ORIGINAL ARTICLE

Daily variation in fine and ultrafine particulate air pollution and urinary concentrations of lung Clara cell protein CC16

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Background: Daily variations in ambient particulate air pollution have been associated with respiratory mortality and morbidity.
Aims: To assess the associations between urinary concentration of lung Clara cell protein CC16, a marker for lung damage, and daily variation in fine and ultrafine particulate air pollution.
Methods: Spot urinary samples (n = 1249) were collected biweekly for six months in subjects with coronary heart disease in Amsterdam, Netherlands (n = 37), Erfurt, Germany (n = 47), and Helsinki, Finland (n = 47). Ambient particulate air pollution was monitored at a central site in each city.
Results: The mean 24 hour number concentration of ultrafine particles was 17.3×10^3 cm^{-3} in Amsterdam, 21.1×10^3 cm^{-3} in Erfurt, and 17.0×10^3 cm^{-3} in Helsinki. The mean 24 hour $PM_{2.5}$ concentrations were 20, 23, and 13 μg/m^3, respectively. Daily variation in ultrafine particle levels was not associated with CC16. In contrast, CC16 concentration seemed to increase with increasing levels of $PM_{2.5}$ in Helsinki, especially among subjects with lung disorders. No clear associations were observed in Amsterdam and Erfurt. In Helsinki, the CC16 concentration increased by 20.2% (95% CI 6.9 to 33.5) per 10 μg/m^3 increase in $PM_{2.5}$ concentration (lag 2). The respective pooled effect estimate was 2.1% (95% CI −1.3 to 5.6).
Conclusion: The results suggest that exposure to particulate air pollution may lead to increased epithelial barrier permeability in lungs.

Ambient air particulate pollution has been associated with adverse respiratory health effects in several studies.[1 2] Health endpoints have usually been measured as changes in lung function, reporting of symptoms, or hospital admissions or mortality due to respiratory diseases.[3] No time series studies have used biomarkers of lung damage. The mechanisms of the observed adverse effects are still largely unknown. Inflammatory processes are suspected to play a key role in the pathomechanisms leading from deposition of particles to the exacerbation of respiratory diseases.

Clara cell protein (CC16) is a 16–17 kD lung epithelium specific protein secreted in the respiratory tract by the non-ciliated Clara cells, known for their vulnerability to toxic insults. CC16 secreted in the respiratory tract diffuses passively across the bronchoalveolar-blood barrier into serum; it is eliminated by the kidneys. In human and experimental animal studies, it has been shown that the concentration of CC16 in extrapulmonary fluids such as serum and urine can be used to evaluate the integrity of the lung epithelial barrier.[4–8]

Serum concentrations of CC16 show considerable variations in healthy subjects. Baseline concentrations reflect the number of Clara cells and the variation of the concentration in time reflects the integrity of the lung epithelial barrier. Serum concentrations slightly increase with aging, whereas a reduction of CC16 in serum of people exposed to tobacco smoke has been observed due to decreased density of CC16 positive cells in the lungs.[4 9] In addition, subjects with chronic obstructive pulmonary disease or lung cancer have a significant reduction of CC16 in serum, whereas increased levels have been found in subjects with sarcoidosis.[4] With respect to environmental exposures, increased CC16 levels in serum have been reported in firemen after exposure to smoke[10 11] and in cyclists in association with two hours' exercise during an ozone (O_3) episode.[12] In addition, exposure to nitrogen trichloride, a gas used in the air of indoor pools, has been associated with increased levels of CC16 in serum, both in humans and in experimental animals.[13] In contrast, in an experimental animal study, nose only exposure to diesel exhaust enriched concentrated $PM_{2.5}$ did not result in increased CC16 levels in blood in rats.[14]

The aim of the present study was to test the hypothesis that increases in daily ambient concentrations of ultrafine and fine particles are associated with increases in urinary concentrations of CC16. The study was a part of the ULTRA study on short term effects of ultrafine and fine particulate air pollution on health among subjects with coronary artery disease.

METHODS

The ULTRA study was carried out in three European cities: Amsterdam, Netherlands, Erfurt, Germany, and Helsinki, Finland. The study periods were: in Amsterdam, 3 November 1998 to 18 June 1999; in Erfurt, 14 October 1998 to 4 April 1999; and in Helsinki, 2 November 1998 to 30 April 1999.

The study protocol was approved by ethical committees in each study centre. A written consent was obtained from all subjects.

In each city, a panel of subjects with coronary heart disease was followed up for six months with biweekly clinical visits and daily symptom diaries. Subjects with coronary heart disease were chosen, as the main aim of the ULTRA study was to investigate effects of air pollution on cardiovascular health. The clinical visit included a collection of a spot urinary sample for analyses of CC16, spirometric measurement of lung function, and recording of ambulatory ECG. For each subject, the visit was scheduled to be always on the

Main messages

- Exposure to particulate air pollution may increase epithelial barrier permeability in lungs, but the response differs in different study centres.
- The reason for the differences in the association between centres is not yet understood, but may provide important insights into factors affecting response to particulate air pollution, such as panel characteristics and composition of particles.

Policy implications

- The result underlines the importance of carefully standardised, multicentre studies in air pollution epidemiology.
- Efforts to decrease concentration of ambient air pollutants should continue, as harmful health effects are observed even at low concentrations.

same weekday at the same time. The daily medication of the subjects was not changed for the clinical visit. In Amsterdam and Helsinki, a field worker visited the subject's home just before the visit. During the study period, concentrations of ambient air pollutants were measured at a fixed monitoring site, with a special emphasis on measurements of particle number concentrations. All methods used in the ULTRA study were conducted according to standard operating procedures (SOP) developed for the ULTRA study.[15]

Altogether, there were 37 panellists in Amsterdam and 47 panellists in both Erfurt and Helsinki. The subjects were characterised by a questionnaire and recording of a 12 lead standard resting ECG.[15] The criteria for being included in the study were: a self report of a doctor diagnosed coronary artery disease, for example, angina pectoris; a past myocardial infarction (MI), PTCA (percutaneous transluminal coronary angioplasty), or coronary bypass surgery; being a non-smoker; age of 50 years or more; and being able to perform spirometry in an acceptable way. The exclusion criteria were a recent (less than three months) MI, stroke, or bypass surgery, unstable angina pectoris, having a cardiac pacemaker, inability to perform an exercise challenge test, type 1 diabetes, and poor cooperation.[15] Table 1 presents the characteristics of the final study population in the three study centres.

Spot urinary samples were collected during the clinical visit or just before the visit at home. As prostatic secretions may contaminate the sample, mid-stream samples were collected from the male subjects. All urinary samples from the three study centres were sent to one laboratory for the analyses. CC16 concentrations were measured by an automated latex immunoassay.[16] From all samples, urinary creatinine concentrations were also determined. The coefficient of variation between the duplicate samples was 22.6% for ln(CC16) and 4.6% for urinary creatinine measurements.

In each city, concentrations of ambient air pollutants were measured at a fixed monitoring site representing urban background levels according the ULTRA SOPs.[15] Particle number concentrations (NC) in different size classes were measured with aerosol spectrometers.[17–19] Fractions of the measured size distributions were determined to form one size class for ultrafine particles (particles in the size range 0.01–0.1 μm), and one for accumulation mode particles (particles in the size range 0.1–1.0 μm). These particles are referred as NC 0.01–0.1 and NC 0.1–1, respectively. For quality control purposes, condensation particle counter (CPC) TSI 3022A was used in all centres to measure the total number concentration of particles with a lower detection limit of 0.007 μm, and the data were used to input missing NC 0.01–0.1 data.[15] The percentages for the imputed hourly values are 6.8%, 1.1%, and 3.3% in Amsterdam, Erfurt, and Helsinki, respectively. $PM_{2.5}$ was measured with Harvard impactors.[20] Data on meteorological variables, PM_{10}, NO_X, CO, SO_2, and O_3 were collected from existing networks (Amsterdam: the Royal Dutch Meteorological Institute and the National Institute of Public Health and the Environment; Erfurt: Thüringer Landesanstalt für Umwelt; Helsinki: Helsinki Metropolitan Area Council). All variables are 24 hour means from noon to noon.

Statistical analyses

In the analyses, CC16 levels were divided by urinary creatinine concentration to account for diuresis. Further, for the analyses the ratio was log transformed. Subjects with CC16 concentrations at the detection limit (CC16 = 1.0 μg/l) were nearly always excluded from the analyses. This resulted in exclusion of four subjects from the Amsterdam panel, one subject from the Erfurt panel, and five subjects from the Helsinki panel. They were all female subjects. Data were analysed by using the statistical packages S-Plus and SAS (SAS Institute Inc., Cary, NC, USA).[21 22]

Adjusted geometric mean values of CC16/creatinine were calculated using the GLM procedure in SAS. For this, individual mean values for ln(CC16/creatinine) and level of spirometric lung function (FEV_1/FVC) were calculated first.

For the exposure variables, lag 0 was defined as the 24 hour period from the previous day noon to the noon of the day of the clinical visit. The five day average was calculated as the mean of lags 0–4.

For the analyses an association between particulate air pollution and CC16, a basic model (GAM) for each panel was built first in S-Plus separately. The following covariates were considered: a dummy for each subject, long term time trend, temperature (lags 0–3), relative humidity (lags 0–3), barometric pressure (lags 0–3), and the weekday of the visit. The basic model was build by entering covariates into the model one by one according to the order above. In each step the association of the lastly entered covariate was evaluated and the most appropriate form of the covariate was included in the following steps. The shape and lags of these covariates were explored using non-parametric functions based on locally weighted least squares, starting from a span of 0.3. Criteria for building the basic model were AIC and exposure-response plots. At each phase the model with the lowest AIC was selected.[15]

Based on the shape of the association explored in S-Plus, variables were entered in the final basic model as linear terms or as both linear and squared terms. The basic model for the Amsterdam panel included linear variables for time trend, temperature (lag 1) and relative humidity (lag 3), linear and squared terms for barometric pressure (lag 1), and weekday as a categorical variable. The basic model for the Erfurt panel included linear terms for time trend, relative humidity (lag 2) and barometric pressure (lag 0), linear and squared terms for temperature (lag 1), and weekday as a categorical variable. The basic model for the Helsinki panel included linear terms for time trend, temperature (lag 3), relative humidity (lag 0), and barometric pressure (lag 3), and weekday as a categorical variable.

In final statistical analyses, individual pollutants were added to the basic model one at a time. A mixed model was used (PROC MIXED in SAS) taking into account repeated

Table 1 Description of the final study population

	Amsterdam	Erfurt	Helsinki
No. of subjects	33	46	42
No. of urinary samples	376	471	402
Sex, n (%)			
Female	9 (27)	3 (7)	18 (43)
Male	24 (73)	43 (93)	24 (57)
Age, years, mean (SD)	70.8 (8.5)	64.5 (8.1)	68.0 (6.4)
Body mass index, kg/m^2, mean (SD)	27.1 (3.3)	27.3 (2.5)	28.9 (4.1)
Asthma, n (%)	1 (3)	0 (0)	8 (19)
COPD*, n (%)	7 (21)	1 (2)	0 (0)
Chronic bronchitis, n (%)	4 (12)	2 (4)	3 (7)
Emphysema, n (%)	2 (6)	1 (2)	1 (2)
Chronic respiratory disorder†, n (%)	16 (48)	19 (41)	29 (69)
FEV_1/FVC, %, mean (SD)	69.8 (9.7)	75.7 (6.5)	75.0 (7.2)
Smoking, n (%)			
Currently non-smoker	33 (100)	46 (100)	42 (100)
Never-smoker	4 (12)	9 (20)	18 (43)
Ex-smoker	29 (88)	37 (80)	24 (57)
Environmental tobacco smoke at home, n (%)	4 (12)	8 (17)	0 (0)
Regular daily medication, n (%)			
Bronchodilator	2 (6)	1 (2)	2 (5)
Inhalable corticosteroids	2 (6)	1 (2)	8 (19)

*COPD, chronic obstructive pulmonary disease.
†Chronic respiratory disorder: diagnosis of asthma, COPD, chronic bronchitis, emphysema, or a report of presence of cough, phlegm, or wheeze not associated with colds

observations and assuming constant correlation within a subject.

A pooled effect estimate was calculated as a weighted average of the centre specific estimates using the inverse of the centre specific variances as weights. The heterogeneity between centres was tested with a χ^2 test.[23] When significant heterogeneity ($p < 0.1$) between the centres was observed, a pooled effect estimate was calculated using a random effects model.[24]

To further explore the association between particulate air pollution and urinary CC16, subgroup analyses were done. These subgroups included gender, not having environmental tobacco smoke at home, being an ex-smoker or never-smoker, having a chronic respiratory disorder (a questionnaire report of a doctor diagnosed asthma, COPD, chronic bronchitis, or emphysema, or presence of cough or phlegm, or wheeze without a cold), and having a doctor diagnosed asthma. The last was possible only in the Helsinki panel due to the low number of subjects with a doctor diagnosed asthma in the other centres.

Two-pollutant models were also explored. In addition, models without adjustment for relative humidity and barometric pressure were examined.

RESULTS

A total of 1352 urinary samples were obtained, and after exclusions, the results of 1249 samples are used in the present analyses. The male subjects had higher CC16 concentration than the female subjects (table 2). The unadjusted mean (SD) urinary CC16 concentrations of the male subjects were 30.9 (56.6), 51.5 (83.0), and 30.8 (40.7) μg/l in Amsterdam, Erfurt, and Helsinki, respectively. The corresponding values for female subjects were 5.5 (7.4), 3.6 (4.4), and 16.1 (24.2) μg/l. Subjects with a diagnosis of asthma had a lower CC16 level than those without (table 2). CC16 concentration increased with age (data not shown). Body mass index and the level of FEV_1/FVC were not associated with urinary CC16 concentration (data not shown).

Table 2 Geometric mean values of (CC16/creatinine) (μg/g) adjusted for all variables listed in the table, and centre, age, body mass index, and level of spirometric lung function (as FEV_1/FVC)

	Yes	No	p value*
Female	4.2		
Male	9.4		0.01
Having asthma	3.9	10.2	0.04
Having COPD	7.3	5.6	0.59
History of myocardial infarction	6.3	6.4	0.99
Current exposure to environmental tobacco smoke at home	5.0	7.9	0.22
Being ex-smoker	5.3	7.6	0.19

*Between the two groups.

The number concentrations of ultrafine particles (NC 0.01–0.1) were rather similar in all study centres, whereas the particle mass concentrations differed from each other; in particular, Helsinki had lower values than Amsterdam and Erfurt (table 3).

There was a low correlation between ultrafine particles and $PM_{2.5}$ in Amsterdam and Helsinki, whereas these two particle measures correlated more strongly in Erfurt (table 4). In all centres, accumulation particles and $PM_{2.5}$ were highly correlated.

In the pooled analyses, no significant associations were found between particulate air pollution, NO_2, CO, and urinary CC16 concentrations, but the estimates tended to be positive (table 5). Significant heterogeneity was found between the centres, however. Ultrafine particles were not significantly associated with urinary CC16 concentration in any of the centres. Increased $PM_{2.5}$ and NC 0.1–1 concentrations were associated with increased concentration of urinary CC16 in the Helsinki panel. The shape of the association was close to linear (fig 1). In Helsinki, excluding the days with $PM_{2.5}$ levels above the 95th centile of pollution had little effect on the effect estimates of the lag 3 (estimate: 21.4%, 95% confidence interval (CI) −0.6% to 43.3%) and five day average (36.0%, 95% CI 2.3% to 69.7%). The estimates for lags 0–2 became somewhat smaller (lag 2: 12.9%, 95% CI −10.3% to 36.2%). In Amsterdam and Erfurt, no significant associations were observed between $PM_{2.5}$ or NC 0.1–1 and urinary CC16.

In the stratified analyses, the pooled estimates were non-significant, and significant heterogeneity between the centres existed (table 6). In Amsterdam and Erfurt, there were no

Table 3 Descriptive statistics of 24 hour mean levels of air pollutants and temperature

	n	Mean	Range	25%–75%
NC 0.01–0.1, 1/cm^3				
Amsterdam	216	17338	5699–37195	12614–21322
Erfurt	177	21124	3867–96678	12401–27933
Helsinki	182	17041	2305–50306	11052–20879
NC 0.1–1, 1/cm^3				
Amsterdam	202	2131	413–6413	1212–795
Erfurt	177	1829	303–6848	964–2237
Helsinki	176	1390	344–3782	909–1672
$PM_{2.5}$, μg/m^3				
Amsterdam	228	20.0	3.8–82.2	10.4–23.9
Erfurt	161	23.1	4.5–118.1	10.5–27.4
Helsinki	181	12.7	3.1–39.8	8.1–16.0
NO_2, μg/m^3				
Amsterdam	237	42.7	8.5–93.5	30.8–53.9
Erfurt	177	28.9	6.7–81.7	18.5–36.8
Helsinki	182	31.1	10.7–67.5	22.8–35.5
CO, mg/m^3				
Amsterdam	237	0.6	0.4–1.6	0.5–0.7
Erfurt	176	0.4	0.1–2.5	0.2–0.5
Helsinki	173	0.4	0.1–1.0	0.3–0.6
Temperature, °C				
Amsterdam	237	7.8	−4.0–20.1	4.6–11.6
Erfurt	177	3.7	−7.8–13.6	0.8–6.7
Helsinki	182	−1.7	−24.3–11.5	−4.6–2.2

significant associations between $PM_{2.5}$ and CC16, including the subgroup of subjects with no environmental tobacco smoke at home. In Helsinki, $PM_{2.5}$ was associated with CC16 in male subjects, ex-smokers, and in subjects with chronic respiratory disorders. In Helsinki, there were no subjects with environmental tobacco smoke at home. In the Helsinki panel, most ex-smokers (75%) were men, and thus the effect of gender and smoking status is hard to separate. Half of the subjects with chronic respiratory disorders were male and half female. The significant positive association between $PM_{2.5}$ and CC16 was observed among both genders in this subgroup (data not shown). Among subjects with no chronic respiratory disorders, there were no significant associations between particulate air pollution and urinary CC16 in any of the three panels.

Models without adjustment for relative humidity and barometric pressure were also explored. This did not affect the pollution estimates. In the two-pollutant model analyses for $PM_{2.5}$, adjusting for CO, NO_2, NC 0.01–0.1, or O_3 had little effect on the $PM_{2.5}$ estimates. Similarly, the effect estimates for NC 0.01–0.1 were little affected when adjusting for $PM_{2.5}$. CO, NO_2, and O_3 were not statistically significantly associated with urinary CC16 concentrations in these two-pollutant models.

DISCUSSION

In the present study, concentrations of ultrafine particle numbers, NO_2, or CO were not associated with urinary concentration of CC16. In Helsinki, CC16 concentration increased with increasing levels of $PM_{2.5}$, especially among male subjects and subjects with lung disorders. No such associations were observed in Amsterdam and Erfurt. The pooled estimates tended to be positive, but they all were non-significant and there was significant heterogeneity between the centres.

To our knowledge, this is the first time series study on the association between particulate air pollution and CC16. In

Table 4 Spearman correlations between particulate air pollution, temperature, and relative humidity

	NC 0.1–1.0	$PM_{2.5}$	NO_2	CO	Temperature °C
NC 0.01–0.1, 1/cm^3					
Amsterdam	0.16	−0.15	0.49	0.22	−0.18
Erfurt	0.67	0.62	0.82	0.72	−0.34
Helsinki	0.53	0.14	0.72	0.35	−0.55
NC 0.1–1, 1/cm^3					
Amsterdam		0.80	0.67	0.60	−0.10
Erfurt		0.84	0.82	0.78	−0.36
Helsinki		0.80	0.72	0.51	−0.17
$PM_{2.5}$, μg/m^3					
Amsterdam			0.49	0.58	−0.14
Erfurt			0.82	0.77	−0.44
Helsinki			0.35	0.40	−0.07
NO_2, μg/m^3					
Amsterdam				0.76	−0.49
Erfurt				0.86	−0.42
Helsinki				0.32	−0.29
CO, mg/m^3					
Amsterdam					−0.59
Erfurt					−0.62
Helsinki					−0.08

All correlations >0.14 and <−0.13 statistically significant at p=0.05 level.

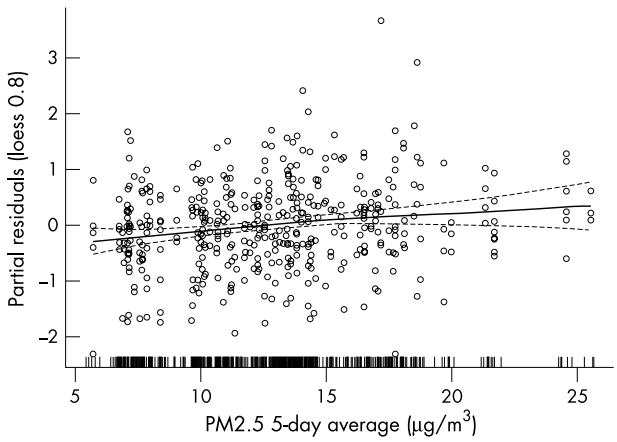

Figure 1 Association between $PM_{2.5}$ (five day average) and urinary CC16 (as ln(CC16/creatinine)) in Helsinki.

experimental studies, it has been shown that CC16 in extrapulmonary fluids is a marker of epithelial permeability.[4–8] In addition, it has been shown that there is short term variation in CC16 levels in relation to variation in exposures such as to ozone and combustion products.[10–12] A transient increase of an average magnitude of 238% in serum CC16 concentration has been reported in firefighters immediately after exposure to smoke. After 10 days the CC16 concentrations were returned to control levels.[10] An acute increase of 38–52% in serum CC16 concentration was observed in firefighters after an exposure to combustion products during an overhaul. A dose-response relation was observed.[11] In cyclists, increased serum levels of CC16 have been reported in association with two hours' exercise during an O_3 episode.[12] In addition, exposure to nitrogen trichloride, a gas used in the air of indoor pools, has been associated with increased levels of CC16 in serum, both in humans and in experimental animals.[13] In rats, increased serum and urinary CC16 concentrations have also been reported after exposure to O_3.[25] In contrast, an exposure to diesel exhaust enriched concentrated $PM_{2.5}$ did not result in increased CC16 levels in serum in rats.[14] In the present study, an increase up to 38.8% in urinary CC16 concentration was observed in Helsinki, the magnitude of which effect is in accordance with the previous studies.

Our study was performed in three cities using the same study protocol. However, only in Helsinki, in which the $PM_{2.5}$ concentrations were the lowest, was a significant effect observed between $PM_{2.5}$ and urinary CC16 concentration. It has been shown among these same study subjects in Amsterdam and Helsinki that the fixed site 24 hour $PM_{2.5}$ measurements correlate well with 24 hour personal exposure. The median Pearson's correlation coefficients between personal and outdoor $PM_{2.5}$ measurements were 0.79 in Amsterdam and 0.76 in Helsinki.[20] Therefore, the $PM_{2.5}$ exposure measurements used in the present analyses describe well the real variations in personal exposure. In addition, exposure to environmental tobacco smoke at home did not confound the associations.

One could argue that the observed association between $PM_{2.5}$ and urinary CC16 is due to chance as it is observed only in one study centre. However, in the ULTRA study, associations between $PM_{2.5}$ and cardiac health endpoints (heart rate variability, ischaemic changes in ECG) have also been found, especially in Helsinki.[26 27] Moreover, the odds ratio for the association between $PM_{2.5}$ and incidence of shortness of breath symptom was larger in Helsinki (1.32) than in

Table 5 Associations between different lags of particulate air pollution, NO_2, CO, and urinary CC16; percentage change (95% CI) in ln(CC16/creatinine) per change in pollutant concentration

	Amsterdam	Erfurt	Helsinki	Pooled estimate
NC 0.01–0.1 (10000/cm^3)				
lag 0	9.1 (−6.5 to 24.7)	1.2 (−6.7 to 9.0)	−1.6 (−14.0 to 10.7)	1.7 (−4.4 to 7.8)
lag 1	1.9 (−13.5 to 17.2)	−4.9(−13.4 to 3.6)	2.7 (−10.3 to 15.7)	−1.8 (−8.3 to 4.6)
lag 2	11.3 (−4.3 to 26.9)	−0.9 (−10.2 to 8.4)	−1.7 (−16.9 to 13.6)	1.5 (−5.6 to 8.6)
lag 3	7.3 (−8.7 to 23.2)	−1.0 (−10.4 to 8.3)	6.3 (−8.4 to 20.9)	2.3 (−4.8 to 9.3)
5-day mean	18.4 (−8.0 to 44.8)	−5.8 (−19.8 to 8.1)	12.4 (−14.0 to 38.9)	1.8 (−9.4 to 13.0)
NC 0.1–1 (1000/cm^3)				
lag 0	1.7 (−6.6 to 9.9)	3.7 (−5.4 to 12.7)	15.5 (0.001 to 30.9)*	4.3 (−1.4 to 10.0)
lag 1	6.6 (−2.0 to 15.3)	1.5 (−7.2 to 10.2)	10.8 (−4.2. 25.8)	5.1 (−0.6 to 10.7)
lag 2	6.0 (−2.2 to 14.1)	2.0 (−5.1 to 9.1)	10.5 (−4.1 to 25.1)	4.5 (−0.5 to 9.6)
lag 3	−0.05 (−7.2 to 7.2)	−1.0 (−7.0 to 5.1)	17.4 (3.4 to 31.4)*	1.6 (−3.5 to 6.7)§
5-day mean	7.8 (−6.2 to 21.9)	0.4 (−10.4 to 11.2)	43.2 (17.4 to 69.0)†	13.1 (−4.3 to 30.5)¶
$PM_{2.5}$ (10 μg/m^3)				
lag 0	2.2 (−3.8 to 8.1)	1.4 (−3.4 to 6.1)	23.3 (6.3 to 40.3)†	2.8 (−1.1 to 6.7)¶
lag 1	3.9 (−2.5 to 10.3)	2.1 (−2.4 to 6.5)	6.4 (−8.2 to 21.1)	2.9 (−0.6 to 6.5)
lag 2	3.0 (−3.6 to 9.5)	−0.1 (−4.4 to 4.2)	20.2 (6.9 to 33.5)†	5.0 (−2.4 to 12.4)¶
lag 3	1.5 (−7.6 to 4.7)	−1.5 (−5.9 to 2.8)	17.6 (4.3 to 30.9)†	1.6 (−4.7 to 7.9)¶
5-day mean	1.9 (−7.0 to 10.9)	0.7 (−5.6 to 7.0)	38.8 (15.8 to 61.8)‡	9.7 (−6.0 to 25.4)**
NO_2 (10 μg/m^3)				
lag 0	3.5 (−2.3 to 9.3)	3.8 (−3.6 to 11.2)	3.2 (−5.1 to 11.4)	3.5 (−0.5 to 7.5)
lag 1	2.3 (−3.4 to 8.0)	2.1 (−5.3 to 9.6)	1.2 (−7.3 to 9.6)	2.0 (−2.0 to 6.0)
lag 2	5.0 (−0.4 to 10.4)	1.1 (−6.0 to 8.3)	−1.0 (−10.3 to 8.4)	2.8 (−1.1 to 6.7)
lag 3	1.4 (−4.1 to 6.9)	−1.2 (−7.5 to 5.1)	9.2 (0.1 to 18.3)*	1.8 (−1.9 to 5.6)
5-day mean	6.3 (−3.3 to 15.9)	0.1 (−10.5 to 10.7)	13.1 (−4.1 to 30.4)	4.9 (−1.6 to 11.5)
CO (mg/m^3)				
lag 0	28.2 (−25.5 to 81.8)	3.3 (−25.0 to 31.5)	56.8 (−10.1 to 124)	14.6 (−8.8 to 37.9)
lag 1	30.9 (−29.5 to 91.2)	−9.9 (−40.3 to 20.4)	11.8 (−50.8 to 74.4)	0.4 (−24.4 to 25.2)
lag 2	48.4 (−10.2 to 107)	−8.1 (−42.7 to 26.5)	15.0 (−47.5 to 77.6)	8.1 (−18.7 to 34.9)
lag 3	24.0 (−30.5 to 78.4)	−5.5 (−34.7 to 23.7)	34.5 (−23.5 to 92.5)	6.6 (−16.8 to 30.1)
5-day mean	60.8 (−28.7 to150)	−11.9 (−52.9 to 29.0)	65.0 (−25.2 to 155)	10.1 (−24.2 to 44.4)

*, $p<0.05$; †, $p<0.01$; ‡, $p<0.001$.
§Test for heterogeneity between centres, $p<0.1$.
¶Test for heterogeneity between centres, $p<0.05$.
**Test for heterogeneity between centres, $p<0.01$.

Table 6 Association between $PM_{2.5}$ (lag 2) and urinary CC16; stratified analysis; percentage change (95% CI) in ln(CC16/creatinine) per 10 μg/m^3 change in $PM_{2.5}$

	Amsterdam	Erfurt	Helsinki	Pooled estimate
Male subjects	5.6 (−3.1 to 13.9)	0.8 (−3.7 to 5.2)	30.6 (14.2 to 47.0)‡	9.9 (−2.8 to 22.6)¶
Female subjects	−1.0 (−11.6 to 9.6)	na	6.2 (−16.3 to 28.8)	0.3 (−9.2 to 9.8)
No environmental tobacco smoke at home	3.6 (−3.3 to 10.4)	−0.4 (−5.2 to 4.4)	20.2 (6.9 to 33.6)§	5.3 (−2.5 to 13.1)¶
Ex-smokers	4.5 (−2.5 to 11.4)	−1.7 (−6.6 to 3.2)	30.6 (14.1 to 47.1)‡	8.8 (−5.1 to 22.7)**
Never smokers	na†	7.6 (−2.3 to 17.5)	6.8 (−15.6 to 29.3)	7.5 (−1.4 to 16.4)
Subjects with chronic respiratory disorder*	6.4 (−4.2 to 17.1)	−2.1 (−9.9 to 5.8)	27.9 (11.9 to 43.9)‡	8.9 (−4.2 to 22.0)**
Asthma	na	na	29.4 (−12.6 to 71.4)	na

*Chronic respiratory disorder: asthma, COPD, chronic bronchitis, or emphysema, or a report of presence of cough, phlegm, or wheeze not associated with colds.
†na, not applicable.
‡$p<0.001$; §$p<0.01$.
¶Test for heterogeneity between centres, $p<0.05$.
**Test for heterogeneity between centres, $p<0.01$.

Amsterdam (1.16) and Erfurt (1.08).[28] These observations support the fact that the observed harmful effect is not due to chance. Further, the association between $PM_{2.5}$ and urinary CC16 was observed among subjects with chronic lung disorders who are thought to be more susceptible to respiratory effects of air pollution than healthy subjects. The reason why these effects were observed only in Helsinki is not yet understood. The composition of particulate air pollution differs between the centres. In context with this same study, we have shown that long range transported particles form a larger proportion of $PM_{2.5}$ in Helsinki (50%) than in the two other centres (32% and 34%).[29] The correlations between different measures of particulate air pollution were also clearly higher in Erfurt than in Amsterdam or Helsinki, suggesting a difference in the air pollution mixture or meteorological conditions. There are also climatic differences during winter time between the centres, Helsinki being clearly the coldest city of the three. There are also some differences in the panel characteristic. In addition to a higher prevalence of chronic lung disorders, subjects in the Helsinki panel experienced more ischaemic changes in the ECG during a light exercise test compared to the other two panels, suggesting that the disease status was different in subjects in Helsinki.[27]

The mean urinary concentrations of CC16 agreed well with the previous studies.[4] Asthmatic subjects have a lower baseline concentration as well as those who have previously been smokers, due to decreased density of CC16 positive cells in the lungs. Male subjects have a higher concentration because of prostate gland secretion of CC16.[4] However, it is not likely that prostate gland secretion of CC16 could confound the present analysis because it is unlikely that daily variations in prostate gland secretion are correlated with daily variation in particulate air pollution.

The present results from Helsinki suggest that exposure to particulate air pollution may lead to increased epithelial barrier permeability in lungs. However, the association was observed only in one study centre out of three. The reason for this is not yet understood, but it can be due to differences in the panel characteristics, climate, and composition of ambient particulate air pollution between the study centres.

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