**Association of novel metrics of particulate matter with vascular markers of inflammation and coagulation in susceptible populations –results from a panel study.**

Regina Rückerla, Alexandra Schneiderb, Regina Hampelb, Susanne Breitnerb, Josef Cyrysa, Jianwei Gua, David Diaz-Sanchezc, Robert B Devlinc, Jens Soentgend, Wolfgang Koenige, Annette Petersf

**a) Regina Rückerl** (regina.pickford@helmholtz-muenchen.de), **Josef Cyrys** (cyrys@helmholtz-muenchen.de), **Jianwei Gu** (jianwei.gu@helmholtz-muenchen.de)

ESC-Environmental Science Center, University of Augsburg, Universitätsstr. 2, 86135 Augsburg, Germany and Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for

Environmental Health, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany

**b) Regina Hampel** (regina.hampel@helmholtz-muenchen.de), **Susanne Breitner** (susanne.breitner@helmholtz-muenchen.de), **Ute Kraus** (ute.kraus@helmholtz-muenchen.de)**, Alexandra Schneider** (alexandra.schneider@helmholtz-muenchen.de)

Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany

**c) Robert B Devlin (**devlin.robert@epamail.epa.gov**)**, **David Diaz-Sanchez (**diaz-sanchez.david@epamail.epa.gov**)**

Environmental Public Health Division, National Health and Environmental Effects Research Laboratory, Environmental Protection Agency, Research Triangle Park, Durham, NC 27711, USA

**d) Jens Soentgen (**soentgen@wzu.uni-augsburg.de**)**

ESC-Environmental Science Center, University of Augsburg, Universitätsstr. 2, 86135 Augsburg, Germany

**e) Wolfgang Koenig** (koenig@dhm.mhn.de)

Klinik für Herz-& Kreislauferkrankungen, Deutsches Herzzentrum München, Technische Universität München, Lazarettstr. 36, 80636 Munich, Germany. DZHK (German Centre for Cardiovascular Research), partner site Munich Heart Alliance, Munich, Germany

**f)** **Annette Peters** (peters@helmholtz-muenchen.de),Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Ingolstädter Landstr. 1, 85764 Neuherberg, Germany and German Center for Diabetes Research (DZD e.V.), München, Germany

Corresponding author: Regina Rückerl

Email: regina.pickford@helmholtz-muenchen.de

Postal address: Helmholtz Zentrum München

Institute of Epidemiology II

Ingolstädter Landstr. 1

85764 Neuherberg

Phone: +49 89 3187 3660

Fax: +49 89 3187 3380

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List of abbreviations:

AIC: Akaike’s information criterion

APS: Aerodynamic Particle Sizer

BET: Brunauer, Emmet and Teller Method

CPC: Condensation Particle Counter

CRP: C-reactive protein

DCPS: Diffusion Charging Particle Sensor

DMA: differential mobility analyser

*Dp:* mobility equivalent diameter

EAD: Electric Aersol Detector

**EDTA: ethylenediaminetetraacetic acid**

**ELISA: enzyme-linked immunosorbent assay**

FDMS: Filter Dynamics Measurement Systems

GSTM1: glutathione S-transferase M1

IL-6: interleukin-6

IGT: impaired glucose tolerance

IQR: interquartile range

KORA studies: Cooperative Health Research in the Region Augsburg

LC: particle length concentration

LC (EAD): particle length concentration measured by EAD

MPO: myeloperoxidase

MI: myocardial infarction

NC: number concentration

NO: nitric oxide

**PM2.5: particulate matter (mass) with a size range of <2.5 μm in aerodynamic diameter**

**PM10: particulate matter (mass) with a size range of <10 μm in aerodynamic diameter**

**PSD: particle size distribution**

**SC: particle surface concentration**

**SC (DCPS): particle surface concentration** measured by **DCPS**

TDMPS: Twin Differential Mobility Particle Sizer

TEOM: Tapered Element Oscillating Microbalances

T2D: type 2 diabetes

UCPC: ultrafine Condensation Particle Counter

UDMA: ultrafine Differential Mobility Analyser

**UFP: ultrafine particles, particle number concentration, with a size range of <0.1μm in diameter**

*ρ*2.5: Apparent density for **PM2.5**

*ρ*10: Apparent density for P**M10**

Abstract

Background and Aims: Epidemiological studies have shown an adverse effect of ambient air pollutants on health with inflammation and oxidative stress playing an important role. We examine the association between blood biomarkers of inflammation and coagulation and physical attributes of particulate matter which are not routinely measured such as particle length or surface area concentration and apparent density of PM.

Methods: Between 3/2007 and 12/2008 187 non-smoking individuals with type 2 diabetes mellitus (T2D) or impaired glucose tolerance (IGT) were examined. In addition, 87 participants with a potential genetic predisposition on detoxifying and inflammatory pathways defined by the null polymorphism for *glutathione S-transferase M1* in combination with a certain single nucleotide polymorphism on the *C-reactive protein (CRP)* gene(rs1205) or the *fibrinogen* gene (rs1800790) were examined. Participants had blood drawn up to seven different times, resulting in 1,765 blood samples. Air pollutants were collected at a central measurement station and individual 24-hour averages calculated. Associations between air pollutants and high sensitivity CRP, myeloperoxidase (MPO), interleukin (IL)-6 and fibrinogen were analysed using additive mixed models.

Results: For the panel with genetic susceptibility, increases were seen for CRP and MPO with most attributes, specifically particle length and active surface concentration. The %change of geometric mean and 95% confidence intervals for the 5-day average exposure for CRP and MPO were 34.6% [21.8;48.8] and 8.3% [3.2;13.6] per interquartile range increase of particle length concentration and 29.8% [15.9;45.3] and 10.4 [4.4; 16.7] for active surface area. Results for the panel of T2D and IGT and the other blood biomarkers were less conclusive.

Conclusions: Particle length concentration and active surface concentration showed strong positive associations with blood biomarkers reflecting inflammation. These air pollution metrics might reflect harmful aerosol properties better than particulate mass or number concentration and therefore be important for epidemiological studies.

Introduction:

Epidemiological studies have shown that ambient air pollutants can negatively affect human cardiovascular health (Ruckerl et al. 2011) (Brook et al. 2010). It has been reported that some subpopulations may be more at risk from the harmful effects of particulate air pollution than the general population. These subpopulations include e.g. patients with chronic obstructive pulmonary disease, previous myocardial infarction (MI) or diabetes (Brook et al. 2010).

Traditionally, particles are classified by size, such as PM10, PM2.5 (particulate matter of particles with less than 10 µm and 2.5 µm in aerodynamic diameter, respectively) and ultrafine particles (UFP), which have a diameter below 100 nm. The size classification of PM mass concentration into PM2.5 and PM10 has been mostly driven by the different behaviour of the two particle fractions in human respiratory system: PM2.5 reach terminal bronchioles and alveoli region, whereas the coarse particles (in the size range 2.5 – 10 µm) tend to deposit in the upper (thoracic) regions of the human respiratory system.

Particulate matter is a complex mixture of solid and liquid particles from various sources. Their physical and chemical properties change depending on the relative contributions from sources such as vehicle exhaust, household and industry emissions, road dust, forest fires or wind-blown soil. For example, combustion particles consist of an elemental carbon core surrounded by a layer of chemicals that include organic hydrocarbons, metals, nitrates and sulphates. The carbon core as well as the enclosing chemicals determine the toxicity of the particle (Donaldson and Tran 2002; Nel 2005). Combustion particles also have characteristic size distributions in ambient settings as suggested by source apportion approaches ([Gu et al. 2012](#_ENREF_10)). Depending on size, surface area and chemical composition particles might vary in their ability to illicit pathophysiological responses as well as where they are deposited in the respiratory tract.

However, the roles of specific physical and chemical properties of particles remain still rather unclear and are not reflected by studying PM2.5 and PM10 alone ([Schlesinger et al. 2006](#_ENREF_36)). Long-term continuous measurements of particle properties such as particle number concentration (PNC), particle size distribution (PSD), chemical composition of particles, surface area concentration, particle length concentration and other particulate variables are needed to assess their relevance for health effects ([Gu et al. 2012](#_ENREF_10)).

In order to collect an enhanced PM measurement data set and to study the effects of different attributes of particulate pollution on human health, we established a fixed monitoring station in an urban background area of Augsburg, Germany ([Pitz et al. 2008a](#_ENREF_28)). This monitoring station has been designed for the collection of a number of physical and chemical particulate variables. Empirically, this data can be used to derive number concentrations, surface area concentrations and mass concentrations in various size ranges and relate it to health outcomes (Peters et al 1997). In order to validate these calculations done under rather strong assumptions, we also measured active (Fuchs) surface by a Diffusion Charging Particle Sensor (DCPS) and particle length concentration by an Electric Aersol Detector (EAD). In addition, we used the data in combination with PM2.5 and PM10 to calculate apparent density of PM2.5 and PM10 (*ρ*2.5 and *ρ*10, respectively) providing an indirect measures of chemical composition of the particles.

In earlier analyses, we assessed the association between a range of blood biomarkers of coagulation/fibrinolysis and inflammation and UFP, PM2.5, PM10, coarse particles (PM2.5-10), black carbon (BC), nitric oxide (NO), nitrogen dioxide (NO2) and carbon monoxide (CO) in susceptible populations ([Ruckerl et al. 2014](#_ENREF_34)). We found clear positive associations for the inflammatory markers myeloperoxidase (MPO) and high sensitivity C-reactive protein (hsCRP) for a five-day average exposure to PM2.5 and BC, especially in a population that contained the null polymorphism for *glutathione S-transferase M1* (GSTM1) in combination with a specific single nucleotide polymorphism on the *C-reactive protein* (CRP) or the *fibrinogen* gene.

In this publication, we explore the role of novel attributes of particulate matter as discussed above. We concentrated on the four blood markers which we have previously shown to have significant associations with PM2.5 and PM10, namely hsCRP, MPO, interleukin (IL)-6 and fibrinogen, to facilitate comparison between traditional measurers of PM and novel physical attributes. We hypothesised that the associations for the analysed blood biomarkers would generally show positive associations, as we previously reported for PM2.5 or PM10 mass ([Ruckerl et al. 2014](#_ENREF_34)), but that specifically, measures of the surface area of particles would display stronger associations as suggested previously ([Stoeger et al. 2006](#_ENREF_38)).

Methods

**Study population**

**The details of the study have been described in detail elsewhere (**[**Ruckerl et al. 2014**](#_ENREF_34)**). Briefly, our study comprised individuals with** type 2 diabetes (T2D, n=83), impaired glucose tolerance (IGT, n=104) and patients with **potential genetic susceptibility in a** detoxifying **pathway (n=87). These latter p**atients did not have known IGT or diabetes but had to have the null-polymorphism for *GSTM1* **and either two major alleles of the single nucleotide polymorphism (SNP) rs1205 located in the *CRP* gene** ([Kolz et al. 2008](#_ENREF_17), [Sunyer et al. 2008](#_ENREF_39)) **or at least one minor allele of the SNP rs1800790 located in the *fibrinogen* gene *FGB* (**[**Peters et al. 2009**](#_ENREF_27)**,** [**Jacquemin et al. 2008**](#_ENREF_14)**), or both.**

**Exclusion criteria for the study were 1) current smoking, 2) intake of platelet aggregation inhibitors except for acetylsalicylic acid, 3) a myocardial infarction and/or interventional procedures (percutaneous coronary intervention, PCI; coronary artery bypass grafting, rheumatoid arthritis) less than six months before the recruitment for the study, and 4) chronic inflammatory diseases such as Crohn’s disease, or colitis ulcerosa. Information on life-style, additional diseases and medication intake were collected at baseline and throughout the study. All individuals participated in up to seven repeat visits scheduled every four to six weeks on the same weekday and the same time of the day resulting in a total of 1,766 blood samples (522, 675 and 569 for diabetes patients, IGT and participants with potential genetic susceptibility, respectively).**

Written informed consent was obtained from all participants. The study protocol was approved by the local Ethics Committee (“Bayerische Landesaerztekammer”).

**Blood withdrawal and processing**

For analyses of the blood biomarkers, venous blood samples were collected into tubes that contained ethylenediaminetetraacetic acid (EDTA) and citrate (Becton Dickinson, Franklin Lakes, NJ, USA) at each visit. Samples were centrifuged at 4°C and plasma aliquots were immediately stored at -80°C until shipment on dry ice to collaborating laboratories.

Interleukin (IL)-6 was analysed using an IL-6 ultra-sensitive kit **(Meso Scale Diagnostics, Gaithersburg, Maryland, USA)**. HsCRP and MPO were analysed by a Mesoscale discovery kit, (Meso Scale diagnostics, Gaithersburg, MD, USA) and fibrinogen was quantified by immunonephelometry (Dade Behring, Marburg, Germany).

If a participant had a cold/influenza, surgery, a dental intervention or an acute infection of the urogenital/gastro-intestinal/respiratory tract during the three days prior to the visit, the respective blood sample was excluded from the analyses. Only individuals with at least two valid blood samples were considered for analysis.

**Air pollutants**

**Ambient air pollution data were collected at a fixed monitoring site in Augsburg throughout the study period. The site was located approximately 1 km south-east of the city centre and is considered as representative for the urban background in Augsburg (**[**Cyrys et al. 2008**](#_ENREF_6)**).** Particle size distribution (PSD) ranging from 3 nm to 10 µm was measured by a twin differential mobility particle sizer (TDMPS). The TDMPS consists of three sub-systems: 1) an Ultrafine Differential Mobility Analyser (UDMA) combined with an ultrafine Condensation Particle Counter (UCPC), which measures PSD from 3-20 nm; 2) a differential mobility analyser (DMA) combined with a Condensation Particle Counter (CPC), which measures PSD from 10-800 nm; and 3) an aerodynamic particle sizer (APS, Model 3321, TSI Inc., Shoreview, MN, U.S.), which measures PSD from 500 nm – 10 µm. The sub-systems overlap at certain size ranges. We merged the final PSD into 3 nm – 10 µm as our final data set.

Particle mass concentration of PM2.5 and PM10 was measured by two independent Tapered Element Oscillating Microbalances (TEOM, model 1400ab, Thermo Fisher Scientific Inc., Waltham, MA, U.S.). For further details see ([Pitz et al. 2008a](#_ENREF_28)). Particle density for PM2.5 (*ρ*2.5) and PM10 (*ρ*10) was calculated as the ratio of the mass concentration and the corresponding volume concentration, assuming that particles were spherically shaped. For details see ([Pitz et al. 2008b](#_ENREF_29)).

Data on particle length concentration and particle surface concentration were acquired with two different approaches:

1) by using diffusion charging technology. Diffusion charging is a process in which particles are exposed to a unipolar ion atmosphere, in which ions undergoing Brownian motion attach to particle surface, imparting an electrical charge to the particle ([Baron and Willeke 2001](#_ENREF_2)).

LC(EAD), the length concentration of particles between 10nm and 1000nm in aerodynamic diameter (or 10nm to 800nm in mobility equivalent diameter, *Dp*) was measured by an electric aerosol detector (EAD, model 3070A, TSI Inc., Shoreview, MN, U.S.). Active (Fuchs) surface of the particles, SC(DCPS) in the size range <1 µm was measured by a Diffusion Charging Particle Sensor (DCPS) (model LQ1, Matter Aerosol AG, Wohlen, Swizerland). Results obtained by Ku and Maynard (2005, 2006) and Jung and Kittelson (2005) showed that the LQ1 response corresponds well with the theoretical active surface for particles with diameters in a 20–200 nm range. Theoretical active surfaces were calculated based on ([Fuchs 1964](#_ENREF_8)), ([Gäggeler et al. 1989](#_ENREF_9)) and ([Rogak, Baltensperger and Flagan 1991](#_ENREF_32)). Jung and Kittelson ([Jung and Kittelson 2007](#_ENREF_15)) report that the responses of a LQ1-DC and an EAD to singlets (NaCl) particles are proportional to *Dp*1.36 and *Dp*1.13, respectively. The response of LQ1-DC agrees with Fuchs surface area, which is proportional to *Dp*1.39 within 2.4% error. The response of the EAD is almost proportional to diameter, *Dp*. The total length of particles in a given air volume is obtained by summing the particle diameters in a certain amount of time i.e. LC(EAD) reflects the length of an imaginary chain of particles.

2) by calculating particle length concentration and surface area concentration based on the measurements of the PSD dataset. For these parameters, size segregated measures were calculated assuming a spherical shape of the particles. While the number concentration of particles does not take the particle diameter into account, particle length concentration equals the particle number concentration times the diameter of the particle within a certain size range and surface area concentration equals the particle number concentration times the squared diameter of the particle within a certain size range. Equations (2)-(4) show the calculation for number concentration (NC), length concentration (LC) and surface concentration (SC) where (di) = the particle diameter within the relevant size bin and d1 and d2 are the lower and upper edge of the size range, respectively.

, (1)

**(2)

. (3)

More details for these calculations can be found in Gu et al. ([2012](#_ENREF_10)).

For each participant and visit, individual 24-hour averages of exposure preceding blood sampling were calculated if more than two thirds of the hourly measurements were available. We considered the 24 h immediately before blood sampling (lag 0: 0 to 23 h) up to 4 days before the blood sampling (lag 1: 24 to 47 h, lag 2: 48 to 71 h, lag 3: 72 to 95 h, lag 4: 96 to 119 h), and the 5-day average exposure (0 to 119 h).

**Statistical analyses**

Longitudinal data were analysed using additive mixed models with a random participant effect. We assumed a compound symmetry structure for the covariance matrix to model the correlation between repeated measures in each participant.

For each blood marker a separate confounder model was built taking all participants together. Continuous confounders were added to the model linearly, as polynomial, or smoothly as a penalised spline. The decision of which lag and shape of the variable to be used was based on the goodness-of-fit, according to Akaike’s information criterion (AIC). Long-term time trend, air temperature and relative humidity were forced into the models. Barometric pressure and weekday were only included if it improved the model fit. We used the same confounder models as in our previous analyses ([Ruckerl et al. 2014](#_ENREF_34)). The specific models for each blood marker are given in the supplemental materials (Table A). The blood biomarkers were log-transformed before the analyses to fulfil the model assumption of residual normality. For hsCRP and fibrinogen one single blood measurement was excluded as the residual plots showed implausibly large values on visual inspection. Air pollution effects were calculated for the panel of T2D and IGT combined; however, as **sensitivity analysis we also looked at the panels of T2D and IGT separately.**

We also tested for independent effects of particle metrics by using two-pollutant models for selected pollutants in the panels which showed the strongest associations. To avoid collinearity, these analyses were only conducted when the pollutants’ Spearman inter-correlation was <0.60. Lags with the absolute greatest single-day effect were included in these two-pollutant models.

All results are presented as %change of the geometric mean of the outcome per increase in one interquartile range (IQR) of the respective air pollutant attribute.

Results

**Participants and blood markers**

In total, 274 participants took part in the study, 187 of which belonged to the group of T2D (n=83) or IGT (n=104) while the genetically susceptible individuals comprised 87 participants (Table 1). There were slightly more males in all groups and participants had a mean age of approximately 63 years, with the combined group of T2D and IGT being significantly older than the group of genetically susceptible participants. The T2D/IGT group also contained slightly more ex-smokers and a higher percentage reported a history of coronary heart disease; however only history of hypertension and previous MI differed statistically significantly in both groups. Accordingly, the reported intake of medication was also higher in the group of T2D/IGT. Blood marker concentrations were significantly lower in the panel of genetically susceptible participants (Table 1). HsCRP, IL-6 and Fibrinogen showed a correlation coefficient of around 0.5. The other coefficients of correlation were below 0.2 (data not shown).

**Air pollutants**

The descriptive statistics of daily average concentrations of selected air pollutants and meteorological parameters can be found in Table 2.

LC(EAD) was somewhat smaller than the calculated size segregated particle length. Particle length in the larger size fractions was larger than for the smaller size fractions. A similar picture was seen for particle surface concentration.

Spearman correlation coefficients between selected air pollutants are shown in Figure 1. LC(EAD) and SC(DCPS) were highly correlated (r=0.93). Correlation coefficients for LC(EAD) were around 0.7 with UFP, PM2.5 and PM10. Results were similar for SC(DCPS).

The correlation between LC(EAD) and the LC10-800nm calculated from the PSD dataset was 0.85. The size range 10 – 800nm (mobility or stokes diameter) in the PSD dataset equals an aerodynamic diameter of 10-1000nm, the range of the EAD. SC10-800nm showed a correlation of 0.80 with SC(DCPS). *ρ*2.5 and *ρ*10 showed a correlation of 0.72, but were not highly correlated to any of the other air pollution markers. The lower number of observations for *ρ*2.5 and *ρ*10 is due to the calculation method. For the calculation of *ρ*2.5 and *ρ*10, both particle mass measured by TEOM and data from the TDMPS need to be available. Thus, if either of the measurements were missing, or where observations for TEOM mass were imputed by TDMPS mass, no observations for *ρ*2.5 and *ρ*10 are available.

Null or slightly negative correlations were seen between most of the pollutants and the meteorological variables.

**Table 1. Description of patient characteristics.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|   |   | **All n=274** | **T2D or IGT** **n=187** | **Gen. Susc.****n=87** | **P value** |
|   | **Arithmetic Mean** | **SD** | **Arithmetic Mean** | **SD** | **Arithmetic Mean** | **SD** |  |
| **Age** (years) |  | 63.1 | 10.9 | 66.4 | 8.5 | 55.9 | 12.1 | <0.01d |
| **BMI**a (kg/m²) |  | 29.3 | 5.5 | 30.5 | 5.4 | 26.8 | 5.0 | <0.01d |
|   |   | **N** | **%** | **N** | **%** | **N** | **%** |  |
| **Gender** | Male | 157 | 57 | 108 | 58 | 49 | 56 | 0.82e |
|  |  |  |  |  |  |  |  |  |
| **Smoking** | Never smoker | 132 | 48 | 89 | 11 | 43 | 49 | 0.25e |
|  | Ex smoker | 133 | 49 | 94 | 88 | 39 | 45 |  |
|  | Occasional smoker | 9 | 3 | 4 | 2 | 5 | 6 |  |
| **History of** | Coronary heart disease | 25 | 9 | 18 | 10 | 7 | 8 | 0.67e |
|  | Myocardial infarction | 23 | 8 | 20 | 11 | 3 | 3 | 0.04e |
|   | Hypertension | 169 | 62 | 135 | 72 | 34 | 39 | <0.01e |
|  | Diabetes | 187 | 68 | 187 | 100 | 0 | 0 | <0.01e |
| **Medication use** | Antidiabetics | 52 | 19 | 50 | 27 | 2 | 2 | <0.01e |
|  | Statins | 67 | 24 | 54 | 29 | 13 | 15 | 0.012e |
|  | Anti-inflammatory agentsi | 60 | 22 | 49 | 26 | 11 | 13 | 0.012e |
|  | Acetyl salicylic acid | 65 | 24 | 53 | 28 | 12 | 14 | <0.01e |
|  | Beta-blockers | 84 | 31 | 69 | 37 | 15  | 17 | <0.01e |
|  | Anti-thrombotic medic.k | 73 | 27 | 60 | 32 | 13 | 15 | <0.01e |
| **Blood biomarkers** |  | **N** | **Arithmetic Meanf** | **SDh** | **Geometric meang** | **N** | **Arithmetic Meanf** | **SD** | **Geometric meang** | **N** | **Arithmetic Meanf** | **SD** | **Geometric meang** |  |
|  | hsCRPb (mg/l) | 1765 | 2.1 | 2.8 | 1.0 | 1196 | 2.5 | 3.3 | 1.2 | 569 | 1.4 | 1.4 | 0.8 | <0.01d |
|  | MPOc (ng/ml) | 1766 | 15.7 | 5.3 | 14.3 | 1197 | 16.2 | 3.7 | 15.1 | 569 | 14.5 | 7.5 | 12.7 | <0.01d |
|  | IL-6d (pg/ml) | 1761 | 1.4 | 2.9 | 1.0 | 1193 | 1.7 | 3.5 | 1.2 | 568 | 1.0 | 0.7 | 0.8 | <0.01d |
|  | Fibrinogen (g/l) | 1765 | 3.6 | 0.6 | 3.5 | 1196 | 3.7 | 0.6 | 3.6 | 569 | 3.3 | 0.5 | 3.3 | <0.01d |

a: body mass index, b: high sensitivity C-reactive protein, c: myeloperoxidase, d: interleukin-6; d: t-test; e: chi² test; f: arithmetic mean of participants’ arithmetic means; g: geometric mean of participants’ geometric means; h: Standard deviation; i: anti-inflammatory agents comprise e.g. corticosteroids, cortisol, non-steroid anti-inflammatory agents; k: anti-thrombotic medication comprises e.g. Vitamin K antagonists, medication from the heparin group, new oral anti-coagulants

**Table 2: Description of air pollutants and meteorological variables (24h averages) for the study period (19.03.2007 – 17.12.2008).**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **N** | **Mean** | **SD**g | **Min** | **25%** | **Median** | **75%** | **Max** | **IQR** | **IQR**h **5-day average** |
| **UFP**a[n/m³] | 611 | 9,537 | 4,417 | 1,897 | 6,305 | 8,890 | 12,027 | 26,503 | 5,722 | 4,279 |
| **PM**b**2.5** [µg/m³] | 644 | 13.7 | 10.0 | 1.6 | 6.7 | 11.3 | 17.8 | 65.8 | 11.1 | 9.4 |
| **PM10** [µg/m³] | 645 | 18.3 | 12.0 | 2.1 | 10.0 | 15.8 | 24.0 | 86.5 | 13.9 | 10.6 |
| **NC**c**3-10** [µg/m³] | 611 | 839 | 383 | 151 | 566 | 788 | 1,047 | 3,030 | 481 | 390 |
| **NC10-30** [µg/m³] | 611 | 3,798 | 1,715 | 894 | 2,512 | 3,416 | 4,852 | 10,966 | 2,341 | 1,707 |
| **NC30-50** [µg/m³] | 611 | 2,666 | 1,321 | 500 | 1,736 | 2,400 | 3,484 | 8,316 | 1,748 | 1,251 |
| **NC50-100** [µg/m³] | 611 | 3,072 | 1,674 | 488 | 1,874 | 2,719 | 3,961 | 11,459 | 2,088 | 1,546 |
| **LC(EAD)** [mm/cm³] | 569 | 0.6 | 0.3 | 0.1 | 0.4 | 0.5 | 0.7 | 1.7 | 0.4 | 0.3 |
| **LC**d**10-800** [mm/cm³] | 611 | 0.8 | 0.4 | 0.1 | 0.5 | 0.7 | 0.9 | 2.8 | 0.5 | 0.4 |
| **LC3-10** [mm/cm³] | 610 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.02 | 0.00 | 0.00 |
| **LC10-30** [mm/cm³] | 611 | 0.07 | 0.03 | 0.02 | 0.05 | 0.07 | 0.09 | 0.20 | 0.04 | 0.03 |
| **LC30-50** [mm/cm³] | 611 | 0.10 | 0.05 | 0.02 | 0.07 | 0.09 | 0.13 | 0.31 | 0.07 | 0.05 |
| **LC50-100** [mm/cm³] | 611 | 0.21 | 0.12 | 0.03 | 0.13 | 0.18 | 0.27 | 0.78 | 0.15 | 0.11 |
| **SC(DCPS)** [µm²/cm³] | 592 | 37.5 | 19.9 | 6.9 | 23.4 | 32.7 | 47.9 | 115.3 | 24.5 | 22.3 |
| **SC**e**10-800** [µm²/cm³] | 611 | 305.3 | 183.3 | 38.5 | 174.2 | 276.4 | 385.0 | 1,516.5 | 210.8 | 168.9 |
| **SC3-10** [µm²/cm³] | 611 | 0.14 | 0.06 | 0.03 | 0.09 | 0.13 | 0.17 | 0.49 | 0.08 | 0.06 |
| **SC10-30** [µm²/cm³] | 611 | 4.7 | 2.2 | 1.1 | 3.1 | 4.3 | 6.1 | 12.9 | 3.0 | 2.06 |
| **SC30-50** [µm²/cm³] | 611 | 12.0 | 6.0 | 2.3 | 7.8 | 10.9 | 15.8 | 38.0 | 8.0 | 5.7 |
| **SC50-100** [µm²/cm³] | 611 | 47.0 | 26.0 | 7.2 | 28.1 | 41.4 | 61.4 | 175.6 | 33.2 | 24.7 |
| ***ρ***f**2.5** [g/cm3] | 367 | 1.2 | 0.3 | 0.6 | 0.9 | 1.1 | 1.4 | 2.3 | 0.5 | 0.4 |
| ***ρ*10** [g/cm3] | 432 | 1.4 | 0.3 | 0.8 | 1.1 | 1.4 | 1.6 | 2.3 | 0.4 | 0.4 |
|  |  |  |  |  |  |  |  |  |  |  |
| **Air temperature** [°C] | 636 | 10.9 | 7.3 | -5.8 | 5.0 | 11.2 | 17.0 | 27.0 | 12.0 | 11.4 |
| **Relative humidity** [%] | 636 | 77.0 | 12.6 | 32.4 | 68.1 | 77.6 | 86.9 | 100.0 | 18.8 | 13.1 |
| **Barometric pressure** [hPa] | 636 | 961.3 | 7.6 | 933.9 | 957.1 | 961.3 | 965.9 | 983.5 | 8.9 | 7.4 |
| a: ultrafine particles, number concentration of particles <100nm, b: particulate matter with aerodynamic diameter<=2.5µm and <=10µm, respectively; c: number concentration of particles with 3-10nm, 10-30nm, 30-50nm, and 50-100nm of mobility diameter, respectively; d: length concentration of particles with 3-10nm, 10-30nm, 30-50nm, and 50-100nm of mobility diameter, e: surface concentration of particles with 3-10nm, 10-30nm, 30-50nm, and 50-100nm of mobility diameter, respectively; f: apparent density for PM2.5 and PM10, respectively; g: SD: standard deviation, h:IQR: interquartile range |



**Figure 1. Spearman correlation coefficients of selected air pollutants for the study period (19.03.2007 – 17.12.2008).**

**Air pollution and blood markers**

Associations between blood biomarkers and various metrics of particulate matter for all lags are given in the supplemental materials (Tables B.1 to C.3).

Panel of genetically susceptible participants

For the panel of genetically susceptible participants we found clear increases in **hsCRP** in association with air pollutants. The strongest associations were seen for the 5-day average exposure. Additionally lags 0 to 2 showed statistically significant associations (Tables B.1-B.3, supplemental material). The strongest effects were seen for particle length concentration, followed by particle surface area concentration. Associations for the calculated particle length concentration were clearly smaller than for LC(EAD). Similarly, the associations for the calculated surface area concentration were smaller than for SC(DCPS) (Figure 2). For apparent density of particles with 2.5 or 10µm aerodynamic diameter, respectively, we found no consistent associations with hsCRP (Figure 2). The various size ranges of particle number concentration, particle length concentration and particle surface concentration showed positive associations (supplemental material, Figure A1).

A similar picture was seen for **MPO**; in general, effect estimates were smaller than for hsCRP, with the exception of apparent density, for which clear negative associations were seen, especially for *ρ*2.5 (Figure 3). Positive associations for NC; LC and SC were seen for the size ranges 30-50nm and 50-100nm but not for the smallest one (supplemental material, Figure A2).

**IL-6** concentrations decreased unexpectedly in association with air pollutants for most lags. The largest effects were detected for the 5-day average exposure. Again, LC(EAD) and SC(DCPS) showed the strongest association -9.2 95% CI:[-15.4;-2.5] and -9.9 [-16.5;-2.9], respectively. Associations for LC(EAD) were similar to LC10-800nm (-9.2 [-14.2;-3.8]). Also, some of the size fractions of PNC showed significant associations, e.g. NC30-50nm and NC50-100nm (-10.4[-16.1;-4.4] and -9.2[-14.0;-4.2], respectively). No consistent associations were seen for apparent density (supplemental material Tables B.1-B.3).

For **fibrinogen** we found only few statistically significant associations in the panel of genetically susceptible patients. For lag 1, fibrinogen increased mainly in association with the larger size ranges of particle surface area concentration (SC30-50: 0.9[0.1;1.7], SC50-100: 0.8[0.0;1.5]) and particle length concentration (LC30-50: 0.9[0.0;1.8], LC50-100: 0.8[0.0;1.5]) (supplemental material Tables B.1-B.3). In addition, positive associations were seen for lag 3 for SC30-50 (1.2[0.2;2.1]), SC50-100 (1.0[0.1;1.8]), LC30-50 (1.3[0.2;2.3]) and LC50-100 (1.0[0.0;1.9]).



**Figure 2**: hsCRP in association with the 5-day average exposure of ambient air pollutants in the panel of genetically susceptible participants. Light colours indicate physical attributes; dark colours indicate traditionally measured air pollutants.
UFP: ultrafine particles, number concentration of particles <100nm, PM: particulate matter with aerodynamic diameter<=2.5µm and <=10µm, respectively; LC( EAD): length concentration measured by EAD; LC10-800nm: length concentration of particles with 10-800nm; SC(DCPS): surface concentration measured by DCPS; SC10-800nm: surface concentration of particles with 10-800nm; RHO: apparent density for PM2.5 and PM10, respectively;



**Figure 3**: MPO in association with the 5-day average exposure of ambient air pollutants in the panel of genetically susceptible participants. Light colours indicate physical attributes; dark colours indicate traditionally measured air pollutants.
UFP: ultrafine particles, number concentration of particles <100nm, PM: particulate matter with aerodynamic diameter<=2.5µm and <=10µm, respectively; LC( EAD): length concentration measured by EAD; LC10-800nm: length concentration of particles with 10-800nm; SC(DCPS): surface concentration measured by DCPS; SC10-800nm: surface concentration of particles with 10-800nm; RHO: apparent density for PM2.5 and PM10, respectively;

Panels of T2D and IGT

In the combined panels of T2D and IGT hardly any associations were seen for hsCRP or MPO apart from *ρ*2.5 which showed clear negative associations (Figures 4 and 5). For fibrinogen, positive associations were seen for several air pollutants especially with lag 1 and the 5-day average exposure (supplemental material, Tables C.1-C.3).





**Figure 4**: Associations of hsCRP with the 5-day average exposure of ambient air pollutants in the panel of genetically susceptible participants (light colours) and the panel of metabolic dysfunction (T2D and IGT, dark colours).
T2D: type 2 diabetes mellitus, IGT: impaired glucose tolerance, LC (EAD): length concentration measured by EAD; LC: length concentration of particles with 10-800nm of mobility diameter, SC (DCPS): surface concentration measured by DCPS; SC: surface concentration of particles with 310-800nm of mobility diameter; RHO: apparent density





**Figure 5**: Associations of myeoloperoxidase (MPO) with the 5-day average exposure of ambient air pollutants in the panel of genetically susceptible participants (light colours) and the panel of metabolic dysfunction (T2D and IGT, dark colours).

T2D: type 2 diabetes mellitus, IGT: impaired glucose tolerance, LC (EAD): length concentration measured by EAD; LC: length concentration of particles with 10-800nm of mobility diameter, SC (DCPS): surface concentration measured by DCPS; SC: surface concentration of particles with 310-800nm of mobility diameter; RHO: apparent density

**Sensitivity analysis**

In general, results for the separate analyses of the panels of T2D and IGT were similar to the joint analyses. Only fibrinogen showed clear positive associations for the panel of IGT with most pollutants.

**Two pollutant models**

Of the selected air pollutants, only apparent density fulfilled the criterion of a correlation coefficient <0.60 with LC(EAD) and SC(DCPS).

For hsCRP, MPO and IL-6 associations for the two-pollutant models were similar to the one pollutant models. Only a few associations were slightly stronger in the two-pollutant models, e.g. IL-6 with LC(EAD) and SC(DCPS), when adjusted for *ρ*2.5, and some slightly weaker, e.g. hsCRP with SC(DCPS) when adjusted for *ρ*2.5 (Figures B, C and D supplemental materials). For fibrinogen, associations were somewhat inconclusive for lag 4 in the two-pollutant models as we found that the associations for both, LC(EAD) and SC(DCPS) turned from positive to negative, when adjusted for ρ2.5 or ρ10 (Figure D, supplemental materials). The results for ρ2.5 and ρ10 on the other hand remained unchanged in the adjusted models; however air pollution data for lag 4 did not differ from the other lags.

Discussion

**Summary**

We examined the association between novel physical attributes of particulate air pollution and four blood biomarkers reflecting inflammation and coagulation in potentially susceptible participants. We found clear associations between increased levels of inflammatory markers hsCRP and MPO and LC(EAD) and SC(DCPS). in the panel of genetically susceptible participants. MPO showed a decrease in association with apparent density in this group. In addition, positive associations were seen for fibrinogen in the T2D/IGT panel. For the other blood markers and panels, the results were less clear.

**Comparison of association of vascular biomarkers with traditional pollutant measurements and physical attributes of PM.**

The general picture of the associations between the blood biomarkers and the PM attributes is similar to the associations with traditionally measured air pollutants. We found very few associations between PM2.5 or PM10 concentration and vascular biomarkers for the panel of IGT/T2D. However, clear increases for hsCRP and MPO were seen in the panel of genetically susceptibles, and small positive associations for fibrinogen, while IL-6 decreased, mainly with later lags ([Ruckerl et al. 2014](#_ENREF_34)).

A closer look at the association between **hsCRP** and PM physical attributes in the panel of genetically susceptibles shows the by far strongest associations for particle length concentration. In particular, LC(EAD) and SC(DCPS) showed the strongest associations, potentially integrating the observations for all size fractions ranging from the very ultrafine to the border of the fine particles. For the 5-day average exposure this association was in the same range as NO and BC with an increase of more than 30% in geometric mean per increase in IQR of air pollutant; however, the associations for the single lags were even higher for LC(EAD). The association for SC(DCPS) was similar to those of PM10 and PM2.5, but lower in the first lags and higher in the later ones.

For **MPO**, LC(EAD) and SC(DCPS) showed a similar strength of the association compared to UFP larger than 30 nm. The main difference between traditional air pollutants and PM physical attributes was that while the traditional pollutants showed positive associations mainly with lag 0 and/or 1 and the 5-day average exposure, the associations for LC(EAD) and SC(DCPS) were positive for all lags except for lag 4.

The unexpected decreases we found for **IL-6** ([Ruckerl et al. 2014](#_ENREF_34)) were confirmed with the PM physical attributes. A similar decrease was found in a European study on myocardial infarction survivors; however the decrease was limited to later lags and/or only some of the cities that were part of the study ([Ruckerl et al. 2007](#_ENREF_33)). In this panel, the associations for LC(EAD) and SC(DCPS) were in the same range as NO, NO2 and UFP which showed the strongest associations we found for IL-6 with the traditional pollutants. While most of the other blood biomarkers were not strongly associated with UFP, IL-6 had its strongest decrease with UFP. This finding was confirmed with the PM physical attributes, as the strongest decreases were found for the smaller size ranges of the surface area concentration. However, pollutants were highly correlated.

The results for **fibrinogen** were comparable with an increase of about 1% in geometric mean per interquartile range of air pollutant.

**Health effects of particle length concentration and particle surface concentration**

In our analyses, the direct measurements of particle length concentration and active surface concentration, LC(EAD) and SC(DCPS), showed stronger associations with the blood markers than the calculated parameters. The general direction and lag structures were similar, however. The results indicate that these integrated measures of particle properties in particular are advantageous, if all size ranges in the ultrafine fraction are associated with the outcomes of interest. If only certain sizes are relevant, these summary measures may lack the ability examine the size segregated health effects.

Surface area concentration is considered as one of the most influential parameters regarding biological effects such as inflammatory injury or oxidative damage (Valavanidis et al. 2008). However, while associations between blood biomarkers, especially hsCRP, and particle mass and/or particle number concentration have been demonstrated repeatedly in epidemiological studies ([Ruckerl et al. 2011](#_ENREF_35), [Huttunen et al. 2012](#_ENREF_13), [Delfino et al. 2008](#_ENREF_7), [Wang et al. 2015](#_ENREF_42), [Lanki et al. 2015](#_ENREF_19), [Schneider et al. 2010](#_ENREF_37)), to date, not many publications have examined health effects in association with particle length concentration or particle surface concentration in human populations.

Stoeger et al. ([2006](#_ENREF_38)) found particle surface area to be the best parameter for evaluating the inflammatory potential of particles in mice. However, the authors measured particle surface area with a different approach namely by assessing the specific surface area from the quantity of a gas that is absorbed in multi-molecular layers on the surface of the particle (Brunauer, Emmet and Teller Method, BET ([Brunekreef B and Hughes E 2009.](#_ENREF_5), [Brunauer 1938](#_ENREF_4))). Other authors also report that particle surface area is a key factor regarding inflammatory reactions and oxidative stress in rats and human epithelial cells ([Monteiller et al. 2007](#_ENREF_23), [Brown et al. 2001](#_ENREF_3), [Tran 2000](#_ENREF_40), [Oberdörster 1996](#_ENREF_26)), however, again, surface area was recorded with different methods. Maynard and Maynard ([2002](#_ENREF_22)) analysed the association between surface area estimated from mass-based time-series data and historic mortality data from London. They found a linear relationship between ambient surface area and mortality. The authors pointed out, however, that many simplifying assumptions underlie the estimated surface area. Mooshammer et al. ([2003](#_ENREF_24)) measured SC(DCPS) data for six weeks in a school in Austria. They found that several lung function parameters in school children decreased significantly with an increased 8-h morning mean of SC(DCPS), and that most of the results remained stable even after adjustment for PM10. Moreover, asthma-like symptoms, which were captured in a diary in children with a history of asthma or a pathological lung function test, were positively related to the same day daily mean of SC(DCPS).

The importance of particle length concentration in our data is further supported by Figure 6. It depicts the %change of the geometric mean of hsCRP (Panel A) and MPO (Panel B) for the 5-day average exposure in the panel of genetically susceptible participants, for selected air pollutants plotted with the coefficient of determination (R²) of these pollutants with LC(EAD). The observed increases in the blood markers are the stronger, the closer this correlation is. For hsCRP, panel A indicates that LC(EAD) and SC(DCPS) are likely to capture the same underlying aerosol properties. In addition, there are also three clusters detectable. Cluster I above the dotted line comprises the cluster of NO, CO and BC in the top right corner, and cluster II is composed of the smallest size fractions for NC, LC and SC (left side). These clusters show stronger effects than would be predicted. The larger size fractions, on the other hand, form cluster III showing smaller associations than one would expect. For MPO, panel B indicates that all measured air pollutants are likely to capture the same underlying aerosol properties with the exception of cluster IV made up by particle density.

Measurements of UFP, which are also characterised by their large surface area, pose a variety of challenges to the devices and the staff. Some devices need a radioactive source and use butanol, which can cause problems to other measurement devices if allocated closely to each other. Also, they are laborious and require intensive calibration and/or quality assurance ([UFIREG-report 2014](#_ENREF_41)). The EAD device, on the other hand, is simple to operate ([Woo et al. 2001](#_ENREF_45)) and runs reliably ([Westerdahl et al. 2005](#_ENREF_43)). Wilson et al. ([Wilson et al. 2007](#_ENREF_44)) deduced from their comparison of techniques which measure parameters related to the surface area of fine particles that the continuous measurements acquired by the EAD may provide a useful indicator of the particle surface area deposited in the lungs and is therefore useful for epidemiological studies as well as monitoring occupational exposures.

A



PM2.5

PM10

NC3-10nm



I

III

II

B



SC(DCPS)

IV

**Figure 6**: Coefficient of determination with LC(EAD) and %change in hsCRP (Panel A) and MPO (Panel B) for the 5-day average exposure in the panel of genetically susceptible participants.
UFP: ultrafine particles, number concentration of particles <100nm, PM: particulate matter with aerodynamic diameter<=2.5µm and <=10µm, respectively; NC: number concentration of particles with 3-10nm, 10-30nm, 30-50nm, and 50-100nm of mobility diameter, respectively; LC: length concentration of particles with 10-800nm, 3-10nm, 10-30nm, 30-50nm, and 50-100nm of mobility diameter, SC (DCPS): surface concentration measured by DCPS; SC: surface concentration of particles with 10-800nm, 3-10nm, 10-30nm, 30-50nm, and 50-100nm of mobility diameter, respectively; RHO: apparent density for PM2.5 and PM10, respectively; NO: nitric oxide, NO2: nitrogen dioxide;

**Apparent particle density**

Health effects of ambient air pollution not only depend on particle size and concentration, but also on the chemical composition. Collecting detailed information on the chemical composition for the measured particles is costly and labour intensive and can only be conducted for larger time scales such as days or weeks. Apparent particle density might serve as a crude marker for chemical composition for epidemiological studies as it takes material density and particle shape into account, both reflecting chemical composition ([Pitz et al. 2008a](#_ENREF_28), [Pitz et al. 2008b](#_ENREF_29), [Hasheminassab et al. 2014](#_ENREF_12)). The particle density of freshly emitted combustion particles is usually below 1.0 g cm-3 ([Ntziachristos and Samaras 2006](#_ENREF_25)) due to the agglomerate nature of soot particles but can go up to 2.0 g cm-3 if compaction processes are involved ([Pitz et al. 2008b](#_ENREF_29)). Diesel particles have been shown to range between 0.4 and 0.6 cm-3 ([Lapuerta, Armas and Gomez 2003](#_ENREF_20)). Naturally generated particles on the other hand, such as crustal materials, for example, have a density of about 2.9 g cm-3 ([Hänel 1977](#_ENREF_11)). In accordance with our hypothesis we found mostly negative associations in our data, indicating an increase in blood markers with a decrease in particle density. Up to date, no other studies seem to have examined health endpoints in association with apparent particle density.

**Biological mechanisms**

The results of these analyses point towards inflammation and oxidative stress as pathways that link pollution and cardiovascular disease. It is hypothesized that circulating pro-oxidative and/or pro-inflammatory mediators such as CRP and IL-6, which are released from the lungs in response to air pollution exposure, induce a systemic inflammatory response ([Araujo and Nel 2009](#_ENREF_1), [Ruckerl et al. 2011](#_ENREF_35)). Persistently high levels of these mediators as well as acute changes have been associated with an increased risk of cardiovascular events in large cohort studies ([Koenig et al. 1999](#_ENREF_16), [Ridker et al. 1997](#_ENREF_31)) and might therefore explain the link between exposure to ambient air pollution and increased cardiovascular diseases and T2D ([Rajagopalan and Brook 2012](#_ENREF_30), [Liu et al. 2013](#_ENREF_21)).

**Future implications**

This is one of the first papers to look at a variety of novel attributes of particulate matter in association with health outcomes. Our data indicate that there may be air pollution parameters which are able to capture adverse health effects better than the routinely measured parameters such as PM2.5 and PM10. This might be a limitation not only to research but also for the protection of public health. We therefore propose that additional pollutants or characteristics should be included into a comprehensive air quality monitoring strategy, in line with the recommendations of the projects UFIREG (Ultrafine Particles – an evidence based contribution to the development of regional and European environmental and health policy; <http://www.ufireg-central.eu/index.php/results>) and AirMonTech (Air Pollution Monitoring Technologies for Urban Areas; <http://www.airmontech.eu/> ([Kuhlbusch et al. 2014](#_ENREF_18))). The focus of these networks should be broad enough to include an assessment of compliance with EU standards in background and hotspot sites, and the assessment of population-based exposure appropriate for health effect studies. Permanent “research sites” measuring a large range of pollutants in carefully chosen sites should be integrated into the air quality monitoring networks as national monitoring networks have aims beyond compliance monitoring, such as clarification of health effects, source apportionment, and abatement assessment. Before these additional markers can be recommended for epidemiological studies, exposure studies should establish the temporal and spatial variability within cities, like it has been done for UFP and PM2.5 in the past ([Cyrys et al. 2008](#_ENREF_6)).

**Conclusions**

In our analyses, the direct measurements of particle length concentration, LC(EAD) and active surface concentration, SC(DCPS), showed strong associations with blood biomarkers reflecting inflammation and oxidative stress. In general, direction and lag structures were similar to those of traditionally measured pollutants. In addition, we found that the associations for blood biomarkers and apparent particle density, especially for PM2.5 were going in the expected direction.

These novel air pollution metrics might reflect harmful aerosol properties better than particulate mass or number concentration and could therefore be important for epidemiological studies. As this is one of the first epidemiological studies examining health outcomes in association with these air pollution parameters, however, results need to be confirmed in other studies and with additional health outcomes.

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