

PM₁₀-induced Hospital Admissions for Asthma and Chronic Obstructive Pulmonary Disease

The Modifying Effect of Individual Characteristics

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Background: Evidence suggests that oxidative stress is a unifying feature underlying the toxic actions of particulate matter (PM). We have investigated whether individual plasma antioxidant concentrations (uric acid and vitamins C, A, and E) and 10 antioxidant genes modify the response to PM with respect to hospital admissions for chronic obstructive pulmonary disease (COPD) or asthma.

Methods: Using a bidirectional, hospital-based, case-crossover study, 209 patients admitted for asthma or COPD to the Chelsea and Westminster Hospital (London), with 234 admissions, were recruited between May 2008 and July 2010. PM₁₀ levels in the area of Kensington and Chelsea at the time of admission were compared with the levels 14 days before and 14 days after the event. Conditional logistic regression was used to estimate the effect of PM₁₀ at several temporal lags, while controlling for confounders.

Results: An increase in asthma/COPD admission rate was related to a 10 µg/m³ increase in PM₁₀, with the highest effect noted 0–3 days before the exacerbation (for lag 0–3, odds ratio = 1.35 [95% confidence interval = 1.04–1.76]). Serum vitamin C modified the effect of PM₁₀ on asthma/COPD exacerbations. A similar (although weaker) influence was observed for low levels of uric acid and vitamin E, whereas vitamin A showed no effect modification. *GSTP1* (rs1695), *SOD2* (rs4880), and *Nrf2* (rs1806649) were asso-

ciated with a trend toward an increased risk of hospital admissions during periods of high PM₁₀ levels.

Conclusions: Our study suggests that the concentration of antioxidants in patients' serum modifies the short-term effects of PM₁₀ on asthma and COPD exacerbations.

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Daily variations in outdoor air pollution, especially particulate matter (PM), have been associated with increased mortality¹ and morbidity, with more emergency visits and hospital admissions due to respiratory complaints.^{2–5} Recent research has identified oxidative stress as a potential mechanism underlying the toxic actions of air pollution.^{6,7} Oxidative stress is the imbalance of biologic pro-oxidant and antioxidant processes,⁸ occurring when the generation of oxidant molecules, or free radicals, exceeds the available antioxidant defenses.⁹ On entering the lung, ambient pollutants come into direct contact with the fluid layer that covers the respiratory epithelium, and they can cause oxidative damage through either production of free radicals or induction of inflammation.⁶ Antioxidants such as vitamins A (retinol), C (ascorbate), and E (α-tocopherol), as well as uric acid, can play a protective role against oxidative stress induced by oxidant pollutants.¹⁰

Epidemiologic studies have shown a positive association between dietary concentrations of vitamin C and pulmonary function values.^{11,12} Asthmatics and people with chronic obstructive pulmonary disease (COPD) have been shown to have markedly decreased concentrations of vitamin C in respiratory tract lining fluid compared with healthy control subjects.¹³ Among these groups of patients, those with lower antioxidant concentrations might be more prone to experience exacerbations in the presence of particulate air pollution.

There is little information in the literature on the impact of antioxidants on the acute effects of air pollution exposure in humans.⁷ Experimental studies suggest that antioxidant supplementation could modulate the acute changes in lung function observed among people exposed to photo-oxidant chemicals.^{14–17} In contrast, a recent chamber study did not

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support such findings,¹⁸ and 2 studies that investigated the modulation of acute respiratory effects of air pollution by serum antioxidants¹⁹ and by antioxidant supplementation²⁰ did not show a clear effect in relation to PM levels.

Genetic factor may also contribute in explaining the individual's susceptibility to the respiratory effects of air pollution, as suggested in a recent systematic review.²¹

We hypothesized that the risk of COPD and asthma admissions would be positively associated with traffic-related air pollution in subjects with a pre-existing respiratory condition, and that this association would be modified by concentrations of antioxidants (uric acid and vitamins C, A, and E) measured in the patients' blood and polymorphisms in genes related to defense against oxidative stress. We also considered other individual characteristics that can lead to increased risk of PM-related health effects, namely sex, age, asthma versus COPD diagnosis, and smoking status.

We studied the association of outdoor levels of PM with a diameter $<10\ \mu\text{m}$ (PM₁₀) with emergency hospital admissions for exacerbations of asthma and COPD in London, using a case-crossover design and exploring several lag times between PM₁₀ exposure and exacerbation.

METHODS

Study Design

We performed a bidirectional, hospital-based, case-crossover study, which is a special case-control design where every case serves as its own control, and control periods are defined as days on which the subject did not experience the study outcome. Control days were selected as the same day of the week as case days using a symmetric design, proposed by Bateson and Schwartz,²² which consisted of taking 2 days as controls, one before and one after the event, equidistant from the latter (± 14 days). This provides adequate control for long-term trends, seasonality, and day of the week.

Patient Data

All adult patients aged 18 years or more who underwent an emergency admission due primarily to a reasonable suspicion of an exacerbation (triggered by any cause, including infection) of pre-existing (diagnosed or undiagnosed) asthma or COPD (including chronic bronchitis and emphysema) at the Chelsea and Westminster Hospital (London), between May 2008 and July 2010, were invited to take part in the study. A trained research nurse administered a questionnaire on characteristics of the exacerbation, other environmental risk factors such as active and passive smoking and occupation, and use of medicines for breathing and medical services in the past 12 months. She also took blood samples, measured exhaled nitric oxide using NIOX MINO monitor (Aerocrine, Bedfordshire, United Kingdom), assessed sensitization to common allergens (*Dermatophagoides pteronyssinus*, group B2 grass pollens, cat fur, group B3 tree pollens, soya bean,

latex, mold mix [*Aspergillus fumigatus*, *Penicillium notatum*, *Alternaria alternata*, *Mucor mucedo*, *Cladosporium cladosporioides*], and English weed mix) using skin prick tests, and distributed a self-administered questionnaire on dietary habits.

Blood samples were collected for genotyping and for the measurement of serum total cholesterol and specific markers of antioxidant concentrations, namely vitamin C, uric acid, vitamin A, and vitamin E. Total cholesterol concentration was analyzed using an InfinityTM kit (ThermoTrace, Victoria, Australia) and involved a Trinder-type color reaction measured on a multiplate reader (SpectraMax 190—Molecular Devices, Berkshire, United Kingdom). Plasma vitamin C and uric acid concentrations were analyzed in aliquots (preacidified for storage) using a reverse-phase high-performance liquid chromatography system (Gilson, United Kingdom) with an electrochemical detector, following a modified method of Iriyama et al.²³ The lipid-soluble antioxidants, vitamin E and A, concentrations were analyzed in nonacidified aliquots using a separate Gilson high-performance liquid chromatography system with ultraviolet detection, following a modified method of Kelly et al.²⁴ Because vitamins E and A are bound to lipoproteins and their concentration is affected by cholesterol levels, their measurements were corrected for serum total cholesterol. Measurements were performed using duplicate incubation with internal and external standards. All assays were run with an in-house serum quality control to check for intra- and interassay variation and minimum detection limit. Inter- and intra-assay coefficients of variability were $<10\%$ for all antioxidants.

We genotyped 12 single nucleotide polymorphisms (SNPs) in 10 genes related to oxidative stress. Eight of these genes—*GSTP1*, glutathione S-transferase Mu 1 (*GSTM1*), *GSTT1*, *SOD2*, *GPX1*, *CAT*, *NQO1*, and *HMOX1*—were selected based on published evidence identified using HuGE navigator “Gene Prospector” and the search term “oxidative stress.”²⁵ All of them had been previously investigated in relation to the effect of gene-pollution interactions on the respiratory system.²¹ Two other genes (*SLC2A9* and *Nrf2*) were added based on their strong biologic rationale, despite more limited previous evidence—*SLC2A9* for its regulation of serum uric acid levels²⁶ and *Nrf2* for its key role in regulating cellular response against oxidative stress, mainly by activating the transcription of antioxidant genes with upregulation of several antioxidants.²⁷ DNA was extracted from ethylenediaminetetraacetic acid-buffered whole blood. All genotyping was performed by KBioscience, Hertfordshire, United Kingdom (www.kbioscience.co.uk). SNPs and insertion/deletion polymorphisms were genotyped using KBiosciences Competitive Allele-Specific PCR genotyping system, a homogeneous fluorescent resonance energy transfer-based system, coupled with competitive allele-specific

polymerase chain reaction. Blind duplicates and Hardy-Weinberg equilibrium tests were used as quality control tests.

Environmental Data

We downloaded data from the Air Quality London database (period 2008–2010) (<http://www.londonair.org.uk/london/asp/default.asp>) on 24-hour PM₁₀ from 3 fixed monitoring sites, representing concentrations in residential areas near the Chelsea and Westminster Hospital (Earls Court-roadside ~0.6 mile from the hospital, Cromwell Road-roadside ~0.9 mile from the hospital, and North Kensington-background ~2.8 mile from the hospital). For completeness, ambient air quality measurements of daily 8-hour maximum ozone (O₃) and 24-hour PM_{2.5} concentrations, available only at the background station (North Kensington), and 24-hour nitrogen dioxide (NO₂) (Cromwell Road, North Kensington) were also obtained. The effect of O₃ concentrations in warm months (April–September) was also analyzed.

Daily information on temperature, humidity, and barometric pressure was provided by the Met Office for St James and Kew Gardens stations (period 2008–2010).

Missing data on the aggregate level (daily 24 hours average) for each monitoring site were replaced using a formula adapted from the APHEA (Air Pollution and Health—A European Approach) method.²⁸ In particular, when the daily information for a station was missing, it was imputed as a weighted mean of values from other stations. When the daily data of a pollutant were missing for all available stations, the value was left missing. Finally, we used the combined average of 24-hour values measured at each site for the analysis.

Data Analyses

The concentration of each pollutant at the time of admission for each case (case period) was compared with concentrations obtained in a specified period before and after the exacerbation (control period)—specifically, 14 days before and 14 days after the event. Sensitivity analyses were also conducted considering the 2 control periods as 7 days before and after the event, to better control for seasonal variability.

We explored several lag periods by considering the mean pollutant concentrations during the 24-hour period ending at midday on the day of exacerbation (lag 0), on the previous day (lag 1), 2 days previously (lag 2), and 3 days previously (lag 3), as well as cumulative exposure over the previous 0–1 days (lag 0–1) and 0–3 days (lag 0–3).

We used the χ^2 test to compare the characteristics of the patients with the information on serum antioxidants with those without these data. Conditional logistic regression was used to estimate the effect of each pollutant while controlling for confounders (temperature and humidity); time trends and day of the week were controlled for by design. Natural cubic splines of temperature calculated at the same lag of pollutant

were used, with knots located at the 25th and the 75th percentiles of the distribution. Barometric pressure was modeled as a linear term.

Recurrent events (subjects admitted and recruited more than once) were included in the analysis under the assumption that within-subject correlation had been completely accounted for by study design, which controls for observed and unobserved subject-specific variables. For subjects with recurrent hospital admissions, we checked that there was no overlap between case and control periods. However, to assess the impact of any residual confounding effect associated with multiple hospital admissions, we also performed a sensitivity analysis by considering only the first admission of each patient.

Results from the analyses were reported as the odds ratio (OR) and 95% confidence interval (CI) for asthma/COPD admission associated with a 10 $\mu\text{g}/\text{m}^3$ increase in pollutant concentration (a value close to the interquartile range of the PM₁₀ and PM_{2.5} distributions). Results were reported as OR of asthma/COPD admission associated with the interquartile range for O₃ and NO₂.

Individual characteristics tested for possible effect modification were as follows: antioxidant concentrations (uric acid and vitamins C, A, and E) measured in serum; SNPs in antioxidant genes; age (18–34, 35–54, 55+ years); sex; a previous diagnosis of asthma, COPD, or both; and smoking status (current/former/never). Antioxidants were categorized into high and low concentrations, with a cutoff point at the median serum concentration of each antioxidant. For each genetic variant, we assumed a dominant genetic model, thus comparing the pollution effect for carriers of the minor allele (either homozygotes or heterozygotes) versus homozygotes for the major allele (wild type), due to the small number of nonwild homozygotes for most SNPs.

The PM₁₀ effect was evaluated in the categories of each individual covariate, and statistical tests were performed to test for effect modification (likelihood ratio test). The relative excess risk due to interaction (RERI), a measure of interaction on an additive scale that is more meaningful in the public health context, was also calculated, and the 95% CI for RERI was estimated as the 2.5th and 97.5th percentiles of the resulting 10,000 bootstrap sampling distribution.^{29,30} RERI > 0 indicates additive interaction.

All analyses were performed with Stata IC 10.1 (Stata software version 10.1; Stata Corp., College Station, TX).

RESULTS

Two hundred and nine patients admitted for exacerbations of asthma or COPD to the Chelsea and Westminster Hospital, London, on 234 occasions, were recruited between 2008 and 2010, with a similar number of patients recruited in each season: January/March ($n = 62$, 27%), April/May ($n = 53$, 22%), June/August ($n = 62$, 26%), and September/

December ($n = 59$, 25%). Blood samples for specific antioxidant concentrations from 141 patients with 158 exacerbations were available, and blood samples for genotyping from 154 individuals with 176 exacerbations were available. At the time of their admission, about 85% of the subjects lived in the South Western area of London around the Chelsea and Westminster Hospital, which covers approximately 20 square miles.

Table 1 shows the characteristics of the recruited subjects with and without blood samples for specific antioxidant concentrations and genotyping. No differences were observed between the groups with regard to sex, age, smoking status, and a previous diagnosis of asthma, COPD, or both. A similar number of men and women were recruited. The majority of

subjects were 54–74 years of age, had a previous diagnosis of COPD, and were former smokers.

Descriptive statistics on antioxidant levels are reported in eTable 1 (<http://links.lww.com/EDE/A586>). Serum vitamin C levels ($<13 \mu\text{mol/L}$ vs. $\geq 13 \mu\text{mol/L}$) were not different between age-groups, between current and never smokers, or between participants with asthma and COPD. However, women had higher serum levels of vitamin C than men. High levels of vitamin C were associated with high levels of vitamin E, whereas no correlation was observed between the levels of vitamin C and other antioxidants.

Table 2 describes concentrations of PM_{10} for each year in the study period. The current European standard of $50 \mu\text{g}/\text{m}^3$ of PM_{10} level was exceeded on 20 days in 2008 and 10 days in 2010. O_3 and PM_{10} levels were not correlated during the study period ($r = -0.14$), whereas PM_{10} level was highly correlated with $\text{PM}_{2.5}$ levels ($r = 0.92$) and moderately correlated with NO_2 ($r = 0.55$) (correlation between NO_2 and $\text{PM}_{2.5}$: $r = 0.65$).

Asthma/COPD admission rate was related to a $10\text{-}\mu\text{g}/\text{m}^3$ increase in PM_{10} concentration, with the highest effect noted 0–3 days before the exacerbation (lag 0–3, OR = 1.35 [95% CI = 1.04–1.76]) (Table 3). The sensitivity analyses including only the first admission from each patient confirmed the findings from the main analyses, with associations of similar magnitude at lag 1, 3, 0–1, 0–2, and 0–3.

No effect was found for daily maximum 8-hour average O_3 concentrations, either using the whole data or restricting the analysis to the summer months (April–September). The associations with $\text{PM}_{2.5}$ and NO_2 were similar to the association with PM_{10} (eTable 2, <http://links.lww.com/EDE/A586>). However, $\text{PM}_{2.5}$ data were available only from December 2008 (for 151/234 exacerbations in analysis), and therefore, we considered PM_{10} as the primary PM indicator to determine possible effect modifications of individual characteristics.

Data were stratified by a number of possible effect modifiers, as shown in Table 4. Only the results for lag 0–3 are reported. Although no statistically significant effect modification by age was observed, the effect of PM_{10} was higher in people older than 75 years of age. A significant effect

TABLE 1. Characteristics of the 209 Subjects by Available Serum and Genetic Information

Characteristic	Subjects With Serum and Genetic Data (n = 141) No. (%)	Subjects With Genetic Data Only (No Serum) (n = 14) No. (%)	Subjects Without Any Serum or Genetic Data (n = 55) No. (%)
Sex			
Men	72 (51)	7 (54)	21 (38)
Women	69 (49)	6 (46)	34 (62)
Age (years)			
18–54	35 (25)	7 (54)	15 (14)
55–74	61 (43)	6 (46)	29 (42)
75+	45 (32)	0 (0)	24 (44)
Previous diagnosis			
Asthma	41 (29)	3 (23)	14 (25)
COPD	57 (40)	3 (23)	26 (47)
Both asthma and COPD	27 (19)	5 (39)	11 (20)
None	16 (11)	2 (15)	4 (7)
Smoking status			
Current smokers	46 (33)	5 (38)	20 (36)
Exsmokers/nonsmokers	94 (67)	8 (61)	35 (64)

Approximately 85% of the subjects lived, at the time of the admission, in a South Western area of London defined by 6 contiguous postcodes (SW1, SW3, SW5, SW6, SW7, SW10). This area covers approximately 25 square miles. The Chelsea and Westminster Hospital is located in the middle of this area.

TABLE 2. Descriptive Statistics of the Outdoor PM_{10} Concentration at the 3 Monitoring Stations,^a by Year

Year	Minimum	Percentiles			Maximum	Mean (SD)	No. Days $>50 \mu\text{g}/\text{m}^3$
		25th	50th	75th			
2008	8.7	21	26	33.8	108.7	29.2 (12.1)	20
2009	15.3	22	25.3	32	75.7	28.4 (9.6)	15
2010	11.7	22	25.5	31.0	59.3	27.6 (8.5)	10

^aNorth Kensington (background station, ~2.8 miles from Chelsea and Westminster Hospital), Earls Court (roadside, ~0.6 mile from the Hospital), Cromwell Road (roadside, ~0.9 mile from the Hospital).

TABLE 3. Adjusted^a Associations of COPD/Asthma Exacerbations With Each 10- $\mu\text{g}/\text{m}^3$ Increase in PM_{10} by Temporal Lags, for 234 Chelsea and Westminster Hospital Admissions, 2008–2010

Lag	OR (95% CI)
lag 0	1.26 (1.04–1.52)
lag 1	1.15 (0.95–1.40)
lag 0–1	1.25 (1.00–1.55)
lag 2	1.15 (0.94–1.41)
lag 3	1.20 (0.99–1.46)
lag 0–3	1.35 (1.04–1.76)

^aModels adjusted for temperature and humidity.**TABLE 4.** Adjusted^a Association of COPD/Asthma Exacerbations for Each 10- $\mu\text{g}/\text{m}^3$ Increase in PM_{10} at Lag 0–3, by Individual Characteristics, for 234 Chelsea and Westminster Hospital Admissions, 2008–2010

Effect Modifier	OR (95% CI)	Test for Interaction
Sex		$P = 0.258$
Men	1.49 (1.02–2.18)	
Women	1.21 (0.83–1.76)	
Age (years)		$P = 0.388$
18–54	1.14 (0.64–2.04)	
55–74	1.31 (0.88–1.95)	
75+	1.72 (1.07–2.77)	
Asthma/COPD		$P = 0.903$
Only asthma	1.33 (0.76–2.32)	
Only COPD	1.42 (0.93–2.16)	
Asthma/COPD	1.25 (0.72–2.17)	
Smoking status		$P = 0.043$
Current smokers	1.87 (1.11–3.16)	
Exsmokers/nonsmokers	1.19 (0.87–1.62)	

^aModels adjusted for temperature and humidity.

modification was seen with smoking status (dichotomized as smokers and exsmokers/nonsmokers) (P value = 0.04). Among smokers, a 10 $\mu\text{g}/\text{m}^3$ increase in PM_{10} concentration was associated with an 87% increased risk of airway exacerbations, whereas in nonsmokers and exsmokers, no excess risks were observed. Sex and a previous diagnosis of asthma or COPD (or both) did not show evidence of effect modification.

Modification of the effect of PM_{10} by vitamin C was not dependent on the cutpoint chosen, and the interaction was also statistically significant (P interaction = 0.03) when the antioxidant was analyzed as a continuous variable (Table 5). This effect was still present after the exclusion of smokers and elderly subjects, implying that the “protective” effect of this antioxidant was not explained by smoking or age. The relative risk of exacerbation for each 10 $\mu\text{g}/\text{m}^3$ increase in PM_{10} concentration was 1.2 times higher in subjects with low

TABLE 5. Adjusted^a Association of COPD/Asthma Exacerbations for Each 10- $\mu\text{g}/\text{m}^3$ Increase in PM_{10} at Lag 0–3, by Individual Antioxidant Capacity, for 158 Chelsea and Westminster Hospital Admissions, 2008–2010

Effect Modifier	OR (95% CI)	Test for Interaction
Vitamin C		$P = 0.007$
<13 $\mu\text{mol}/\text{L}$	2.17 (1.38–3.43)	
≥ 13 $\mu\text{mol}/\text{L}$	0.90 (0.56–1.46)	
Uric acid		$P = 0.238$
<236 $\mu\text{mol}/\text{L}$	1.83 (1.18–2.84)	
≥ 236 $\mu\text{mol}/\text{L}$	1.13 (0.72–1.77)	
Vitamin A		$P = 0.697$
<2.2 $\mu\text{mol}/\text{L}$	1.37 (0.90–2.07)	
≥ 2.2 $\mu\text{mol}/\text{L}$	1.69 (1.04–2.75)	
Vitamin A (corrected for cholesterol)		$P = 0.153$
<0.5 $\mu\text{mol}/\text{L}$	1.18 (0.78–1.78)	
≥ 0.5 $\mu\text{mol}/\text{L}$	2.02 (1.22–3.36)	
Vitamin E		$P = 0.062$
<23.7 $\mu\text{mol}/\text{L}$	1.79 (1.19–2.68)	
≥ 23.7 $\mu\text{mol}/\text{L}$	1.12 (0.66–1.90)	
Vitamin E (corrected for cholesterol)		$P = 0.256$
<5.5 $\mu\text{mol}/\text{L}$	1.64 (1.09–2.47)	
≥ 5.5 $\mu\text{mol}/\text{L}$	1.37 (0.83–2.26)	

^aModels adjusted for temperature and humidity.

levels of vitamin C (<13 $\mu\text{mol}/\text{L}$) than in subjects with high levels (≥ 13 $\mu\text{mol}/\text{L}$) (RERI = 1.19 [95% CI = 0.25–5.59]). A similar association was seen among participants with low levels of uric acid and vitamin E, whereas vitamin A did not show evidence of effect modification.

Similar effect modification by vitamin C was seen in relation to the effects of NO_2 on asthma and COPD exacerbations (eTable 3, <http://links.lww.com/EDE/A586>). No significant effect modification was observed regarding O_3 using the whole data (eTable 4, <http://links.lww.com/EDE/A586>). A significant modification of the effect of O_3 by levels of vitamin E was observed only when limiting the analysis to the warm months, with the highest effect noted in those participants with low levels of vitamin E (OR = 5.99 [95% CI = 1.07–33.46]) (eTable 5, <http://links.lww.com/EDE/A586>). However, this result should be considered cautiously due to the small number of observations (114 exacerbations between April and September).

The genotype and allele frequency for each SNP is given in eTable 6 (<http://links.lww.com/EDE/A586>). The genotype frequencies for all SNPs did not deviate from Hardy–Weinberg equilibrium.

Associations of PM_{10} with respiratory hospital admissions were not statistically significantly modified by genotypes (Table 6). *GSTP1* (rs1695), *SOD2* (rs4880), and *Nrf2* (rs1806649) were associated with a marginally increased risk of PM_{10} -induced hospital admissions ($P < 0.15$), with subjects with wild genotype more susceptible to the effect of PM.

TABLE 6. Adjusted^a Association of COPD/Asthma Exacerbations for Each 10- $\mu\text{g}/\text{m}^3$ Increase in PM_{10} at Lag 0–3, by Gene Polymorphisms, for 176 Chelsea and Westminster Hospital admissions, 2008–2010

Effect Modifier	OR (95% CI)	Test for Interaction
<i>GSTP1</i> (rs1695)		$P = 0.149$
AA	1.78 (1.14–2.80)	
AG, GG	1.15 (0.77–1.73)	
<i>GSTP1</i> (rs1138272)		$P = 0.486$
CC	1.50 (1.10–2.05)	
CT, TT	0.92 (0.34–2.47)	
<i>GSTM1</i>		$P = 0.252$
Present	1.70 (1.08–2.68)	
Null	1.19 (0.80–1.77)	
<i>GSTT1</i>		$P = 0.383$
Present	1.30 (0.93–1.82)	
Null	2.11 (1.07–4.16)	
<i>SOD2</i> (rs4880)		$P = 0.074$
CC	1.85 (1.02–3.37)	
CT, TT	1.21 (0.85–1.72)	
<i>GPX1</i> (rs1050450)		$P = 0.558$
CC	1.65 (1.04–2.60)	
CT, TT	1.33 (0.88–1.99)	
<i>CAT</i> (rs1001179)		$P = 0.877$
G/G	1.41 (1.00–2.00)	
G/A A/A	1.48 (0.83–2.62)	
<i>NQO1</i> (rs1800566)		$P = 0.247$
CC	1.51 (1.04–2.19)	
CT, TT	1.37 (0.78–2.41)	
<i>HMOX1</i> (rs2071746)		$P = 0.568$
AA	1.45 (0.81–2.59)	
AT, TT	1.43 (1.01–2.02)	
<i>SLC2A9</i> (rs734553)		$P = 0.384$
AA	1.59 (1.11–2.28)	
AC, CC	1.18 (0.68–2.05)	
<i>SLC2A9</i> (rs733175)		$P = 0.404$
TT	1.57 (1.08–2.28)	
TC, CC	1.16 (0.70–1.91)	
<i>Nrf2</i> (rs1806649)		$P = 0.107$
CC	1.74 (1.13–2.67)	
CT, TT	1.14 (0.75–1.73)	

^aModels adjusted for temperature and humidity.

Results of effect modification by genetic variants concerning NO_2 and O_3 , as well as O_3 in warm months, are shown in eTables 7–9 (<http://links.lww.com/EDE/A586>). For NO_2 , a significant effect modification was seen for the 2 variants of *GSTP1*, and a trend toward statistical significance for *GSTT1* deletion ($P = 0.07$) and *Nrf2* ($P = 0.14$). A significant interaction between *NQO1* and O_3 was observed, with the effect of O_3 larger in subjects with wild genotype (*Pro*).

We also studied whether benefits of antioxidant levels (vitamin C) differed according to genetic factors associated with increased susceptibility to oxidative stress. No statisti-

cally significant three-way interaction (gene $\times$ pollution $\times$ vitamin C) was observed. Despite the limited sample size, a 3-fold elevated risk of asthma/COPD admission was seen in subjects carrying the Ile (wild) homozygous genotype and with low vitamin C concentration related to a 10- $\mu\text{g}/\text{m}^3$ increase in PM_{10} concentration (OR = 3.3 [95% CI = 1.5–7.4]). Similarly for *Nrf2*, people with wild genotype and low vitamin C concentration were more susceptible to PM_{10} adverse effect (OR = 3.1 [95% CI = 1.5–6.3]). Results were similar for NO_2 , with an increased adverse effect of NO_2 in subjects with low vitamin C levels and wild genotype of *GSTP1* (OR = 7.6 [CI = 1.5–38]) and *Nrf2* (OR = 6.7 [CI = 1.4–30]).

DISCUSSION

A 10- $\mu\text{g}/\text{m}^3$ increase in PM_{10} concentration was associated with a 35% increased risk of exacerbations requiring hospital admission in asthmatic or COPD patients, with the effect being greater in the elderly subjects, in smokers, and in subjects with low concentrations of serum vitamin C. Our results also suggest that the presence of certain polymorphisms of antioxidant genes may further modify the effect of PM_{10} . The identification of factors that can modify a person's susceptibility to the respiratory effects of air pollution is important, because overall estimates of pollution effects in the population can greatly underestimate the effect in the most susceptible subgroups.³¹ The short-term effect of PM_{10} on the risk of hospitalization in patients with asthma and COPD found in our study is in line with numerous epidemiologic studies showing increased susceptibility to PM exposure in subjects with pre-existing respiratory diseases.³²

Association of PM and NO_2 with exacerbation of chronic lung disease is supported by a recent study that showed that in patients with COPD living in London, outdoor pollution (particularly PM_{10} , black smoke, and NO_2) worsens symptoms and exacerbations.³³ The negative result concerning O_3 was not surprising, as O_3 is predominantly a seasonal pollutant and is generally low in the United Kingdom compared with the United States and parts of Europe. Furthermore, concentrations of O_3 are usually lower in urban areas such as central London because it reacts rapidly with nitrogen oxides from traffic exhaust. A significant effect of summer O_3 was observed among participants with low levels of vitamin E, although this finding should be interpreted with caution, bearing in mind the limitations of conducting a subgroup analysis in such a small data set.

Although age did not significantly modify the effect of air pollution in these analyses, the increase in the risk of exacerbation associated with high levels of PM_{10} was mainly seen in older participants (72% higher in subjects older than 75 years of age). The elderly subjects are considered a susceptible subgroup because the capacity to neutralize toxic effects declines with age,³⁴ as demonstrated by the increased

risk of cardiovascular morbidity from short-term PM exposure in older adults.³² Some evidence suggests higher susceptibility to the respiratory effects of air pollution in the elderly subjects due to a weaker immune system³⁵ and a likely reduction of antioxidant defenses in the respiratory tract lining fluid.⁶ However, despite some reports of an increase in respiratory-related hospital admissions,^{5,36} epidemiologic studies that have examined respiratory-related effects of PM exposure have not consistently shown associations between respiratory-related effects and PM exposure among the elderly subjects.

In smokers, the risk of exacerbation associated with PM₁₀ increased by 87%, with no evidence of increase among nonsmokers and exsmokers (possibly due to lack of power, given the low proportion of patients in this category). Our results are consistent with a previous time-series study that showed that male smokers aged 30 years or older had an increased risk of cardiorespiratory mortality associated with PM₁₀.³¹ A number of mechanisms could explain the positive interaction between smoking and ambient particulate pollution on mortality. For instance, smoking is associated with oxidation and decreased concentrations of the major endogenous antioxidant, glutathione,³⁷ which would exacerbate oxidative stress induced by particulate air pollution.

The most important finding of our study is that patients' antioxidant concentrations, mainly vitamin C but also possibly vitamin E, appear to modify the short-term effects of PM₁₀ on asthma and COPD exacerbations. As vitamin C and E concentrations were highly correlated, we also created an index combining the 2 antioxidants (having both vitamin C and vitamin E deficiency, only vitamin C or vitamin E deficiency, vs. none). The increase in risk of airway exacerbations in subjects with both vitamin C and vitamin E deficiency (OR = 2.6 [95% CI = 1.44–4.68]) was not substantially different from that in subjects with low levels of vitamin C.

Although studies with larger sample sizes are needed to confirm this, a protective effect of vitamin C against the effects of air pollution in this clinical subgroup has strong biologic plausibility. Oxidative stress occurs when there is imbalance between oxidants and antioxidants. Because many antioxidants are derived from food, increased attention is being paid to the quality of diet and how diet may help protect the population from an oxidizing environment. Using a food frequency questionnaire, a recent case-crossover study on 23,484 deaths in 1998 in Hong Kong observed consistently negative RERI between outdoor pollutants and regular consumption of vegetables, fruits, and soy. The effects of PM₁₀, NO₂, and O₃ were smaller in subjects who regularly consumed fruits.³⁸

Unfortunately, plasma vitamin C is only an approximate indicator of vitamin C intake.³⁹ Dietary questionnaires were returned by only 55 subjects (23%), and therefore, we

were not able to test whether antioxidant intake, as measured using a food frequency questionnaire, modifies the effects of air pollution. Because vitamin C levels tend to vary seasonally with intake of fresh fruits, we also considered possible effect modification by season, but found no evidence for this. Further investigations should compare effect modification of plasma versus dietary antioxidants on air pollution-induced respiratory exacerbations.

Despite a plausible mechanistic model linking air pollution, oxidative stress, and dietary supplementation, and results from animal studies suggesting that supplementation with vitamin C and vitamin E modulates the pulmonary response to O₃ and NO₂,⁷ evidence from experimental studies in humans is not conclusive.^{14–20} These studies (none of which uses a crossover design) have limited sample sizes and therefore low statistical power to detect interactions. Furthermore, although chamber studies have reported protection against O₃ with vitamin supplementation, the end points for detecting protection were not consistent, and the magnitude of the protective effect was small.

Particulate pollution, in contrast with gaseous pollutants, can cross the respiratory tract lining fluid and lead to oxidative reactions.⁶ Little has been published on possible PM effect modification by antioxidant food intake or supplementation in humans. This gap in knowledge has been highlighted in a recent systematic review.⁴⁰ An observational study conducted among 227 subjects 50–70 years of age with chronic respiratory symptoms showed some differences in PM effects between groups with high versus low intake of vitamin C and β -carotene.¹⁹ A randomized controlled trial of antioxidant supplementation (vitamins E and C), conducted among 158 asthmatic children resident in Mexico City, did not show important changes in lung function parameters associated with ambient PM₁₀ in either the placebo or the supplement group, although it did suggest that antioxidant supplementation might modulate the adverse effect of O₃.²⁰ Interestingly, this trial found a three-way interaction between antioxidant supplementation, *GSTM1* gene polymorphism, and O₃ on lung function,⁴¹ suggesting that children with genetic reduction of antioxidant defenses are at increased risk of O₃-related pulmonary impairment, and that benefits of antioxidant supplementation may differ among people according to their genetic susceptibility.

In our data, *GSTM1* polymorphism did not modify the effects of PM₁₀. However, other genetic variants (namely, *SOD2* [rs4880], *Nrf2* [rs1806649], and *GSTP1* [Ile105Val, rs1695]) showed marginally significant modification of the effect of PM₁₀ on hospital admissions. *Nrf2* is a transcription factor that appears to contribute to the regulation of antioxidant defenses²⁷ and has been shown in vitro to protect against the oxidizing effects of diesel exhaust chemicals.⁴² Studies on animals show that deletion of the *Nrf2* gene can modify susceptibility to oxidative stress,⁴³ but, to our knowledge,

there has been no published report on the combined effects of *Nrf2* polymorphisms and pollution in humans.

Previous results on the *GSTP1* Ile105Val polymorphism from 11 studies summarized in a recent systematic review were conflicting, with the risk allele being the valine allele in 7 studies and the isoleucine allele in 3.²¹ Persons with *GSTP1* wild genotype (Ile/Ile) were more susceptible to the adverse effect of NO₂.

Susceptible subjects, such as those with a pre-existing respiratory disease, are most likely to benefit from a nutritional intervention. The median vitamin C concentration in our population with asthma and COPD was about one-third of the concentrations observed in a previous study of young people living in the United States who consumed a vitamin C-deficient diet for 2 weeks, and about one-fifth of the concentration observed in the same people after 2 weeks of a vitamin C-rich diet.¹⁶

Although time-series and case-crossover analysis are the most commonly used methods for assessing the short-term effects of air pollution, to our knowledge, ours is the first such study to examine possible effect modification by antioxidant concentrations and genotyping through investigation of interactions between fixed individual factor and time-varying concentrations of air pollution. Previous time-series studies had looked at effect modification of sex, age, and pre-existing diseases,³² whereas few studies have been able to investigate the possible effect modification of smoking status³¹ and of diet,³⁸ as this information is generally absent from the available hospital records. Although attenuated by the crossover design, the greatest limitation of our study is probably the modest sample size, which limits the precision of effect estimates.

In summary, PM₁₀ outdoor concentrations were associated with increased hospital admissions for exacerbation of asthma or COPD among adults in London. The short-term effect of PM₁₀ on the risk of exacerbation was modified by smoking and age, as well as patients' antioxidant concentrations. Antioxidant genes' polymorphisms might also play a role in modifying the adverse respiratory effects of PM₁₀ and the protection from such effects provided by high antioxidant concentrations. This finding has public health relevance, given the possibility of implementing targeted preventive interventions aimed at protecting sensitive population groups from the adverse effects of air pollution. Further studies with larger sample sizes are recommended to confirm these findings and elucidate the underlying mechanisms.

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