Focused Exposures to Airborne Traffic Particles and Heart Rate Variability in the Elderly

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Background: Exposure to airborne particles may increase cardiac risk by altering autonomic balance. Because these risks may be particularly great for traffic-related particles, we examined associations between particles and heart rate variability as 44 subjects participated in 4 repeated trips aboard a diesel bus.

Methods: Twenty-four hour electrocardiograms were correlated with continuous particle concentrations using generalized additive models controlling for subject, weekday, time, apparent temperature, trip type, activity, medications, and autoregressive terms. Associations were assessed for short- and medium-term moving averages of measured concentrations.

Results: Heart rate variability was negatively associated with fine particulate matter. Positive associations were demonstrated with heart rate and the low-to-high frequency power ratio. Associations were strongest with 24-hour mean concentrations, although strong short-term associations also were found during bus periods, independent of daily exposures. Overall, associations were greatest for high-frequency power with the following effects per interquartile change in the 24-hour mean concentrations: –15% (95% confidence interval = –17% to –14%) for $PM_{2.5}$ (4.6 $\mu g/m^3$); –19% (–21% to –17%) for black carbon (459 ng/m^3); and –14% (–15% to –12%) for fine particle counts (39 pt/cm^3). For each interquartile change in the 5-minute $PM_{2.5}$ concentration (10 $\mu g/m^3$) aboard the bus, an 11% (95% confidence interval = –14% to –8%) decrease in high-frequency power was observed.

Conclusions: This investigation indicates that fine particles are negatively associated with heart rate variability, with an overall trend towards reduced parasympathetic tone. Although daily associations were evident for all particles, short-term associations were predominantly limited to traffic-related particles.

(*Epidemiology* 2007;18: 95–103)

Submitted 8 December 2005; accepted 21 July 2006.

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Supported by NIEHS (ES09825/ ES00002), USEPA (R827353), and EPRI (W09207).

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ISSN: 1044-3983/07/1801-0095
DOI: 10.1097/01.ede.0000249409.81050.46

Airborne particles consistently have been associated with adverse cardiovascular health outcomes.[1] Although the mechanisms by which these particles impact cardiovascular health are not well understood, findings from recent studies suggest that autonomic function is likely an important biologic pathway. Particulate air pollution has been linked, for example, with increased dysrhythmia,[2–5] changes in blood pressure,[6] heart rate,[7–9] and mortality caused by heart failure.[3] Alterations in the autonomic balance of the heart as measured by heart rate variability also have been documented in several panel studies,[10–16] cross-sectional studies,[17,18] and an experimental study with controlled exposures to concentrated ambient air pollution particles.[19] These effects have been shown to occur relatively quickly after exposure, further supporting an autonomic component in particle mediated toxicity.[20]

Accumulating epidemiologic evidence suggests that exposures to traffic-related particles may be partly responsible for the observed increases in cardiac problems. In a recent case-crossover study, German investigators found that the odds of a myocardial infarction were nearly 3 times larger after direct exposures to traffic.[21] Similarly, living near major roadways has been shown to be a predictor of nonaccidental mortality in both the Netherlands and Canada.[22,23] Other research has documented adverse cardiovascular effects in association with ambient concentrations of traffic-related pollutants, such as black carbon and nitrogen dioxide.[2,24,25] One recent investigation even documented stronger associations between heart rate variability and black carbon rather than regional particles, suggesting the importance of traffic-related particles for autonomic function.[26]

If traffic-related pollution is at least partly responsible for the cardiovascular effects of air pollution, the public health implications would be substantial, because of the heavy use of motor vehicles for transportation throughout the world. For example, the 2004 U.S. Census reported that approximately 93% of working Americans commute via motorized vehicles and spend, on average, 50 minutes each day in transit to and from work.[27] This time in transit may result in sizeable personal exposures to traffic-related particles because greater-than-average concentrations of particulate pollution have been documented inside buses, cars, and other forms of public transportation.[28–30] To date, however, there has been very limited epidemiologic research on the health effects of time spent in traffic, and no investigations to our knowledge that directly examine the

health effects of traffic-related exposures on the elderly, a sensitive subpopulation.[31]

Data for this investigation come from a study designed to enhance the magnitude and variability of exposures to traffic through the use of field trips aboard a diesel-powered bus. By using a real-world high-exposure activity that is commonly experienced by independent senior adults, we were able to enhance exposures to a cohort of individuals who generally are not included in controlled-exposure studies. As a result, we were able to investigate the short- and medium-term relationships between traffic-related particulate pollution and autonomic function in a potentially susceptible subpopulation. Because the critical induction window for traffic-related health effects remains unknown, we also examined differences in the short-term toxicity of ambient and fresh traffic-related particles by assessing if bus exposures had stronger short-term associations with heart rate variability than nonbus exposures.

METHODS

Study Population

Data were collected from 44 nonsmoking seniors (≥60 years old) with the approval of the Harvard School of Public Health Human Subjects Committee. All participants were independently mobile and lived in one of 4 independent senior residences in suburban St. Louis, Missouri. Because our outcome of interest was heart rate variability, individuals with atrial flutter, atrial fibrillation, or a paced rhythm were excluded from participation. Similarly, individuals with left bundle branch blocks were selected only if their heart rate variability could be ascertained. Persons with unstable angina and those unable to provide written informed consent also were excluded.

Study Design

All study subjects were asked to participate in 4 group trips into downtown St. Louis between March and June of 2002. Each trip included 2 standardized 1-hour periods aboard a diesel-powered shuttle bus, dominated by highway driving. Additional time spent aboard the bus while waiting for departure was classified as bus time but was not counted toward the 1-hour driving period. After the first bus trip, which typically occurred between 9:30 and 10:30 am, subjects participated in an activity (ie, a theater performance, Omni movie, outdoor band concert, or Mississippi River boat cruise), followed by lunch. Subjects then reboarded the bus for a 1-hour return trip to their residence beginning between 1:00 and 2:30 pm, depending on the activity.

Before each trip, participants were outfitted with a continuous Holter electrocardiogram monitor containing a SEER data card (General Electric Medical Systems: Waukesha, WI). Electrocardiogram data from the V5 and AVF axes were collected during a 24-hour period using a modified 5-lead placement. Because installation and removal of the Holter monitors occurred between 8:00 and 9:00 am, we excluded from data analysis measurements collected during this time period. Health status and medication use were ascertained before each trip using daily technician-administered questionnaires. Height, weight, and reports of doctor diagnoses were collected during a baseline evaluation before the study.

Continuous measurements of traffic-related particles were collected during the 48 hours surrounding each trip. Twenty-four hours before each trip, air pollutant concentrations were measured using 2 portable carts that were placed in a central location inside the participants' living facilities. On the day of the trip, the monitoring carts followed the participants throughout their day, beginning in the health testing room during electrocardiogram placement, then aboard the bus, to the group activity, and lunch. At the end of the trip the carts were returned to the central location in the living facility, where they remained until the conclusion of the health testing on the following morning.

Heart Rate Variability Analyses

All electrocardiograms were analyzed by trained technicians using well-established methodology for assessing heart rate variability.[32] After removal of regions with noise and artifact, all normal-to-normal intervals from the 24-hour recordings were analyzed for time and frequency domain parameters in 5-minute and 24-hour epochs using standard, validated algorithms on a General Electric Marquette MARS 8000 Workstation. The time domain parameters for this investigation included the standard deviation of all normal-to-normal intervals (SDNNs), the square root of the mean squared difference between adjacent normal-to-normal intervals (rMSSDs), and the percentage of adjacent normal-to-normal intervals that differed by more than 50 milliseconds (pNN50). The frequency domain measures included high-frequency power (HF; in the range of 0.15–0.4 Hz), low frequency power (LF; in the range of 0.04–0.15 Hz), and the ratio of these 2 metrics (LF/HF). Average heart rate also was reported. All epochs containing at least 50% of valid data were included for analysis (>99% of data).

Exposure Measurements

Two portable carts containing continuous sampling instrumentation were used to monitor exposures to traffic-related pollutants including fine particulate mass ($PM_{2.5}$), black carbon, and size-specific particle counts. $PM_{2.5}$ concentrations were measured using a TSI DustTrak aerosol monitor Model 8520 (Shoreview, MN) with a Perma Pure Nafion diffusion dryer (Toms River, NJ). Integrated samples of $PM_{2.5}$ mass also were collected using a Harvard Impactor (Air Diagnostics and Engineering, Inc., Harrison, ME) for daily calibration of the trip and facility periods. Continuous black carbon concentrations were measured using a portable Magee Scientific Aethalometer (Berkeley, CA) with a 2.5-μm impaction inlet. Particle counts were measured using a Climet Model CI500 (Redlands, CA) with a modified flow rate of 0.1 cubic feet per minute. Prior to analysis, data from the Climet were aggregated by aerodynamic diameter and expressed as fine (0.3 to 2.5 μm) and coarse (2.5 to 10 μm) particle counts. Temperature and relative humidity were recorded with a Telaire 7001 (Goleta, CA) and used to calculate apparent temperature, a biologic weather stress index.[33]

For each pollutant, we calculated the 4- and 24-hour moving average concentrations preceding each 5-minute epoch of heart rate variability. These averaging periods were

selected based on the findings of past investigations.[12–14,17] We also calculated shorter moving averages of 5, 30, and 60 minutes preceding each epoch, to examine the specific impact of the bus exposures. In addition, hourly lags were created to evaluate consistency with our main findings. Average air pollutant concentrations were considered to be valid for analysis if data capture during the time period of interest was greater than 75%. This resulted in more than 94% completeness for $PM_{2.5}$, over 95% completeness for black carbon, and 96% completeness for fine and coarse particle counts across the 5-, 30-, and 60-minute moving averages as well as our 4- and 24-hour moving averages.

Statistical Analysis

Initially, we calculated descriptive statistics across all 5-minute epochs for each outcome and exposure measure. We then stratified the data by location to compare levels between periods spent at the facility, aboard the bus, at lunch, and at the activity. Analysis of variance tests, which accounted for autocorrelation among repeated measures, were programmed in SAS (Version 8.02; SAS Institute; Cary, NC) to test for differences in exposure between the 5-minute averages measured during these time periods at the 95% confidence level. Spearman correlations also were calculated to evaluate the relationships between $PM_{2.5}$, black carbon, fine particle count, and coarse particle count concentrations during different study periods.

Before statistical modeling, all outcome variables were transformed logarithmically as each was highly skewed. For pNN50, which could have the value of 0, the logarithm was calculated for pNN50+1. Associations of air pollution with each transformed outcome were then assessed using general additive models in R Version 2.0.1 (The R-Project for Statistical Computing; available at http://www.r-project.org/). All models controlled for subject, hour of day, day of week, trip (as a proxy of activity type and season), current location (ie, bus, activity, lunch, or facility), and apparent temperature averaged during the previous 4 hours. Our models also adjusted for cardiac medication use by including terms for daily use of beta-blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, and sympathomimetics. Subject, day, trip, location, and cardiac medications were included as categorical variables, whereas apparent temperature and hour of day were evaluated using penalized splines with 3 and 8 degrees of freedom, respectively. Because our main models used all 5-minute epochs of heart rate variability, we also included 1.5 hours of residuals as linear terms to account for correlation among neighboring epochs of approximately 0.4. This number of residual terms was selected using partial autocorrelation function plots and a comparison of generalized cross-validation scores for various models. Sensitivity analyses with the 24-hour epochs did not include these autocorrelation terms. None of our final models controlled for heart rate since models with and without heart rate produced qualitatively similar findings.

Single pollutant models were first run to examine the impact of short (5-, 30-, and 60- minute) and medium-term (4- and 24-hour) moving averages of particulate pollution on each heart rate variability outcome. Because these individual models do not account for any correlation between the short- and medium-term averages, we also investigated the independent impacts of short- and medium-term exposures by including separate terms for the previous 5-minute and remaining 23-hour-and-55-minute moving averages into our models. Based on our hypothesis that exposures from the bus may have stronger short-term associations with heart rate variability than nonbus exposures, we also ran these models including interaction terms for bus periods with the 5-minute average. For presentation purposes, all effect estimates (β) were transformed into percent changes per interquartile range (IQR) of a pollutant using the formula: $(e^{(\beta * IQR)} - 1) \times 100$. Estimates are presented with their 95% confidence intervals.

RESULTS

Study Population

A total of 158 person-trips were conducted during this study, with the majority (35 of 44) of subjects participating on all 4 trips. Table 1 presents selected personal characteristics of our 44 nonsmoking study subjects. Participants were predominantly white females with a median age of 80 years. Subjects often were overweight (median body mass index of 29 kg/m^2) but generally regarded themselves as in good health (91%). Approximately 11% had a history of myocardial infarction, 82% were hypertensive, and 18% were diabetic.

TABLE 1. Participant Characteristics and Outcome Measures (n = 44)

Participant Characteristics	**Number (%)**
Female	36 (82)
Age (yrs)	
62–79	20 (45)
80–94	24 (55)
Race/ethnicity	
White	41 (93)
Black	3 (7)
Smoking history*	
Never	23 (52)
Former	21 (48)
Body mass index (kg/m^2)	
18–29	29 (66)
30–49.6	15 (34)
Hypertension	36 (82)
Past myocardial infarction	5 (11)
Outcome Measures	**Mean ± SD**
Heart rate (beats/min)	74 ± 14
SDNN (ms)	45 ± 31
rMSSD (ms)	36 ± 38
pNN50 (%)	10 ± 16
LF (ms^2)	487 ± 1784
HF (ms^2)	369 ± 876
LF/HF (none)	2.0 ± 1.9

*All participants were nonsmokers at the time of the study.

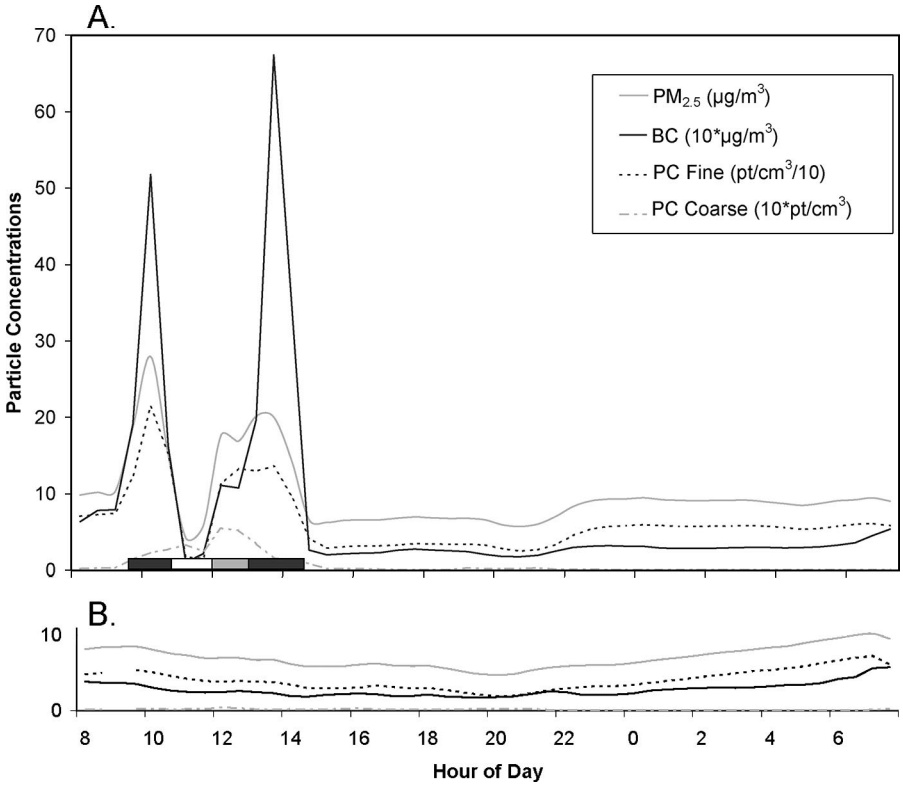

FIGURE 1. Particulate concentrations during (A) a sample trip day and (B) the previous nontrip day. Dark boxes at bottom of graph A represent any time periods during which the one-hour bus trips occurred. Lunch was demarcated using a light shaded box, whereas the activity (an Omni movie) was highlighted with the clear box.

Heart Rate Variability Measurements

Descriptive statistics of our heart rate variability measurements for the different time periods are presented in Table 1. Crude levels of heart rate variability did not differ qualitatively between bus and facility periods, although modest differences were found in our multivariate models. In those models, the fixed effects for location predicted decreases in LF, SDNN, the LF-to-HF ratio, and heart rate aboard the bus as compared with facility periods. The largest changes were observed for LF and SDNN, with 10% and 15% decreases predicted aboard the bus, respectively. Changes to the LF-to-HF ratio and heart rate aboard the bus were more modest, with decreases of 5% or less as compared with facility levels.

Exposure Measurements

$PM_{2.5}$, black carbon, and fine particle count concentrations were systematically higher during periods when participants were aboard the bus, whereas coarse particle counts were elevated throughout the entire trip period. These trends are illustrated for one example trip in Figure 1. Across all trips, black carbon, a common indicator for traffic, was strongly enhanced by the bus trips with a 10-fold increase in the median concentration over median levels at the living facilities (Table 2). $PM_{2.5}$, fine particle count, and coarse particle count concentrations also were elevated aboard the bus with 2.5-, 3-, and 16-fold increases over periods spent at the living facilities, respectively. Despite increases during bus periods, coarse particle count concentrations had the highest

TABLE 2. Median (Inter-quartile Range) Levels for Exposure and Meteorological Parameters by Period

	Type of Period				
	All	**Facility**	**Bus**	**Activity**	**Lunch**
$PM_{2.5}$ mass ($\mu g/m^3$)	7.7 (6.8)	6.8 (5.1)	17.2 (10.3)	8.2 (16.1)	11.2 (5.9)
Black carbon (ng/m^3)	330 (337)	285 (270)	2911 (2464)	482 (1168)	434 (276)
Fine particle counts (pt/cm^3)	42 (57)	36 (45)	105 (96)	50 (133)	69 (48)
Coarse particle counts (pt/cm^3)	0.02 (0.11)	0.01 (0.04)	0.16 (0.13)	0.29 (0.26)	0.16 (0.36)
Apparent temperature (°C)	22 (4)	22 (4)	24 (4)	22 (2)	22 (2)

Values are reported for 5-minute averaging periods.

median concentration during the activity periods. In addition to higher median concentrations during the trips, trip periods also demonstrated elevated variability for all particulate metrics as compared with time spent at the facility.

Concentrations of the fine particulate species showed similar trends and were strongly correlated with one another, irrespective of time period. For example, correlations between the 24-hour mean $PM_{2.5}$, black carbon, and fine particle count concentrations ranged from 0.80 to 0.98. Correlations were similarly strong when analyses were limited only to periods spent aboard the bus (r = 0.76 to 0.97). $PM_{2.5}$, black carbon, and fine particle counts concentrations on the bus also were strongly correlated with corresponding 24-hour moving averages (r = 0.55 to 0.86), although 5-minute and 24-hour moving averages were only weakly correlated (r = −0.003 to 0.51). Similar correlation patterns were observed for coarse particle counts as 24-hour mean concentrations were poorly correlated with corresponding 5-minute averages (r = −0.03) but were strongly correlated with average coarse particle counts aboard the bus (r = 0.63). Poor correlations, however, were found between coarse particle count concentrations and all fine particulate measures during all time periods.

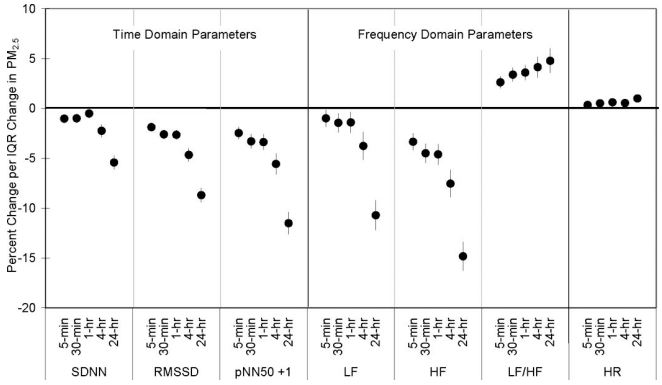

FIGURE 2. Associations between microenvironmental $PM_{2.5}$ and heart rate variability outcomes by averaging period. All models were adjusted for subject, trip, activity, day of week, hour of day, daily cardiac medication use, and the 4-hour mean apparent temperature. Interquartile ranges for $PM_{2.5}$ were 6.8, 6.9, 6.9, 6.8, and 4.5 $\mu g/m^3$ for the 5-minute, 30-minute, 1-hour, 4-hour, and 24-hour average concentrations, respectively.

Associations Between Particles and Heart Rate Variability

Across all averaging periods, $PM_{2.5}$ was associated with decreases in SDNN, rMSSD, pNN50, LF, and HF (Fig. 2). Increases were observed with $PM_{2.5}$ for the LF/HF ratio and heart rate. Although associations were seen for all moving averages, the magnitude of these associations increased with averaging period, with the largest associations consistently found for the 24-hour moving average. A confirmatory investigation of individual lag hours of exposure supported this time course, as associations increased in magnitude beyond 4-hour and generally subsided by 24-hour (results not shown).

Consistent results were found across the various measures of fine particles, with black carbon and fine particle counts also exhibiting negative associations with SDNN, rMSSD, pNN50, LF, and HF and positive associations with the LF/HF ratio and heart rate (Table 3). The largest associations were found with pNN50 and HF, particularly for black carbon, for which an interquartile increase of 459 ng/m^3 in the 24-hour moving average was associated with a –13% (95% confidence interval = –15% to –11%) change in pNN50 + 1 and a −19% (−21% to −16%) change in HF. Of all the pollutants, black carbon also exhibited the largest associations for the LF/HF ratio with a 9% (7% to 11%) increase predicted per interquartile increase. Fine particle exposures had the smallest associations with heart rate, with a 1% or less increase per interquartile increase in the daily moving average. Coarse particle counts also were associated with all outcomes in single-pollutant models, with inverse relationships to those found for $PM_{2.5}$, black carbon, and fine particle counts. In 2 pollutant models, however, control for

TABLE 3. Percent Change (95% Confidence Interval) in Heart Rate Variability per Inter-Quartile Change in the 24-Hour Moving Average of Various Microenvironmental Pollutants

	Single-Pollutant Models				Two-Pollutant Models	
	$PM_{2.5}$	Black Carbon	PC Fine	PC Coarse	$PM_{2.5}$	PC Coarse
SDNN	−5.5 (−6.3 to −4.8)	−5.3 (−6.5 to −4.1)	−5.1 (−5.8 to −4.4)	2.4 (1.3 to 3.6)	−5.7 (−6.5 to −4.9)	−0.7 (−1.9 to 0.6)
RMSSD	−9.1 (−9.8 to −8.4)	−10.7 (−11.9 to −9.5)	−8.0 (−8.7 to −7.2)	3.9 (2.6 to 5.1)	−9.4 (−10.1 to −8.6)	−1.3 (−2.6 to −0.05)
PNN50 +1	−12.2 (−13.3 to −11.1)	−13.2 (−15.0 to –11.4)	−10.2 (−11.3 to −9.0)	2.9 (1.0 to 4.9)	−13.1 (−14.3 to −11.9)	−4.3 (−6.3 to −2.4)
LF	−10.8 (−12.3 to −9.3)	−11.3 (−13.7 to −8.8)	−9.9 (−11.4 to −8.4)	6.4 (3.7 to 9.1)	−10.7 (−12.4 to −9.1)	0.2 (−2.5 to 3.0)
HF	−15.1 (−16.7 to −13.7)	−18.8 (−21.1 to –16.5)	−13.7 (−15.1 to −12.2)	10.2 (7.4 to 13.1)	−14.9 (−16.5 to −13.3)	1.3 (−1.5 to 4.1)
LF/HF	5.1 (3.9 to 6.4)	9.3 (7.2 to 11.4)	4.3 (3.1 to 5.5)	−3.3 (−5.0 to −1.6)	4.9 (3.6 to 6.2)	−0.9 (−2.7 to 1.0)
HR	1.0 (0.9 to 1.2)	1.0 (0.8 to 1.3)	0.9 (0.8 to 1.1)	−1.1 (−1.3 to −0.8)	0.9 (0.7 to 1.1)	−0.6 (−0.9 to −0.4)

All models were controlled for subject, trip type, current location, day of week, time of day, the 4-hour moving average of apparent temperature, and cardiac medications. Initial results are from single pollutant models whereas the last 2 columns present results from a 2-pollutant model containing both $PM_{2.5}$ and coarse particle count concentrations. Models used the following interquartile ranges: 4.5 $\mu g/m^3$ for $PM_{2.5}$, 459 ng/m^3 for black carbon, 39 pt/cm^3 for fine particle counts, and 0.066 pt/cm^3 for coarse particle counts.

$PM_{2.5}$ generally resulted in a weakening or an actual change in direction of the relationships with coarse particle counts. Associations between $PM_{2.5}$ and heart rate variability, on the other hand, were relatively unchanged by control for coarse particle counts.

Despite sometimes-modest correlations with daily exposures, short-term averages remained associated with heart rate variability after concurrent control for concentrations measured during the remainder of the day, as shown in Table 4 for $PM_{2.5}$ and black carbon. When all 5-minute periods were examined, the strengths of these associations were weak as compared with those for the 24-hour moving averages. When the analyses were limited to bus periods, however, associations between 5-minute pollutant concentrations and heart rate variability (as measured by SDNN, RMSSD, pNN50, LF, and HF) were substantially larger than corresponding associations for nonbus periods (Table 5). These short-term associations for particles from bus periods generally were similar to those reported for 24-hour mean exposures, whereas associations for nonbus particles were generally lower by an order of magnitude.

Further exploration of these short-term associations indicated that for $PM_{2.5}$, bus periods were associated with stronger short-term changes in heart rate variability than nonbus periods due to larger concentrations aboard the bus as well as larger associations per unit change in pollution. This was confirmed by our finding of statistically significant effect modification of $PM_{2.5}$ associations by bus periods for all outcome parameters with the exception of the LF/HF ratio. In contrast, concentrations of black carbon during bus periods were clearly associated with larger changes in heart rate variability as a result of higher concentrations but actual differences in the magnitude of the associations per unit change in pollution were less consistent for this pollutant. For both $PM_{2.5}$ and black carbon, no differences in association were observed for 5-minute mean concentrations during the lunch or activity periods, nor did periods of sleep substantially modify the observed short-term associations.

TABLE 4. Independent Short- and Medium-Term Associations With Heart Rate Variability Across All Time Periods (% Change Per IQR (95% CI))

	$PM_{2.5}$	Black Carbon
SDNN		
5-min mean	−0.5 (−0.8 to −0.1)	−0.3 (−0.5 to −0.1)
23:55-hr mean	−4.6 (−5.3 to −4.0)	−4.7 (−5.9 to −3.5)
RMSSD		
5-min mean	−0.9 (−1.3 to −0.5)	−0.3 (−0.5 to −0.1)
23:55-hr mean	−7.5 (−8.1 to −6.8)	−9.3 (−10.5 to −8.1)
PNN50 +1		
5-min mean	−1.1 (−1.7 to −0.5)	−0.3 (−0.6 to −0.1)
23:55-hr mean	−9.9 (−10.9 to −8.9)	−10.5 (−12.3 to −8.7)
LF		
5-min mean	0.4 (−0.5 to 1.2)	−0.5 (−0.9 to −0.1)
23:55-hr mean	−10.0 (−11.4 to −8.6)	−9.8 (−12.4 to −7.2)
HF		
5-min mean	−1.5 (−2.3 to −0.6)	−0.9 (−1.2 to −0.5)
23:55-hr mean	−12.9 (−14.2 to −11.5)	−15.4 (−17.8 to −12.9)
LF/HF		
5-min mean	1.9 (1.3 to 2.4)	0.3 (0.1 to 0.6)
23:55-hr mean	3.2 (2.1 to 4.3)	6.5 (4.5 to 8.6)
HR		
5-min mean	0.1 (0.1 to 0.2)	0.1 (0.1 to 0.2)
23:55-hr mean	0.8 (0.7 to 0.9)	0.4 (0.2 to 0.7)

Short- and medium-term averages of exposure were entered simultaneously into the model, resulting in estimates of association between all heart rate variability measurements and the previous 5 minutes of exposure controlling for all other exposures during the preceding 24 hours (23:55-h mean). Models also were controlled for subject, trip type, current location, day of week, time of day, the 4-hour moving average of apparent temperature, and cardiac medications. The inter-quartile ranges for the 5-minute means were 6.8 $\mu g/m^3$ and 337 ng/m^3 whereas the 23:55-hour means were 4.2 $\mu g/m^3$ and 490 ng/m^3 for $PM_{2.5}$ and black carbon, respectively.

DISCUSSION

In this investigation, we found fine particles to be consistently associated with decreased heart rate variability, as assessed using the time-domain measures of SDNN, rMSSD, and pNN50 and the frequency measures of LF and HF. For each heart rate variability measure, associations were strongest for $PM_{2.5}$, black carbon, and fine particle counts. Given our study design (which specifically enhanced exposures to traffic-related pollution) and the strong associations observed for the traffic pollutants of black carbon and fine particle counts, our results suggest that traffic-related particles affect autonomic function. This impact may be mediated through an enhanced decline in the parasympathetic control to the heart, as indicated by the observed increases in heart rate and the ratio of LF to HF.[32]

Associations generally were largest in magnitude when using the 24-hour moving average of pollution, although strong and independent associations also were observed with short-term averages on the order of 5 minutes. These short-term associations were largest while participants were riding the bus, in part because of elevated concentrations during these time periods. Such associations also were enhanced aboard the bus independent of concentrations for all fine particles as measured by $PM_{2.5}$, but less clearly for the more specific marker of traffic, black carbon. These findings seem to suggest that traffic-related particles may have a stronger influence on rapid-acting pathways than ambient particles of other origins. This new evidence is an interesting, given that Peters et al[21] reported increased odds of myocardial infarction in the hour immediately after periods spent in traffic.

Although our exposure monitors were with participants during the trip periods but not at the living facilities, it is unlikely that measurement error alone is responsible for weaker associations for $PM_{2.5}$ during nonbus periods. If this were the case, we would anticipate a similar bias toward the null for black carbon during nonbus periods. This was not, however, consistently observed across the various heart rate variability outcomes. In addition, concentrations measured during the activities and lunch (when monitors were placed alongside the participants) also demonstrated weaker associations than the bus periods. Despite these assurances, how-

TABLE 5. Independent Associations of Short-Term Averages (5-Minute Means) of $PM_{2.5}$ and Black Carbon With Heart Rate Variability by Bus and Nonbus Periods

	$PM_{2.5}$			Black Carbon		
	Effect Estimate	*P* Value of Interaction	% Change Per IQR (95% Confidence Interval)	Effect Estimate	*P* Value of Interaction	% Change Per IQR (95% Confidence Interval)
SDNN						
Bus	−0.0052		−5.0 (−6.3 to −3.7)	−0.02		−4.6 (−6.1 to −3.0)
Nonbus	−0.00093	<0.0001	−0.5 (−0.9 to −0.2)	0.003	<0.0001	−0.1 (−0.3 to 0.1)
RMSSD						
Bus	−0.005		−4.8 (−6.2 to −3.5)	−0.01		−2.6 (−4.2 to −0.9)
Nonbus	−0.0013	<0.0001	−0.7 (−1.1 to −0.4)	−0.01	0.64	−0.3 (−0.5 to −0.1)
pNN50 +1						
Bus	−0.0066		−6.3 (−8.4 to −4.2)	−0.008		−2.0 (−4.5 to 0.5)
Nonbus	−0.0015	<0.0001	−0.8 (−1.4 to −0.3)	−0.02	0.34	−0.5 (−0.8 to −0.1)
LF						
Bus	−0.0073		−7.0 (−9.8 to −4.1)	−0.02		−6.0 (−9.3 to −2.5)
Nonbus	0.0011	<0.0001	0.6 (−0.1 to 1.4)	−0.006	0.028	−0.2 (−0.7 to 0.3)
HF						
Bus	−0.011		−10.7 (−13.5 to −7.9)	−0.02		−5.8 (−9.1 to −2.3)
Nonbus	−0.0013	<0.0001	−0.7 (−1.5 to 0.04)	−0.03	0.50	−0.9 (−1.4 to −0.4)
LF/HF						
Bus	0.0038		3.9 (1.7 to 6.0)	−0.003		−0.8 (−3.1 to 1.7)
Nonbus	0.0024	0.39	1.4 (0.8 to 1.9)	0.03	<0.0001	0.8 (0.5 to 1.1)
HR						
Bus	0.00073		0.7 (0.5 to 1.0)	−0.0003		−0.5 (−0.8 to −0.2)
Nonbus	−0.00002	<0.0001	−0.01 (−0.08 to 0.1)	0.008	<0.0001	0.3 (0.26 to 0.34)

All models were controlled for subject, trip type, current location, day of week, time of day, the 4-hour moving average of apparent temperature, cardiac medications, and the mean $PM_{2.5}$ concentration during the remainder of the 24-hour period. Effect estimates are reported as log(ms)/($\mu g/m^3$) for SDNN and RMSSD, log(%)/($\mu g/m^3$) for pNN50 + 1 and LF/HF, and log(ms^2)/($\mu g/m^3$) for HF and LF. The interquartile range for $PM_{2.5}$ was 10 $\mu g/m^3$ aboard the bus and 5.6 $\mu g/m^3$ during nonbus periods. The interquartile range for black carbon was 2.6 $\mu g/m^3$ aboard the bus and 0.27 $\mu g/m^3$ during other periods.

ever, it is possible that reduced measurement error or a nonlinear dose response relationship might partially explain the stronger associations with bus periods for $PM_{2.5}$.

Because the autonomic nervous system is responsible for regulating the electrical control of the heart, changes to parasympathetic and sympathetic balance can have important clinical significance as they may result in electrical instability and cardiac arrhythmias. Such instability has been documented by several investigators who have found associations between decreased heart rate variability and incidence of ventricular tachycardia[34] as well as increased arrhythmic death.[35] Alterations in the sympathetic–parasympathetic balance also may cause cardiovascular symptoms by influencing the force of cardiac contraction, potentially leading to hemodynamic stress and heart failure among compromised individuals. Evidence of these effects can be seen in several investigations, which have reported the presence of decreased parasympathetic tone among individuals with heart failure.[32] Changes in autonomic control may also have important implications for cardiovascular outcomes as the autonomic nervous system is an important regulator of the immune system and the coagulation cascade.[36] In fact, previous research has demonstrated that the administration of autonomic antagonists can modify the oxidative stress response of airborne particles in rats.[37] This is interesting because we found some evidence of increased inflammation within this study population with increasing levels of air pollution.[38] Although such changes were subclinical within this cohort, a reduction in anti-inflammatory neural input may ultimately favor the onset of strokes, myocardial infarctions, or the progression of atherosclerosis.[39] Through these mechanisms or others, decreased heart rate variability has been shown to be predictive of poor cardiac health as it is associated with an increased incidence of cardiac events including angina pectoris, myocardial infarction, coronary heart disease deaths, and congestive heart failure among individuals with no apparent cardiac disease.[40]

The findings of this investigation are biologically plausible as particles are thought to influence the autonomic nervous system directly by stimulating afferent nerves in the lungs[41] or damaging the myocardium as a result of translocation to the heart.[42] It is also believed that particles may cause indirect effects to the autonomic nervous system via inflammatory pathways.[43] In fact, our results confirm the findings of the majority of studies of heart rate variability in senior adults, which have found relatively consistent relationships between pollution and decreased heart rate variability.[10–19] Our study also is consistent with previous reports in that air pollution appears to result in a greater loss of parasympathetic tone as evidenced by larger reductions in HF

power as compared with LF power. With the addition of this study, evidence for reduced parasympathetic tone has been documented in panel studies,[10,14,15] cross-sectional studies,[17,18] an experimental study with controlled exposures to concentrated ambient air pollution particles,[19] and now a quasi-experimental design. One recently published panel study[44] reported no associations between $PM_{2.5}$ and HF and LF in the elderly; however, that investigation was conducted in the Pacific Northwest, where it has been hypothesized that the composition of particles may be different and less toxic than other parts of the United States.

Despite good general agreement with past investigations of senior adults, our findings conflict with the only other known investigation of heart rate variability and in-vehicle exposures. In that study of young and healthy North Carolina state troopers,[45] positive associations were found between in-vehicle $PM_{2.5}$ concentrations and SDNN, pNN50, and HF power measured on the morning after the work shift. Differences between these findings and ours are likely due to the age and baseline health of the study participants, since several investigations have demonstrated that negative associations between air pollution and heart rate variability are strongest among senior adults and individuals or animals with cardiovascular disease.[10,15,19,46]

As our investigation focused on older adults, these results may not be generalizable to younger individuals. Generalization of our findings also may be limited by the relatively small number of participants, which included 44 individuals across 25 sampling days. Nevertheless, our findings were strong presumably because of reduced exposure error, heightened variability in our exposures, and our use of 24-hour of heart rate variability measured in 5-minute epochs. Although most other investigations have used longer heart rate variability epochs or did not collect a full 24-hour of data, this approach has been used previously to investigate the impact of air pollution on heart rate variability.[15,47] In addition, we were able to account for the lack of independence among our observations by including autocorrelation terms in our model, which reduced the correlation between residuals of neighboring time periods from 0.4 in unadjusted models to less than 0.01 in our final model. An examination of associations with heart rate variability summarized during the entire 24 hours of monitoring also produced findings that were qualitatively similar to those reported here but with substantially less statistical power.

Although we had sufficient power to link fresh traffic particulate pollution to short-term decrements in heart rate variability, it was not possible to conclude definitively that bus exposures alone were responsible for the observed daily associations since electrocardiograms were not collected on days without bus-related exposures. This investigation also cannot distinguish the independent influence of each fine-particulate metric due to high correlations, nor can we eliminate the possibility that our findings for particulate levels are in some degree confounded by gaseous pollutants or highway noise. On the other hand, our results indicate that fine rather than coarse particles are likely to be most important with respect to heart rate variability. In addition, our results highlight the overall importance of traffic as a source of decreased heart rate variability, because exposure to automobiles is known to increase fine particulate matter, gaseous pollutants,[30,48] and noise.[49]

In summary, this study documented elevated concentrations and variability of particulate matter during periods aboard a diesel-powered bus and correlated these changes with alterations in heart rate variability. Our findings indicate that increasing levels of fine particulate matter are linked to decreasing heart rate variability in older adults with the strongest reductions in parasympathetic tone. Although associations with daily mean concentrations were evident for all particles, our findings suggest the presence of short-term associations for traffic-related particles.

ACKNOWLEDGMENTS

The authors thank Doug Dockery, Petros Koutrakis, Frank Speizer, Antonella Zanobetti, Eric Rimm, Jay Turner, Sara Forrester, Colleen Peter, Gail McCallum, Marisa Barr Rubin, Martha Fay, Tanya Kotlov, our field staff, and study subjects.

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