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Time Course of Heart Rate Variability Decline Following Particulate Matter Exposures in an Occupational Cohort

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Although research suggests that particles influence cardiac autonomic response as evidenced by decreases in heart rate variability (HRV), the time course of the response remains unclear. Using a crossover panel study, we monitored 36 male boilermaker welders, occupationally exposed to metal-rich particulate matter (PM) to investigate the temporal trend of hourly HRV subsequent to PM exposure. Ambulatory electrocardiograms were collected over work (exposure) and non-work (control) periods and the mean of the standard deviations of all normal-to-normal intervals for all 5-min segments ($SDNN_i$) was calculated hourly for up to 14-hrs post-work. The exposure-response relationship was examined with linear mixed effects regression models to account for participants monitored over multiple occasions. Models were adjusted for non-work HRV to control for diurnal fluctuations and individual predictors of HRV. The mean (SD) work $PM_{2.5}$ concentration was 1.12 (0.76) mg/m^3. Hourly $SDNN_i$ was consistently lower post-work as compared to the same time period on a non-work day. HRV was inversely associated with work $PM_{2.5}$ exposures in each of the 14-hrs post-work. The hourly associations suggested an early and later phase response, with the largest regression coefficients observed 2–3 hrs ($\beta = -6.86$ (95% CI: -11.91, -1.81) msec/1 mg/m^3 at 3-hrs), and then 9–13 hrs ($\beta = -8.60$ (95% CI: -17.45, 0.24) msec/1 mg/m^3 at 11-hrs), after adjusting for non-work HRV, smoking status, and age. This investigation demonstrates declines in HRV for up to 14 hours following PM exposure and a multiphase cardiovascular autonomic response with immediate (2 hrs) and delayed (9–13 hrs) responses.

INTRODUCTION

Short and long-term exposure to ambient particulate matter (PM) has been linked to increased risk for cardiovascular events (Brook et al., 2004). While the exact mechanism of particle-induced cardiovascular effects remains unclear, one of the potential pathways is through cardiac autonomic control, which is often assessed by heart rate variability (HRV) (Pope & Dockery, 2006). Air pollution studies suggest that declines in HRV are associated with PM exposures averaged over both short time periods (minutes) and long time periods (days) (Pope & Dockery, 2006). Decreased HRV is linked to adverse cardiovascular outcomes, including increased risk of death among chronic heart failure patients (La Rovere et al., 2003) and onset of hypertension (Schroeder et al., 2003; Singh et al., 1998).

Although research suggests that particles influence cardiac autonomic response (Schulz et al., 2005) and that there is an inverse exposure-response relationship between PM and HRV (Pope & Dockery 2006), the time course of the response following exposure remains unclear. Identifying the time course of particle-induced cardiovascular changes is important for understanding the mechanism(s) of response. In air pollution studies,

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it is difficult to disentangle the time course of HRV response due to constant exposure and the accumulation of cardiac effects. Experimental panel studies (Stekiel et al., 2004; Devlin et al., 2003; Gong et al., 2003; Scharrer et al., 2007; Gong et al., 2004) have tried to address this problem by administering particle exposures over a discrete time period. However, these studies differed in the duration of HRV monitoring and none continuously evaluated post-exposure hourly HRV changes.

Using a crossover panel study, our objective was to characterize the time course of HRV response to occupational exposure to particulate matter in a cohort of boilermaker construction workers. Previous investigations among this cohort has observed declines in HRV during (Magari et al., 2001) and subsequent to (Cavallari et al., 2007) exposure to high levels of metal-rich particles. We sought to investigate the time course of the HRV response by continuously monitoring HRV in the 14 hours following a discrete period of exposure to welding fumes.

METHODS

From 1999 to 2006, boilermakers from a local union were recruited into a panel study and monitored for 24 hours over two periods, work and non-work. Work monitoring occurred at a union welding school and at the overhaul of an oil-fired boiler within a power plant. At the welding school, boilermakers received instruction and practiced welding, cutting, and grinding techniques. The boiler overhaul entailed removing and replacing the boiler's interior wall panels and water-circulating tubing. At both locations, boilermakers primarily performed shielded metal arc welding (stick) and gas metal arc welding, most commonly using base metals of mild steel (manganese alloys) and stainless steel (manganese, chromium, and nickel alloys) with electrodes composed mainly of iron with variable amounts of manganese (1–5%). Plasma arc or acetylene torch cutting and grinding also occurred at both work sites. All non-work monitoring occurred within six months of the work monitoring, with 80% within the same week. Workers were allowed to participate in the study on multiple occasions over the seven-year sampling period with work and non-work monitoring at each occasion.

A self-administered questionnaire was used to collect information on medical history and medication use. Individuals were classified as hypertensive if they self-reported doctor diagnosed hypertension or the use of anti-hypertensive medications including ACE inhibitors or beta-blockers. Demographic and lifestyle information including smoking and occupational history was also collected.

Particle Exposure Assessment

Personal, work-shift, particle exposure was continuously monitored using the light-scattering technology of a DustTrak^{TM}Aerosol Monitor (TSI, Inc., St. Paul, MN) fitted with an inlet impactor designed to separate particles with a median aerodynamic diameter of less than 2.5 μm ($PM_{2.5}$). Each aerosol monitor received daily flow and zero calibration checks as well as yearly factory calibration. No correction factor was applied to the DustTrak^{TM} measurements, because a previous study within a similar exposure environment, indicated good agreement between DustTrak^{TM} and gravimetric sample results (Kim et al., 2004). The monitor was placed in a padded pouch, with the inlet tubing secured to the participant's shoulder in the breathing zone area. The DustTrak^{TM} took $PM_{2.5}$ concentration readings every 10 seconds and recorded 1 -min averages. Participants were asked to keep a work log to record information on shift length and respirator use. Mean $PM_{2.5}$ exposures were calculated from the 1-min concentrations over the reported shift length.

Heart Rate Variability Measurement

Participants were fitted with a standard 5-lead ECG Holter monitor (Cardio Data Systems, Dynacord 3-channel device, model 423) at the start of the work shift on work periods and in the morning on non-work periods. To facilitate good lead contacts, the participant's skin was shaved, if necessary, cleansed with an alcohol wipe, and slightly abraded and electrodes were placed in a modified V_1 and V_5 position. Each cardiac tape was sent to Raytel Cardiac Services (Haddonfield, NJ) for processing and analysis using a DelMar Avionic (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, blinded to work and non-work periods, used standard criteria to accept or reject all normal or abnormal findings including any beats with 20% or greater prematurity. Tapes were analyzed in the time domain, and the standard deviation of all normal-to-normal intervals (SDNN) was calculated for all 5-min segments. We calculated the SDNN index ($SDNN_i$), from the mean of the SDNN intervals for all 5-min segments (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996) in hourly increments starting at the time work ended on work periods and over the same time of day on non-work periods for the 14-hours post-exposure. Heart rate was continuously monitored and summarized over all 5-min segments. We averaged heart rate over the work-shift on workdays and over the equivalent time period on non-work days.

Statistical Analysis

To account for correlated outcomes among boilermakers who participated on multiple occasions, we used linear mixed-effects regression models with random intercepts and unstructured covariance to investigate the associations between hourly $SDNN_i$ and either dichotomous or continuous $PM_{2.5}$ exposure. For the dichotomous exposure model, work and non-work HRV measures were treated as the outcome. The mean difference and 95% confidence intervals were calculated from the dichotomous exposure variable. Continuous work $PM_{2.5}$ exposures were investigated by modeling each hourly work HRV outcome while adjusting for hourly non-work HRV measured at the same time of day. By including non-work HRV (baseline) in each model,

each participant served as his own control and thus personal factors (including cardiac risk factors) that did not change between work and non-work measurements were unlikely to introduce bias due to confounding. We also adjusted for age and smoking status (dichotomous), which are predictors of HRV. In a sensitivity analysis, we included the average heart rate over the work shift as a surrogate for both activity and stress, which are potential correlates of $PM_{2.5}$ exposure. Hourly $SDNN_i$ summarized over the 14 hours following work gave a total of 14 exposure-response models. Distributional assumptions were verified by plotting the residuals against the predictors. To identify potentially influential points, we used a Cook's distance > 1 (Cook & Weisberg 1982). All analyses were performed using in SAS version 9.1 (Gary, NC).

RESULTS

The study population consisted of 36 males with mean age of 40 years of whom 29 (81%) were white (Table 1). The majority of workers (72%) were monitored once and 10 (28%) were monitored on multiple occasions with eight monitored twice, one monitored three times, and one monitored four times for a total of 48 paired work and non-work observations. Due to limitations in the ECG recording length and malfunctions, 47 paired observations were available at the 1st hour post exposure and 43 paired observations at the 14th hour past exposure. Twelve participants were smokers at study enrollment and two had quit smoking by subsequent participation. Five participants reported cardiac conditions, including two myocardial infarctions, one stent, one murmur and one arrhythmia. Six participants reported either doctor diagnosis of hypertension or were using medication for hypertension on at least one measurement occasion. Two individuals reported both hypertension and cardiac conditions. Two participants reported the use of statins and three used ace inhibitors; no one reported using beta-blockers, calcium channel blockers, or diuretics.

TABLE 1
Study Population (n = 36) and Exposure Characteristics

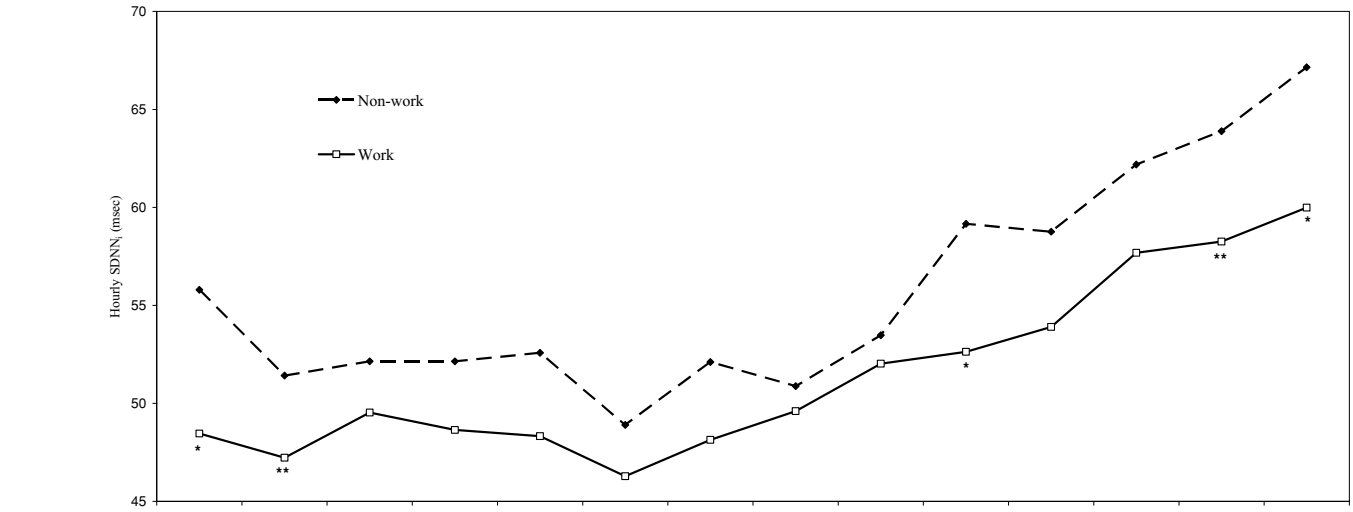
Characteristic	Mean ± SD or Number (%)
Age (years)[a]	40 ±11
Range	22–63
Race	
White	29 (81)
Black	3 (8)
Hispanic	3 (8)
Asian	1 (3)
Male	36 (100)
Current smoker[a]	12 (33)
Hypertensive	6 (17)
Seniority (years as boilermaker)[a]	10.4 (11.7)
Work shift $PM_{2.5}$ exposure (mg/m^3)[b]	1.12 ± 0.76
Range	0.12–3.99
Work shift respirator use[b]	12 (25)

[a]At first participation.
[b]Over 48 measurement occasions.

$PM_{2.5}$ exposures ranged from 0.12 to 3.99 mg/m^3 with a mean (SD) of 1.12 (0.76) mg/m^3 (Table 1) over a mean (range) work-shift length of 5.33 (2.67–9.67) hours. On average, the participant's work-shifts ended at 14:35, and ranged from 11:25–17:13. Only 4 (8%) of the 48 monitored work periods occurred at the power plant location. Workers reported using a respirator on 12 (25%) of the work shifts monitored including 8 reports of a paper dust mask and 4 reports of a half-face respirator. Information on the duration of respirator use was unavailable. Mean (SD) heart rate over the work shift, 97(12) bpm, was higher on workdays as compared to mean (SD) heart rate over the same time of day on non-workdays, 87(11) bpm.

Ambulatory ECG data were collected for a total of 96 person-days. Work $SDNN_i$ was consistently lower than non-work $SDNN_i$ over the same time period (Figure 1). Hourly HRV followed the typical circadian rhythm with increased $SDNN_i$ in the evening on both work and non-work periods. The difference between work and non-work $SDNN_i$ varied with each hour past exposure (Figure 1). The initial difference between work and non-work was large and statistically significant in the first two hours following exposure. In the subsequent hours, this difference was attenuated, until reaching 10 hours post exposure where the difference increased and remained substantial, reaching the largest difference at 14 hours past exposure.

The exposure-response associations were investigated in models adjusted for non-work HRV, smoking status and age as well as models additionally adjusted for heart rate at work. A similar pattern was observed in both analyses (Table 2). The majority of the mixed model regression analyses revealed a negative association between work $PM_{2.5}$ and hourly $SDNN_i$; elevated $PM_{2.5}$ levels were consistently associated with declines in $SDNN_i$ at each hour following exposure, after adjusting for HRV during matched time-periods of non-working days and other covariates (Table 2). When we adjusted for heart rate during work, the effect estimates increased in the 1st through 9th hours and 14th hour following exposure and decreased in the 10th through 13th hour following exposure. The magnitude of the exposure-response association varied by hours since exposure in each model with a large and statistically significant decline reached within the first three hours followed by a slight increase in the association with a decline in the 9th hour past exposure reaching a nadir in the 11th hour past exposure (Figure 2). To further examine the associations observed between 9 to 14 hours past exposure, we performed a pooled analysis, using all data points within the time period for a total of 285 hourly post-work observations. The exposure-response association persisted with −7.48 (95% CI: −10.31, −1.62) msec change in hourly $SDNN_i$ between 9 and 14 hours past exposure per 1 mg/m^3increase in $PM_{2.5}$ after adjusting for non-work HRV, age and smoking status.



Time since exposure (hrs)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mean time of day	14:35	15:35	16:35	17:35	18:35	19:35	20:35	21:35	22:35	23:35	0:35	1:35	2:35	3:35
SDNN$_i$ Difference (msec)	-7.34	-4.19	-2.62	-3.52	-4.25	-2.62	-3.97	-1.28	-1.45	-6.53	-4.87	-4.51	-5.63	-8.36
(95% CI)	(-14.0, -0.70)	(-9.19, 0.18)	(-7.10, 1.85)	(-8.61, 1.57)	(-9.66, 1.15)	(-8.07, 2.84)	(-8.85, 0.92)	(-6.65, 4.09)	(-5.71, 2.81)	(-10.4, -2.66)	(-11.3, 1.60)	(-12.2, 3.19)	(-12.1, 0.89)	(-16.0, 0.76)

FIG. 1. Mean hourly $SDNN_i$ by time since exposure for work periods, matched by time of day for non-work periods. Time of day represents the mean time that each period begins. While hourly $SDNN_i$ is matched by time of day on work and non-work periods for each individual, it differs between individuals due to different work shift end times. The gradual increase in $SDNN_i$ by time since exposure represents the circadian variation in HRV with increases in the evening. $SDNN_i$ difference represents the difference between non-work and work hourly $SDNN_i$ and was calculated using a dichotomous exposure variable for the work period with mixed regression models with random intercepts to account for multiple participation times. $^{*}p < 0.05$ $^{**}p < 0.10$.

Examination of residual plots confirmed model assumptions. Likewise, examination of Cook's distance showed no evidence of influential points for all models with the exception of the model for the 1st hour following exposure. When the potentially influential point was removed, the effect estimate in the first hour following exposure was −0.14 (95% CI: −5.92, 5.65) msec/(1 mg/m^3) after adjusting for baseline non-work $SDNN_i$, age and smoking status.

DISCUSSION

Our results confirm previous findings of an inverse exposure-response relationship between particle exposure and HRV and further suggest that declines in HRV persist for at least 14-hours following exposure. Furthermore, over the 14-hours following exposure there was heterogeneity in the HRV response with a stronger decline in hourly $SDNN_i$ in the first few hours post-exposure followed by a plateau and a second period of decline in the 9–10 hours post-exposure. The multiphase pattern was observed both in the comparison between work and non-work hourly HRV as well as the exposure-response models with continuous $PM_{2.5}$ exposure.

The inverse association between HRV and PM exposure is consistent with studies of air pollution exposures (Pope and Dockery 2006; Brook et al., 2004) as well as previous research within this population of boilermaker welders (Magari et al., 2001; Cavallari et al., 2007). The time course of changes in HRV following exposure has been investigated indirectly in experimental panel studies and in occupational cohorts. However, findings in these studies have been inconsistent, including the direction of the exposure-response relationship. A chamber study of both young and elderly participants exposed for 2 hours to concentrated ambient particles (CAPs) observed decreased HRV both immediately and 24 hours post-exposure, but only among the elderly participants (Devlin et al., 2003). A study of healthy participants and participants with asthma exposed for 2 hours to CAPs found no change in hourly HRV and small changes in 5-min HRV immediately, 4-hrs and 2-days post-exposure (Gong et al., 2003). A lack of an immediate post-exposure HRV change was also observed in a study of young, healthy participants exposed for 1 hour to welding fumes (Scharrer et al., 2007). Results are also mixed among occupational studies where among vehicle maintenance workers, an association between work $PM_{2.5}$ exposures and post-shift HRV was not observed (Eninger & Rosenthal, 2004), however among highway patrol troopers, positive associations were observed between in-vehicle work $PM_{2.5}$ concentrations and SDNN post-shift and waking (Riediker et al., 2004).

Unlike the previous experimental panel or occupational studies, this present study systematically followed the HRV response in the 14 hours following exposure. Differences in results between previous studies and our study may be due to the timing of outcome measures. The current study observed heterogeneity

TABLE 2
Independent Associations of Work $PM_{2.5}$ and Hourly $SDNN_i$ by Time Since Exposure

Time since exposure (hrs)	n	Model 1[a]	Model 2[b]
		β[c](95% CI)	β[c](95% CI)
1	47	−1.44 (−7.75, 4.87)	4.10[e] (−0.39, 8.60)
2	48	−5.33[e] (−10.97, 0.31)	−3.21 (−8.78, 2.37)
3	48	−6.86[d] (−11.91, −1.81)	−6.45[d](−1 1.59, −1.31)
4	48	−2.17 (−9.33, 4.99)	−0.01 (−6.96, 6.94)
5	48	−4.73 (−11.99, 2.53)	−2.03 (−8.27, 4.22)
6	48	−3.52 (−9.89, 2.84)	−1.99 (−8.46, 4.48)
7	48	−1.59 (−7.53, 4.35)	−0.34 (−6.22, 5.54)
8	48	−0.72 (−7.63, 6.20)	0.72 (−6.35, 7.78)
9	48	−5.55[d] (−10.65, −0.45)	−5.26[e] (−10.62, 0.11)
10	48	−3.66 (−8.85, 1.53)	−3.68 (−9.17, 1.80)
11	48	−8.60[e] (−17.45, 0.24)	−9.41[d] (−18.60, −0.23)
12	48	−5.98 (−14.67, 2.70)	−6.45 (−15.62, 2.72)
13	48	−8.27[e] (−17.00, 0.46)	−7.33 (−16.55, 1.89)
14	43	−4.19 (−12.71, 4.33)	−4.75 (−13.81, 4.32)

[a]Adjusted for baseline hourly $SDNN_i$ on a non-work day measured at the same time of day as work HRV, smoking status, and age.
[b]Adjusted for all variables in Model 1 and heart rate during work.
[c]Effect estimates are reported as msec/(mg/m^3)
[d]$p < 0.05$
[e]$p < 0.10$

in the exposure-response association over the hours following exposures suggesting that timing is an important factor to consider in quantifying the exposure-response relationship. Studies monitoring HRV immediately following exposure may not be capturing the correct response time period. In addition, particle concentration or composition may also play a role in eliciting a cardiovascular response. For the experimental CAPs studies, participant exposures included 2 hours of air pollution-based exposures in concentrations of 5–200 μg/m^3 (Devlin et al., 2003; Gong et al., 2003). While composition may have been similar to ours in the experimental panel study with welding fume exposures (Scharrer et al., 2007), exposures occurred for only 2 hours rather than over a work shift. The occupational studies also differed in exposure characteristics where mean $PM_{2.5}$ exposures were two orders of magnitude lower in the highway patrol workers (0.024 mg/m^3) and vehicle maintenance workers (0.060 mg/m^3) compared to our study (1.12 mg/m^3), and were from combustion rather than metal-rich sources.

As in the epidemiologic literature, results from toxicological studies on the cardiovascular effects of PM exposures are mixed. The association appears to vary by animal model: among dogs, increases in HRV (Godleski et al., 2000) and no response (Muggenburg et al., 2003) were found following PM exposure; however, decreases in HRV were observed among rodent models including cardio-compromised rats with residual oil fly ash (Wellenius et al., 2002) and CAP exposures (Chang et al., 2005), healthy rats, after instillation of ambient PM (Rivero et al., 2005), aged spontaneously hypertensive rats exposed to ultrafine traffic particles (Elder et al., 2007), and apolipoprotein deficient mice exposed to particles (Lippmann et al., 2005; Corey, Baker, & Luchtel 2006). The post-exposure declines in HRV among rodents were observed in both short (0–120 minutes) (Rivero et al., 2005; Wellenius et al., 2002; Elder et al., 2007) and long periods (1–4 days) (Corey, Baker, & Luchtel 2006). Few studies have examined the HRV-PM effects over multiple hours post-exposure. In a study of aged spontaneously hypertensive rats exposed to ultrafine traffic particles and monitored for 2 days following exposure, as compared to rats exposed to filtered air, a brief, yet statistically significant, decline in SDNN was observed in the first 2 hours post-exposure (Elder et al., 2007). Chang et al. monitored HRV in rats exposed to CAPs for 15 hours past-exposure and found an inverse exposure-response following exposure, with a leveling off to null after 4 hours (Chang et al., 2005). Using heart rate rather than HRV, studies demonstrate a multiphase cardiac response to particle exposures. In a study of pulmonary hypertensive rats with 6-hr inhalation exposure to CAPs, a statistically significant bi-modal positive dose-response association with post-exposure heart rate was observed with peaks at the 3rd and 7th hour (Cheng et al., 2003). Particle exposure in healthy rats also produced significant biphasic, dose-related responses with early phase (0–6 hrs) and later phase (12–72 hrs) bradychardia following instillation (Campen et al., 2001).

Proposed mechanisms of metal-induced cardiovascular responses also lend evidence to the pattern of HRV responses that

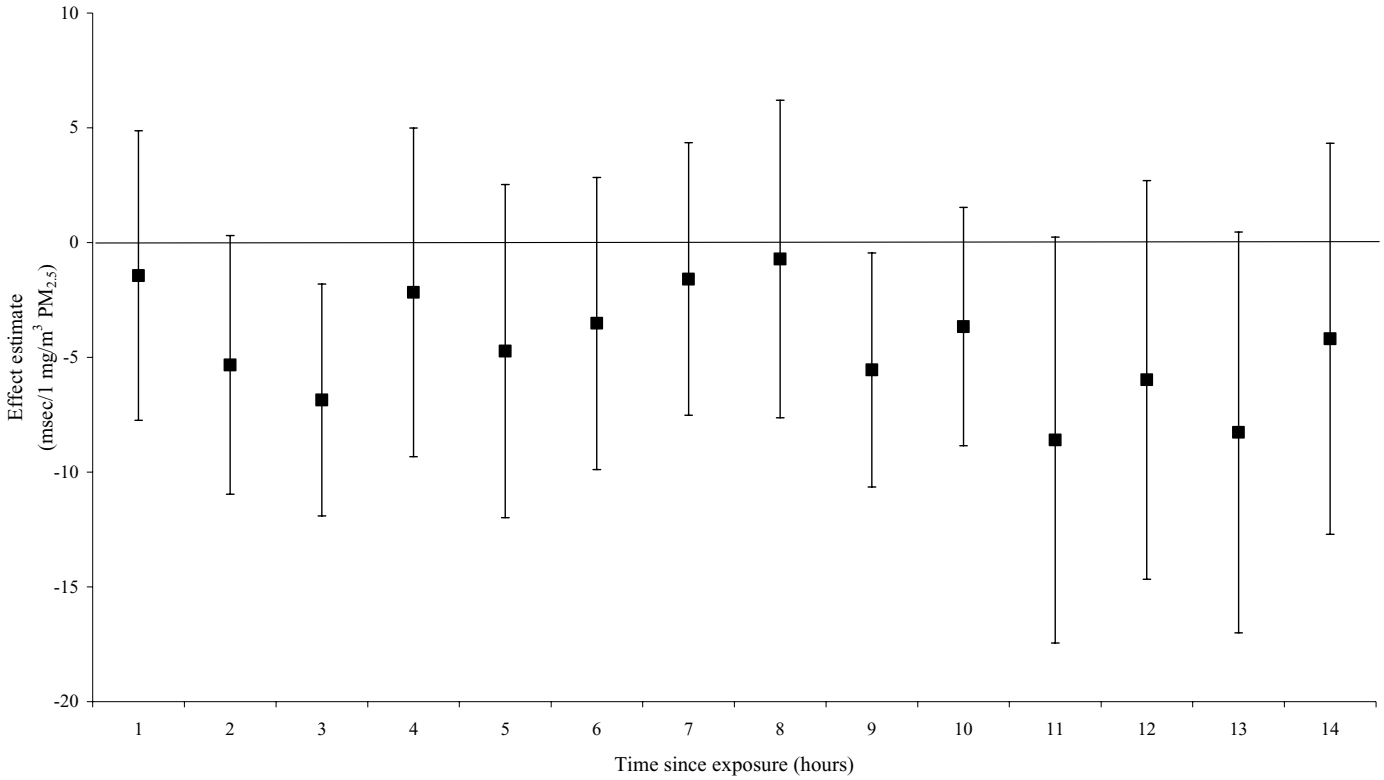


FIG. 2. Effect estimates from linear mixed effects regession models assessing the association between hourly $SDNN_i$ and work $PM_{2.5}$ exposure in individual models where time is the hours since the end of work. Estimates are in units of msec hourly $SDNN_i$ per 1 mg/m^3 increase in work $PM_{2.5}$ after adjusting for non-work $SDNN_i$ over the same time period, smoking status, and age.

we observed within this study. In healthy rats, instilled vanadium produced immediate bradychardia, while nickel elicited similar, yet delayed effects (Campen et al., 2002). While the precise mechanism is unknown, once the transition metal components of $PM_{2.5}$ are delivered to the airways, they may catalyze the production of reactive oxygen species (ROS), which cause airway injury and inflammation and start a cascade of pulmonary and cardiac responses (Gonzalez-Flecha, 2004). This proposed mechanism is supported in part by *in vitro* and *in vivo* studies showing an association between transition metal exposures and generation of ROS (Maciejczyk and Chen, 2005), and an association between transition metal exposures and cardiovascular autonomic changes in mice (Lippmann et al., 2006) and rats (Campen et al., 2001) yet not dogs (Muggenburg et al., 2003). Alternately, other metals such as zinc has been shown to trigger effects by directly interacting with cellular proteins (Tal et al., 2006; Haase and Maret, 2005). The different mechanisms by which metals elicit response may account for the different time course and heterogeneity in cardiovascular autonomic response observed among the different occupational cohorts and toxicological studies. While toxicological evidence supports the role of metals, oxidative stress and inflammation in the observed HRV response, other mechanisms cannot be ruled out. Non-specific particle effects, independent of particle composition, may be responsible for the observed decline, especially in the early phase response. While the exact mechanism is unknown, the results of this study suggest that multiple mechanisms may be contributing to the varying time course of cardiovascular autonomic responses that we observed in this cohort.

While numerous studies demonstrate that low HRV is a predictor of cardiac and all-cause mortality in the general population (Kors, Swenne, and Greiser 2007), the clinical significance of the declines in HRV that we observed within this healthy cohort is unclear, as is the importance of the timing of the decline. Since the participants work shift ended by 14:35 on average, the second phase of response between the 9th–14th hours after exposure began at 23:35 and continued to 5:35. When the hourly outcomes from the 9th to 14th hours were combined, a statistically significant exposure-response association was observed, at a time when most workers are sleeping. The decline in hourly $SDNN_i$ observed in the night period is consistent with our previous study of long-duration HRV among this cohort (Cavallari et al., 2007).

Strengths of this study include detailed exposure assessment with personal $PM_{2.5}$ exposure measurements to reduce exposure misclassification. While the number of participants available for our study was small, the efficiency of the study was improved by the crossover design. Each participant served as his own control by providing baseline, non-work HRV measures over the same time period as work measures. In addition

to accounting for the inherent circadian variation of HRV, baseline measures also controlled for potential confounding by time-invariant, individual factors such as smoking, health status or general physical activity levels. The majority (80%) of baseline monitoring occurred within one week of workday monitoring and when the analysis was restricted to these individuals, the observed trend remained with the largest declines in HRV seen in the 3rd, 11th, and 13th hours post-exposure though the confidence intervals for the effect estimates widened. The study is also strengthened by study design; separating the exposure period (occurring during work) and the outcome period (occurring in the hours after work) by time limits the potential for confounding. In addition to being time-varying, potential confounders must also be associated with exposure during the work time period and be predictors of HRV in the time following exposure. For example, while ventilation during work may be associated with exposure during the work period, it is not associated with HRV several hours after exposure occurs. Although we were unable to account for predictors of HRV that might vary between the work and non-work periods, such as quantity of caffeine, alcohol and cigarette consumption, for the exposure-response analysis, we hypothesize that neither caffeine nor alcohol consumption were correlated with work $PM_{2.5}$ exposure; therefore the potential for confounding bias is small. We adjusted for smoking by using a binary variable, but were unable to account for the quantity of cigarettes consumed. However, workers were allowed to smoke on the job, and it is unlikely that the quantity of cigarettes consumed differed between work and non-work periods. Thus, the likelihood of such a bias is low.

Since activity, specifically work activity, which may be associated with exposure, may have varied between work and non-work periods, we investigated whether work effects, quantified using mean heart rate during work, could explain the association we observed. In general, adjustment for work heart rate resulted in the blunting of effect estimates for 1 to 8 hours post-exposure. HRV in the first hour following exposure was most affected. Nonetheless even after adjustment, we observed a similar trend in the exposure-response relationship with statistically significant associations at 9 and 11 hours post-exposure. Furthermore, while work activity may be associated with exposure, it is unlikely that activity at work is a predictor of HRV several hours after the activity occurred, as HRV appears to return to baseline levels within 5 to 60 minutes following exercise (Perini and Veicsteinas, 2003).

We attempted to account for respirator use by adding an interaction term to the models of HRV (data not shown). Workers reporting no respirator use had a larger decline in HRV with work $PM_{2.5}$ exposure; however, the confidence intervals for the effect estimates among respirator users and non-respirator users overlapped. A limitation of the study is that potential co-pollutant gases including ozone, nitrogen oxides or carbon monoxide were not measured. However, confounding by co-pollutants, such as ozone or carbon monoxide, is also unlikely as previous measurements among a subset of these participants found no correlation with $PM_{2.5}$ (Liu et al., 2005) and these concentrations are low in similar welding operations (Burgess, 1995).

The small sample size limited our ability to investigate the potentially modifying effects of smoking status, cardiac compromise, and high blood pressure. Due to limitations in the ECG recorder used, we were unable to monitor the cohort past 14 hours following exposure and we were unable to obtain additional time or frequency domain HRV measures. Future studies should monitor over long time periods, as it appears that the response does not level off 14 hours past exposure. Studies should also obtain additional time and frequency domain HRV measures to investigate role of the sympathetic and parasympathetic system with regard to the temporal complexity of the response.

In summary, these results support the cardiotoxicity of occupational particulate matter exposures. The results suggest a multiphase, cardiovascular autonomic response subsequent to exposure to high levels of work particles, including an early response, 3 hours post-exposure, followed by a later response, 9–13 hours post-exposure. The results highlight the importance of continually monitoring HRV subsequent to particle exposures to further our understanding of the temporal complexity of the cardiovascular autonomic response to particle exposures.

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