Inflammatory markers and particulate air pollution: characterizing the pathway to disease

Ariana Zeka,[1]* James R Sullivan,[1] Pantel S Vokonas,[2] David Sparrow[2] and Joel Schwartz[1]

Background	Increased concentrations of particles in air have been related to changes in inflammatory markers that in turn are hypothesized in mediating the particle effects on cardiovascular disease. The present work examined this association in an elderly cohort in the Greater Boston area and addresses the relative role of particles from different sources.
Methods	The study included 710 subjects, active members of the VA Normative Aging Study cohort with measurements of blood markers. Concentrations of particle number (PN), black carbon (BC), fine particulate matter ($PM_{2.5}$), and sulphates were measured at a central site near the examination site.
Results	Positive associations were found between traffic-related particles (PN and BC) and inflammatory markers, but only suggestive associations were found with exposures to $PM_{2.5}$ and sulphates. The particle effect on the inflammatory markers was greater among subjects older than 78 years and among obese. A suggestion for a greater effect of particles on inflammatory markers among *GSTM1*-null subjects and non-users of statin drugs was also seen.
Conclusions	The findings of the study support the hypothesis that particles can induce cardiovascular disease through inflammatory pathways, suggestive of a greater toxicity of traffic-related particles.
Keywords	Air pollution, C-reactive protein, blood sedimentation, fibrinogen, inflammation, sulphates, white blood cells

The consistent evidence for the positive association between airborne particles, morbidity and mortality has led several investigators to explore the underlying mechanisms that lead to exposure-related responses. It has been hypothesized that particles in air induce inflammation, which in turn could lead to cardiovascular effects.[1–7] High concentration of particles in air have been associated with increased blood clotting factors such as serum levels of fibrinogen,[1,8–11] inflammatory factors such as C-reactive protein,[6,12,13] increased plaque formation and decreased plaque stability,[14] vasoconstriction, impaired flow mediated dilation, temporary coronary occlusion,[15–17] and ischaemia,[14] all suggested as potential mechanisms leading to cardiovascular morbidity.

Attention has also recently focused on the relative toxicity of particles from different sources and the potential that different biological pathways are influenced by different types of particles. In particular some studies have suggested greater effects of particles from traffic sources than from coal burning power plants (sulphates) on some cardiovascular endpoints such as heart rate variability,[18] while for others, such as flow mediated dilation, sulphates have been equipotent.[17] Identifying relative toxicity from different sources of particles is particularly important in allowing the best targeting of control strategies to improve public health.

Given this evidence, the present study was carried out with the hypothesis that increases in particle concentrations are related to changes in inflammatory markers, such as white

---

[1] Exposure, Epidemiology, and Risk Program, Department of Environmental Health, Harvard School of Public Health, Boston, MA, USA.

[2] VA Normative Aging Study, VA Boston Healthcare System and the Department of Medicine, Boston University School of Medicine, Boston, MA, USA.

* Corresponding author. Exposure, Epidemiology, and Risk Program, Department of Environmental Health, Harvard School of Public Health, 401 Park Drive, Suite 415 W, PO Box 15677, Boston, MA 02115, USA. E-mail: azeka@hsph.harvard.edu

blood cell (WBC) count, C-reactive protein levels and sediment rate, and thrombotic factors such as serum levels of fibrinogen, that may be potential risk factors for cardiovascular disease. The study also aimed to evaluate the relative toxicity of sulphate particles vs particles from traffic on these intermediary markers of cardiovascular disease.

Methods

Study population

The present work included subjects from an ongoing study of aging established by the Veterans Administration in 1963, enrolling 2280 community-dwelling men from the Greater Boston area (ages 21–80 years), who were confirmed to be free of known chronic medical conditions (The Normative Aging Study—NAS).[19] Participants in the study are examined every 3 years. For the present work we included 710 currently active subjects who were examined between November 14, 2000 and December 31, 2004.

The examination of the study subjects took place in the study centre, in the morning, after an overnight fast and abstinence from smoking. Blood drawings were taken at that time by a technician. Later in the morning, following the clinical examination, the subjects' height and weight were also measured. On the same day, subjects were asked to respond to a questionnaire concerning cigarette smoking, alcohol consumption, medical conditions, and medication use. The responses to this questionnaire were then confirmed by a physician interview. Temperature of the room where the examination was performed was kept within 24–25°C.

Markers of inflammation

The markers of inflammation/thrombosis obtained for this study were WBC count, C-reactive protein, sediment rate, and fibrinogen. The WBC count was performed using a standard automated electrical impedance hematology analyzer (Coulter Counter Model S-Plus Series—Coulter Electronics). C-reactive protein concentrations were determined using an immuno-turbidimetric assay on the Hitachi 917 analyzer (Roche Diagnostics—Indianapolis, IN), using reagents and calibrators from Denka Seiken (Niigata, Japan).[20] Sediment rate was measured with the Wintrobe Method, which is performed in a 100 mm tube (Wintrobe tube) containing oxalate as the anticoagulant.[21] MDA Fibriquick method was used for fibrinogen level measurements.[22]

Air pollution

A key aim of this study was to examine the effects of particles from different sources. We used continuous measurements of concentrations of particle number (PN), black carbon (BC), and particulate matter of aerodiameter ≤2.5 μm ($PM_{2.5}$), and 24 h integrated measurements of particulate sulphate (SO_4^{2-}), all measured at the Harvard School of Public Health monitoring sites: on the roof of the School of Public Health Building 2 (677 Huntington Avenue, Boston, MA), and Countway Library (10 Shattuck Street, Boston, MA), ~1 km away from the examination site. Because the two sites are within 30 m of each other, we combined the data received from either one.

There was no period when we had data from both sites simultaneously.

PN (particle count) was measured using a TSI Condensation Particle Counter (CPC) Model 3022a, and is a measure of the number of particles (0.007–3 μm) detected per cubic centimetre. BC measurements were carried out using a Magee Scientific Aethalometer (AE-16). Concentration of BC was expressed as ng/m^3. Measurements were taken every 5 min, and then averaged to 30 min concentrations. All BC concentrations <400 ng/m^3 and >10 000 ng/m^3 were excluded from the original exposure data. $PM_{2.5}$ was measured using a Tapered Element Oscillating Microbalance (TEOM), Rupprecht & Pataschnick 1400A model, operated at 50°C with two 4LPM-$PM_{2.5}$ impactors before the inlet. Measurements were recorded in 30 min averages and expressed in $μg/m^3$. Because TOEM monitors can lose semi-volatile particles owing to heating of the sample filter to 50°C, a season-specific calibration factor was applied to compensate for this loss.[23] Particulate sulphate (SO_4^{2-}) was measured using the Harvard/EPA Denuder System (HEADS), which samples inorganic gaseous and particulate species in air. Concentrations of SO_4^{2-} were calculated from the net sulphate ion concentration on the Teflon filter and the net volume of ambient air sampled. Samples were collected for 24 h periods (9 a.m. to 9 a.m.), and the date given was the starting date of the sample. Concentrations of SO_4^{2-} were expressed in $μg/m^3$.

Depending on the mechanism, the effects of airborne particles may take time to develop, and many studies have implicated exposure over periods from several days[24] to more than a month[25] as relevant to cardiovascular mortality. An animal study in Boston, using chemiluminescence, showed that following transfer of animals from Boston air to filtered air, reactive oxygen species in the heart, lung, and liver declined continuously for 5 days (end of experiment).[26] This suggests long averaging times may be appropriate. The MONICA study[6] showed the association between particles and C-reactive protein with a 6 day average. Therefore, one would also expect to see similar associations between longer-term average exposures and these intermediary markers of disease. Consequently, we calculated moving averages for each pollutant concentration for the 48 h, 1 and 4 weeks preceding each subject's examination, using hourly measurements for PN, BC, and $PM_{2.5}$, and daily measurements for SO_4^{2-}. Lagged exposure concentrations were matched on the hour of the blood measurement for PN, $PM_{2.5}$, and BC and on the day of examination for SO_4^{2-}.

Missing daily values of each pollutant were estimated using non-linear regression models that included local meteorological data obtained from the United States Surface Airways and Airways Solar Radiation hourly data (including extinction coefficient,[27] a measure of light scattering by fine particles).

Statistical methods and analyses

Measurements of markers of inflammation were natural log-transformed to normalize the distribution of the outcome and stabilize the variance. Linear regression analyses were carried out to evaluate the relationship between inflammatory markers and different lagged averages of air pollutants. We controlled all analyses for important factors such as age

(quadratic function), body mass index (BMI—calculated as weight in kilograms/height in meters square—kg/m^2), season (used as an indicator variable, with winter the reference). We controlled additionally for the effect of weather by including in models temperature, relative humidity, and barometric pressure. We also examined non-linear effects of age, BMI, and season using non-parametric regression models.

We assessed potential confounding by use of anti-hypertensive or cardiac medications; the presence of hypertension defined as systolic blood pressure of ⩾140 mm Hg, diastolic blood pressure of ⩾90 mm Hg, or reported use of anti-hypertensive medication[28]; smoking (indicator variable—current, former vs never); alcohol drinking (yes/no); and fasting glucose levels.

We examined for effect modification of the air pollutant-marker associations by age (dichotomized into ⩾78 years—the cut point for the upper quartile of age distribution among study subjects, or <78 years); obesity (BMI ⩾ 30 kg/m^2); use of anti-hypertensive and cardiac medications including anti-arrhythmics, angiotensin-converting enzyme inhibitors, beta-blockers, and calcium-channel blockers; whether the subject was homozygous for the deletion of glutathione-S-transferase M1 (GSTM1)-null statin drugs use; high blood pressure defined as either diastolic blood pressure >82 mm Hg, or systolic blood pressure >140 mm Hg (75th percentiles of the distribution among study subjects); and hypertension defined as above.

We tested for statistically important differences between effect estimates of strata of a potential effect modifier (for example the difference between the two categories of age) by calculating the 95% confidence interval (95% CI) as shown below:

$$\left(\hat{Q}_1 - \hat{Q}_2\right) \pm 1.96\sqrt{S\hat{E}_1 \pm S\hat{E}_2}$$

where, $\hat{Q}_1$ and $\hat{Q}_2$ are the estimates for the two categories of an effect modifier and $S\hat{E}_1$ and $S\hat{E}_2$ their respective standard errors.[29,30] Regardless of significance, we considered modification of effect by a factor of 2 or more to be important and discuss it as such.

Two-pollutant models were also carried out to examine the sensitivity of the marker-pollutant associations in the presence of other pollutants.

Results

The study included elderly subjects with a mean age of 73 years (Table 1). Almost a quarter of the study subjects were above the defined limit for obesity (BMI ⩾ 30 kg/m^2). The distribution of the inflammatory markers was skewed—hence were log-transformed for the subsequent analyses. About 53% of the study subjects were currently using anti-hypertensive or cardiac medication, and 36% were users of statin drugs. GSTM1 was present in 41% of the study subjects. Current smokers were only a small proportion in our study, while almost two-thirds of subjects had a smoking history.

The distribution of air pollutants in our study is presented in Table 2. Particle concentration distributions for the pollutants were moderately skewed. Sufficient variability was present for concentrations of the four pollutants among the study

Table 1 The VA Normative Aging Study subjects' characteristics between November 2000 and December 2004

	Mean	SD	25th%	50th%	75th%	90th%
Age (years)	73.0	6.7	68	73	78	82
BMI (kg/m^2)	28.3	4.1	25.6	27.8	30.4	33.4
White blood cell count (000/mm^3)	6.4	3.2	5.2	6.0	7.1	8.4
Fibrinogen (mg/dl)	348.5	88.5	294	332	387	457
C-reactive protein (mg/dl)	0.35	0.85	0.08	0.17	0.35	0.65
Sediment rate (mm/h)	13.0	9.4	6	11	20	27

	n	% of total				
Use of anti-hypertensive and cardiac medication	373	52.5				
Anti-arrhythmics	76	10.7				
ACE-inhibitors[a]	57	8.0				
Beta-blockers	273	38.5				
Ca+ channel blockers	92	13.0				
Statin use	256	36.1				
GSTM1 present	292	41.1				
Smoking status						
Never smoker	195	27.5				
Current smoker	32	4.5				
Former smoker	460	65.0				
Missing information	21	3.0				

[a] Angiotensin converting enzyme.

subjects. No important correlations were observed between pollutant pairs.

Changes in inflammatory markers and thrombotic risk were measured by the change in fibrinogen, C-reactive protein, sediment rate, and WBC count in relation to changes in concentrations of particles (Table 3). All the results are reported as percentage increase in the marker for 1 SD increase in the concentration of the air pollutant.

PN concentration in the prior 48 h was associated with increased fibrinogen levels (4.19%; 95% CI = 2.04–6.34%), as were concentrations the week before (2.14%; 95% CI = 0.05–4.23%). A suggestive finding was seen for an association with C-reactive protein levels for PN concentrations 48 h prior. For sedimentation rate, in contrast, the only association was with the average over the preceding 4 weeks (43.65%; 95% CI =15.47–71.83%). No effect was found for this pollutant and WBC count.

Effects of BC concentrations on fibrinogen and sediment rate, and a suggestive effect on C-reactive protein were consistently seen with a 4 week moving average, while no effect was seen for WBC count. The relative change for 1 SD change in the concentration level to this pollutant for fibrinogen was 1.78% (95% CI = 0.19–3.36%). For C-reactive protein, a marginally precise association was seen (5.41%; 95% CI = −1.00 to 11.81%), whereas a statistically important association was seen for sediment rate (21.65% increase; 95% CI = 1.48–41.82%).

Table 2 The distribution of particle components for lagged averaged concentrations at 48 hours between November 2000 and December 2004

Pollutant	Total number of measurements[a]	% Missing[b]	Mean	SD	50th%	75th%	90th%	PN[d]	BC[e]	PM$_{2.5}$	SO$_4^{2-}$
								Correlations between pollutant pairs[c]			
Particle number (count/cm^3)	35 101	10.4	27 160	15 851	24 200	37 153	49 390	1.0	0.30	−0.02	−0.15
Black carbon (ng/m^3)	43 320	3.0	0.77	0.63	0.61	1.00	1.51		1.0	0.52	0.30
PM$_{2.5}$ (µg/m^3)	42 391	8.1	11.16	7.95	9.39	14.57	21.48			1.0	0.50
SO$_4^{2-}$ (µg/m^3)	1491	0	2.29	1.62	1.84	2.81	4.10				1.0

[a] Total number of measurements after filling in missing values as described in text.
[b] Percentage missing from the total number of measurements after filling in missing values.
[c] Spearman correlation coefficients.
[d] Particle number.
[e] Black carbon.

Table 3 The association between thrombotic and inflammatory markers and concentrations of particle components

Pollutant	Fibrinogen %[a]	Fibrinogen 95% CI[a]	C-reactive protein %	C-reactive protein 95% CI	Sediment rate %	Sediment rate 95% CI	WBC count %	WBC count 95% CI
Particle number								
48 h	**4.19**	**2.04–6.34**	7.29	−1.63 to 16.21	21.24	−6.94 to 49.42	0.84	−1.64 to 3.32
1 week	**2.14**	**0.05–4.23**	−2.33	−10.96 to 6.30	23.45	−4.13 to 51.03	−1.07	−3.51 to 1.37
4 weeks	0.12	−2.00 to 2.24	−7.16	−16.31 to 1.98	**43.65**	**15.47–71.83**	0.26	−2.25 to 2.78
Black Carbon								
48 h	0.84	−0.63 to 2.31	4.51	−2.03 to 11.06	−4.56	−25.55 to 16.43	−0.63	−2.45 to 1.19
1 week	0.60	−0.95 to 2.15	1.07	−5.55 to 7.68	1.98	−18.15 to 22.11	−0.13	−1.87 to 1.60
4 weeks	**1.78**	**0.19–3.36**	5.41	−1.00 to 11.81	**21.65**	**1.48–41.82**	−0.55	−2.36 to 1.26
PM$_{2.5}$								
48 h	−0.18	−1.93 to 1.57	−4.88	−13.29 to 3.53	−16.91	−43.66 to 9.84	−3.18	−5.39 to −0.97
1 week	−1.39	−3.46 to 0.67	−1.37	−10.44 to 7.71	−18.89	−47.48 to 9.70	−0.51	−3.02 to 2.00
4 weeks	1.14	−0.60 to 2.88	4.36	−3.25 to 11.96	**24.93**	**0.68–49.18**	−0.03	−2.17 to 2.10
SO$_4^{2-}$								
48 h	0.60	−1.23 to 2.42	1.57	−7.13 to 10.27	4.05	−23.26 to 31.36	−0.12	−2.35 to 2.11
1 week	0.03	−1.93 to 1.99	0.21	−8.27 to 8.69	−5.87	−32.39 to 20.64	−0.48	−2.87 to 1.90
4 weeks	1.12	−0.52 to 2.77	5.29	−1.91 to 12.49	−1.60	−25.24 to 22.04	0.75	−1.30 to 2.80

In bold are presented precisely estimated effects.
[a] Estimates are presented as percentage increase in inflammatory marker per 1 SD increase in the pollutant. All models are adjusted for Age, Age^2, BMI, and season (spring, summer, and fall as indicator variables—reference winter).

High concentrations of PM$_{2.5}$ 4 weeks prior showed a suggestive positive association with fibrinogen levels (1.14% increase; 95% CI = −0.60 to 2.88%) and a positive association with sediment rate (24.93% increase; 95% CI = 0.68–49.18%).

No statistically precise associations were found between SO$_4^{2-}$ concentrations and any of the inflammatory outcomes. However, suggestive evidence was seen for an association with fibrinogen (1.12%; 95% CI = −0.52 to 2.77%) and C-reactive protein (5.29%; 95% CI = −1.91 to 12.49%) for concentrations 4 weeks prior.

The use of non-parametric models with spline terms for age, BMI, and season, control for weather, or the presence of other pollutants in the model did not change the effects of exposure on inflammatory markers. We found no confounding of the marker–pollutant associations by use of anti-hypertensive or cardiac medications, the presence of hypertension, smoking, alcohol drinking (yes/no), or fasting glucose levels.

Effect modification

Although no statistically important difference was found for any category of effect modifiers, there was suggestion that older age (78 years or older) increased the effect of PN concentrations on fibrinogen and C-reactive protein levels (Table 4). Almost all the effect of BC on sediment rate was seen in the younger group.

About a 2-fold relative effect was seen for the association of PN with fibrinogen among the obese. A ~4-fold difference was seen for the association between BC and C-reactive protein in the presence of obesity. There was also a suggestion for effect modification by obesity of the association between BC and sediment rate.

We observed a suggestive greater effect of BC on C-reactive protein among *GSTM1*-null subjects (9.73%; 95% CI = 1.48–17.98%) vs *GSTM1*-present subjects (−2.97%; 95% CI = −14.05 to 8.10%) for concentrations 4 weeks prior. A similar,

Table 4 Effect modification of the inflammatory marker–traffic-related air pollutant association by age and obesity

Inflammatory markers and air pollutants	Age				BMI			
	<78 years old		≥78 years old		<30		≥30	
	%[a]	95% CI[a]	%	95% CI	%	95% CI	%	95% CI
Fibrinogen								
Particle number								
48 h	3.98	1.55–6.41	5.18	0.71–9.66	**3.50**	0.97–6.04	**6.64**	2.45–10.84
1 week	**1.24**	−1.09 to 3.58	**5.13**	0.66–9.61	2.14	−0.19 to 4.47	1.93	−2.76 to 6.62
Black carbon								
4 weeks	1.65	−0.10 to 3.40	1.70	−1.91 to 5.32	1.56	−0.18 to 3.30	2.71	−0.99 to 6.40
C-reactive protein								
Particle number								
48 h	**5.45**	−4.99 to 15.89	**10.78**	−6.43 to 27.99	7.88	−2.96 to 18.72	6.32	−9.55 to 22.20
Black Carbon								
4 weeks	6.32	−0.86 to 13.49	5.20	−8.94 to 19.34	**3.41**	−3.92 to 10.75	**14.16**	0.68–27.65
Sediment rate								
Particle number								
4 weeks	37.64	11.19–64.09	27.25	−27.08 to 81.57	37.33	10.04–64.61	31.18	−18.21 to 80.57
Black Carbon								
4 weeks	**24.58**	5.07–44.09	**0.72**	−35.15 to 36.60	**15.50**	−3.36 to 34.35	**30.79**	−10.05 to 71.64

In bold are presented differences in effects of a factor of 2 or greater.

[a] Estimates are presented as percentage increase in inflammatory marker per 1 SD increase in the pollutant. All models are adjusted for Age, Age^2, BMI, and season (spring, summer, and fall as indicator variables—reference winter).

but weaker signal was seen for PN (data not shown). A stronger effect of BC on sediment rate was seen among non-users of statins (36.01%; 95% CI = 13.88 to 58.13%) compared with users (−12.29%; 95% CI = −39.13 to 14.55%).

Increased diastolic or systolic blood pressure, or current use of anti-hypertensive and cardiac medication did not modify the associations between inflammatory markers and air pollutants (data not shown).

Discussion

The present study found effects of ambient concentrations of traffic-source particles and suggestive findings for effects of sulphates on intermediary markers of cardiovascular disease when averaged over medium timescales (generally several days to few weeks). The findings of this study extend previous epidemiological observations by examining the role of particles from different sources. Sulphates showed weakly suggestive evidence for an association with increased level of fibrinogen and C-reactive protein, and no association with sediment rate or WBC count. Traffic particles, in contrast, were associated with thrombotic and inflammatory markers. Such a finding supports previous evidence that exposure to increased concentrations of particles is associated with observable changes in response of inflammatory markers, which may in part explain the observed association between air pollution and cardiovascular events. None of these associations were confounded by other factors influencing cardiovascular disease or changed for non-linear terms of age, BMI, season, or control for weather.

We found a positive effect for PN concentrations with fibrinogen, a thrombotic factor, a positive effect for sediment rate, and only a suggestive association with C-reactive protein, but not with WBC count—inflammatory markers, although the lag structure was less consistent. BC showed similar positive associations with a consistent lag structure. Similarly, PM$_{2.5}$ showed a suggestive positive association with fibrinogen and a positive association with sediment rate for concentrations 4 weeks prior.

The suggestive finding for an association of sulphates with thrombotic and inflammatory potential, via their weak correlation with fibrinogen and C-reactive protein levels, adds to the recent evidence showing an association with flow mediated dilation[17] and may partially suggest some mechanistic pathway for the observed association between cardiovascular mortality and sulphate levels.[31] However, since the finding of our study was weak for this pollutant, caution may be used in interpretation.

PN including the fine range particles represents freshly generated local traffic particles. In contrast, BC can represent both aged and fresh traffic particles, and includes transported as well as locally generated traffic particles. For example, using back trajectory analyses to determine the location of air masses 36 h before measurements of pollution concentrations in Boston, we found the highest BC concentrations in Boston occurred when the air masses had come from the New York metropolitan area (results not shown). The finding that the association with PN was generally with the shorter averaging time may be related to this distinction, but may also be due to chance. SO_4^{2-} concentrations are markers for transported particles from coal burning power plants. Supporting this, back trajectory analyses showed higher concentrations of sulphates with air masses coming from the south-west, the coal burning facilities.

Although much remains to be understood about the mechanisms leading to cardiovascular disease and mortality from exposure to particles, studies have shown that systemic inflammation[6,32–34] and pro-thrombotic activity may be key factors of the physio-pathological processes leading to such events. Such processes can occur either through the release of mediators—pro-inflammatory cytokines due to injury of the pulmonary alveoli[33,35,36] or the direct activation of blood cells due to deposition of particles in the lung, or due to the infiltration of the smallest particles directly into the blood stream,[37–39] where these may react with platelets and the endothelium.[7,40,41] In addition, low-grade inflammation caused by continuous exposure to air particles has been hypothesized to be associated with increased blood coagulability, a risk factor for cardiovascular disease.[40,42]

Increased fibrinogen levels, a thrombogenic factor, have been linked to increased risk of cardiovascular disease, partly through increased plasma viscosity and partly as an independent factor.[1] Toxicology and epidemiological studies have shown the link between increased fibrinogen levels or plasma viscosity and high concentrations of air pollutants.[1,3,9–11] Positive associations between C-reactive protein and particulate matter have been observed by epidemiological studies.[6,40,43] A positive correlation of C-reactive protein and coronary artery disease, which could be explained by the atherogenic effects of chronic inflammation, is well known.[44,45] Reports have suggested that C-reactive protein may be an independent predictor of cardiovascular disease in both men and women.[46] Although C-reactive protein levels in our study were close to what is considered clinically normal in a healthy human,[47] the association we found with ambient particles may suggest possible cardiovascular risk at low-level inflammation. This was also supported by the association found with moderately elevated sediment rate among the study subjects. A study by Peters[6] showed an association between air pollution and C-reactive protein levels comparable with that of our study.

The lagged structure of exposure effect on the inflammatory and vascular markers in the present study suggests delayed effects of particles on the endpoint outcomes—cardiovascular disease morbidity and mortality. Lagged exposure effects on these mediatory mechanisms were also seen in the MONICA study (6). Further epidemiological evidence on delayed effects of air pollution on morbidity and mortality has been limited. The only study that looked at longer lags of exposure in association with mortality events by Zanobetti *et al.*[25] found increased cardiovascular mortality with particle exposures occurring more than 1 month before the event.

The effects of traffic particles on the inflammatory markers were modified by age and obesity in our study. Although the mean age among our study subjects was 73 years, therefore an elderly population, we were still able to see an indication of effect modification by older age (those >78 years old). In support of this finding, several studies have reported a greater effect of air pollution among the elderly, possibly explained by the greater susceptibility in these population sub-groups.[48–53]

Obesity, defined in our study as a BMI of 30 kg/m^2 or greater, was associated with a 2- to 4-fold increased effect of traffic-related particulate pollution on the inflammatory markers. Obesity represents a pro-inflammatory state that may be related to the release of pro-inflammatory cytokines from fat cells,[54–56] higher circulating C-reactive protein,[57] or as result of an inflammatory response to the poor ventilation and performance of the lungs due to mechanical constriction of the bronchial tree.[58,59] Considering these mechanisms, overweight may increase susceptibility to air pollution by enhancing the response to inflammatory stimuli. In support of these suggested mechanisms, higher C-reactive protein levels were seen among obese subjects in our study (mean C-reactive protein = 0.42 mg/dl; SD = 0.52 mg/dl vs mean C-reactive protein = 0.33 mg/dl; SD = 0.93 mg/dl, among those with BMI < 30 kg/m^2). Obesity was also shown to be associated with an increased response of heart rate variability to particles in this cohort.[60]

There was a suggestion for a greater effect of BC on the inflammatory markers among *GSTM1*-null subjects and among non-users of statin drugs. An earlier study of the same cohort saw a similar effect of these two factors on reduced heart rate variability from high concentrations of fine particles.[60] Reactive oxygen species (ROS) are important factors in the pathogenesis of cardiovascular disease[61] and have been suggested to influence the adverse effect of particles.[62] Genetic polymorphisms of glutathione-*S*-tranferases—key factors in cellular defenses against ROS, have been shown to modify the response to air pollutants.[63] Statins are a class of drugs commonly prescribed for their lipid-lowering properties, with substantial anti-inflammatory and antioxidant activity.[63–65] Our findings support such evidence for increased inflammatory responses in the absence of *GSTM1* and statin use.

We recognize the limitation that in this study we were not able to measure personal exposure to air pollutants. Exposure to air particles was measured at a central monitoring site, thus, introducing a potential for measurement error. Because of the non-differential nature of such error, we would expect an underestimation of the effects of air pollution observed in the present study. Also, sulphate data were available for fewer days in our study, and concentrations were not that high, limiting our power to examine this source. However, the study showed that increases in particle emissions from mobile sources, and to a lesser extent sulphates, affected markers of potential pathways that lead to cardiovascular disease. Such effects were delayed from several days to several weeks. The greater effects of air pollutants among the elderly and obese subjects should lead to more attention toward susceptible population sub-groups.

Acknowledgements

KEY MESSAGES

- High concentrations of particles are associated with cardiovascular morbidity and mortality.

- Inflammatory markers and thrombotic factors are known as mediating the particle effect on cardiovascular disease.

- High concentrations of particles were associated with changes in inflammatory and thrombotic markers, with suggestion for greater toxicity of traffic-related particles.

References

[1] Peters A, Doring A, Wichmann HE, Koenig W. Increased plasma viscosity during an air pollution episode: a link to mortality? *Lancet* 1997;**349**:1582–87.

[2] Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. *JAMA* 1998;**279**:1477–82.

[3] Pekkanen J, Brunner EJ, Anderson HR, Tiittanen P, Atkinson RW. Daily concentrations of air pollution and plasma fibrinogen in London. *Occup Environ Med* 2000;**57**:818–22.

[4] Ishii H, Fujii T, Hogg JC *et al*. Contribution of IL-1 beta and TNF-alpha to the initiation of the peripheral lung response to atmospheric particulates (PM10). *Am J Physiol Lung Cell Mol Physiol* 2004;**287**:L176–83.

[5] Fujii T, Hayashi S, Hogg JC, Vincent R, Van Eeden SF. Particulate matter induces cytokine expression in human bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2001;**25**:265–71.

[6] Peters A, Frohlich M, Doring A *et al*. Particulate air pollution is associated with an acute phase response in men; results from the MONICA-Augsburg Study [comment]. *Eur Heart J* 2001;**22**:1198–204.

[7] Utell MJ, Frampton MW, Zareba W *et al*. Cardiovascular effects associated with air pollution: potential mechanisms and methods of testing. *Inhal Toxicol* 2002;**14**:1231–347.

[8] Ghio AJ, Hall A, Bassett MA, Cascio WE, Devlin RB. Exposure to concentrated ambient air particles alters hematologic indices in humans. *Inhal Toxicol* 2003;**15**:1465–78.

[9] Huang YC, Ghio AJ, Stonehuerner J *et al*. The role of soluble components in ambient fine particles-induced changes in human lungs and blood. *Inhal Toxicol* 2003;**15**:327–42.

[10] Ghio AJ, Kim C, Devlin RB. Concentrated ambient air particles induce mild pulmonary inflammation in healthy human volunteers. *Am J Respir Crit Care Med* 2000;**162**:981–88.

[11] Schwartz J. Air pollution and blood markers of cardiovascular risk. *Environ Health Perspect* 2001;**109(Suppl 3)**:405–09.

[12] Pope CA 3rd, Hansen ML, Long RW *et al*. Ambient particulate air pollution, heart rate variability, and blood markers of inflammation in a panel of elderly subjects. *Environ Health Perspect* 2004;**112**:339–45.

[13] Brook RD, Brook JR, Rajagopalan S. Air pollution: the ''Heart'' of the problem. *Curr Hypertens Rep* 2003;**5**:32–39.

[14] Suwa T, Hogg JC, Quinlan KB, Ohgami A, Vincent R, van Eeden SF. Particulate air pollution induces progression of atherosclerosis. *J Am Coll Cardiol* 2002;**39**:935–42.

[15] Wellenius GA, Coull BA, Godleski JJ *et al*. Inhalation of concentrated ambient air particles exacerbates myocardial ischemia in conscious dogs. *Environ Health Perspect* 2003;**111**:402–08.

[16] Gurgueira SA, Lawrence J, Coull B, Murthy GG, Gonzalez-Flecha B. Rapid increases in the steady-state concentration of reactive oxygen species in the lungs and heart after particulate air pollution inhalation. *Environ Health Perspect* 2002;**110**:749–55.

[17] O'Neill MS, Veves A, Zanobetti A *et al*. Diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and endothelial function. *Circulation* 2005;**111**:2913–20.

[18] Schwartz J, Litonjua A, Suh H *et al*. Traffic related pollution and heart rate variability in a panel of elderly subjects. *Thorax* 2005;**60**:455–61.

[19] Bell B, Rose C, Damon A. The Normative Aging Study: an interdisciplinary and longitudinal study of health and aging. *Aging Hum Dev* 1972;**3**:4–17.

[20] Roberts WL, Moulton L, Law TC *et al*. Evaluation of nine automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. Part 2. *Clin Chem* 2001;**47**:418–25.

[21] Bedell SE, Bush BT. Erythrocyte sedimentation rate. From folklore to facts. *Am J Med* 1985;**78**:1001–09.

[22] Claus A. Rapid physiological coagulation method for the determination of fibrinogen. *Acta Haematol* 1957;**17**:237.

[23] Allen G, Sioutas C, Koutrakis P, Reiss R, Lurmann FW, Roberts PT. Evaluation of the TEOM method for measurement of ambient particulate mass in urban areas. *J Air Waste Manag Assoc* 1997;**47**:682–89.

[24] Schwartz J. The distributed lag between air pollution and daily deaths. *Epidemiology* 2000;**11**:320–26.

[25] Zanobetti A, Schwartz J, Samoli E *et al*. The temporal pattern of respiratory and heart disease mortality in response to air pollution. *Environ Health Perspect* 2003;**111**:1188–93.

[26] Evelson P, Gonzalez-Flecha B. Time course and quantitative analysis of the adaptive responses to 85% oxygen in the rat lung and heart. *Biochim Biophys Acta* 2000;**1523**:209–16.

[27] Ozkaynak H, Schatz AD, Thurston GD. Relationships between aerosol extinction coefficients derived from airport visual range observations and alternative measures of airborne particle mass. *J Air Pollut Control Assoc* 1985;**35**:1176–85.

[28] Liao D, Duan Y, Whitsel EA *et al*. Association of higher levels of ambient criteria pollutants with impaired cardiac autonomic control: a population-based study. *Am J Epidemiol* 2004;**159**:768–77.

[29] Payton M, Greenstone M, Schenker N. Overlapping confidence intervals or standard error intervals: what do they mean in terms of statistical significance? *J Insect Sci* 2003;**3**:34–39.

[30] Schenker N, Gentleman J. On judging the significance of differences by examining the overlap between confidence intervals. *Am Stat* 2001;**55**:182–86.

[31] Dockery DW, Pope ACd, Xu X *et al*. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993;**329**:1753–59.

[32] Salvi SS, Nordenhall C, Blomberg A *et al*. Acute exposure to diesel exhaust increases IL-8 and GRO-alpha production in healthy human airways. *Am J Respir Crit Care Med* 2000;**161**:550–57.

[33] Salvi S, Blomberg A, Rudell B *et al*. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. *Am J Respir Crit Care Med* 1999;**159**:702–09.

[34] van Eeden SF, Tan WC, Suwa T et al. Cytokines involved in the systemic inflammatory response induced by exposure to particulate matter air pollutants (PM(10)). Am J Respir Crit Care Med 2001; **164:**826–30.

[35] Ross R. Atherosclerosis is an inflammatory disease. Am Heart J 1999;**138:**S419–20.

[36] Libby P. Inflammation in atherosclerosis. Nature 2002;**420:**868–74.

[37] Suwa T, Hogg JC, Quinlan KB et al. Particulate air pollution induces progression of atherosclerosis. J Am Coll Cardiol 2002;**39:**935–42.

[38] Nemmar A, Hoet PH, Vanquickenborne B et al. Passage of inhaled particles into the blood circulation in humans. Circulation 2002;**105:**411–14.

[39] Nemmar A, Vanbilloen H, Hoylaerts MF, Hoet PH, Verbruggen A, Nemery B. Passage of intratracheally instilled ultrafine particles from the lung into the systemic circulation in hamster. Am J Respir Crit Care Med 2001;**164:**1665–68.

[40] Seaton A, Soutar A, Crawford V et al. Particulate air pollution and the blood. Thorax 1999;**54:**1027–32.

[41] Utell MJ, Frampton MW. Acute health effects of ambient air pollution: the ultrafine particle hypothesis. J Aerosol Med 2000;**13:** 355–59.

[42] Seaton A, MacNee W, Donaldson K, Godden D. Particulate air pollution and acute health effects. Lancet 1995;**345:**176–78.

[43] Ruckerl R, Ibald-Mulli A, Koenig W et al. Air pollution and markers of inflammation and coagulation in patients with coronary heart disease. Am J Respir Crit Care Med 2006;**173:**432–41.

[44] Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC. C reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. BMJ 1996;**312:**1061–65.

[45] Vogel CF, Sciullo E, Wong P, Kuzmicky P, Kado N, Matsumura F. Induction of proinflammatory cytokines and C-reactive protein in human macrophage cell line U937 exposed to air pollution particulates. Environ Health Perspect 2005;**113:**1536–41.

[46] Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA 2001;**285:**2481–85.

[47] Pepys MB. The acute phase response and C-reactive protein. In: Weatherall DJ, Ledingham JGG, Warell DA (eds). Oxford Textbook of Medicine. Oxford, UK: Oxford University Press, 1995, pp. 1527–33.

[48] Fischer P, Hoek G, Brunekreef B, Verhoeff A, van Wijnen J. Air pollution and mortality in The Netherlands: are the elderly more at risk? Eur Respir J Suppl 2003;**40:**34S–38S.

[49] Goldberg MS, Bailar JC 3rd, Burnett RT et al. Identifying subgroups of the general population that may be susceptible to short-term increases in particulate air pollution: a time-series study in Montreal, Quebec. Res Rep Health Eff Inst 2000;**97:**7–113.

[50] Filleul L, Le Tertre A, Baldi I, Tessier JF. Difference in the relation between daily mortality and air pollution among elderly and all-ages populations in southwestern France. Environ Res 2004;**94:**249–53.

[51] Filleul L, Baldi I, Dartigues JF, Tessier JF. Risk factors among elderly for short term deaths related to high levels of air pollution. Occup Environ Med 2003;**60:**684–88.

[52] Schwartz J. What are people dying of on high air pollution days? Environ Res 1994;**64:**26–35.

[53] Zeka A, Zanobetti A, Schwartz J. Short term effects of particulate matter on cause specific mortality: effects of lags and modification by city characteristics. Occup Environ Med 2005;**62:**718–25.

[54] Bullo M, Garcia-Lorda P, Megias I, Salas-Salvado J. Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. Obes Res 2003;**11:**525–31.

[55] Kopp HP, Kopp CW, Festa A et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. Arterioscler Thromb Vasc Biol 2003;**23:**1042–47.

[56] Lyon CJ, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. Endocrinology 2003;**144:**2195–200.

[57] Nieman DC, Henson DA, Nehlsen-Cannarella SL et al. Influence of obesity on immune function. J Am Diet Assoc 1999;**99:**294–99.

[58] Biring MS, Lewis MI, Liu JT, Mohsenifar Z. Pulmonary physiologic changes of morbid obesity. Am J Med Sci 1999;**318:**293–97.

[59] Litonjua AA, Sparrow D, Celedon JC, DeMolles D, Weiss ST. Association of body mass index with the development of methacholine airway hyperresponsiveness in men: the Normative Aging Study. Thorax 2002;**57:**581–85.

[60] Schwartz J, Park SK, O'Neill MS et al. Glutathione-S-transferase M1, obesity, statins, and autonomic effects of particles: gene-by-drug-by-environment interaction. Am J Respir Crit Care Med 2005;**172:** 1529–33.

[61] Dhalla NS, Temsah RM, Netticadan T. Role of oxidative stress in cardiovascular diseases. J Hypertens 2000;**18:**655–73.

[62] Donaldson K, Stone V, Seaton A, MacNee W. Ambient particle inhalation and the cardiovascular system: potential mechanisms. Environ Health Perspect 2001;**109(Suppl 4):**523–27.

[63] Haughton G. Environmental justice and the sustainable city. J Plan Educ Res 1999;**18:**233–43.

[64] Albert MA, Danielson E, Rifai N, Ridker PM. Effect of statin therapy on C-reactive protein levels: the pravastatin inflammation/CRP evaluation (PRINCE): a randomized trial and cohort study. JAMA 2001;**286:**64–70.

[65] Sanguigni V, Pignatelli P, Caccese D et al. Increased superoxide anion production by platelets in hypercholesterolemic patients. Thromb Haemost 2002;**87:**796–801.