Traffic-related Particles Are Associated with Elevated Homocysteine

The VA Normative Aging Study

Sung Kyun Park[1], Marie S. O'Neill[1,2], Pantel S. Vokonas[3,4,5], David Sparrow[3,4,5], Avron Spiro III[3,5], Katherine L. Tucker[6], Helen Suh[7], Howard Hu[1,2,7], and Joel Schwartz[7]

[1]Department of Environmental Health Sciences, and [2]Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan; [3]VA Normative Aging Study, Veterans Affairs Boston Healthcare System, Boston, Massachusetts; [4]School of Medicine, and [5]School of Public Health, Boston University, Boston, Massachusetts; [6]Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts; and [7]Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts

***Rationale*: Recent epidemiologic studies have shown that homocysteine, a sulfur-containing amino acid formed during the metabolism of methionine, is a risk factor for atherosclerosis, myocardial infarction, stroke, and thrombosis. Particulate air pollution has been related to cardiovascular death and hospital admission, but the underlying mechanisms are not fully elucidated.**

***Objectives*: We examined the associations between ambient particulate air pollution and plasma concentrations of homocysteine among 960 community-residing older men (mean age, 73.6 ± 6.9 yr).**

***Methods*: Total homocysteine in plasma, measured using high-performance liquid chromatography with fluorescence detection, was regressed on each ambient particulate pollutant (black carbon, organic carbon, sulfate or $PM_{2.5}$), and effect modification by plasma and dietary B vitamins (folate, B6, and B12) was examined.**

***Measurements and Main Results*: The median concentration of total homocysteine was 10.6 μmol/L. Statistically significant positive associations of total homocysteine were observed with traffic-related particles (black carbon and organic carbon). No association was observed with sulfate, an indicator of coal combustion particles, or $PM_{2.5}$ (particulate matter ≤ 2.5 μm in aerodynamic diameter). The effects of black carbon and organic carbon were more pronounced in persons with low concentrations of plasma folate and vitamin B12.**

***Conclusions*: Exposures to ambient particles, particularly from traffic, are associated with elevated plasma total homocysteine. Homocysteine may be a component or biological marker of the oxidation pathways underlying the effect of ambient particles on the cardiovascular system.**

Keywords: air pollution; folate; homocysteine; traffic particles; vitamin B12

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Only one epidemiologic study has examined associations between air pollutants and homocysteine, but no experimental study has been conducted.

What This Study Adds to the Field

Particles generated from traffic vehicles may elevate concentrations of plasma homocysteine. Higher concentrations of plasma folate and vitamin B12 may reduce the adverse effects of particles.

Numerous epidemiologic studies have shown a consistent association between particulate air pollution exposure and early death, particularly from cardiovascular disease (1–3). Several plausible biological mechanisms have been suggested, including direct effects on the myocardium, disturbances of the cardiac autonomic nervous system, and pulmonary and systemic oxidative stress and inflammatory responses that trigger endothelial dysfunction, atherosclerosis, and coagulation/thrombosis (4). However, the exact mechanistic pathways are still not understood. Identifying these potential pathways is one of the most important research priorities in air pollution epidemiology.

Over the last decades, several studies have demonstrated that homocysteine, a sulfur-containing amino acid formed during the metabolism of methionine, is a risk factor for atherosclerosis, myocardial infarction, stroke, and thrombosis (5–8). Elevated blood concentrations of total homocysteine (tHcy) produce endothelial cell injury, primarily through oxidation (9). High tHcy concentrations may suppress the vasodilator nitric oxide (10), and trigger proliferation of vascular smooth muscle cells (11), which may, in turn, increase the risk of cardiovascular disease. In addition, homocysteine is related to increased proinflammatory markers, such as C-reactive protein, fibrinogen, and interleukin-6 (12–14). Although recent randomized clinical trials have failed to reduce the rates of cardiovascular events using homocysteine-lowering therapy (treatment with folic acid, vitamin B12, and vitamin B6) among patients with acute myocardial infarction, stroke, or diabetes (15–17)—possibly because these trials were too late to observe a remedial effect—a large body of evidence from animal and prospective observational studies suggests that homocysteine is an atherogenic determinant.

Particulate air pollution has also been associated with proinflammatory markers (18–20). However, the biological mechanism that connects particulate air pollution exposure with alterations in plasma tHcy concentration is not yet established. Cigarette smoking has been associated with increased plasma tHcy concentrations (21, 22), and even short-term smoking

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Correspondence and requests for reprints should be addressed to Sung Kyun Park, Sc.D., Department of Environmental Health Sciences, University of Michigan School of Public Health, 109 S. Observatory Street, SPH II-M6240, Ann Arbor, MI 48109. E-mail: sungkyun@umich.edu

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cessation significantly decreased tHcy concentrations (23). The latter finding suggests that, because particle pollution exposure may have similarities to active cigarette smoking, short-term changes in particle exposure may influence plasma tHcy. A recent epidemiologic study conducted in Lombardia, Italy, observed that particulate matter $\leq$ 10 μm in aerodynamic diameter (PM_{10}) was associated with increased plasma tHcy in smokers but not in nonsmokers (24). This finding suggests a possible biological mechanism linking air pollution exposure to increased risk of cardiovascular events, but it needs to be confirmed in other studies.

We hypothesized that plasma tHcy concentrations would be elevated among older people as ambient particle concentrations increase because ambient particle exposure is associated with increased reactive oxygen species generation, which might cause an elevation in tHcy. Furthermore, particle components, such as transition metals and carbonaceous materials, may disrupt methionine metabolism, which may also lead to increased tHcy. Increased interest is now focusing on the relative toxicity of particle composition and sources. This can be assessed by examining associations with different particle components.

The purpose of this study was to examine the association between ambient particulate air pollution (black carbon [BC], organic carbon [OC], sulfate [SO_4^{2-}], and particulate matter $\leq$ 2.5 μm in aerodynamic diameter [$PM_{2.5}$]) and plasma tHcy concentrations among community-residing men from the Normative Aging Study (NAS). This allowed us to evaluate the relationship with particles from different sources, particularly traffic-related sources or regional coal combustion sources. We also investigated whether the plasma B vitamins folate, vitamin B6, and vitamin B12, which are involved in homocysteine metabolism and associated with lower tHcy concentrations (25), modified the associations. Some of the results of this study have been previously reported in the form of an abstract (26).

METHODS

Study Population

The NAS is a longitudinal study of aging established by the Veterans Administration in 1963, when 2,280 men from the greater Boston area free of known chronic medical conditions were enrolled (27). Participants have undergone detailed examination every 3 to 5 years, including routine physical examinations, laboratory tests, collection of medical history information, and completion of questionnaires on smoking history, education level, food intake, and other factors that may influence health. Since April 5, 1993, these detailed examinations have also included assessments of plasma tHcy and B vitamins. From January 1995 through June 2005, when air pollution data were available, tHcy concentrations were measured for 967 subjects. We excluded seven subjects with missing values of the potential confounding factors. $PM_{2.5}$ concentrations between January 21, 1998, and March 15, 1999, and BC concentrations between March 30, 1997, and March 31, 1999, were not available. For these extended periods, it was not possible to replace missing values. Thus, tHcy concentrations measured before 1995 and during these periods were excluded. This resulted in 960 subjects for $PM_{2.5}$ (total observations = 1,832) and 843 subjects for BC (total observations = 1,597). We evaluated the associations between $PM_{2.5}$ or BC and plasma tHcy cross-sectionally as well as longitudinally. Most recent tHcy measurements were used for cross-sectional associations with $PM_{2.5}$ and BC to avoid the periods when $PM_{2.5}$ and BC were not available. OC and SO_4^{2-} were measured at the Harvard School of Public Health monitoring site between June 18, 1999, and February 2, 2004 (OC [n = 449], SO_4^{2-} [n = 427]). Because the number of repeated measurements of tHcy was small during this period, cross-sectional associations with the first tHcy measurements were examined for OC and SO_4^{2-}.

Plasma Analysis of B Vitamins and Homocysteine and Assessment of Dietary Intake

Folate, vitamin B12, vitamin B6, and tHcy in fasting plasma were measured by radioassay with the use of a commercially available kit, an enzymatic method using tyrosine decarboxylase, and high-performance liquid chromatography with fluorescence detection, respectively. Dietary intake was assessed with a version of the Willett semiquantitative food frequency questionnaire. Further details are found elsewhere (28) and in the online supplement.

Air Pollution and Weather Data

Hourly $PM_{2.5}$ and BC were measured at a stationary ambient monitoring site, 1 km away from the exam site, using a tapered element oscillating microbalance (model 1400A; Rupprecht and Pataschnick Co., Albany, NY), an aethalometer (Magee Scientific, Berkeley, CA), and a condensation particle counter (model 3022A; TSI, Inc., Shoreview, MN), respectively. Twenty-four-hour integrated OC and SO_4^{2-} were measured using the Partisol model 2300 sequential sampler (Rupprecht and Pataschnick Co.), and the Harvard-EPA annular denuder system sampler, respectively. Weather variables (air temperature and dew point temperature—the temperature at which the liquid water [dew] evaporates at the same rate at which it condenses) were obtained from the Boston airport weather station. Apparent temperature, defined as a person's perceived air temperature and calculated from the dew point and air temperature together (29), was used to adjust for potential effects of weather.

Physiologic responses to air pollution exposure can occur not only on the same day of exposure but also on several subsequent days (30). To evaluate those lagged effects, we used moving averages up to 7 days before the examination day. Because the average time of blood drawing was around 8:00 A.M., we defined concurrent day concentration of hourly measured particles as 24-hour averages ending at 8:00 A.M. on that day. The concentration of the previous day was therefore defined as 24-hour averages ending at 8:00 A.M. on the previous day. In the same manner, the 2-day moving average and 7-day moving averages are 48- and 168-hour averages ending at 8:00 A.M. on that day. For daily measures of particles (OC and SO_4^{2-}), which were collected from 9:00 A.M. of the previous day, we also used daily moving average up to 7 days before the day of the specimen collection. We excluded the concurrent day from those analyses because most of the air pollution monitoring on the day of exam occurred after the blood draw.

Statistical Analysis

Generalized additive models were constructed to estimate the effects of each air pollutant. tHcy was log-transformed to improve normality and stabilize the variance. We identified *a priori* the following variables as important determinants of tHcy (21, 22): age, serum creatinine, body mass index (BMI), systolic blood pressure (SBP), smoking status (never, former, current), pack-years of cigarettes smoked (0, >0–29, 30–59, ≥60), alcohol consumption (≥2 drinks/d; yes/no), and plasma folate, vitamin B6, and vitamin B12. There were a decreasing trend and a seasonal variation of ambient particle concentrations over the study period. Therefore, long-term time trend (calendar date), season (spring/summer/fall/winter), and apparent temperature were considered potential confounders. To determine whether the association between continuous covariates and log-transformed tHcy was linear, we modeled the continuous covariates as penalized splines using R software (R Foundation for Statistical Computing, http://www.r-project.org). Age, serum creatinine, plasma folate and vitamin B12, long-term time trend, and apparent temperature were nonlinearly associated with tHcy, and thus fitted as penalized splines (*see* Figure E1 of the online supplement).

To assess confounding, we determined percentage change for an increase of the interquartile range (IQR) for each air pollutant, with 95% confidence intervals (CIs) after adjustment for age, long-term time trend, season, and apparent temperature (model 1), after adding adjustment for BMI, SBP, smoking status, pack-years of cigarettes smoked, and alcohol consumption (model 2), and finally after further adjustment for serum creatinine, plasma folate, vitamin B6, and vitamin B12 (model 3). We also ran regression models with and without an indicator for folate fortification (after August 1997 or no folate fortification) to assess whether folate fortification confounded those associations. This is because folate fortification was phased in between

TABLE 1. CHARACTERISTICS OF STUDY PARTICIPANTS INCLUDED IN THE BLACK CARBON ANALYSIS (n = 843) IN THE NORMATIVE AGING STUDY

	Mean ± SD	Median	Range
Age, yr	73.6 ± 6.9	74	51–100
Body mass index, kg/m^2	28.0 ± 4.0	27.5	17.0–52.6
Systolic blood pressure, mm Hg	130.2 ± 17.5	129	77–195
Diastolic blood pressure, mm Hg	74.5 ± 10.8	75.0	41–119
Serum creatinine, mg/dl	1.1 ± 0.32	1.0	0.6–5.5
Plasma measures			
Folate, nmol/L	17.3 ± 18.1	13.5	1.5–318
Vitamin B6, nmol/L	111 ± 112	74.3	5.1–1,556
Vitamin B12, pmol/L	543 ± 359	480	74.0–7252
Total homocysteine, μmol/L	11.3 ± 3.6	10.6	4.0–42.2
Dietary intake			
Folate, μg/d	534 ± 296	486	1.3–1732
Vitamin B6, mg/d	11.3 ± 27.5	3.4	0.05–213
Vitamin B12, μg/d	17.7 ± 20.9	10.7	0.05–175
Energy, kcal/d	1,964 ± 624	1,868	816–4,133
		n (%)	
Smoking status			
Never smoker		248 (29.4)	
Former smoker		559 (66.3)	
Current smoker		36 (4.3)	
Pack-years of cigarettes			
0		248 (29.4)	
>0–29		318 (37.7)	
30–59		179 (21.2)	
⩾60		71 (8.4)	
Missing		27 (3.2)	
Alcohol intake (⩾2 drinks/d)		161 (19.1)	
Diabetes mellitus		124 (14.7)	
Hypertension		609 (72.2)	
Ischemic heart disease		269 (31.9)	

October 1996 and August 1997, and it had a major effect on folate concentrations and probably on tHcy concentrations.

We also examined the associations of $PM_{2.5}$ and BC with repeated measures of tHcy using hierarchical mixed-effects regression models. A random intercept model was used to account for subject-specific intercept that reflects unexplained heterogeneity in subjects' overall concentration of tHcy. The same fixed covariates were included as in the cross-sectional analysis and the generalized additive mixed model (GAMM) in R was used. Two error covariance matrices were considered, no within-subject correlation and the first-order autoregressive covariance structures, but the former was selected based on better fit of the model as assessed by the Akaike information criterion.

To test whether B vitamins modify the effects of pollutants, participants were divided into two groups by median values of plasma concentrations or dietary intakes of B vitamins (low vs. high). We introduced interaction terms between dichotomized B vitamins and each air pollutant in the models. We also evaluated effect modification by serum creatinine and preexisting conditions, including diabetes, hypertension, and ischemic heart disease. Additional detail on methods is provided in the online supplement.

RESULTS

Table 1 shows the distribution of the characteristics of the participants included in the BC analysis (n = 843). The study population was all men, predominantly white, and older (mean age, 73.6 yr; SD, 6.9 yr). Mean BMI was in the overweight range (i.e., 28 kg/m^2). Of subjects, 14.7, 72.2, and 31.9% were identified as having diabetes, hypertension, and ischemic heart disease, respectively. The median concentration of tHcy was 10.6 μmol/L. Mean plasma tHcy and B vitamin concentrations were all within normal ranges and mean dietary intakes met National Research Council recommendations (31). Table 2 summarizes air pollution and apparent temperature levels during the study period. All air pollution concentrations during the study period were within the National Ambient Air Quality Standards. The correlations of $PM_{2.5}$ with other particle mass pollutants were very high, especially with SO_4^{2-}, which represents secondary transported particles (Spearman correlation coefficient [ρ], 0.85). Daily concentrations of traffic-related particles (BC and OC) were moderately correlated (ρ = 0.51). Apparent temperature was also highly correlated with all particulate pollutants.

The associations of tHcy with particulate air pollutants are presented in Table 3. After controlling for aforementioned potential confounders, BC was consistently significantly associated with elevated tHcy in both base models and fully adjusted models. The strongest effects were observed with a 4-day average of BC in models 1 and 2, but with the concurrent day's BC in model 3. After controlling for all potential confounders, an IQR increase in concurrent day BC (0.66 μg/m^3) was related to a 3.13% (95% CI, 0.76–5.55%) increase in tHcy. We also found marginally significant associations between elevated tHcy and a 7-day average OC in all three models. $PM_{2.5}$ and SO_4^{2-} were not associated with tHcy in any lags (*also see* Figure E2). We assessed whether folate fortification confounded those associations, but further adjustment for folate fortification did not change the results (data not shown). We also examined the associations of longitudinal measures of tHcy with $PM_{2.5}$ and BC, and confirmed that BC was significantly associated with elevated tHcy in several lagged models, especially with a 4-day average of BC in all three models (e.g., an IQR increase in 4-d BC [0.52 μg/m^3] was associated with a 2.39% [95% CI, 0.63–4.18%] increase in tHcy in model 3), but no statistically significant association was found between $PM_{2.5}$ and tHcy (*see* Table E1).

We assessed whether homocysteine-lowering factors, including folate, vitamin B6, and vitamin B12 modified the effects of BC and OC on tHcy (Figure 1). For simplicity, we used the averaging periods that showed the highest percentage changes in tHcy for each pollutant for this analysis (24-h average for BC;

TABLE 2. CONCENTRATIONS OF AIR POLLUTION AND APPARENT TEMPERATURE (24-h OR 1-d) AND SPEARMAN CORRELATION COEFFICIENTS WITH PLASMA TOTAL HOMOCYSTEINE

					Spearman Correlation Coefficient				
	No. of Days	Mean ± SD	Median	Range	tHcy	$PM_{2.5}$	BC	OC	SO_4^{2-}
$PM_{2.5}$, μg/m^3	567	12.0 ± 6.6	10.6	2.0 to 62.0	0.06	1			
BC, μg/m^3	477	0.99 ± 0.56	0.87	0.07 to 3.7	0.03	0.51*	1		
OC, μg/m^3	262	3.5 ± 1.8	3.1	0.29 to 11.8	0.14†	0.51*	0.51*	1	
SO_4^{2-}, μg/m^3	241	3.2 ± 3.0	2.4	0.39 to 29.0	0.03	0.85*	0.50*	0.41*	1
Apparent temperature, °C	567	11.4 ± 9.5	10.4	−9.4 to 35.7	0.02	0.22*	0.29*	0.44*	0.24*

Definition of abbreviations: BC = black carbon; OC = organic carbon; $PM_{2.5}$ = particulate matter ⩽ 2.5 μm in aerodynamic diameter; tHcy = total homocysteine.
* $P < 0.0001$.
† $P < 0.05$.

TABLE 3. ESTIMATED PERCENTAGE CHANGE (95% CONFIDENCE INTERVAL) IN TOTAL HOMOCYSTEINE FOR AN INTERQUARTILE RANGE INCREASE IN PARTICULATE AIR POLLUTANTS

Lag Model	$PM_{2.5}$	BC	OC	SO_4^{2-}
Concurrent day				
IQR, μg/m³	7.66	0.66		
Model 1	1.32 (−0.83 to 3.52)	2.64 (−0.12 to 5.48)*	—‡	—‡
Model 2	1.55 (−0.77 to 3.91)	2.62 (−0.17 to 5.48)*	—	—
Model 3	1.57 (−0.38 to 3.56)	3.13 (0.76 to 5.55)†	—	—
1-Day previous				
IQR, μg/m³	6.91	0.66	2.00	2.61
Model 1	−1.43 (−3.51 to 0.69)	1.46 (−0.98 to 3.96)	2.12 (−0.98 to 5.31)	0.91 (−0.77 to 2.62)
Model 2	−1.41 (−3.53 to 0.76)	1.32 (−1.14 to 3.85)	1.69 (−1.51 to 5.00)	0.99 (−0.94 to 2.95)
Model 3	−1.28 (−3.12 to 0.60)	0.95 (−1.12 to 3.05)	1.87 (−0.81 to 4.62)	0.91 (−0.72 to 2.57)
2-Day moving average				
IQR, μg/m³	6.47	0.60	1.93	2.10
Model 1	0.04 (−2.13 to 2.26)	2.75 (−0.18 to 5.76)*	−0.39 (−3.67 to 3.01)	−0.25 (−2.07 to 1.60)
Model 2	−0.07 (−2.26 to 2.17)	2.63 (−0.33 to 5.67)*	−0.88 (−4.26 to 2.61)	−0.29 (−2.35 to 1.82)
Model 3	0.25 (−1.69 to 2.22)	2.59 (0.10 to 5.14)†	1.05 (−1.86 to 4.06)	0.05 (−1.74 to 1.86)
3-Day moving average				
IQR, μg/m³	5.83	0.57	1.68	1.73
Model 1	−0.64 (−2.92 to 1.69)	2.95 (−0.44 to 6.46)*	0.53 (−2.66 to 3.83)	−0.15 (−1.97 to 1.69)
Model 2	−0.74 (−3.04 to 1.61)	2.97 (−0.46 to 6.51)*	0.14 (−3.15 to 3.54)	−0.17 (−2.23 to 1.93)
Model 3	−0.59 (−2.63 to 1.49)	3.12 (0.21 to 6.11)†	1.32 (−1.44 to 4.16)	−0.01 (−1.78 to 1.80)
4-Day moving average				
IQR, μg/m³	5.21	0.52	1.64	1.64
Model 1	−0.63 (−2.94 to 1.72)	3.94 (0.24 to 7.78)†	1.57 (−1.89 to 5.15)	−0.69 (−2.74 to 1.41)
Model 2	−0.86 (−3.19 to 1.52)	3.76 (0.02 to 7.64)†	1.42 (−2.14 to 5.12)	−0.60 (−2.95 to 1.81)
Model 3	−0.73 (−2.78 to 1.37)	3.00 (−0.13 to 6.22)*	1.89 (−1.15 to 5.03)	−0.58 (−2.63 to 1.51)
5-Day moving average				
IQR, μg/m³	4.68	0.49	1.60	1.60
Model 1	−0.51 (−2.79 to 1.83)	3.26 (−0.60 to 7.27)*	2.27 (−1.49 to 6.16)	−1.14 (−3.53 to 1.30)
Model 2	−0.82 (−3.13 to 1.54)	2.64 (−1.23 to 6.67)	2.11 (−1.77 to 6.15)	−0.90 (−3.64 to 1.92)
Model 3	−0.84 (−2.85 to 1.22)	2.38 (−0.89 to 5.77)	2.12 (−1.29 to 5.65)	−1.09 (−3.48 to 1.36)
6-Day moving average				
IQR, μg/m³	4.50	0.44	1.43	1.40
Model 1	−0.91 (−3.32 to 1.56)	1.63 (−1.99 to 5.38)	2.83 (−0.74 to 6.52)	0.00 (−2.39 to 2.44)
Model 2	−1.32 (−3.76 to 1.17)	1.03 (−2.62 to 4.80)	2.78 (−0.90 to 6.60)	0.36 (−2.36 to 3.16)
Model 3	−1.44 (−3.58 to 0.74)	0.93 (−2.15 to 4.11)	2.53 (−0.59 to 5.74)	0.41 (−2.01 to 2.89)
7-Day moving average				
IQR, μg/m³	4.20	0.44	1.23	1.30
Model 1	−0.84 (−3.27 to 1.64)	1.38 (−2.45 to 5.36)	2.75 (−0.41 to 6.02)*	−0.16 (−2.51 to 2.24)
Model 2	−1.19 (−3.64 to 1.33)	0.69 (−3.16 to 4.70)	2.55 (−0.71 to 5.92)	0.30 (−2.37 to 3.04)
Model 3	−1.69 (−3.84 to 0.51)	0.45 (−2.81 to 3.83)	2.55 (−0.21 to 5.39)*	0.07 (−2.25 to 2.43)

Definition of abbreviations: BC = black carbon; IQR = interquartile range; OC = organic carbon; $PM_{2.5}$ = particulate matter ⩽ 2.5 μm in aerodynamic diameter.

Model 1: adjustment for season (spring, summer, fall, winter) and penalized splines for age, long-term time trend (date) and apparent temperature (N = 960). Model 2: further adjustment for BMI, systolic blood pressure, smoking status (never, former, current), pack years of cigarettes (0, 30 <, 30-59, 60 ⩾), and alcohol consumption (⩾ 2 drinks/day). 29 men were excluded from these analyses due to missing data for pack years of cigarettes (N = 931). Model 3: further adjustment for penalized splines for serum creatinine and plasma concentrations of folate, vitamin B6, and vitamin B12. 28 men were additionally eliminated from these models due to missing data for plasma B vitamins (N = 903).

* $P < 0.1$.

† $P < 0.05$.

‡ Estimated percent changes for concurrent day OC and SO_4^{2-} were not computed because concurrent day concentrations of OC and SO_4^{2-} were not available. See details of air pollution monitoring in METHODS.

7-d average for OC in model 3). The median values were used to divide participants into low and high groups. The median values of plasma concentration of folate, vitamin B6, and vitamin B12 are 9 nmol/L, 63.3 nmol/L, and 439 pmol/L, respectively, and the median intakes of dietary folate, vitamin B6, and vitamin B12 are 474 μg/day, 3.4 mg/day, and 10.4 μg/day, respectively. In subjects with low concentrations of plasma folate and vitamin B12, BC was associated with 5.31% (95% CI, 2.29–8.42%) and 5.06% (95% CI, 2.03–8.17%) increases in tHcy, respectively, whereas almost null associations were found in subjects with high concentrations (P values for interaction term < 0.05). However, no difference in effect of BC by plasma vitamin B6 was seen. The effect of BC was reduced in subjects with high dietary intakes of B vitamins as compared with those with low intakes, but the differences were not statistically significant. In addition, the effect of OC was significantly lower in persons with a high concentration of plasma vitamin B12 than in those with a low concentration. OC was associated with a 5.23% (95% CI, 1.59–9.01%) increase in persons with low vitamin B12 concentration, but no association was found in persons with a high concentration (P value for interaction = 0.04). However, high vitamin B6 or folate status either as plasma or dietary measures did not mitigate the effect of OC. There was also no significant difference in effect of $PM_{2.5}$ and SO_4^{2-} between persons with low and high plasma or dietary B vitamins (data not shown).

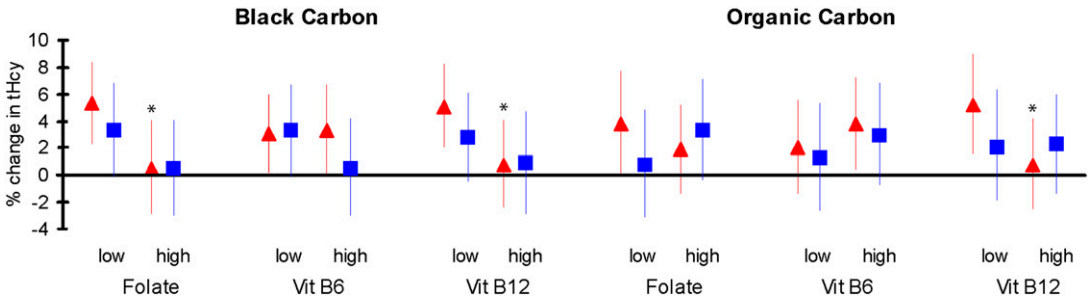

Figure 1. Estimated percentage change (95% confidence intervalCI) in total homocysteine for an interquartile range increase in black carbon (24-h average) and organic carbon (7-d average) by status of plasma and dietary intakes (from food frequency questionnaires) of B vitamins (Vit). The median values were used to divide participants into low and high groups. The median values of plasma concentration of folate, vitamin B6, and vitamin B12 are 13.5 nmol/L, 74.3 nmol/L, and 480 pmol/L, respectively, and the median intake of dietary folate, vitamin B6, and vitamin B12 are 486 μg/day, 3.4 mg/day and 10.7 μg/day, respectively. All models were adjusted for body mass index, systolic blood pressure, smoking status, pack-years of cigarettes smoked, alcohol consumption, and season, and penalized splines for age, serum creatinine, long-term time trend, and apparent temperature. For models of plasma measures of B vitamins, penalized splines for folate, vitamin B6, and/or vitamin B12 were further adjusted. For models of dietary measures of B vitamins, total energy intake and dichotomized folate, vitamin B6, and/or vitamin B12 were further adjusted. *Significantly different from low group (*P* for interaction < 0.05). *Triangles*, plasma measures; *squares*, dietary measures.

We also evaluated effect modification by serum creatinine and preexisting diseases (diabetes, hypertension, ischemic heart disease), but there were no statistically significant differences in effects of air pollution by these variables (data not shown).

DISCUSSION

Ambient particle pollutants were significantly associated with an increase in plasma tHcy concentration, a well-known predictor of cardiovascular disease, in a large community-based cohort. These findings were independent of other risk factors for elevated plasma tHcy, including age, serum creatinine, BMI, SBP, cigarette smoking, alcohol consumption, and plasma B vitamins. We observed significant associations between elevated plasma tHcy and BC, which is an indicator of traffic-related particles. OC, another secondary particle, but primarily derived from transported exhaust from motor vehicles, was associated with tHcy as well. However, no significant association was found with SO_4^{2-}, a marker of secondary particles from coal combustion. The observed associations with BC and OC were stronger in subjects with low plasma folate and vitamin B12, whereas persons with high plasma folate ($\geq$13.5 nmol/L) and vitmin B12 ($\geq$480 pmol/L) were not affected by exposure to BC and OC, which suggests that higher intakes of folate and vitamin B12 may reduce the cardiovascular impact of traffic-related particles. There was no evidence of effect modification by vitamin B6 or preexisting diseases.

The current study showed that the constituents of $PM_{2.5}$ may have different impacts on plasma tHcy. $PM_{2.5}$ is typically a complex of soot, sulfate and nitrates, and transition metals. BC, also called elemental carbon or soot, is generated directly from traffic combustion sources, but reflects both local and aged transported traffic particles. OC can be emitted both from primary emission sources and from secondary chemical reactions of gaseous organic precursors (e.g., polycyclic aromatic hydrocarbons) (32). SO_4^{2-} represents mainly secondary long-range transported particles generated from coal-burning power plants and constitutes the largest component of $PM_{2.5}$ in Boston (33). Recent studies have demonstrated that traffic particles were more strongly associated with cardiovascular events than secondary coal-burning particles (34–36). Studies conducted in the Boston area found that BC was significantly associated with emergency admission due to myocardial infarction (34), and reduced heart rate variability (35), which is linked with ventricular arrhythmias and myocardial infarction (37, 38). Laden and colleagues also showed in the Six Cities Study that the effect of combustion factors from mobile sources was larger than that of particles from coal-fired power plants (36). The results of the current study are in agreement with these findings.

A recent epidemiologic study conducted in Lombardia, Italy, investigated the association between air pollution and tHcy among 1,213 healthy subjects with a mean age of 43.5 years (24). Baccarelli and coworkers (24) found no association between PM_{10} and tHcy in the entire population, but found significant effect modification by cigarette smoking. An IQR increase in the 24-hour average of PM_{10} (32.5 $\mu g/m^3$) was associated with a 6.3% (95% CI, 1.3–11.6%) increase in fasting tHcy in smokers (n = 343), whereas no association was found in nonsmokers. This finding suggests that cigarette smoking and related oxidative stress and inflammation may play an important role in the association of air pollution with homocysteine metabolism. However, we could not examine effect modification by smoking because of an insufficient number of current smokers (n = 43). In the present study, we found significant main effects of particle pollutants. This may be because our study population comprised older people with a mean age of 74 years, whereas only 52 elderly people (>65 yr) were included in Baccarelli and colleagues' study. The difference between our findings suggests that older people may be more susceptible to particle-associated elevation in tHcy.

Several possible physiologic mechanisms may explain the link between particle exposure from ambient air and increased tHcy. Cigarette smoking causes an increase in plasma tHcy concentrations (21, 22), and particle pollution may act similarly. Homocysteine is produced intracellularly by demethylation of methionine. Particle exposure, especially with transition metals bound to particles, may directly inactivate the enzymes involved in homocysteine remethylation, such as methionine synthase (39). Another possible pathway is that reactive oxygen species generated by particle exposures, which lead to oxidative stress (40), may cause increased plasma tHcy. Pretreatment with antioxidant vitamin E and vitamin C has been shown to block impacts of hyperhomocysteinemia, suggesting an oxidative mechanism (9, 41, 42). Hyperhomocysteinemia is associated with enhanced lipid peroxidation *in vivo* (43).

We observed significant effect modification by plasma vitamin B12 and folate in the relation between BC and OC and plasma tHcy. This may relate to the ability of these vitamins to reduce toxicity of compounds known to be in diesel exhaust. An essential amino acid, methionine, can be provided in the diet as well as regenerated from homocysteine via methionine synthase with methyltetrahydrofolate and vitamin B12 as cofactors (25). Low concentrations of plasma folate and vitamin B12 could result in less methylenetetrahydrofolate available for the methyl

donation, leading to hypomethylation (44, 45). Therefore, we might expect that people with low plasma vitamin B12 and folate concentrations would be more responsive to surrogates for particles from diesel exhaust, such as BC and OC, compared with people with high plasma B12 and folate concentrations.

There was no solid evidence of effect modification by vitamin B6. High intake of vitamin B6 mitigated the effect of BC, but not OC. Low vitamin B6 could impair homocysteine removal because vitamin B6 is required when homocysteine is metabolized into cysteine by the transsulfuration pathway (25). The current finding suggests that traffic particles may elevate the tHcy concentration through interruption of the remethylation pathway, but not the transsulfuration pathway. Further studies are needed to confirm our findings.

There are several limitations to this study. One limitation is that we used air pollution concentrations from a single ambient monitoring site as a surrogate for recent pollution exposures. However, ambient particles have relatively uniform spatial distribution across urban areas and the longitudinal correlation between the monitor's readings and personal exposure was high (46). In fact, $PM_{2.5}$ and SO_4^{2-} concentrations in Boston were very spatially homogeneous (47, 48), so it is reasonable that $PM_{2.5}$ and SO_4^{2-} from a single monitoring site could characterize personal exposures to them. By contrast, BC concentration is considerably more spatially variable, depending on traffic exposure. We examined correlations between 24-hour concentrations at our monitoring location and concentrations at 14 monitoring stations operated for a year at a range of metropolitan Boston locations ranging from downtown to rural. Although the association was always significant, the correlation coefficients ranged from 0.54 to 0.94 (median, 0.8), suggesting greater exposure error for that exposure. However, due to the nondifferential nature of such error, any possible exposure misclassification would be expected to bias the results toward the null. We did not have information on the participants' time spent inside versus outside, physical activity, or proximity to heavy traffic, which are also important determinants of exposure to the pollutants of interest.

We controlled many potential confounding factors in the models, including age, serum creatinine, BMI, SBP, smoking status, alcohol consumption, plasma concentrations of B vitamins, long-term time trend, season, and apparent temperature. We also considered other factors, including blood glucose and histories of diabetes, hypertension, and ischemic heart disease, but these did not confound the observed associations. Therefore, bias due to confounding factors should be minimized, although residual or unmeasured confounding cannot be excluded. Our findings need to be confirmed in a study with better assessment of particle exposures, including, potentially, personal monitoring, as well as confounding factors.

The NAS consists of all males of predominantly white race with a mean age of 74 years. Age, sex, and race are important determinants of plasma tHcy. A recent study found an association between inflammatory state and tHcy only among subjects aged 65 or older (12). Furthermore, dietary patterns and behaviors that influence tHcy concentration, such as cigarette smoking, vary by sex and race (21, 22, 49). Therefore, these results may not be generalizable to younger people, women, or other racial/ethnic groups.

In conclusion, exposures to traffic-related particles were associated with an increase in plasma tHcy among community-dwelling older people. Higher concentrations of plasma folate and vitamin B12 appear to protect against increases in plasma tHcy related to exposure to traffic particles. The results of this study suggest a possible mechanism by which particle exposure may lead to adverse cardiovascular events through elevation in plasma tHcy.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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