO_2 ($\mu g/m^3$)	6.8	1.19 (-5.03, 7.83)	1.29 (-4.95, 7.93)
NO_x ($\mu g/m^3$)	8.7	3.45 (-1.48, 8.61)	3.59 (-1.34, 8.76)

CI: confidence interval; hs-CRP: high sensitivity C-reactive protein; IQR: interquartile range; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.

[a] Adjusted for age, sex, BMI, smoking status, month of blood draw, waist–hip ratio and high density lipoprotein (HDL) cholesterol.

[b] Adjusted for age, sex, BMI, smoking status, month of blood draw, waist–hip ratio, high density lipoprotein (HDL) cholesterol, educational level and physical activity.

(*) Effect estimates with p-values < 0.1.

Supplemental Table A 5. Sensitivity Analysis on an extended model including environmental tobacco smoke (ETS) exposure as a further covariate to the main confounder model: effect estimates and 95% CI for the association of air pollutants and hs-CRP, presented as percent change of hs-CRP scaled per IQR of air pollutant. Participants incorporating a missing value for ETS were excluded for both regression model (N=1,866).

Pollutant (unit)	IQR	Percent change per IQR (95% CI)	
		Main model[a]	Extended Model[b]
PNC ($10^3/cm^3$)	2.0	4.50 (-0.45, 9.69)	4.21 (-0.72, 9.38)
PM_{10} ($\mu g/m^3$)	2.1	5.55 (-0.88, 12.41) (*)	5.48 (-0.94, 12.31) (*)
PM_{coarse} ($\mu g/m^3$)	1.4	4.58 (-1.78, 11.35)	4.78 (-1.57, 11.55)
$PM_{2.5}$ ($\mu g/m^3$)	1.4	-0.62 (-6.60, 5.73)	-0.38 (-6.35, 5.97)
$PM_{2.5}$abs ($10^{-5}/m$)	0.3	1.90 (-4.77, 9.04)	2.16 (-4.52, 9.29)
NO_2 ($\mu g/m^3$)	6.8	0.15 (-6.67, 7.46)	0.50 (-6.32, 7.83)
NO_x ($\mu g/m^3$)	8.7	3.04 (-2.34, 8.71)	2.95 (-2.41, 8.59)

CI: confidence interval; hs-CRP: high sensitivity C-reactive protein; IQR: interquartile range; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.

[a] Adjusted for age, sex, BMI, smoking status, month of blood draw, waist–hip ratio and high density lipoprotein (HDL) cholesterol.

[b] Adjusted for age, sex, BMI, smoking status, month of blood draw, waist–hip ratio, high density lipoprotein (HDL) cholesterol and environmental tobacco smoke (ETS).

(*) Effect estimates with p-values < 0.1.

Supplemental Table A 6. Sensitivity Analysis on an extended model including noise as a further covariate to the main confounder model: effect estimates and 95% CI for the association of air pollutants and hs-CRP, presented as percent change of hs-CRP scaled per IQR of air pollutant. Participants incorporating a missing value for ETS were excluded for both regression model (N=2,001).

Pollutant (unit)	IQR	Percent change per IQR (95% CI)	
		Main model[a]	Extended Model[b]
PNC ($10^3/cm^3$)	2.0	3.62 (-1.1, 8.57)	3.42 (-2.02, 9.16)
PM_{10} ($\mu g/m^3$)	2.1	4.52 (-1.54, 10.97)	4.53 (-2.83, 12.45)
PM_{coarse} ($\mu g/m^3$)	1.4	3.85 (-2.17, 10.24)	2.63 (-4.49, 10.27)
$PM_{2.5}$ ($\mu g/m^3$)	1.4	0.59 (-5.19, 6.72)	-0.17 (-6.34, 6.39)
$PM_{2.5}$abs ($10^{-5}/m$)	0.3	3.58 (-2.87, 10.46)	2.35 (-5.01, 10.28)
NO_2 ($\mu g/m^3$)	6.8	2.22 (-4.32, 9.22)	1.07 (-6.6, 9.37)
NO_x ($\mu g/m^3$)	8.7	3.77 (-1.38, 9.19)	3.68 (-2.27, 10)

CI: confidence interval; hs-CRP: high sensitivity C-reactive protein; IQR: interquartile range; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.

[a] Adjusted for age, sex, BMI, smoking status, month of blood draw, waist–hip ratio and high density lipoprotein (HDL) cholesterol.

[b] Adjusted for age, sex, BMI, smoking status, month of blood draw, waist–hip ratio, high density lipoprotein (HDL) cholesterol and noise.

Supplemental Table A 7. Sensitivity Analysis on participants who did not change residence: effect estimates and 95% CI for the association of air pollutants and hs-CRP, presented as percent change of hs-CRP scaled per IQR of air pollutant for the full analysis population and the subpopulation without participants who moved within the recent 5 to 8 years

Pollutant (unit)	IQR	Percent change per IQR (95% CI)	
		Study population (N=2,252) [a]	Subpopulation of non-movers (N=1,866) [a]
PNC (10^3/cm³)	2.0	3.63 (-0.86, 8.33)	3.28 (-1.55, 8.35)
PM_{10} (µg/m^3)	2.1	5.15[(*)] (-0.69, 11.33)	4.44 (-1.75, 11.03)
PM_{coarse} (µg/m^3)	1.4	4.45 (-1.36, 10.59)	3.59 (-2.58, 10.15)
$PM_{2.5}$ (µg/m^3)	1.4	-0.29 (-5.75, 5.49)	0.84 (-5.09, 7.15)
$PM_{2.5}$abs (10^{-5}/m)	0.3	2.80 (-3.31, 9.29)	4.02 (-2.58, 11.07)
NO_2 (µg/m^3)	6.8	1.19 (-5.03, 7.83)	2.48 (-4.23, 9.65)
NO_x (µg/m^3)	8.7	3.45 (-1.48, 8.61)	3.49 (-1.78, 9.03)

CI: confidence interval; hs-CRP: high sensitivity C-reactive protein; IQR: interquartile range; NO_2: nitrogen dioxide; NO_x: nitrogen oxides; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm, respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.

[a] Adjusted for age, sex, body mass index (BMI), smoking status, month of blood draw, waist–hip ratio and high density lipoprotein (HDL) cholesterol.

[(*)] Effect estimates with p-values < 0.1.

Supplemental Table A 8. Descriptive statistics of the study population (N = 2,252) stratified by diabetes status.

	No diabetes[a] (N = 1079)	Pre-diabetes[a] (N = 765)	Diabetes[a] (N = 325)
Personal characteristics			
Age (years)	55.7 ± 11.3	62.9 ± 11.6	69.3 ± 10.1
Sex (male)	409 (37.9)	464 (60.7)	188 (57.8)
Socio-economic covariates			
Marital status			
Single	71 (6.6)	59 (7.7)	19 (5.8)
Married or living with partner	812 (75.3)	561 (73.3)	228 (70.2)
Divorced or separated	124 (11.5)	78 (10.2)	28 (8.6)
Widowed	72 (6.7)	67 (8.8)	50 (15.4)
Education (years)	12.2 ± 2.6	11.8 ± 2.7	11.4 ± 2.4
Occupational status			
Employed, self-employed or in training	696 (64.6)	329 (43.0)	66 (20.3)
Unemployed	13 (1.2)	11 (1.4)	5 (1.5)
Homemaker	88 (8.2)	56 (7.3)	10 (3.1)
Retired	281 (26.1)	369 (48.2)	244 (75.1)
Income, per capita (Euro)	1353.9 ± 688.6	1322.7 ± 637.6	1216.6 ± 598.6
SES (points, Helmert)	15.5 ± 5.0	14.7 ± 5.3	13.9 ± 4.9
Neighborhood SES (%)	26.3 ± 17.9	28.3 ± 18.0	30.2 ± 16.9
Lifestyle covariates			
BMI (kg/m^2)	26.2 ± 4.4	28.9 ± 4.7	31.1 ± 5.3
Waist circumference (cm)	91.0 ± 12.7	101.0 ± 12.6	107.2 ± 12.8
Waist–hip ratio	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
Physical activity			
Low (none)	243 (22.5)	222 (29.0)	146 (44.9)
Medium (~1 h/week)	506 (46.9)	367 (48.0)	121 (37.2)
High (>2 h/week)	330 (30.6)	176 (23.0)	58 (17.8)
Smoking status			
Non-smoker	456 (42.3)	308 (40.3)	141 (43.4)
Ex-smoker	433 (40.1)	342 (44.7)	157 (48.3)
Smoker	190 (17.6)	115 (15.0)	27 (8.3)
Cumulative smoking exposure (pack-years)	13 ± 17.4	17.2 ± 21.5	15.5 ± 24.3
Alcohol consumption (g/d)	3.1 ± 0.9	3.0 ± 0.8	3.0 ± 0.8
Clinical characteristics			
Hs-CRP (mg/L; arithmetic mean)	1.8 ± 2.8	2.9 ± 5.0	3.4 ± 4.8
Total cholesterol (mg/dL)	216.6 ± 37.5	220.2 ± 40.3	205.4 ± 40.8
HDL cholesterol (mg/dL)	70.0 ± 18.9	62.1 ± 17.5	57.8 ± 16.5
LDL cholesterol (mg/dL)	133.5 ± 34.1	139.3 ± 35.1	126.8 ± 36.8
Triglycerides (mg/dL)	104.3 ± 61.1	132.9 ± 73.2	155.3 ± 84.1
Medical history and medication			
Cardiovascular disease[b]	315 (29.2)	393 (51.4)	250 (76.9)
Intake of medication affecting CRP[c]	287 (26.6)	372 (48.6)	252 (77.8)

BMI: body mass index; HDL: high density lipoprotein; hs-CRP: high sensitivity C-reactive protein; KORA: Cooperative Health Research in the Region of Augsburg; LDL: low density lipoprotein; N: total number; SD: standard deviation; SES: socio-economic status.

[a] Mean ± SD or N (%). Percentages are calculated based on observations with available information.

[b] History of myocardial infarction, angina pectoris or hypertension.

[c] Intake of non-steroidal anti-inflammatory drugs, lipid-lowering drugs (including statins).

Supplemental Table A 9. Descriptive statistics of the study population (N = 2,252) stratified by smoking status.

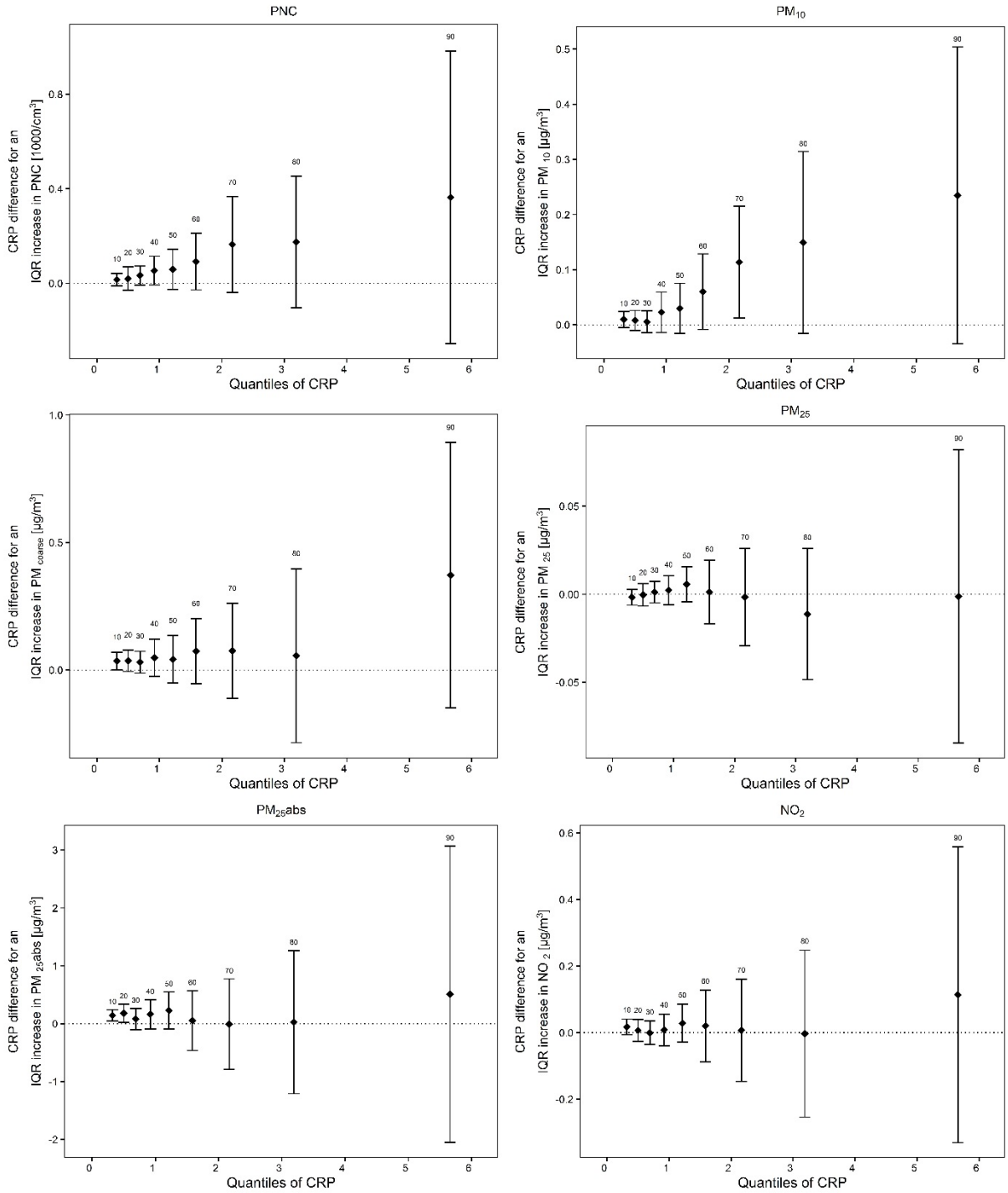
	Non-smoker[a] (N = 938)	Ex-smoker[a] (N = 965)	Smoker[a] (N = 349)
Personal characteristics			
Age (years)	61.5 ± 12.8	61.1 ± 12.3	54.8 ± 9.5
Sex (male)	358 (38.2)	551 (57.1)	182 (52.1)
Socio-economic covariates			
Marital status			
Single	63 (6.7)	54 (5.6)	36 (10.3)
Married or living with partner	679 (72.4)	744 (77.1)	232 (66.5)
Divorced or separated	80 (8.5)	98 (10.2)	62 (17.8)
Widowed	116 (12.4)	69 (7.2)	19 (5.4)
Education (years)	11.9 ± 2.8	12.0 ± 2.6	11.7 ± 2.4
Occupational status			
Employed, self-employed or in training	436 (46.5)	451 (46.8)	237 (67.9)
Unemployed	9 (1.0)	12 (1.2)	10 (2.9)
Homemaker	79 (8.4)	57 (5.9)	26 (7.4)
Retired	414 (44.1)	444 (46.1)	76 (21.8)
Income, per capita (Euro)	1302.3 ± 665.9	1336.4 ± 646.7	1328.7 ± 678.6
SES (points, Helmert)	14.9 ± 5.2	15.2 ± 5.2	14.7 ± 4.7
Neighborhood SES (%)	29.6 ± 18.1	27.8 ± 17.5	26.8 ± 18.0
Lifestyle covariates			
BMI (kg/m2)	27.7 ± 4.8	28.3 ± 5.3	26.9 ± 4.7
Waist circumference (cm)	95.2 ± 13.3	99.2 ± 15.1	95.0 ± 13.3
Waist–hip ratio	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Physical activity			
Low (none)	258 (27.5)	263 (27.3)	119 (34.1)
Medium (~1 h/week)	453 (48.3)	425 (44.0)	149 (42.7)
High (>2 h/week)	227 (24.2)	277 (28.7)	81 (23.2)
Cumulative smoking exposure (pack-years)	0 ± 0	18.7 ± 22.0	28.8 ± 20.2
Alcohol consumption (g/d)	11.9 ± 16.1	16.7 ± 21.8	16.7 ± 23.1
Clinical characteristics			
Hs-CRP (mg/L; arithmetic mean)	2.3 ± 3.6	2.5 ± 4.5	2.7 ± 4.1
Total cholesterol (mg/dL)	219.6 ± 40.5	212.9 ± 38.3	214.9 ± 38.6
HDL cholesterol (mg/dL)	68.0 ± 18.8	65.0 ± 18.4	60.9 ± 19.0
LDL cholesterol (mg/dL)	136.7 ± 35.6	131.5 ± 34.7	135.4 ± 35.3
Triglycerides (mg/dL)	115.5 ± 64.2	125.3 ± 74.3	127.2 ± 83.7
Medical history and medication			
Diabetes			
No diabetes	456 (50.4)	433 (46.5)	190 (57.2)
Pre-diabetes	308 (34.0)	342 (36.7)	115 (34.6)
Diabetes	141 (15.6)	157 (16.8)	27 (8.1)
Cardiovascular disease[b]	417 (44.5)	471 (48.8)	108 (30.9)
Intake of medication affecting CRP[c]	399 (42.6)	448 (46.5)	97 (27.9)

BMI: body mass index; HDL: high density lipoprotein; hs-CRP: high sensitivity C-reactive protein; KORA: Cooperative Health Research in the Region of Augsburg; LDL: low density lipoprotein; N: total number; SD: standard deviation; SES: socio-economic status.

[a] Mean ± SD or N (%). Percentages are calculated based on observations with available information.

[b] History of myocardial infarction, angina pectoris or hypertension.

[c] Intake of non-steroidal anti-inflammatory drugs, lipid-lowering drugs (including statins).



Supplemental Figure A. 1. Quantile regression results: associations between air pollutants and quantiles of high sensitivity C-reactive protein (hs-CRP) presented as difference (95% CI) of hs-CRP per IQR increase in air pollutant. The numbers above each confidence interval indicate the deciles. Adjusted for the main confounder model.

CI: confidence interval; IQR: interquartile range; NO_2: nitrogen dioxide; PM_{10}, PM_{coarse}, $PM_{2.5}$: particulate matter with aerodynamic diameter < 10 µm, 2.5-10 µm and < 2.5 µm respectively; $PM_{2.5}$abs: absorbance of $PM_{2.5}$; PNC: particle number concentration.