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Association of criteria pollutants with plasma hemostatic/inflammatory markers: a population-based study

DUANPING LIAO,[a] GERARDO HEISS,[b] VERNON M. CHINCHILLI,[a] YINKANG DUAN,[a] AARON R. FOLSOM,[c] HUNG-MO LIN[a] AND VEIKKO SALOMAA[d]

[a]*Department of Health Evaluation Sciences, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA*
[b]*Department of Epidemiology, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA*
[c]*Division of Epidemiology, School of Public Health, University of Minnesota, Minneapolis, Minnesota, USA*
[d]*Department of Epidemiology and Health Promotion, KTL-National Public Health Institute, Helsinki, Finland*

To elucidate the health effects of air pollution, the short-term association of criteria pollutants (particles $<10\,\mu$m in diameter [PM_{10}], O_3, CO, NO_2, and SO_2) with hemostatic and inflammatory markers were examined using a population-based sample of 10,208 middle-age males and females of the biracial cohort of Atherosclerosis Risk in Communities (ARIC) study. For each participant, we calculated the following pollutant exposures 1–3 days prior to the randomly allocated cohort examination date: PM_{10}, CO, NO_2, and SO_2 as 24-h averages, and O_3 as an 8-h average of the hourly measures. The hemostatic/inflammatory factors included fibrinogen, factor VIII-C, von Willebrand factor (vWF), albumin, and white blood cell count (WBC). Linear regression models were used to adjust for cardiovascular disease (CVD) risk factors, demographic and socioeconomic variables, and relevant meteorological variables. One standard deviation (SD) increment of PM_{10} (12.8 $\mu g/m^3$) was significantly ($P<0.05$) associated with 3.93% higher of vWF among diabetics and 0.006 g/dl lower of serum albumin among persons with a history of CVD. One SD increment of CO (0.60 p.p.m.) was significantly ($P<0.01$) associated with 0.018 g/dl lower of serum albumin. Significant curvilinear associations, indicative of threshold effects, for PM_{10} with factor VIII-C, O_3 with fibrinogen and vWF, and SO_2 with factor VIII-C, WBC, and serum albumin were found. This population-based study suggest that the hemostasis/inmflammation markers analyzed, which are linked to higher risk of CHD, are associated adversely with environmentally relevant ambient pollutants, with the strongest associations in the upper range of the pollutant distributions, and in persons with a positive history of diabetes and CHD.
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Introduction

Considerable numbers of epidemiological studies have linked short-term changes in fine particulate air pollution with changes in daily morbidity and mortality from cardiopulmonary diseases (Dockery et al., 1993; Burnett et al., 1995; Schwartz and Morris, 1995; US Environmental Protection Agency (US EPA), 1996; Pope III et al., 2002). The effects of gaseous criteria pollutants (including O_3, CO, NO_2, and SO_2) on cardiovascular disease risk have also been reported in some studies (Moolgavkar and Luebeck, 1996; Health Effects Institute, 1997; Schwartz, 1999). The underlying biological mechanisms linking air pollution and cardiopulmonary disease continue to be a subject of research. Recently, several research teams have reported associations between air pollution exposure and the alteration of circulatory regulations, such as heart rate variability, a measure of cardiac autonomic control (Liao et al., 1999, 2004; Pope III et al., 1999; Gold et al., 2000; Creason et al., 2001), plasma viscosity, which is largely influenced by plasma fibrinogen and immunoglobin levels (Peters et al., 1997), systemic inflammation (Peters et al., 2001; Panagiotakos et al., 2004; Pope III et al., 2004), and hematological variable (Seaton et al., 1999).

To elucidate the health effects of criteria pollutants on blood hemostatic and inflammatory responses, and to explore potential injury mechanisms, which may explain the association between air pollution and cardiovascular mortality and morbidity, we conducted this population-based study to examine the association between the short-term (1–3 day) exposure to ambient criteria pollutants and the markers of blood coagulation/systemic inflammation. Our operational concepts/hypotheses are that higher levels of short-term exposure to criteria pollutants are associated with higher levels of blood inflammatory markers (fibrinogen, factor VIII coagulant activity (VIII-C), von Willebrand factor (vWF), and white blood cell count (WBC), and lower levels of albumin, which is inversely associated with the burden of systemic inflammation.

1. Address all correspondence to: Dr. Duanping Liao, Department of Health Evaluation Sciences, Pennsylvania State University College of Medicine, A210, 600 Centerview Dr., Hershey, PA 17033, USA.
Tel.: +1-717-531-4149. Fax: +1-717-531-5779. E-mail: DLiao@psu.edu
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Population and methods

Source Population

Study subjects were selected from the Atherosclerosis Risk in Communities (ARIC) study cohort. The design and objectives of ARIC study have been reported in detail (ARIC Investigators, 1989). Briefly, ARIC is a population-based longitudinal study of atherosclerosis and its sequelae sponsored by the National Heart, Lung and Blood Institute. The ARIC cohort was selected as a probability sample of 15,792 men and women between the ages of 45–64 years at entry from four study centers in the United States, three of which enumerated and enrolled populations reflective of their respective ethnic composition (Washington County, MD, USA; Forsyth County, NC, USA; and selected suburbs of Minneapolis, MN, USA). The fourth quarter of the ARIC cohort was sampled from black residents of Jackson, Mississippi. Eligible participants were interviewed at home, and then invited to a baseline clinical examination (conducted in 1987–1989). The date for the baseline clinical examination was assigned at random. The cohort underwent three follow-up clinical examinations every 3 years after baseline. The follow-up examinations were scheduled according to the anniversary of the baseline examination. Of the 15,792 cohort members inducted during the baseline survey, 27% are African American.

The hemostasis/inflammation variables collected during the baseline examination were used in combination with the air pollution data assessed during the same period for this study. Since there was no air quality monitor in the Washington County, MD field center, participants ($N = 4020$) from that center were excluded from this study. Additionally, due to the small number, persons with ethnicities other than European or African American ($N = 36$) were excluded. PM_{10} data were not recorded on a daily basis, and SO_2 and NO_2 data from the Jackson center were not recorded during the entire study period. For some individuals, the pollutants data were not available for the three days prior to their clinical examination. Consequently, the effective sample sizes for the analyses of PM_{10}, CO, O_3, SO_2, and NO_2 were 7705, 10,208, 8639, 6979, and 7014, respectively.

Air Pollution Data

We abstracted criteria pollutants data for the four ARIC study field centers between 1996 and 1998 from the US EPA's Aerometric Information Retrieval System (AIRS) database. AIRS is a computer-based repository of information about airborne pollution in the United States. The AIRS system is administered by the US EPA, Office of Air Quality Planning and Standards, Information Transfer and Program Integration Division. AIRS databases were maintained and updated by the US EPA, and the databases are physically stored in a network of computers in EPA facilities in North Carolina. The AIRS database contains measurements of ambient concentrations of air pollutants from thousands of monitoring stations operated by EPA, or states and local agencies. These monitoring sites conform to uniform criteria of site selection, instrumentation, and quality assurance. The directly measured daily ambient air pollution data were sent to the AIRS system for storage and analysis. The AIRS database also contains descriptive information about each monitoring station, including its location and operator (US EPA, 1990, 1993, 1995).

The gaseous pollutant data obtained from the AIRS database were hourly monitor-specific measures. From these hourly monitor-specific measures, monitor-specific daily average concentrations were calculated as either the 8-h average (10 00 to 18 00 hours) for O_3 or 24-h averages for CO, SO_2, and NO_2. The PM_{10} data obtained from the AIRS database were monitor-specific daily (24-h) averages. From these monitor specific daily averages, county specific-daily average (CSDA) pollutant exposures were calculated for each pollutant by averaging all available monitor-specific daily averages from all operating monitors within a county on any calendar date.

From the National Weather Center, we obtained data on relative humidity (in %), temperature (in K), and sky cloud cover (the fraction of the celestial dome covered by clouds on a scale from 0 to 10 where 0 indicated very clear and 10 indicated a totally obscured sky), with calendar date and county/state identifiable. In this report, the ''daily meteorological variables'' were defined as the relative humidity, temperature, and sky cloud cover in the county at 14 00 hours.

We linked the individual level cardiovascular disease (CVD) risk factor data and the coagulation /inflammation data with the CSDA air pollution and daily meteorological data, by the clinical examination date and the state and county of each participant's residence. Thus, the individual residential level air pollution and meteorological parameters 1, 2, and 3 days prior to clinical examination (blood draw date) were combined with individual level CVD risk factors and blood coagulation/inflammation data to form the analytic database.

Hemostasis/Inflammation Data

Using a standardized protocol (Clauss, 1957; NHLBI, 1989; Papp et al., 1989; Wu et al., 1990; Nguyen et al., 1995), ARIC technicians drew fasting blood samples into vacuum tubes containing serum-separating gel, sodium citrate, or EDTA. The technicians centrifuged samples at $-4^{\circ}C$. They then froze plasma and serum samples at $-70^{\circ}C$ until analyzed. For this study, the markers of hemostatic factors and inflammatory factors included fibrinogen, VIII-C, vWF, WBC count, and serum albumin. The assays were performed according to standardized protocols with regular quality control procedures in place (National Heart, Lung and Blood Institute (NHLBI), 1989).

Other Covariates

Information on age, ethnicity, gender, education levels, alcohol consumption, and cigarette smoking status were obtained by standardized questionnaires administered by trained and certified interviewers. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. Diabetes mellitus was defined as fasting (8 h) serum glucose ≥ 140 mg/dl, nonfasting glucose ≥ 200 mg/dl, history of physician diagnosed diabetes, or use of an oral hypoglycemic agent or insulin. Sitting blood pressure was measured three times with a random zero sphygmomanometer, after a 5 min rest, by trained technicians following a standardized protocol (NHLBI, 1989). The average of the second and the third readings were used to define hypertension status as diastolic blood pressure ≥ 90 mmHg, systolic blood pressure ≥ 140 mmHg, or self-reported use of antihypertensive medications. For this study, history of a CVD was defined as having hypertension, coronary heart disease (CHD), or stroke, and history of chronic pulmonary disease was defined as having chronic bronchitis, emphysema, or asthma.

Statistical Analysis Methods

Multiple linear regression models were used to assess the associations between each individual pollutant measured 1–3 days to the blood draw and each hemostatic/inflammatory factors and to adjust for relevant confounding factors. The population characteristics were described as mean levels and standard deviations or as proportions. All regression models were adjusted for age, sex, ethnicity-center, education, smoking, drinking status, BMI, history of chronic respiratory disease, humidity, season, cloud cover, and temperature. Histories of CVD and diabetes were also adjusted for when they were not effect modifiers in a particular model. Regression coefficients standardized to 1 SD increment of each pollutant were reported. Statistical interactions between each pollutant and major covariates were evaluated by the inclusion of an interaction term in the regression models, and $P \leq 0.10$ was used to identify statistically significant interaction terms. In the presence of a statistical interaction, stratum-specific regression coefficients were calculated. Higher ordered terms of each pollutant were introduced into the models to identify potential curvilinear associations between the pollutants and the hemostatic/inflammatory factors. We followed the conventional model building process — in the initial model, we always started with a cubic term, a quadratic term, and a linear term of a variable. If the cubic term was not significant, we eliminated it and repeated the process to include only the quadratic and the linear terms in the model, and so forth. In general, if the higher ordered term was significant, we also retained all of its lower terms regardless whether the lower terms were significant. To elucidate the time-course of air pollution exposure and hemostasis/inflammatory responses, lagged regression models were fitted by including into the models primary measure of exposure (concentrations of pollutants one day prior to blood draw) and 1-day and 2-day lagged measure of exposures (concentrations of pollutants 2 days and 3 days prior to blood draw, respectively). All statistical computations were performed using SAS software version 8.2 (The SAS Institute, Cary, NC, USA).

Results

The average participant age was 54 years (Table 1), 57% were female and 66% were white participants. The prevalence of hypertension, diabetes, CHD, stroke, and chronic respiratory disorder were 34%, 9%, 3%, 2%, and 16% respectively. The pollutant levels were negatively associated relative humidity, cloud cover score, and temperature, except for PM_{10} and O_3, which were positively associated with temperature (Table 1). There was no clear pattern of association between the criteria pollutants and CVD risk factors and comorbidity, except for about 8% higher of alcohol use among higher SO_2, NO_2, and CO exposures groups (all $P < 0.05$). Since SO_2 and NO_2 data from the Jackson center (exclusive African-American center) were not recorded during the entire study period, the proportions of white people and less than high school education from the sample available for SO_2 and NO_2 analysis are markedly different from that in other samples in Table 1.

Presented in Table 2 are the summary statistics of the major hemostatic/inflammatory factors and the five criteria pollutants at the participant's residence 1 day prior to blood drawing. These distributional data indicate that exposures to these five criteria pollutants in this population were mostly at the environmentally relevant ambient levels (below current national standards set by US EPA), and the hemostatic / inflammatory markers were within the normal range of healthy individuals. The univariate comparison of blood biomarkers according to the quartiles of the pollutants did not reveal a consistent pattern of association.

Regression coefficients from multivariable linear regression models relating criteria pollutants and hemostatic/inflammatory markers are presented in Table 3. After adjusting for the individual-level CVD risk factors, demographic and socioeconomic variables, and relevant meteorological variables, PM_{10} was significantly ($P < 0.05$) associated with vWF among diabetics and with serum albumin among persons with a history of CVD: 1 SD increment of PM_{10} (12.8 $\mu g/m^3$) was associated with 3.93% increase of vWF among diabetics and with 0.006 g/dl decrease of serum albumin among persons with a history of CVD. In these data, PM_{10} was not significantly associated with fibrinogen and WBC. Higher levels of CO 1 day prior to blood draw were significantly ($P < 0.01$) associated with serum albumin (g/dl): 1 SD increment of CO (0.60 p.p.m.) was associated with 0.018

Table 1. Mean levels (SD) or proportion of selected variables, in the entire study sample and stratified by quartiles of gaseous criteria pollutants.

Variable	All ($N = 10{,}208$)[a]	Quartile of PM_{10} ($N = 7705$)		Quartile of O_3 ($N = 8639$)		Quartile of SO_2 ($N = 6979$)[b]		Quartile of NO_2 ($N = 7014$)[b]		Quartile of CO ($N = 10{,}208$)	
		Q1–3	Q4	Q1–3	Q4	Q1–3	Q4	Q1–3	Q4	Q1–3	Q4
Age (years)	54 (5.8)	54 (5.7)	54 (5.8)	54 (5.7)	54 (5.9)	54 (5.8)	54 (5.8)	54 (5.8)	54 (5.7)	54 (5.8)	54 (5.7)
Women (%)	57	56	57	57	57	54	54	54	54	57	56
White (%)	66	68	67	65	67	92	96	92	96	58	73
Less than high school (%)	21	20	20	20	22	13	10	13	10	24	16
Current smoker (%)	27	26	28	27	28	28	25	28	25	28	26
Current alcohol user (%)	60	62	60	61	57	67	74	66	75	54	66
Hypertension (%)	34	35	34	35	32	25	25	25	25	37	32
Diabetes (%)	9	9	9	9	8	6	6	6	6	10	9
Prevalent CHD (%)	3	3	3	3	3	2	3	2	3	3	3
Stroke (%)	2	2	2	2	2	2	2	2	2	3	2
Chronic lung disease (%)	16	15	17	16	16	18	16	18	16	16	14
BMI (kg/m^2)	27.6 (5.4)	27.6 (5.3)	27.5 (5.3)	27.8 (5.4)	27.2 (5.1)	26.6 (4.7)	26.8 (4.7)	26.6 (4.7)	26.8 (4.7)	27.8 (5.5)	27.5 (5.2)
Relative humidity (%)	81 (12.5)	81 (12.8)	78 (12.9)	82 (12.0)	77 (13.1)	81 (13.2)	78 (13.2)	81 (13.3)	78 (13.1)	82 (12.7)	80 (13.1)
Temperature (K)	287 (11.5)	283 (11.7)	289 (10.5)	285 (12.2)	293 (5.7)	284 (10.5)	276 (12.0)	284 (10.5)	276 (11.9)	286 (10.8)	281 (12.2)
Cloud cover score	4.4 (3.5)	4.8 (3.5)	3.9 (3.5)	5.0 (3.4)	2.6 (3.1)	4.7 (3.5)	4.5 (3.5)	4.7 (3.4)	4.3 (3.5)	4.6 (3.5)	4.3 (3.6)

[a]From the effective sample for the study of CO.
[b]Individuals from Jackson center were excluded due to lack of the SO_2 and NO_2 data during the entire study period.

Table 2. Mean levels (SD) of hemostatic/inflammatory markers and gaseous criteria pollutants, in the entire study sample and stratified by the quartiles of gaseous criteria pollutants.

Variable	Total ($N = 10{,}208$)[a]	Quartile of PM_{10} ($N = 7705$)		Quartile of O_3 ($N = 8639$)		Quartile of SO_2 ($N = 6979$)[b]		Quartile of NO_2 ($N = 7014$)[b]		Quartile of CO ($N = 10{,}208$)	
		Q1–3	Q4	Q1–3	Q4	Q1–3	Q4	Q1–3	Q4	Q1–3	Q4
WBC ($\times 10^3/mm^3$)[a]	6.0 (1.9)	6.1 (2.0)	6.1 (1.9)	6.1 (2.0)	6.0 (1.9)	6.3 (1.9)	6.3 (2.1)	6.3 (2.0)	6.3 (1.9)	6.0 (1.9)	6.2 (2.1)
Factor VIII C (%)[a]	130.6 (39.9)	131 (39.8)	128.8 (38.4)	131.4 (40.3)	128.0 (38.9)	125.3 (36.5)	124.0 (32.6)	125.5 (36.5)	124.2 (33.2)	133.0 (41.5)	130.6 (40.2)
Fibrinogen (mg/dl)[a]	302.1 (65.0)	302.4 (65.9)	300.5 (64.7)	304.1 (66.0)	296.0 (61.6)	296.9 (62.9)	296.8 (60.7)	296.3 (63.2)	296.5 (59.8)	304.7 (667)	302.0 (66.1)
VWF (%)[a]	118.1 (48.4)	116.4 (47.5)	117.3 (48.8)	117.4 (48.4)	120.1 (48.5)	112.0 (43.9)	109.0 (39.7)	112.0 (44.0)	108.5 (39.9)	119.2 (49.5)	114.0 (46.3)
Albumin (g/dl)[a]	3.86 (0.28)	3.88 (0.26)	3.86 (0.27)	3.86 (0.27)	3.84 (0.26)	3.88 (0.26)	3.89 (0.25)	3.88 (0.26)	3.89 (0.25)	3.85 (0.27)	3.86 (0.26)
PM_{10} ($\mu g/m^3$)	29.9 (12.8)	24.0 (6.96)	47.3 (10.11)								
O_3 (p.p.m.)	0.04 (0.02)			0.03 (0.01)	0.06 (0.01)						
SO_2 (p.p.m.)	0.005 (0.004)					0.005 (0.003)	0.006 (0.005)				
NO_2 (p.p.m.)	0.02 (0.008)							0.01 (0.004)	0.03 (0.006)		
CO (p.p.m.)	1.4 (0.6)									1.1 (0.3)	2.2 (0.5)

[a]From the effective sample for the study CO.
[b]Individuals from Jackson center were excluded due to lack of the SO_2 and NO_2 data during the entire study period.

Table 3. Multivariable adjusted[a] regression coefficients (SE) for the associations between 1 SD increment of criteria pollutions and hemostatic/inflammatory markers.

Pollutant	Dependent variable				
	Fibrinogen (mg/dl)	Factor VIII-C (%)	vWF (%)	WBC ($\times 103/mm^3$)	Albumin (g/dl)
PM_{10} (SD = 12.8 $\mu g/m^3$)	0.163 (0.755)	Figure 1	Diabetics: 3.93 (1.80)* Nondiabetics: −0.54 (0.58)	0.021 (0.019)	CVD: −0.006 (0.003)* Non-CVD: 0.029 (0.017)
CO (SD = 0.60 p.p.m.)	−0.16 (0.67)	0.45 (0.42)	−0.29 (0.50)	0.003 (0.017)	−0.018 (0.003)**
NO_2 (SD = 0.008 p.p.m.)	−1.45 (0.78)	−0.22 (0.46)	−0.21 (0.56)	0.017 (0.021)	−0.004 (0.003)
O_3 (SD = 0.017 p.p.m.)	Figure 2	−0.29 (0.56)	Figure 3	−0.039 (0.023)	−0.002 (0.004)
SO_2 (SD = 0.004 p.p.m.)	−0.40 (0.94)	Figure 4	0.65 (0.67)	Figure 5	Figure 6

[a]Adjusted for age, sex, ethnicity-center, education, smoking, drinking status, BMI, history of chronic respiratory disease, history of CVD and history of diabetes when appropriate, humidity, season, cloud cover, and temperature.
*$P<0.05$.
**$P<0.01$.

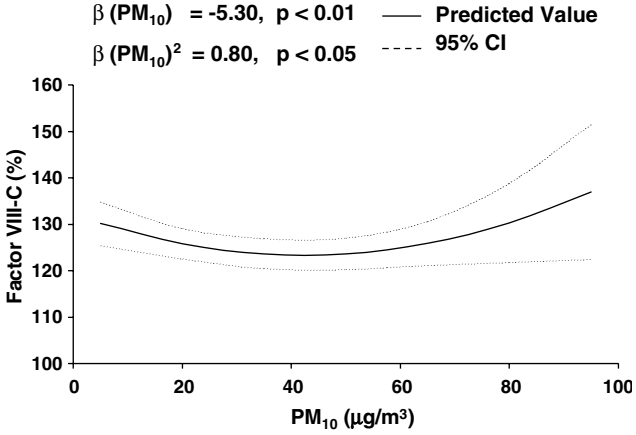

Figure 1. Multivariable adjusted relationship between PM_{10} and factor VIII-C from the entire study sample, with regression coefficients (β) per 1 SD increase of PM_{10} (12.8 $\mu g/m^3$) and P for $\beta = 0$ inserted. Adjusted for age, sex, ethnicity-center, education, smoking, drinking status, BMI, history of CVD, diabetes, and chronic respiratory disease, humidity, season, cloud cover, and temperature.

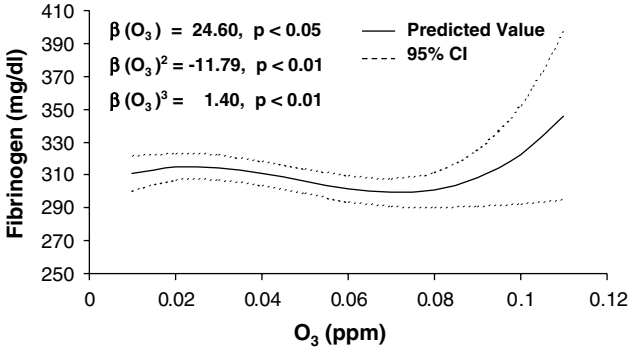

Figure 2. Multivariable adjusted relationship between O_3 and fibrinogen in persons with a history of CVD, with regression coefficients (β) per 1 SD increase of O_3 (0.017 p.p.m.) and P for $\beta = 0$ inserted. Adjusted for age, sex, ethnicity-center, education, smoking, drinking status, BMI, history of diabetes and chronic respiratory disease, humidity, season, cloud cover, and temperature. For persons without a history of CVD, the regression coefficients per one standard deviation increase of O_3 (P for $\beta = 0$) were −2.03 ($P = 0.81$), −0.65 ($P = 0.84$), and 0.08 ($P = 0.83$) for βO_3, βO_3^2, and βO_3^3 respectively.

(SE = 0.003) g/dl decrease of serum albumin. In these data, NO_2, measured 1-day prior to the blood draw, was not significantly associated with any of the hemostaic/inflammatory factors analyzed. The multivariable-adjusted predicted mean values and 95% confidence intervals (CI) of the hemostatic/inflammatory factors at different levels of air pollution, and the corresponding regression coefficients (β) and P values for testing the significant associations ($\beta = 0$) are presented in Figures 1–6, for the pollutants that were significantly associated with hemostatic/inflammatory factor in a pattern reflective of a curvilinear relationship. In general, PM_{10}, O_3 and SO_2 levels, measured 1-day prior to the blood draw, were associated positively and significantly with several hemostatic/inflammatory markers. A history of CVD modified some of the associations: specifically, the associations were more pronounced among persons with a history of CVD. The associations of PM_{10}, O_3 and SO_2 with the dependent variables were in general more pronounced at higher levels of the pollutants. For instance, the adjusted means (SE) of vWF were 118 (0.79), 117 (0.86), and 124 (1.97) % at O_3 levels of <0.04, 0.04–0.07, and >0.07 p.p.m. respectively. Interactions between gaseous pollutants and history of chronic respiratory conditions were not statistically significant at $P \leq 0.10$. It should be noted that the regression coefficients presented in Table 3 and Figures 1–6 were in general small in magnitude per 1 SD change of gaseous pollutants, indicating weak associations.

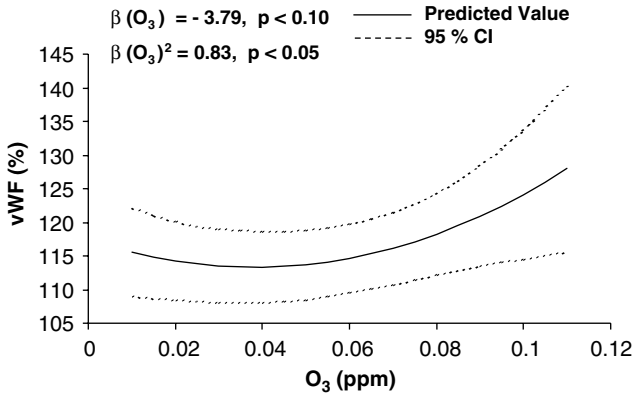

Figure 3. Multivariable adjusted relationship between O_3 and vWF from the entire study sample, with regression coefficients (β) per 1 SD increase of O_3 (0.017 p.p.m.) and P for $\beta = 0$ inserted. Adjusted for age, sex, ethnicity-center, education, smoking, drinking status, BMI, history of CVD, diabetes, and chronic respiratory disease, humidity, season, cloud cover, and temperature.

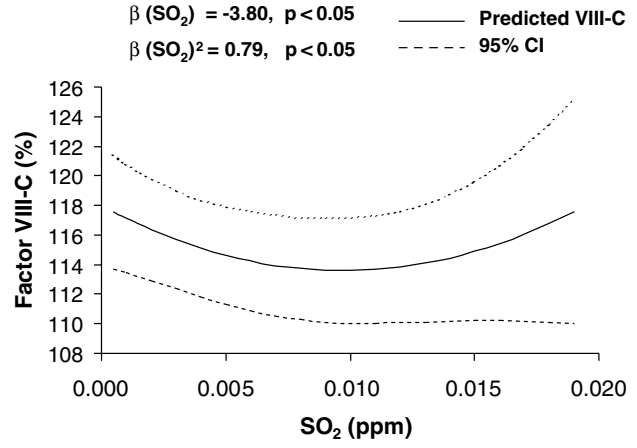

Figure 4. Multivariable adjusted relationship between one standard deviation (0.004 p.p.m.) increase of SO2 and plasma factor VIII-C levels from the entire study sample, with regression coefficients (β) and P for $\beta = 0$ inserted. Adjusted for age, sex, ethnicity-center, education, smoking, drinking status, BMI, history of CVD, diabetes, and chronic respiratory disease, humidity, season, cloud cover, and temperature.

To elucidate the time-course of air pollution and hemostatic/inflammatory responses, 1-day and 2-day lags (pollutant concentrations measured 2 and 3 days prior to blood draw, respectively) were included into the multivariable models in addition to the pollutant measured 1 day prior to blood draw. We first perform several conventional diagnostic tests for collinearity, and none was statistically significant in these lagged models. The results (data not shown) from these lagged models suggested that lagged exposures (2 or 3 days) were not significantly associated with hemostatic/inflammation variables. Furthermore, adjusting for 2- and 3-day exposures simultaneously did not change the pattern of association between exposures 1 day prior to blood draw and hemostatic/inflammatory variables.

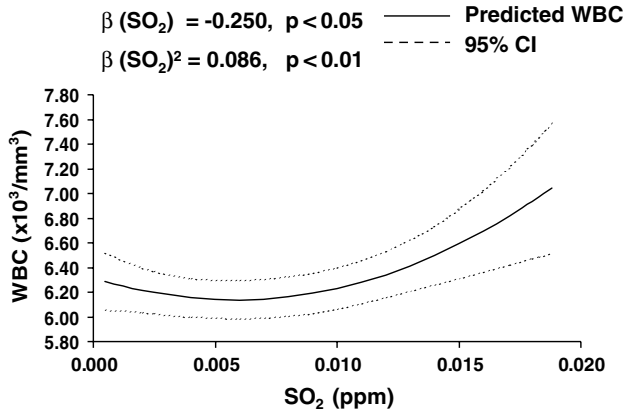

Figure 5. Multivariable adjusted relationship between one standard deviation (0.004 p.p.m.) increase of SO_2 and WBC from persons with a positive history of CVD, with regression coefficients (β) and P for $\beta = 0$ inserted. Adjusted for age, sex, ethnicity-center, education, smoking, drinking status, BMI, history of diabetes and chronic respiratory disease, humidity, season, cloud cover, and temperature. For persons without a history of CVD, the regression coefficients per 1 SD increase of SO_2 (P for $\beta = 0$) were -0.03 ($P = 0.74$) and -0.10 ($P = 0.96$) for βSO_2, and βSO_2^2 respectively.

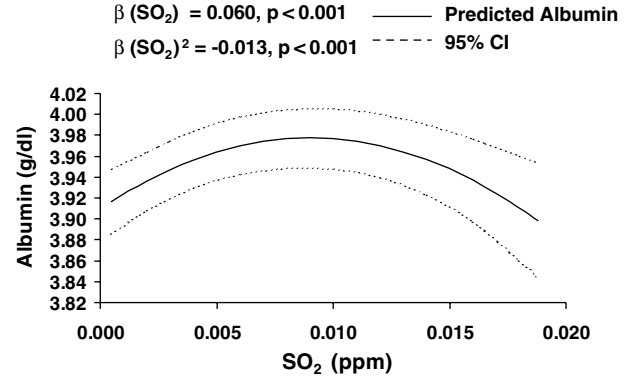

Figure 6. Multivariable adjusted relationship between 1 SD (0.004 p.p.m.) increase of SO_2 and serum albumin levels from the entire study sample, with regression coefficients (β) and P for $\beta = 0$ inserted. Adjusted for age, sex, ethnicity-center, education, smoking, drinking status, BMI, history of CVD, diabetes, and chronic respiratory disease, humidity, season, cloud cover, and temperature.

Discussion

Although numerous studies have demonstrated a significant association between air pollution and the rates of cardiopulmonary disease hospitalizations, morbidity, and mortality, few previous studies measured individual level exposures and outcomes. Consequently, the potential injury mechanisms and potential pathophysiological pathways underlying the association between air pollution and CVD has not been adequately addressed, at least not until recently when several research teams reported associations between air pollution levels and physiological responses of the cardiovascular system ((Peters et al., 1997; Liao et al., 1999, 2004; Pope

III et al., 1999; Gold et al., 2000; Creason et al., 2001). As a result, controversies exist at the levels of science, regulation, and industry as to the causal relationship between air pollution and CVD.

Atherosclerosis, which progresses over decades (Fuster et al., 1992), is the main cause of CVD. Laboratory and pathological data support the idea that inflammation has a role in both the initiation and the progression of atherosclerosis, and anti-inflammatory agents may have a role in the prevention of cardiovascular disease (Munro and Cotran, 1988; Nieminen et al., 1993; Ross, 1993; Alexander, 1994). In past several years, several population-based epidemiological studies have suggested that inflammation is one of the key factors in the initiation and the progression of atherosclerosis (Kuller et al., 1996; Ridker et al., 1997). The mechanism that relates inflammation to atherosclerosis and thrombosis is unclear. It is possible that inflammation markers are surrogates for cellular cytokines (Bataille and Klein, 1992), which help recruit macrophages and monocytes into atherosclerotic plaques (Biasucci et al., 1996). In addition, inflammation can induce monocytes to express tissue factor, a membrane glycoprotein important in initiating coagulation (Cermak et al., 1993). In a related physiological system, hemostatic factors probably have a significant role in the development of cardiovascular disease, via their involvement in the formation of platelet–fibrin thrombi in the setting of atherosclerotic plaque disruptions, or by enhancing the development of fatty streaks and their conversion into fibrosis plaques. Higher levels of plasma fibrinogen level (Wilhelmsen et al., 1984; Meade et al., 1986; Kannel et al., 1987; Yarnell et al., 1991; Ernst and Resch, 1993; Heinrich et al., 1994) and WBCs (Friedman et al., 1974; Grimm et al., 1985; Ernst et al., 1987; Yarnell et al., 1991) are consistent risk factors for cardiovascular disease, and WBCs may interact with fibrinogen (Yarnell et al., 1991). Studies of the prospective relationship between the development of CHD and factor VIII (Meade et al., 1986; Cortellaro et al., 1992), vWF (Jansson et al., 1991; Cortellaro et al., 1992; Thompson et al., 1995), and platelet count (Thaulow et al., 1991) are more limited and inconsistent. In the ARIC Study (Folsom et al., 1997), from which this report obtained the outcome data, it was demonstrated that elevated levels of fibrinogen, WBC, factor VIII-C, and vWF were risk factors for CHD. Possibly through its inverse association with inflammation, serum albumin is consistently and negatively associated with the development of CVD (Phillips et al., 1989; Kuller et al., 1991; Nelson et al., 2000). It should be noted that some markers analyzed in this study are both markers of systemic inflammation and hemostasis, such as fibrinogen, factor VIII, and vWF.

Although the air pollution–inflammation pathway is biologically plausible, no study to date has examined the air pollution–inflammation association in a large population-based sample. Results from this large population-based study, which to our knowledge is the first in this field, suggest that higher levels of criteria pollutants, even at levels far below the current EPA standards, have adverse health effects on the hemostasis and inflammation responses. These findings, if confirmed by others, suggest that airborne pollutants are significant associated with blood coagulation/thrombosis formation and systemic inflammation, and through these potential injury mechanisms, airborne pollution may increase CVD risk.

In this study, with exposures to criteria pollutants assessed 1-day prior to blood drawing, several markers of hemostasis and inflammation were significantly associated with PM_{10}, O_3, and SO_2 levels in a curvilinear pattern, and with CO levels in a linear pattern. The curvilinear associations suggest that any effects of these pollutants on the hemostatic/inflammatory markers are absent at lower levels of exposures and become more pronounced at higher levels of exposures–threshold effects. However, it should be noted that the most pronounced associations in these six figures (Figures 1–6) occurred when the air pollutants were above 2–3 SDs from the mean. Also, we cannot offer a biological explanation, nor can we rule out the role of chance finding for the apparent 'U' and inverse 'U' relationships between SO_2 and factor VIII-C and albumin (Figures 4 and 6, respectively). Therefore, generalization of the statistical significant curvilinear associations should be exercised with caution.

In these data, the associations between PM_{10} and serum albumin, O_3 and fibrinogen, and between SO_2 and WBC were significant only among persons with a history of CVD, but not among persons without such a history. Similarly, the association between PM_{10} and vWF was significant only among persons with a history of diabetes, but not among persons without such a history. These findings are consistent with those observed in studies of the association of ambient fine particle concentrations and cardiac autonomic control (Liao et al., 1999, 2004; Creason et al., 2001), and with the observation of a stronger association between air pollutant exposure and cardiopulmonary mortality among elderly persons with previous history of cardiovascular disease (US EPA, 1996). However, it should be noted that we tested interactions between each of the pollutant and history of CVD, diabetes, chronic pulmonary diseases, age, gender, education, and ethnicity in relationship to each of the hemostasis/inflammatory markers. Testing of multiple interactions was motivated by previous studies that indicated several comorbidities as effect modifiers for air pollution and CVD risk factors (US EPA, 1996; Liao et al., 1999, 2004; Creason et al., 2001). The statistically significant interactions we have identified in these data may be chance findings, while the lack of statistical significance for some potential effect modifiers may be due to limited statistical power. Replication of these interactions by other studies is needed before conclusions of different susceptibility by comorbid conditions

can be made. Several environmental studies have associated with air pollution with C-reactive protein (Peters et al., 2001; Panagiotakos et al., 2004; Pope III et al., 2004), which is a sensitive marker of systemic inflammation. In our study, we did not measure C-reactive protein, thus, we cannot directly associate air pollution measures with this sensitive marker of systemic inflammation. We anticipate that the pattern of associations between air pollutants and C-reactive protein be similar to that between air pollutants and other markers of systemic inflammation measured in this study, since C-reactive protein and has been associated with these variables. Similarly, Seaton et al. (1999) first reported the associations between PM_{10} and blood factors, including fibrinogen, factor VII, C-reactive protein, and WBC in a repeated measured panel study of 112 elderly individuals. They observed a significant positive association between acute PM_{10} exposure and C-reactive protein, a significant, but negative association between acute PM_{10} exposure and fibrinogen and factor VII, and no association for WBC. However, potential curvilinear associations were not tested in this study. Thus, it is difficult to compare the results from our study to that of Seaton et al. because of the design, population, and the analytic differences.

In summary, these data are supportive of the hypothesized air pollution–inflammation–cardiovascular disease pathway at the population level. The magnitude of the estimated effects shown in Table 3 and Figures 1–6, that is, the regression coefficients associated with 1 SD differences in each of the pollutants, are in general small, indicating weak associations. These weak associations suggest that these pollutants are 'minor' risk factors as a putative cause of cardiovascular disease. By contrast, a public health perspective argues that estimates of the magnitude observed in this study would have a significant impact on the population's health, because of the widespread, long-term exposure of the population to low-level ambient air pollutants. In this regard, the pollutant levels for this study derived as daily averages from ambient air monitors such as those used in different locations in the US, and are reflective of the low ambient air exposure levels, even the ''very high'' level of exposures in this study, to which most of the population is exposed on a daily basis.

It should be noted that this is a cross-sectional study, which precludes consideration of a temporal relationship between the air pollutants and hemostasis/inflammation. Individual level time-varying factors cannot be assessed in this study because of the cross-sectional design and we do not have individual level measures several days prior to the clinical examination. Although we were able to assess short-term, prior exposure over the days preceding the blood draw, we assessed ambient exposures to five criteria pollutants by calculating the daily averages from measured data available from several monitors within a county. Although this approach provides the technically most feasible measures of exposure for individual residents we cannot rule out misclassification of the exposures. However, there is no evidence to suggest that such misclassification might be systematic as to the levels of individual dependent variable measures and other CVD risk factors, because clinical examination (and blood draw) dates were assigned at random to all study participants. Following this argument, the associations we observed in this study would have been underestimated due to nondifferential misclassification of exposure. It should also be emphasized that this study was designed to investigate the short-term association between air pollution and hemostasis/inflammation markers in data obtained 1, 2, and 3 days prior to the blood draws, and that we are unable to rule out other patterns of exposure–outcome associations, such as subacute or long-term cumulative effects. This study cannot assess long-term (in term of years) effects of air pollution on the inflammation markers. This is due to the cross-sectional design, and the limited number of study sites (all participants were long time permanent resident from one of the three communities). But more importantly, because the variation of the long-term exposures between individuals who live within a center is very small, the main source of variation can be attributed to the difference between centers. In another words, the 'dummy' coded center variables are in a sense the surrogates of long-term exposures. In all of our statistical models, we adjusted for the 'dummy' coded center variables. Therefore, we feel confident that the short-term effects of air pollution on the inflammation and blood coagulation variables reported in this study are not confounded by the center, nor the long-term. In this study, we statistically adjusted for individual level risk factors for CVD, such as age, sex, smoking, body mass index, histories of CVD, diabetes, and chronic pulmonary diseases, demographic and socioeconomic status, ethnicity-center, education levels, and meteorological factors, such as season, temperature, humidity, and total sky cover. Thus, the results are less likely to reflect bias due to these confounding factors. Although residual confounding by other factors cannot be totally ruled out, we do not believe that minor residual confounding factors could have yielded the consistent findings observed. The main results (Table 3 and Figures 1–6), the regression models were adjusted for ethnicity and center. To further confirm that the significant associations presented in the table and figures were not due to center effect, we performed two additional analyses (data not shown). We first tested center–pollutant interactions in relationship to each of the hemostasis/inflammation markers, and none was statistically significant at $P<0.10$. We also stratified the main models by center, and the patterns of associations were similar across the centers. The lagged analysis in our data indicate that pollutant concentrations, measured 2 or 3 days prior to blood draw, were not significantly associated with the markers of inflammation and hemostasis. Furthermore,

adjusting for 2- and 3-day exposures simultaneously did not change the pattern of association between exposures 1 day prior to blood draw and hemostasis/inflammation. We had also performed three separate analyses (lag day −1, 2, and 3), each of which included only 1 day's air pollution data in the model (data not shown). The results were consistent with that from the three lagged exposures model. Finally, we had to exclude a large number of individuals from our analysis, mostly due to the unavailability of exposure data. This reduces the generalizability of the findings, but is unlikely to have introduced selection bias because of the manner in which the four study cohorts were chosen. Our analysis indicated that persons included were similar to those excluded for major cardiovascular disease risk factors. We would like to point out that although direct comparison of the effect size generated from this cross-sectional study with that from time-series analysis cannot be made because of the design differences, the adverse effects from air pollution on the markers of systemic inflammation observed in this study are consistent with and support of that seen from time-series analysis. The consistent results from different study designs and different populations may provide a more convincing evidence for the associations of interest.

Particulate matter is a complex mixture of suspended particles that vary in size and composition. This study assessed the acute effects of five criteria pollutants; the ability to generalize these findings to other pollutants, such as $PM_{2.5}$, may be limited. Similarly, particle compositions were not available for analysis in this report, thus the inferences from our data can only be made to the mass concentration of PM_{10}, not its chemical composition and proportions of components in the mixture.

In conclusion, the data from this population-based, cross-sectional study suggest that the hemostasis/inflammation markers analyzed are associated adversely with environmentally relevant ambient pollutants, with the strongest associations in the upper range of the pollutant distributions, and in persons with a positive history of CVD and diabetes. Given the established and consistent associations between higher levels of hemostasis/inflammation markers and the development of cardiovascular disease, our findings suggest an injury mechanism and potential underlying pathway by which air pollution could affect the risk of cardiovascular disease morbidity and mortality.

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