

Cardiac Autonomic Dysfunction Effects From Particulate Air Pollution and Protection by Dietary Methyl Nutrients and Metabolic Polymorphisms

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Background—Particulate air pollution is associated with cardiovascular mortality and morbidity. To help identify mechanisms of action and protective/susceptibility factors, we evaluated whether the effect of particulate matter <2.5 μm in aerodynamic diameter ($\text{PM}_{2.5}$) on heart rate variability was modified by dietary intakes of methyl nutrients (folate, vitamins B₆ and B₁₂, methionine) and related gene polymorphisms (C677T methylenetetrahydrofolate reductase [*MTHFR*] and C1420T cytoplasmic serine hydroxymethyltransferase [*cSHMT*]).

Methods and Results—Heart rate variability and dietary data were obtained between 2000 and 2005 from 549 elderly men from the Normative Aging Study. In carriers of [CT/TT] *MTHFR* genotypes, the SD of normal-to-normal intervals was 17.1% (95% CI, 6.5 to 26.4; $P=0.002$) lower than in CC *MTHFR* subjects. In the same [CT/TT] *MTHFR* subjects, each 10- $\mu\text{g}/\text{m}^3$ increase in $\text{PM}_{2.5}$ in the 48 hours before the examination was associated with a further 8.8% (95% CI, 0.2 to 16.7; $P=0.047$) decrease in the SDNN. In [CC] *cSHMT* carriers, $\text{PM}_{2.5}$ was associated with an 11.8% (95% CI, 1.8 to 20.8; $P=0.02$) decrease in SDNN. No $\text{PM}_{2.5}$ -SSDN association was found in subjects with either [CC] *MTHFR* or [CT/TT] *cSHMT* genotypes. The negative effects of $\text{PM}_{2.5}$ were abrogated in subjects with higher intakes (above median levels) of B₆, B₁₂, or methionine. $\text{PM}_{2.5}$ was negatively associated with heart rate variability in subjects with lower intakes, but no $\text{PM}_{2.5}$ effect was found in the higher intake groups.

Conclusion—Genetic and nutritional variations in the methionine cycle affect heart rate variability either independently or by modifying the effects of $\text{PM}_{2.5}$. (*Circulation*. 2008;117:1802-1809.)

Key Words: aging ■ epidemiology ■ heart rate ■ metabolism ■ nervous system, autonomic

Reductions in heart rate variability (HRV), a noninvasive measure of cardiac autonomic dysfunction that independently predicts cardiovascular mortality,¹ have been related to short-term exposure to particulate air pollution (PM), particularly to fine-particulate air pollution of <2.5 $\mu\text{mol}/\text{L}$ in aerodynamic diameter ($\text{PM}_{2.5}$).²⁻⁶ This relation has been investigated to clarify mechanisms underlying the increased risk of cardiovascular disease (CVD) associated with $\text{PM}_{2.5}$ exposure observed in multiple investigations.^{7,8}

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Dietary methyl nutrients, including folate, the B vitamins pyridoxine (B₆) and cyanocobalamin (B₁₂), and methionine, are coenzymes or substrates in the methionine cycle that contribute

to controlling biological processes^{9,10} such as methyl group transfers, homocysteine synthesis, and redox states that may be affected by PM exposure.¹¹⁻¹⁴ The activity of the methionine cycle depends on the availability of dietary methyl nutrients^{15,16} and is modified by genetic variations in metabolic genes.^{17,18} In particular, the CT and TT genotypes of the C677T methylenetetrahydrofolate reductase (*MTHFR*) polymorphism have been associated with reduced enzyme activity^{17,19} and linked, although not consistently, with increased risk of CVD.²⁰ Conversely, the TT genotype of the C1420T cytoplasmic serine hydroxymethyltransferase (*cSHMT*) polymorphism has been associated with higher homocysteine levels²¹ and has been found to interact with *MTHFR* polymorphisms in determining increased CVD risk.¹⁸

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Whether differences in dietary intakes of methyl nutrients or genetic variation in the methionine cycle modify the effects of PM_{2.5} exposure on cardiovascular outcomes has never been tested. In the present study, we examined how the association of PM_{2.5} with HRV in the Normative Aging Study, a repeated-measures investigation of elderly subjects from the Boston metropolitan area, was affected by C677T *MTHFR* and C1420T *cSHMT* polymorphisms and by variations in dietary intakes of folate, vitamin B₆, vitamin B₁₂, and methionine.

Methods

Study Population

Our study population consisted of 549 men from the Normative Aging Study, a longitudinal study of aging established in 1963 by the US Veterans Administration.²² Between November 2000 and June 2005, all participants still presenting for examination (n=676) were evaluated for HRV. Of these, 127 subjects were excluded because of heart arrhythmias, measurement time <3.5 minutes, or missing potential confounding data. The remaining 549 subjects had HRV measured in either 1 (n=363) or 2 (n=186) visits. None of the subjects had a recent (≤6 months) myocardial infarction. The study participants were all male, and 539 of them (97.3%) were white. The present was approved by the Institutional Review boards of all participating institutions, and all participants gave written informed consent to the study.

HRV Measurement

HRV was measured for 7 minutes with a 2-channel (5-lead) ECG monitor (Trillium 3000 model, Forest Medical, East Syracuse, NY) while the subject was seated. The SD of normal-to-normal intervals (SDNN), high frequency (HF; 0.15 to 0.4 Hz), and low frequency (LF; 0.04 to 0.15 Hz) were computed with a fast Fourier transform using software (Trillium-3000, PC-Companion Software, Forest Medical) complying with established guidelines.²³ We selected for the analysis the 4 consecutive minutes of ECG reading with the lowest number of artifacts.

Air Pollution and Weather Data

Continuous PM_{2.5} was measured at a stationary monitoring site on the roof of Countway Library, Harvard University in downtown Boston (Mass) with a tapered-element oscillating microbalance (model 1400A, Rupprecht & Pataschnick Co, East Greenbush, NY). Meteorological data were obtained from the Boston airport weather station. The 48-hour moving average of PM_{2.5} was used as the exposure index because this exposure period has shown the strongest association in previous studies.⁶

Semiquantitative Food-Frequency Questionnaires

Study subjects completed a food-frequency questionnaire referring to intake in the prior year at every visit.¹⁸ Food-frequency questionnaire data were available for 713 of the total 735 visits. Estimates of dietary intake, including folate, vitamin B₆, vitamin B₁₂, and methionine, were derived from the frequency and dosage information on the food-frequency questionnaire using software developed by the Nurses' Health Study²⁴ and processed by Nurses' Health Study operators. Validity and reliability of this food-frequency questionnaire for estimating daily vitamin intakes have been described previously.^{24,25}

Genotyping Methods

We performed genotyping of the C677T *MTHFR* (rs1801133) and C1420T *cSHMT* (rs1979277) polymorphisms on a subset of 362 of the 549 subjects included in the study. These 362 subjects were part of a prior analysis of a nested case-control study of CVD and controls selected by risk set sampling.¹⁸ We estimated that data from the 362 subjects with available genotyping provided us with statis-

tical power to detect effect modifications in the association between PM_{2.5} and HRV equal to 86% for C677T *MTHFR* and 77% for C1420T *cSHMT*. These power calculations were performed on potential PM_{2.5} effects on the HF component of HRV, which was associated with PM_{2.5} exposure in a previous study on this population,¹³ assuming the same HF SD and effect modification size as those observed in our recent work on hemochromatosis (HFE) gene polymorphisms.²⁶

DNA was extracted from stored frozen buffy coat of 7 mL whole blood using the QiAmp DNA blood kits (QIAGEN, Germantown, Md). Genotypes of C677T *MTHFR* and C1420T *cSHMT* were determined by the TaqMan procedure using the allelic discrimination technique (ABI Prism 7900 Sequence Detection System, Applied Biosystems, Foster City, Calif). Details of the genotyping are given elsewhere.¹⁸

Statistical Analysis

HRV measurements were log₁₀ transformed to improve normality. The following potential confounders were chosen a priori and included in the analysis: age, past/current coronary heart disease (CHD), body mass index, mean arterial pressure, fasting blood glucose, cigarette smoking (never/former/current), alcohol consumption (≥2 drinks per day, yes/no), use of β-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, room temperature, season, and 48-hour moving average of outdoor apparent temperature. Potential nonlinearity between apparent temperature and HRV was accounted for by the use of linear and quadratic terms. All independent variables were fitted as time-varying covariates.

Because our data included repeated measures of HRV for many participants, our data may lack independence. Thus, we fit a mixed-effects model (PROC MIXED in SAS version 9.0, SAS Institute Inc, Cary, NC). We assumed the following: $Y_{it} = b_0 + u_i + b_1 X_{lit} + \dots + b_p X_{pit} + \beta \text{Pollution}_{it} + \epsilon_{it}$, where Y_{it} is the logarithm of HRV in subject i at time t , b_0 is the overall intercept, and u_i is the separate random intercept for subject i . In this equation, X_{lit} to X_{pit} are the covariates. We used this model to assess the effect of PM_{2.5} on HRV. To evaluate the effect modification of PM_{2.5} effect by gene polymorphisms or dietary intakes, we added interaction terms to a model that included the main effects for both PM_{2.5} and the genetic/dietary factors. Dietary factors were entered in the model as time-varying variables.

Because *MTHFR* and *SHMT* genotype data were obtained from a convenience sample that represented a subset of our study population, stratum-weighted regression was used to obtain unbiased estimates, as indicated in a recent work on the use of extant case-control data for the analysis of additional outcomes.²⁷ All results including genotype data presented throughout this article are obtained from mixed models that used weights equal to 1 for cases and equal to the reciprocal of the probability of being sampled into the study for controls.²⁷ As a sensitivity analysis, we fitted non-weighted mixed models that also included the original case status variable or were restricted only to the original control series, with no notable differences in the results.

All regression analyses presented here were repeated after heart rate was included as an independent variable. Such adjustment by heart rate did not modify, compared with the results presented here, the significance of the gene polymorphism main effects and of the interactions of PM_{2.5} with gene polymorphisms or dietary intakes.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Table 1 shows the characteristics of the entire study group and of the subjects with and without genotype data. Genotype distributions were in Hardy-Weinberg equilibrium for both C677T *MTHFR* ($P=0.95$) and C1420T *cSHMT* ($P=0.35$). Subjects with genotype data, which included CVD cases and age-matched controls from a previous study on CVD,¹⁹ had

Table 1. Anthropometric and Clinical Characteristics* of the Study Population in the Normative Aging Study

	All Subjects (n=549)	Subjects With Genotype Data† (n=362)	Subjects Without Genotype Data† (n=187)	P‡
CVD incident cases (1961–1998),§ n (%)	141 (25.7)	141 (39.0)	0 (0.0)	<0.001
Age, y	72.8±6.7	74.1±6.6	70.4±6.0	<0.001
Body mass index, kg/m ²	28.1±4.1	28.0±4.0	28.4±4.1	0.06
Systolic blood pressure, mm Hg	130.4±16.3	130.2±16.6	130.8±15.8	0.75
Diastolic blood pressure, mm Hg	74.8±9.7	74.0±9.8	76.3±9.3	0.001
Mean arterial pressure, mm Hg	93.3±10.5	92.7±10.6	94.4±10.2	0.02
Heart rate, bpm	70.7±6.8	70.7±6.5	70.9±7.5	0.79
Fasting blood glucose, mg/dL	107.5±26.8	109.0±27.1	104.7±25.9	0.02
Total cholesterol, mg/dL	194.9±37.8	192.4±38.0	199.8±36.8	0.002
HDL, mg/dL	49.6±13.5	50.0±12.6	50.9±14.9	0.31
Triglycerides, mg/dL	130.9±71.3	133.2±77.7	126.5±56.9	0.35
Smoking status, n (%)				
Never smoker	173 (31.6)	114 (31.5)	59 (31.7)	
Current smoker	29 (5.3)	14 (3.4)	15 (8.1)	
Former smoker	346 (63.1)	234 (64.6)	112 (60.2)	0.11
Alcohol intake (≥2 drinks/d), n (%)	102 (18.6)	69 (19.1)	33 (17.7)	0.73
Diabetes mellitus, n (%)	77 (14.0)	61 (16.9)	16 (8.6)	0.01
History of CHD, n (%)	156 (28.4)	137 (37.9)	19 (10.2)	<0.001
History of stroke, n (%)	35 (6.4)	28 (7.7)	7 (3.7)	0.10
Hypertension, n (%)	383 (69.8)	259 (71.6)	124 (66.3)	0.24
Use of β-blocker, n (%)	183 (33.3)	132 (36.5)	51 (27.3)	0.04
Use of calcium channel blocker, n (%)	73 (13.3)	55 (15.2)	18 (9.6)	0.08
Use of ACE inhibitor, n (%)	116 (21.1)	82 (22.7)	34 (18.2)	0.27
<i>MTHFR</i> 677C>T genotype				
CC	NA	137 (37.8)	NA	
CT	NA	170 (47.0)	NA	
TT	NA	55 (15.2)	NA	
<i>cSHMT</i> 1420C>T genotype				
CC	NA	171 (47.2)	NA	
CT	NA	149 (41.2)	NA	
TT	NA	42 (11.6)	NA	

HDL indicates high-density lipoprotein; ACE, angiotensin-converting enzyme. Values are mean±SD when appropriate.

*Information collected at the time of the first measurement of HRV.

†Subjects with or without genotype data for the C677T *MTHFR* or C1420T *cSHMT* polymorphisms.

‡Probability value for differences between groups with and without genotype data from Fisher's exact test or Wilcoxon (Mann-Whitney) test.

§Incident cases of CVD, including CHD and stroke, diagnosed between 1961 and 1998 were included in the nested case-control study on CVD¹⁸ for which genotypes were originally determined. Controls were sampled from the remaining cohort subjects by risk set sampling.

older age, higher fasting blood glucose, and, presumably because of tighter control or other changes in cases after the CVD event, lower blood pressure and total cholesterol, as also suggested by the more frequent use of β-blockers (Table 1). Consistently, controls from the original study did not show differences compared with subjects without genotype data except for older age and, because body mass index is negatively associated with age in healthy elderly individuals, moderately lower body mass index (Table I of the online Data Supplement). Table 2 shows dietary intakes of methyl nutrients, HRV measures, and environmental data for the 735 visits included in the study. No differences were observed in the subset of visits (n=485) with available C677T *MTHFR*

and C1420T *cSHMT* genotyping compared with the entire study population.

We calculated in multivariate models the adjusted percent change in HRV associated with the C677T *MTHFR* and C1420T *cSHMT* genotypes (Table 3). Subjects carrying the *MTHFR* 677 CT/TT genotypes exhibited a reduction of 17.1% in SDNN (95% CI, −25.4 to −6.5; *P*=0.002), 33.6% in HF (95% CI, −50.7 to −10.4; *P*=0.008), and 36.2% in LF (95% CI, −50.1 to −18.3; *P*<0.001) relative to the CC genotype. *cSHMT* genotypes were not associated with HRV (Table 3).

The association between *MTHFR* genotypes and HRV remained significant after ambient PM_{2.5} was added as an independent variable to the models (supplementary Table III).

Table 2. Dietary Intakes and Environmental Variables of the Study Population in the Normative Aging Study

	All Visits (n=735)	Visits in Subjects With Genotype Data* (n=485)	Visits in Subjects Without Genotype Data* (n=250)	P†
Nutrient intake, geometric mean (95% CI)				
Folate, μg/d	460.2 (438.9 to 482.5)	467.3 (441.8 to 494.3)	447.4 (410.3 to 487.9)	0.33
Vitamin B ₆ , mg/d	4.15 (3.83 to 4.50)	4.17 (3.78 to 4.60)	4.12 (3.59 to 4.72)	0.63
Vitamin B ₁₂ , μg/d	11.9 (11.1 to 12.7)	11.7 (10.8 to 12.7)	12.1 (10.8 to 13.6)	0.83
Methionine, g/d	1.86 (1.80 to 1.92)	1.83 (1.76 to 1.91)	1.90 (1.79 to 2.01)	0.38
Daily intake lower than recommended, ^{34,35} n (%)				
Folate (<400 μg/d)	270 (38.2)	173 (37.8)	97 (39.0)	0.63
Vitamin B ₆ (<1.7 mg/d)	97 (13.6)	65 (14.0)	32 (12.0)	0.79
Vitamin B ₁₂ (<2.4 μg/d)	14 (2.0)	11 (2.4)	3 (1.2)	0.29
Methionine (<19 mg · kg ⁻¹ · d ⁻¹)	234 (33.3)	158 (34.7)	76 (30.9)	0.31
HRV, geometric mean (95% CI)				
SDNN,‡ ms	32.6 (31.2 to 34.0)	32.6 (30.9 to 34.4)	32.6 (30.4 to 34.9)	0.85
HF,‡ ms ²	77.9 (69.8 to 86.9)	80.5 (70.2 to 92.3)	73.1 (60.9 to 87.7)	0.54
LF,‡ ms ²	94.5 (86.5 to 103.2)	91.5 (82.0 to 102.0)	100.6 (86.5 to 117.0)	0.33
Environmental variables, geometric mean (95% CI)				
PM _{2.5} ,§ μg/m ³	10.5 (10.0 to 10.9)	10.4 (9.9 to 11.0)	10.5 (9.8 to 11.4)	0.81
Outdoor temperature,§ °C	11.1 (10.4 to 11.9)	11.7 (10.8 to 12.6)	10.1 (8.9 to 11.3)	0.08
Room temperature, °C	24.0 (23.9 to 24.1)	24.0 (23.9 to 24.2)	24.0 (23.8 to 24.2)	0.99

*Subjects with genotype data for the C677T *MTHFR* or the C1420T *cSHMT* polymorphisms.

†Test for differences between groups with or without genotype data from mixed models or logistic regression with generalized estimating equations.

‡Difference in SDNN and power in HF (0.15 to 0.4 Hz) or LF (0.04 to 0.15 Hz) computed with a fast Fourier transform algorithm.

§Average of hourly measurements of PM_{2.5} and outdoor apparent temperature during the 48 hours before the HRV measurement.

We estimated the association of PM_{2.5} with HRV overall and by C677T *MTHFR* and C1420T *cSHMT* genotypes (Table 4). In all subjects with genotype data, a 10-μg/m³ increase in ambient PM_{2.5} level in the 48 hours before the HRV measurement was negatively but nonsignificantly associated with SDNN, HF, and LF. In the full data set (Table 5), PM_{2.5} was significantly associated with SDNN (-7.1%; 95% CI, -13.2 to -0.6%; P=0.03) and HF (-18.7%; 95% CI, -31.1 to -4.0; P=0.01).

In subjects carrying the *MTHFR* 677 CT/TT genotypes (Table 4), PM_{2.5} level was associated with significant de-

creases in both SDNN (-8.8%; 95% CI, -16.7 to -0.2; P=0.047) and HF (-22.8%; 95% CI, -38.2 to -3.5; P=0.02), whereas no PM_{2.5}-related change was found in *MTHFR* 677 CC subjects. However, the statistical interactions between PM_{2.5} level and C1420T *cSHMT* genotypes were not statistically significant (P≥0.19).

In subjects carrying the *cSHMT* 1420 CC genotype, PM_{2.5} level was associated with significant decreases in SDNN (-11.8%; 95% CI, -20.8 to -1.8; P=0.02) and HF (-30.8%; 95% CI, -46.9 to -9.8; P=0.007), whereas no

Table 3. Adjusted Percent Change in HRV Associated With *MTHFR* 677C>T and *cSHMT* 1420C>T Genotypes

HRV Component*	Genotype	Change, %	95% CI	P
Main effect of <i>MTHFR</i> 677C>T genotype on HRV*				
SDNN	CC	Reference	...	
	CT/TT	-17.1	-26.4 to -6.5	0.002
HF	CC	Reference	...	
	CT/TT	-33.6	-50.7 to -10.4	0.008
LF	CC	Reference	...	
	CT/TT	-36.2	-50.1 to -18.3	<0.001
Main effect of <i>cSHMT</i> 1420C>T on HRV*				
SDNN	CC	Reference	...	
	CT/TT	4.0	-7.5 to 17.0	0.51
HF	CC	Reference	...	
	CT/TT	6.3	-20.7 to 42.5	0.68
LF	CC	Reference	...	
	CT/TT	2.3	-19.8 to 30.5	0.85

*SDNN and power in HF (0.15 to 0.4 Hz) and LF (0.04 to 0.15 Hz) computed with a fast Fourier transform algorithm.

Table 4. Adjusted Percent Change in HRV for Each 10 $\mu\text{g}/\text{m}^3$ of $\text{PM}_{2.5}$ in the 48 Hours Before the Measurement by *MTHFR* 677C>T and *cSHMT* 1420C>T Genotype

HRV Component*	Genotype	Change, %	95% CI	<i>P</i>	<i>P</i> for Interaction
Main effect of $\text{PM}_{2.5}$ on HRV, all subjects with genotype data					
SDNN	All subjects	-6.0	-13.5 to 2.0	0.14	
HF	All subjects	-17.1	-32.3 to 1.6	0.07	
LF	All subjects	-8.2	-22.1 to 8.2	0.31	
Effect of $\text{PM}_{2.5}$ on HRV by <i>MTHFR</i> 677C>T genotype					
SDNN	CC	3.7	-9.8 to 19.2	0.61	
	CT/TT	-8.8	-16.7 to -0.2	0.047	0.19
HF	CC	4.7	-25.7 to 47.5	0.79	
	CT/TT	-22.8	-38.2 to -3.5	0.02	0.37
LF	CC	6.4	-19.3 to 40.1	0.66	
	CT/TT	-12.1	-26.6 to 5.4	0.16	0.27
Effect of $\text{PM}_{2.5}$ on HRV by <i>cSHMT</i> 1420C>T genotype					
SDNN	CC	-11.8	-20.8 to -1.8	0.02	
	CT/TT	-0.1	-10.4 to 11.2	0.98	0.02
HF	CC	-30.8	-46.9 to -9.8	0.007	
	CT/TT	-0.8	-24.0 to 29.3	0.95	0.03
LF	CC	-17.1	-33.2 to 2.9	0.09	
	CT/TT	1.4	-18.2 to 25.8	0.90	0.10

*SDNN and power in HF (0.15 to 0.4 Hz) and LF (0.04 to 0.15 Hz) computed with a fast Fourier transform algorithm.

significant $\text{PM}_{2.5}$ -related change was found in CT/TT subjects (Table 4). The statistical interactions between $\text{PM}_{2.5}$ level and C1420T *cSHMT* genotypes were statistically significant for both SDNN ($P=0.02$) and HF ($P=0.03$).

In our data, the *MTHFR* 677 CT/TT genotypes were associated with increased risk of CVD (supplementary Table IV), as defined for the original case-control study.¹⁸

When subjects were divided according to their dietary intakes of folate, vitamin B₆, vitamin B₁₂, or methionine, we found that the negative effect of $\text{PM}_{2.5}$ on HRV was abrogated in subjects with B₆, B₁₂, or methionine higher than the median daily intake of the study population (Table 5). In particular, the association of $\text{PM}_{2.5}$ with SDDN was significantly modified by B₆, B₁₂, and methionine intakes (P for interaction <0.05). The modification of the association of $\text{PM}_{2.5}$ with HF and LF was statistically significant for differences in methionine intake (P for interaction ≤ 0.03) and only borderline significant for B₆ and B₁₂ (P for interaction, between 0.06 and 0.07). When we evaluated the association of dietary intakes with HRV regardless of $\text{PM}_{2.5}$ exposure (supplementary Table V), vitamin B₆, vitamin B₁₂, and methionine intakes exhibited positive associations, generally nonsignificant, with HRV. A significant increase in SDNN was found in association with methionine intake above the median (11.4%; 95% CI, 2.0 to 21.7; $P=0.02$). Other potential modifiers of the $\text{PM}_{2.5}$ -HRV association such as CHD, obesity, diabetes, or hypertension were not associated with methyl nutrient intakes in this population (supplementary Table VI).

Throughout this article, we have presented modifications in HRV as percent changes. Changes on the original scale are presented in supplementary Tables VII to IX.

Discussion

The present study, based on an elderly population in Boston (Mass), showed that genetic and nutritional variations in the methionine cycle metabolism modified HRV either independently or by modifying the effects of $\text{PM}_{2.5}$. We demonstrated that HRV outcomes were affected by multiple components of the methionine cycle, including polymorphisms in genes coding for enzyme proteins (*MTHFR* and *cSHMT*) and dietary intakes of enzyme cofactors (B₆, B₁₂) and cycle substrates (methionine).

We found that subjects with *MTHFR* 677 CT/TT genotypes had lower HRV than subjects with the CC genotypes. This finding is in the same direction as the results of a comprehensive meta-analysis²⁰ based on 11 162 CHD cases and 12 758 controls from 40 different studies that showed that TT carriers had significantly higher risk of CHD. However, the *MTHFR* 677 TT genotype appeared to be associated with increased CHD risk only in European populations, and there has been speculation as to whether dietary or other characteristics abrogated the C677T *MTHFR* effect in North American populations.²⁰ Our findings in this elderly population suggest that at least some age groups of the US population may not be protected against the negative effects of C677T *MTHFR* on cardiac function.

The negative association between $\text{PM}_{2.5}$ and HRV was modified by both C677T *MTHFR* and C1420T *cSHMT* polymorphisms, although the effect modification was significant for the C1420T *cSHMT* polymorphism only. $\text{PM}_{2.5}$ effects on HRV were stronger in subjects with the *MTHFR* 677 CT/TT and *cSHMT* 1420 CC genotypes, which have been associated with reduced enzyme activity^{17,19,21} and increased risk of CVD.^{18,20} *MTHFR*, although not directly part of the methionine cycle, is

Table 5. Adjusted Percent Change in HRV for Each 10 $\mu\text{g}/\text{m}^3$ of $\text{PM}_{2.5}$ in the 48 Hours Before the Measurement by Folate, Methionine, and Vitamin B₆ and B₁₂ Intake

HRV Component*	Intake Group	Visits, n	Change, %	95% CI	P	P for Interaction
Main effect of $\text{PM}_{2.5}$ on HRV, all subjects						
SDNN	All subjects	713	-7.1	-13.2 to -0.6	0.03	...
HF	All subjects	713	-18.7	-31.1 to -4.0	0.01	...
LF	All subjects	713	-11.8	-23.2 to -1.3	0.08	...
Effect of $\text{PM}_{2.5}$ on HRV by folate intake†						
SDNN	<495.8 $\mu\text{g}/\text{d}$	353	-8.8	-16.4 to -0.4	0.04	
	\geq 495.8 $\mu\text{g}/\text{d}$	354	-5.7	-13.8 to -3.1	0.20	0.57
HF	<495.8 $\mu\text{g}/\text{d}$	353	-18.9	-34.6 to -0.6	0.06	
	\geq 495.8 $\mu\text{g}/\text{d}$	354	-20.0	-35.7 to -0.3	0.05	0.92
LF	<495.8 $\mu\text{g}/\text{d}$	353	-15.7	-29.5 to -0.9	0.06	
	\geq 495.8 $\mu\text{g}/\text{d}$	354	-9.9	-24.9 to -8.2	0.27	0.57
Effect of $\text{PM}_{2.5}$ on HRV by vitamin B ₆ intake†						
SDNN	<3.65 mg/d	357	-13.1	-20.0 to -5.5	0.001	
	\geq 3.65 mg/d	356	1.9	-7.1 to -11.7	0.69	0.006
HF	<3.65 mg/d	357	-27.4	-40.9 to -10.6	0.003	
	\geq 3.65 mg/d	356	-5.2	-24.3 to -18.8	0.65	0.06
LF	<3.65 mg/d	357	-20.0	-32.6 to -4.9	0.01	
	\geq 3.65 mg/d	356	0.2	-17.0 to -20.9	0.99	0.06
Effect of $\text{PM}_{2.5}$ on HRV by vitamin B ₁₂ intake†						
SDNN	<11.1 $\mu\text{g}/\text{d}$	356	-12.2	-19.1 to -4.7	0.002	
	\geq 11.1 $\mu\text{g}/\text{d}$	357	1.3	-7.8 to -11.4	0.78	0.01
HF	<11.1 $\mu\text{g}/\text{d}$	356	-27.3	-40.6 to -11.0	0.002	
	\geq 11.1 $\mu\text{g}/\text{d}$	357	-4.9	-24.6 to -20.1	0.68	0.06
LF	<11.1 $\mu\text{g}/\text{d}$	356	-19.2	-31.8 to -4.4	0.01	
	\geq 11.1 $\mu\text{g}/\text{d}$	357	0.2	-17.5 to -21.6	0.99	0.07
Effect of $\text{PM}_{2.5}$ on HRV by methionine intake†						
SDNN	<1.88 g/d	352	-11.9	-18.9 to -4.1	0.003	
	\geq 1.88 g/d	351	3.0	-6.3 to -13.1	0.54	0.007
HF	<1.88 g/d	352	-25.7	-39.6 to -8.7	0.005	
	\geq 1.88 g/d	351	0.2	-20.4 to -26.0	0.99	0.03
LF	<1.88 g/d	352	-20.9	-33.4 to -6.0	0.008	
	\geq 1.88 g/d	351	4.1	-14.1 to -26.2	0.68	0.02

*SDNN and power in HF (0.15 to 0.4 Hz) and LF (0.04 to 0.15 Hz) computed with a fast Fourier transform algorithm.

†Subjects were divided into the high and low intake groups using the median values of folate, methionine, vitamin B₆, and vitamin B₁₂ intakes of the study population.

the key limiting enzyme required for the conversion of 5-10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the methyl group donor required for the remethylation of homocysteine to methionine.²⁸ *cSHMT* produces the *MTHFR* substrate 5-10-methylenetetrahydrofolate from tetrahydrofolate in a B₆-dependent reaction. Thus, the effects of both the *MTHFR* 677 CT/TT and *cSHMT* 1420 CC genotypes may be mediated through a reduction in the methionine cycle activity.

The reduction in HRV associated with $\text{PM}_{2.5}$ level was abrogated in subjects with intakes of B₆, B₁₂, and methionine higher than the median level of our study population. Conversely, $\text{PM}_{2.5}$ level was negatively associated with all measures of HRV in subjects with lower intakes. These results suggest that lower availability of B₆, B₁₂, or methionine, as well as genetically determined reductions in key enzymatic

activities, may reduce methionine cycle-dependent cell functions that counteract $\text{PM}_{2.5}$ effects. The methionine cycle communicates with different pathways presiding over various cell functions, including DNA methylation and glutathione synthesis.^{9,15} The methyl donors produced by the methionine cycle contribute to DNA methylation as substrates of DNA methyl transferases. Global DNA methylation content in blood leukocytes and other tissues decreases with aging, a finding that has been related to the age-associated increase in cardiovascular risk,²⁹ and oxidative DNA damage such as that following $\text{PM}_{2.5}$ exposure may interfere with methylation processes,³⁰ thus also resulting in genomic hypomethylation. Genetic or dietary factors that increase the production of methyl donors may prevent the potential loss of DNA methylation that may be caused by $\text{PM}_{2.5}$ exposure.

In our previous work in the Normative Aging Study, we showed that the association of PM_{2.5} with reduced HRV was stronger in subjects with glutathione S-transferase deletion, a common polymorphism that impairs glutathione-related responses to oxidative stress.¹³ Glutathione is synthesized from homocysteine, also a substrate of the methionine cycle, through additional B₆-dependent reactions.⁹ Rodents exposed to concentrated urban particles evinced increased reactive oxygen species in both the lung and heart,³¹ an effect muted by preadministration of *N*-acetyl cysteine, a glutathione precursor and potent antioxidant.³² Experimental evidence has shown that a meal rich in methionine shifts the cycle activity toward glutathione production.⁹ Thus, a reduction in methionine cycle activity may represent an additional mechanism modulating particle effects by reducing oxidative stress defenses.

The median intakes in our populations were above the current dietary reference intakes of folate, B₆, B₁₂, and methionine. However, a relatively high percentage of individuals had daily intakes of methionine lower than the dietary reference intakes. The nutritional profile of the study subjects, which also included a large majority showing adequate B₆ and B₁₂ intakes, indicates that the usual diet in the study population was rich in meat with less intake of vegetables and dairy products. Our findings indicate that differences in B₆ and B₁₂ intakes in the range above the current dietary reference intake levels may modify the cardiovascular effects of air pollution. This finding, together with the increased particle-related risk in subjects with lower methionine intake, would warrant, if confirmed, a reassessment of current strategies for methyl nutrient supplementation.

A potential limitation of this study is that we used ambient PM_{2.5} concentrations from a single monitoring site as a surrogate for recent exposure to PM_{2.5}. A recent study comparing ambient concentrations at this site with personal exposures in Boston has shown a high longitudinal correlation³³ between the 2 measurements; the study also reported that PM_{2.5} concentrations were spatially homogeneous over the Boston area. This suggests that our use of ambient concentrations is reasonable and that the resulting exposure error is likely to be nondifferential. In our analyses, we considered several potential confounding factors that may have influenced HRV measures; we adjusted our models for age, existing diagnosis of CHD, body mass index, mean arterial pressure, fasting blood glucose, cigarette smoking, alcohol consumption, room temperature, outdoor apparent temperature, season, and use of β -blockers, calcium channel blockers, and angiotensin-converting enzyme inhibitors. Therefore, chances that the observed associations reflected bias resulting from confounders are minimized.

Our analyses of C677T *MTHFR* and C1420T *cSHMT* polymorphisms were based on a subset of the study population that had previously been included in a case-control study on CVD nested in the Normative Aging Study cohort.¹⁸ The use of such a convenience sample with extant genotype data did not appear to have produced bias in our results because analyses restricted to controls from the original case-control series or adjusted by CVD case status confirmed our findings.²⁷

Our results can be generalized only to an aged population that consists of older men who are almost all white. The effect on women, children, and different ethnic groups should be addressed in future studies, particularly in relation to the exposure of different population groups to PM_{2.5} with various geographical locations, occupations, socioeconomic status, and behavioral characteristics. Other health outcomes of PM_{2.5}, including respiratory responses, also may be modified by genetic variations in the methionine pathway or differences in B₆, B₁₂, and methionine intake. Our findings provide novel hypotheses to pursue further research to investigate the mechanisms of action of air particles and ultimately to identify measures to prevent CVD and to reduce the effects of air pollution in human populations.^{34,35}

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Disclosures

None.

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CLINICAL PERSPECTIVE

Reductions in heart rate variability (HRV), a noninvasive measure of cardiac autonomic dysfunction that independently predicts cardiovascular mortality, have been related to short-term exposure to particulate air pollution (PM), particularly to fine-particulate air pollution of $<2.5 \mu\text{mol/L}$ in aerodynamic diameter ($\text{PM}_{2.5}$). This relation has frequently been investigated to clarify mechanisms underlying the increased risk of cardiovascular disease associated with $\text{PM}_{2.5}$ exposure. In a repeated-measures study of 549 elderly individuals from eastern Massachusetts, we evaluated HRV in relation to genetic polymorphisms (C677T methylenetetrahydrofolate reductase [*MTHFR*] and C1420T cytoplasmic serine hydroxymethyltransferase [*cSHMT*]) and dietary intakes of methyl nutrients that participate in the methionine cycle and contribute to biological processes such as methyl group transfers, homocysteine synthesis, and redox states that are potentially affected by PM exposure. Results from this investigation indicate that genetic and nutritional variations conducive to lower methionine cycle function affect HRV either independently or by enhancing the negative effects of PM. In particular, carriers of [CT/TT] *MTHFR* genotypes exhibited lower HRV, which was decreased further in the presence of higher ambient $\text{PM}_{2.5}$ in the 48 hours before the examination. $\text{PM}_{2.5}$ exposure was associated with lower HRV in individuals with [CC] but not in those with [CT/TT] *cSHMT* genotypes. In addition, the negative effects of $\text{PM}_{2.5}$ on HRV were abrogated in subjects with higher intakes (above the median) of vitamin B₆, vitamin B₁₂, or methionine. These findings provide novel hypotheses to investigate the mechanisms of action of air particles and ultimately to identify measures to reduce the effects of air pollution in human populations.

Cardiac Autonomic Dysfunction: Effects From Particulate Air Pollution and Protection by Dietary Methyl Nutrients and Metabolic Polymorphisms

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