The Association of Particulate Air Metal Concentrations with Heart Rate Variability

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Numerous studies show an association between particulate air pollution and adverse health effects. Particulate matter is a complex mixture of elemental carbon, ammonium, sulfates, nitrates, organic components, and metals. The mechanisms of action of particulate matter ≤ 2.5 μm in mean aerodynamic diameter ($PM_{2.5}$), as well as the constituents responsible for the observed cardiopulmonary health effects, have not been identified. In this study we focused on the association between the metallic component of $PM_{2.5}$ and cardiac autonomic function based on standard heart rate variability (HRV) measures in an epidemiologic study of boilermakers. Thirty-nine male boilermakers were monitored throughout a work shift. Each subject wore an ambulatory electrocardiogram (Holter) monitor and a personal monitor to measure $PM_{2.5}$. We used mixed-effects models to regress heart rate and SDNN index (standard deviation of the normal-to-normal) on $PM_{2.5}$ and six metals (vanadium, nickel, chromium, lead, copper, and manganese). There were statistically significant mean increases in the SDNN index of 11.30 msec and 3.98 msec for every 1 μg/m^3 increase in the lead and vanadium concentrations, respectively, after adjusting for mean heart rate, age, and smoking status. Small changes in mean heart rate were seen with all exposure metrics. The results of this study suggest an association between exposure to airborne metals and significant alterations in cardiac autonomic function. These results extend our understanding of the adverse health effects of the metals component of ambient $PM_{2.5}$. *Key words:* cardiac autonomic function, heart rate variability, metal exposure, occupational exposures, particulate matter.
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Particulate air pollution with a mean aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$) has been associated with several adverse health outcomes, particularly death and hospital admissions from cardiopulmonary diseases (Samet et al. 2000; Schwartz 1997; Schwartz and Dockery 1992). $PM_{2.5}$ is a complex mixture of elemental carbon, ammonium, sulfates, nitrates, organic components, and metals (U.S. EPA 1996; Wilson and Spengler 1996). Studies of individual metals *in vitro* and *in vivo* have demonstrated that the metals component of $PM_{2.5}$ is particularly toxic (Costa and Dreher 1997; Gavett et al. 1997; Grabowski et al. 1999).

The mechanisms responsible for the cardiac morbidity and mortality associated with particulate air pollution have not been fully elucidated (Pope 2000). Furthermore, it is unclear which specific component or components of particulate air pollution are responsible for health effects. Disturbance of the autonomic nervous system in response to $PM_{2.5}$ has been demonstrated in several epidemiologic studies (Gold et al. 2000; Liao et al. 1999; Magari et al. 2001; Pope et al. 1999). These alterations may be induced directly through a sympathetic stress response (Godleski et al. 2000) or indirectly by inflammatory cytokines produced in the lungs and released into the circulation (Monn and Becker 1999; Quay et al. 1998).

The metals present in residual oil fly ash, a component of ambient $PM_{2.5}$, have been shown to induce cytokine proliferation in both *in vitro* and in animal models (Carter et al. 1997; Dreher et al. 1996). Murata and co-workers (Murata and Araki 1991; Murata et al. 1993) reported autonomic nervous system dysfunction in workers exposed to lead, zinc, and copper. They noted decreases in overall measures of heart rate variability (HRV), as well as decreases in specific measures of parasympathetic tone. In a study of autonomic function in manganese alloy workers, Barrington et al. (1998) also noted decreases in the parasympathetic component of the autonomic nervous system. Although previous studies indicate decreases in parasympathetic tone, there were no reports regarding the effects of metals on overall cardiac autonomic tone.

To examine the effects of the metals component of occupationally derived $PM_{2.5}$ on the autonomic nervous system, we designed a short-term longitudinal study in a cohort of young boilermaker construction workers. We explored the cardiac effects of occupational exposure to six metals by investigating alterations in cardiac autonomic function using an ambulatory electrocardiogram (ECG) monitor. In this study, personal particulate measurements were made with a personal environmental monitor (PEM). The SDNN (standard deviation of the normal-to-normal intervals) index was calculated as the mean of the 5-min SDNN means throughout the work shift and was used as the main HRV indicator. The SDNN index is an overall measure of cardiac autonomic tone. We also calculated the mean heart rate as the mean of the 5-min means throughout the work shift. We used mixed-effects models to regress both the SDNN index and the mean heart rate, as well as lags of these outcomes, from 30 min to 8 hr, on the total $PM_{2.5}$ mass, the average $PM_{2.5}$ concentration, and the six metals.

Methods

Study subjects. The Institutional Review Board of the Harvard School of Public Health approved the study. Written informed consent was obtained from each subject before testing began. All testing was in accordance with institutional guidelines. The study population consisted of 39 male (apprentice/journeyman) boilermaker construction workers. A self-administered questionnaire was used to collect information on medical history, including respiratory and

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cardiac systems, and the use of all prescription and nonprescription medications. Subjects reported their smoking history and whether a physician had diagnosed them with hypertension. Subjects were defined as having chronic bronchitis if they either reported that a physician had diagnosed them with it or they met the symptom definition. The symptom definition entails that the subject report a chronic cough that produces thick mucus for at least 3 months and that this cough recurs for at least 2 consecutive years.

Continuous Holter monitoring and tape processing. Continuous heart rate monitoring was performed using a five-lead Holter monitor from Cardio Data Systems, Model Dynacord 3 Channel Model 423 (Haddonfield, NJ). Each participant's skin was shaved if necessary, cleansed, and slightly abraded to ensure satisfactory lead contact. Electrodes were placed in a modified V1 and V5 position. Each subject was given a daily diary to record any symptoms such as chest pain and shortness of breath and to record activities such as eating and sleeping.

Each 24-hr tape was sent to Raytel Cardiac Services (Maspeth, NY) and analyzed using a Delmar Avionics (Irvine, CA) Model Strata Scan 563. Only beats with an RR interval between 0.6 and 1.5 sec and an RR ratio of 0.8–1.2 were included in the analysis. Trained technicians performed all analyses, and all normal and abnormal findings were either accepted or rejected based on standard criteria to ensure quality control. Tapes were analyzed in the time domain, and reports summarizing heart rhythm, rate analysis, and ST changes were generated. We calculated the mean heart rate and SDNN in 5-min segments for the entire recording. The SDNN index was calculated for the duration over which air monitoring took place (the 8- to 10-hr workday) and is calculated as the mean of the SDNN intervals for all 5-min segments within the 8- to 10-hr recording (1996). We also calculated the mean heart rate over the duration of air monitoring as the mean of all 5-min mean heart rates. Lags of 30 min to 8 hr between exposure and the SDNN index and mean heart rate measures were also calculated.

Table 1. Summary population statistics.

No. subjects	39
Current smokers (*n*)	20
Race (*n* white)	36
Hypertension (*n*)	7
Chronic bronchitis (*n*)	8
Age (years)	
Mean ± SD	38.3 ± 12.8
Range	18–59
Years as boilermaker	
Mean ± SD	13.2 ± 13.2
Range	0–40
Heart rate, mean ± SD (beats/min)	94.0 ± 11.0
SDNN index, 5-min mean ± SD (msec)	54.0 ± 18.4

Airborne particulate collection. Personal particle monitoring began at the start of the workday and ended at the completion of the work shift for each subject. A diary of work activity throughout the day was also kept. Particle collection was performed with a PEM (MSP Corporation, Minneapolis, MN) designed to collect particles < 2.5 µm using a GilAir-5 personal pump (Sensidyne Incorporated, Clearwater, FL) drawing air at 4 L/min. Polytetrafluorethylene (PTFE) 37-mm filters from Pall Gelman Corporation (East Hills, NY) with a pore size of 2 µm and a support ring were used inside the PEM to collect the particles. Equipment problems early in the study prevented running the pump at the full 4 L/min required to obtain the 2.5 µm particle cutsize. For the first 13 samples collected, the pump ran at only 2.5 L/min, thereby increasing the mean particle cutsize size to approximately 3.2 µm.

Filters were conditioned in a temperature- and humidity-controlled room for 24 hr and weighed on a Mettler MT5 C31 balance (Mettler Toledo, Columbus, OH) before use. We reconditioned and reweighed filters after 48 hr of use. The difference between pre- and postweight was calculated in milligrams. We determined mean air concentrations by dividing the total weight collected by the volume of air collected.

Metals analysis on air filters. We performed metals analysis for each filter using an adapted acid bomb digestion technique (Loring and Rantala 1992). Briefly, filters were removed from the support ring and placed in Teflon Parr bombs with a 45-mL capacity (Parr, Moline, IL). We added 5 mL of concentrated, trace-metal–free nitric acid and 400 µL of trace-metal–free hydrofluoric acid to the bomb. The bomb was then capped, sealed, and microwaved at 750 W for 3 min. The bombs were allowed to cool in an ice bath for a minimum of 1 hr before opening. After opening, we added 10 mL of 1.5% boric acid to each bomb and microwaved it a second time at 750 W for 3 min. Again, the bombs were allowed to cool in an ice bath and opened. Digested samples were diluted to 50 mL with deionized trace-metal–free water and stored in a dark room while awaiting inductively coupled plasma mass spectrometry (ICP-MS) analysis.

We used mass spectrometry to determine concentrations of nickel, vanadium, lead, chromium, manganese, and copper in the digestate using an Elan ICP-MS Model 5000 (Perkin-Elmer, Norwalk, CT). Indium and bismuth were used as the internal standards for each sample. We assessed accuracy and precision using known additions of standard metals to blank filters and replicate analyses. Filter extraction efficiencies were determined by digesting blank filters loaded with known quantities of NIST 1648 (National Institute of Standards and Technology, Urban Particulate Matter). The limit of detection (LOD) was determined as 3 times the standard deviation of 10 replicate measurements of the filter blank samples. We corrected each sample for blank filter metal content. The metal weight contained on each filter was calculated by comparing ICP-MS results to standard curves generated for each metal. We determined the air metal concentrations by dividing the metal weights contained on each filter by the volume of air collected. Values below the limit of detection (LOD) were used as such and were not assigned one-half the LOD.

Statistical methods. Descriptive statistics were generated and Spearman correlation coefficients were calculated for the $PM_{2.5}$ average concentration and the six metals investigated. We used mixed-effects regression models using SAS software, version 8 (SAS Institute, Cary, NC) to investigate the effects of the air metal concentrations on the SDNN index and the mean heart rate. We also examined the effects of total $PM_{2.5}$ mass and concentration on the SDNN index and the mean

Table 2. Summary exposure measure statistics.

Sample	No. of samples	No. above LOD	LOD[a]	Mean ± SD	25th Percentile	50th Percentile	75th Percentile	Range
PM filter weight (mg)	48	48	6.0×10^{-3}	1.40 ± 1.70	0.43	0.69	1.31	0.15–6.33
PM average concentration (mg/m^3)[b]	48	48	3.13×10^{-3}	1.16 ± 1.61	0.26	0.56	1.05	0.09–7.66
V concentration (µg/m^3)	48	15	8.59×10^{-3}	0.76 ± 1.96	0.05	0.13	0.43	0–11.62
Ni concentration (µg/m^3)	48	27	13.0×10^{-3}	1.33 ± 2.39	0.23	0.60	1.22	0.06–12.61
Cr concentration (µg/m^3)	48	24	6.51×10^{-3}	1.28 ± 3.29	0.05	0.25	0.66	0–16.24
Mn concentration (µg/m^3)	48	39	1.82×10^{-3}	22.17 ± 52.68	0.47	5.13	20.32	0.01–338.34
Cu concentration (µg/m^3)	48	40	3.13×10^{-3}	5.96 ± 17.83	0.43	2.43	3.79	0.04–122.19
Pb concentration (µg/m^3)	48	36	1.30×10^{-3}	0.37 ± 0.56	0.05	0.17	0.52	0.01–3.34

[a]LOD based on 8 hr of collection at 4 L/min. [b]$PM_{2.5}$ weight divided by the volume of air collected.

heart rate. The lagged SDNN indices and lagged mean heart rates were also regressed on the two $PM_{2.5}$ and metal exposure metrics. A random effect for each study subject and fixed covariates such as smoking status, age, and mean heart rate were included in the models. We also investigated the modifying effects of individual characteristics such as hypertension, chronic bronchitis, and smoking. Sensitivity analyses examining the effects of outlying exposure measures on regression results were performed using a subset of the data with these outlying values removed. We also investigated $\log_{10}$ transformations of metal concentrations in the models again to control the effects of outlying values.

Results

A summary of the subject demographics is presented in Table 1. The study population consisted of 39 men, mostly white, 20 of whom were current smokers at the time of monitoring. Their average age was 38.3 years (SD = 12.8 years), with an average of 13.2 years (SD = 13.2) working in the boilermaker trade. The mean of the 5-min mean heart rates during work was 94.0 beats/min (SD = 11.0 beats/min), and the mean SDNN index during work was 54.0 msec (SD = 18.4).

The personal exposures of all participants are summarized in Table 2. Eight of the 39 study subjects participated more than once, yielding a total of 48 samples. Average $PM_{2.5}$ concentrations were skewed, with a mean of 1.16 (SD = 1.61) mg/m^3 and a median of 0.56 mg/m^3. The limit of detection is based on 8 hr of collection with a pump drawing air at 4 L/min. Metal concentrations were also skewed and are presented with their range, mean, and 25th, 50th, and 75th percentile values, using all 48 samples in Table 2. Values below the LOD were used as is and were not assigned one-half the LOD. They were included in these calculations because we did not want to lose any information contained in these values—particularly, the measurement error they represent. All metal concentrations, although well above levels typically found in ambient air measurements taken in Boston (Godleski et al. 2000; Huffman et al. 2000), were below the Occupational Safety and Health Administration permissible exposure limits.

Spearmann correlations between individual metals and the average $PM_{2.5}$ concentration are presented in Table 3. Both the corrected filter weights and average $PM_{2.5}$ concentrations were highly correlated with all metals, except vanadium. The correlation among the six metals studied ranged from 0.53 to 0.85, with the exception of vanadium. Vanadium was not highly correlated with the other five metals, with correlations ranging from 0.12 to 0.43.

Results from models regressing the SDNN index on the $PM_{2.5}$ measures and the six metals are summarized in Table 4. The $PM_{2.5}$ weight and average $PM_{2.5}$ concentration were not predictive of the SDNN index (no lag) throughout the workday, nor for any of the lagged SDNN index measures investigated. Among the individual metals, lead and vanadium were significantly associated with an increased SDNN index. With no lag in the SDNN index, lead was associated with an average 3.98 msec [95% confidence interval (CI), 1.64–6.32] increase in the SDNN index for every 1 μg/m^3 increase in the lead concentration, whereas vanadium was associated with an average 11.30 msec (95% CI, 2.88–19.73) increase in the SDNN index for every 1 μg/m^3 increase in vanadium concentration. These models were adjusted for mean heart rate, age, and smoking status. Small marginal effects of nickel on the SDNN index (no lag) were also observed. We considered $\log_{10}$ transformations of the exposure metrics to minimize any effects outlying exposures may have had on the regression estimates. Using these $\log_{10}$ transformed exposure metrics, we found little change in the statistical significance of the exposure coefficients, although manganese became marginally significant (no lag). To further examine the effects of outlying exposures, we repeated the regression analysis on data sets that eliminated outliers. With outliers removed, there was a slight increase in the regression estimates for each metal, although little qualitative change in their respective statistical significance was observed.

The association of $PM_{2.5}$ weight, average $PM_{2.5}$ concentration, and individual metals with the mean heart rate was also assessed (Table 5). The estimated effects of all the exposure metrics were small, and none attained statistical significance when considering the mean heart rate without any lag. The largest associations were seen between vanadium and lead and the 1-hr lagged mean heart rate, although not statistically significant. Statistically significant associations were, however, seen using a 2-hr lagged mean heart rate. The $PM_{2.5}$ weight and $PM_{2.5}$ average air concentration showed average increases of 1.65 msec (95% CI, –0.06 to 3.36) and 1.67 msec (95% CI, 0.11–3.22) in the mean heart rate, respectively, for every 1 mg increase in the $PM_{2.5}$ weight and 1 mg/m^3 increase in the average $PM_{2.5}$ concentration. Nickel and

Table 3. Correlation matrix for $PM_{2.5}$ and the six metals investigated.

$PM_{2.5}$ weight	Lead	Vanadium	Chromium	Manganese	Nickel	Copper
Lead	0.74	–0.07	0.87	0.82	0.79	0.85
p-Value	< 0.0001	0.66	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Vanadium		0.11	0.74	0.56	0.78	0.76
p-Value		0.44	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Chromium			–0.22	–0.43	0.25	–0.22
p-Value			0.14	0.002	0.09	0.14
Manganese				–0.85	0.78	0.85
p-Value				< 0.0001	< 0.0001	< 0.0001
Nickel					0.53	0.79
p-Value					0.0001	< 0.0001
Copper						0.69
p-Value						< 0.0001

Table 4. Estimated change associated with unit changes in particle and metal indices and 95% CIs for the SDNN index at various lags between exposure and SDNN index.

Predictor variable	No lag[a] (msec)	95% CI	30-min lag[a] (msec)	95% CI	1-hr lag[a] (msec)	95% CI	2-hr lag[a] (msec)	95% CI	3-hr lag[a] (msec)	95% CI
$PM_{2.5}$ mass (mg)	0.23	(−2.36–2.81)	0.63	(−2.15–3.41)	1.01	(−1.93–3.95)	0.32	(−4.82–5.46)	0.36	(−4.52–5.23)
$PM_{2.5}$ avg. conc. (mg/m^3)[b]	–0.77	(−3.60–2.05)	–0.01	(−2.86–2.85)	0.22	(−2.91–3.35)	–0.94	(−6.09–4.21)	–0.79	(−5.66–4.07)
V (μg/m^3)	3.98	(1.64–6.32)	3.67	(0.56–6.79)	3.47	(0.33–6.61)	2.99	(−0.95–6.93)	2.44	(−1.22–6.11)
Ni (μg/m^3)	1.28	(−0.81–3.37)	0.74	(−1.49–2.97)	0.99	(−1.33–3.30)	0.72	(−2.68–4.11)	0.65	(−2.46–3.77)
Cr (μg/m^3)	–0.02	(−1.50–1.46)	–0.01	(−1.35–1.33)	0.17	(−1.26–1.59)	–0.06	(−2.29–2.16)	–0.03	(−2.14–2.09)
Mn (μg/m^3)	–0.04	(−0.14–0.05)	–0.02	(−0.13–0.09)	–0.03	(−0.14–0.09)	–0.05	(−0.22–0.11)	–0.04	(−0.19–0.11)
Cu (μg/m^3)	–0.09	(−0.37–0.20)	–0.08	(−0.44–0.29)	–0.08	(−0.45–0.28)	–0.12	(−0.57–0.34)	–0.11	(−0.52–0.30)
Pb (μg/m^3)	11.30	(2.88–19.73)	8.66	(−2.03–19.35)	8.90	(−1.83–19.62)	8.30	(−5.84–22.44)	7.37	(−5.67–20.41)

Each model adjusted for mean heart rate, age, and smoking (yes/no).
[a]Estimated association. [b]Average $PM_{2.5}$ concentration, mass divided by the volume of air collected.

chromium showed marginally significant associations with the 2-hr lagged mean heart rate. The association of all the exposure metrics with the mean heart rate began to decrease after the lag was increased past 3 hr (data not shown). Log_{10} transformations of each exposure metric were again investigated and regression estimates revealed no changes in statistical significance. Analyses removing outlying exposures again showed little change in the regression estimates or their statistical significance.

We also explored the modifying effects of each metal and $PM_{2.5}$ measure by smoking status, the presence of chronic bronchitis, and hypertension in the SDNN index and mean heart rate models. There was no significant difference in effects between smokers and nonsmokers in all of the SDNN index or mean heart rate models. In addition, there were no significant differences between hypertensive and normotensive subjects.

There were no modifying effects of chronic bronchitis in models regressing the mean heart rate (no lag) on the two $PM_{2.5}$ exposure metrics or any of the six metals. There were, however, marginally significant differences in the association among those with and without chronic bronchitis in the two models that regressed the SDNN index (no lag) on vanadium and copper. Compared to the estimated main association of 3.76 msec (95% CI, 1.44–6.08) among those without chronic bronchitis, those individuals with chronic bronchitis had an additional average increase of 17.02 msec (95% CI, –2.49 to 36.54) in their SDNN index for every 1 μg/m^3 increase in vanadium concentrations. Those individuals without chronic bronchitis had an average decrease of 0.06 msec (95% CI, –0.34 to 0.23), whereas those with chronic bronchitis had an additional average –7.48 msec (95% CI, –16.27 to 1.31) decrease in their SDNN index for every 1 μg/m^3 increase in copper concentrations.

Finally, multiple-metal models were considered (Table 6). A single model regressing the SDNN index (no lag) on nickel, vanadium, and lead concentrations, adjusted for age, mean heart rate, and smoking status, was investigated. We included nickel, vanadium, and lead in the single model because they exhibited the strongest association with the SDNN index (no lag) in the single-metal models. The magnitude of the association of vanadium and lead with the SDNN index (no lag) and the respective statistical significance showed little change, but the association of nickel on the SDNN index changed direction and became less statistically significant.

We also investigated a second multiple-metal model regressing the mean heart rate (no lag) on the same three metals (vanadium, nickel, and lead) adjusted for age and smoking status (Table 6). There was little change in the association of vanadium and nickel with heart rate in the multiple metal model compared to models including only one metal. There was a change in direction of the lead association, but it remained statistically insignificant.

Discussion

As far as we are aware, this study is the first prospective study in humans to investigate the association of personal measurements of individual metals present in $PM_{2.5}$ with cardiac autonomic function in an occupational cohort. This cohort provided a unique opportunity to study the association of relatively high concentrations of six metals, which are typically present in ambient $PM_{2.5}$, with changes in HRV.

Several researchers have noted a general association of ambient $PM_{2.5}$ and alterations in cardiac autonomic function (Gold et al. 2000; Liao et al. 1999; Magari et al. 2001; Pope et al. 1999). These studies have, in general, reported decreases in SDNN measures. In fact, previous work based on this same cohort has shown decreases in the 5-min SDNN and small increases in the 5-min heart rate associated with increases in moving $PM_{2.5}$ averages (Magari et al. 2001). In this study, where we averaged the 5-min SDNN's for 8–10 hr, we found the opposite associations with relatively small average increases in the SDNN index associated with the total $PM_{2.5}$ weight and small average decreases associated with the average $PM_{2.5}$ air concentration. In addition, we found small average decreases in the mean heart rate associated with $PM_{2.5}$ weight and small average increases associated with the $PM_{2.5}$ average air concentration. None of these associations with SDNN index or mean heart rate was near statistical significance, however. There were substantial fluctuations in both $PM_{2.5}$ concentration (measured simultaneously with both a continuous light-scattering monitor and with an integrated PEM measurement) and SDNN during the course of the work shift, and the previous work documented associations among those fluctuations. It was impossible to measure metal concentrations in such short intervals. This is a major limitation of this study. It is also the case that, unlike the study of Pope et al. (1999), which correlated the SDNN computed over 24 hr with particles over a similar period, our SDNN index does not incorporate any long wavelength, low-frequency components. It merely averages the 5-min SDNNs over a longer period. Hence, we may be missing the correlation among low-frequency components of airborne particles and HRV.

A 2- to 4-hr lag between exposure to $PM_{2.5}$ and changes in HRV has been demonstrated in several studies (Gold et al. 2000; Magari et al. 2001; Schwartz et al. 2002) and provided the impetus to investigate several lags in this study. After incorporating lags into the SDNN index, there was little overall change in the magnitude and significance of most of the associations with the $PM_{2.5}$ weight and $PM_{2.5}$ average air concentration. Interestingly, the association between the mean heart rate and both $PM_{2.5}$ weight and average air concentration got stronger and gained statistical significance as the lag time increased. However, the association decreased and lost statistical significance after incorporating lags of mean heart rate over 3 hr (data not shown). These results support the lagged effects reported previously in several studies.

Individual metal models regressing the SDNN index on vanadium and lead revealed the largest average associations with the SDNN index without any lag incorporated. These models revealed average increases in the SDNN index associated with increases in exposure to these metals. Somewhat smaller

Table 5. Estimated change associated with unit changes in particle and metal indices and 95% CIs for the mean heart rate at various lags between exposure and mean heart rate.

Predictor variable	No lag[a] (msec)	95% CI	30-min lag[a] (msec)	95% CI	1-hr lag[a] (msec)	95% CI	2-hr lag[a] (msec)	95% CI	3-hr lag[a] (msec)	95% CI
$PM_{2.5}$ mass (mg)	–0.003	(−1.58–1.58)	0.33	(−0.97–1.64)	0.74	(−0.17–1.64)	1.65	(−0.06–3.36)	1.85	(−0.31–4.02)
$PM_{2.5}$ avg conc (mg/m^3)[b]	0.17	(−1.63–1.97)	0.30	(−1.03–1.62)	0.74	(−0.15–1.63)	1.67	(0.11–3.22)	1.87	(−0.17–3.92)
V (μg/m^3)	0.87	(−1.20–2.94)	0.80	(−1.71–3.30)	1.24	(−1.07–3.55)	1.06	(−1.74–3.86)	0.97	(−1.99–3.93)
Ni (μg/m^3)	0.79	(−0.67–2.25)	0.29	(−0.83–1.41)	0.68	(−0.08–1.45)	1.21	(−0.11–2.53)	1.10	(−0.60–2.80)
Cr (μg/m^3)	0.23	(−0.76–1.23)	0.06	(−0.54–0.67)	0.30	(−0.11–0.71)	0.60	(−0.11–1.31)	0.57	(−0.40–1.54)
Mn (μg/m^3)	0.02	(−0.05–0.08)	0.01	(−0.05–0.06)	0.03	(−0.02–0.07)	0.06	(−0.03–0.16)	0.06	(−0.04–0.16)
Cu (μg/m^3)	0.08	(−0.12–0.29)	0.06	(−0.16–0.28)	0.13	(−0.06–0.31)	0.12	(−0.16–0.40)	0.07	(−0.23–0.37)
Pb (μg/m^3)	0.19	(−6.77–7.16)	0.17	(−6.25–6.59)	2.55	(−2.91–8.02)	1.91	(−7.22–11.05)	0.44	(−9.56–10.44)

avg conc, average concentration. Each model adjusted for age and smoking (yes/no).
[a]Estimated association. [b]Average $PM_{2.5}$ concentration, mass divided by the volume of air collected.

associations were seen between nickel concentrations and increases in the SDNN index, although they were not statistically significant. These results contrast with the decreases in the several-minute to several-hour SDNN measures from our earlier studies on the same cohort and other studies that considered $PM_{2.5}$ as a whole (Gold et al. 2000; Liao et al. 1999; Magari et al. 2001; Pope et al. 1999). It is further contradictory to the results that Murata and co-workers (Murata and Araki 1991; Murata et al. 1993) and Barrington et al. (1998) report in their studies of lead, zinc, copper, and manganese workers. They noted decreases in both overall measures of HRV and decreases in HRV measures of parasympathetic tone. In our study, the only decreases in the SDNN index were associated with increases in copper, manganese, and chromium exposure; however, they were relatively small and not statistically significant at any of the lags investigated. Again, the SDNN index and the several minutes to 24-hr SDNN measures are not perfectly synonymous and may account for some of the discrepancy. Finally, the increases in the SDNN index observed in this study may reflect the fact that the metals component of particulate air pollution that we measured, considered alone, may not be responsible for the cardiac autonomic changes reported in other studies.

The associations between mean heart rate and individual metals revealed consistent increases in heart rate throughout the various lags investigated. The largest average increases in mean heart rate were associated with lead and vanadium after incorporating a 1-hr lag into the mean heart rate. These results are similar in direction to the those increases in mean heart rate associated with $PM_{2.5}$ reported in earlier studies on this same cohort, as well as those reported by Pope et al. (1999). However, Gold et al. (2000) reported average decreases in mean heart rate associated with $PM_{2.5}$ exposure.

We investigated multiple-metal models in this study in an attempt to understand possible interactions between metals. Models with three metals showed little change in estimates from the single-metal models. Murata and Araki (1991) reported the antagonism of the effects of lead on autonomic nervous system dysfunction by zinc, showing an attenuation of effects in workers exposed to both lead and zinc. Moreover, Godleski et al. (2000), in their study of normal canines exposed to lower levels of concentrated particles derived from ambient air, noted little association of exposure to a group of metals (arsenic, barium, copper, manganese, nickel, lead, vanadium, and zinc) with changes in HRV. Recently Laden et al. (2000) reported that the strongest association between $PM_{2.5}$ and cardiovascular deaths was with the fraction of the $PM_{2.5}$ from traffic. Traffic particles have a high organic fraction that may be more responsible for the association with autonomic function. A recent panel study of elderly subjects in Boston has reported a stronger association between these carbonaceous particles and SDNN than for other $PM_{2.5}$ particles (Schwartz et al. 2002). It is important to note, however, that, even though decreases in overall measures of HRV are typically associated with adverse outcomes (Dekker et al. 1997; La Rovere et al. 1998; Lanza et al. 1999), significant increases in overall measures of HRV could indicate parasympathetic dominance, which has been shown to predispose individuals to potentially fatal arrhythmias (Kasanuki et al. 1997).

Several limitations to our study should be considered when interpreting our results. Even though other metals are found in $PM_{2.5}$ and have been associated with adverse health effects, we investigated only six metals. We chose the six metals for investigation in this study on the basis of past evidence of adverse effects, the exposure history of this cohort, and analysis constraints. Additionally, the small sample size in this study limited the investigation of any interactions that may exist between various metals and their association with HRV changes. The database is currently being expanded, and this issue will be given further consideration in future studies. Furthermore, the occupational metal and $PM_{2.5}$ exposures examined in this study are higher than levels typically found in ambient air pollution. It is important to recognize, however, that there is a need to explore exposure–response relationships over a wide range.

Confounding by other copollutants is unlikely in this occupational setting, but it cannot be completely discounted. Confounding by environmental co-pollutants such as SO_2, O_3, and NO_2 are unlikely during this work, as these pollutants do not covary with the dominant metals exposures occupationally derived (data not shown). In addition, highly reactive gases such as O_3 and SO_2 also tend to disappear in enclosed spaces.

Metals, as a component of ambient $PM_{2.5}$, have received much attention in the literature. This study extends observations on the association of metals with alterations in cardiac autonomic function.

Table 6. Estimated change associated with unit changes in metal indices and 95% confidence intervals for the SDNN index and mean heart rates for multiple-metal models.

Outcome variable	Predictor variables included in one model (μg/m³)	Estimated association (msec)	95% CI
SDNN index (msec)[a]			
	V	3.41	(0.56–6.26)
	Ni	–0.84	(−3.19–1.51)
	Pb	8.23	(−1.96–18.42)
Heart rate (beats/min)[b]			
	V	0.71	(−1.81–3.22)
	Ni	0.91	(−1.02–2.84)
	Pb	–3.05	(−12.04–5.94)

[a]Model adjusted for mean heart rate, age, and smoking (yes/no). [b]Model adjusted for age and smoking (yes/no).

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