

PM_{2.5} metal exposures and night heart rate variability: A panel study of welders

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Abstract

Background

To better understand the mechanism(s) of particulate matter (PM) associated cardiovascular effects, research priorities have shifted towards identifying the responsible PM characteristics. Evidence suggests that metals play a role in the cardiotoxicity of fine PM (PM_{2.5}) and in exposure-related decreases in heart rate variability (HRV). We examined the association between daytime exposure to the metal content of PM_{2.5} and night HRV in a crossover panel study of workers exposed to metal-rich welding fumes.

Methods

Twenty-six, male welders were monitored by ambulatory electrocardiogram (ECG) on a workday while exposed to welding fume and a non-workday (baseline). From the ECG, rMSSD (square root of the mean squared differences of successive intervals) was summarized over the night (0:00-7:00). Workday, gravimetric PM_{2.5} samples were analyzed by x-ray fluorescence to determine metal content. We used linear mixed effects models to assess the associations between night rMSSD and PM_{2.5} metal exposures both with and without adjustment for total PM_{2.5}. Matched measurements from a non-workday were used to control for individual cardiac risk factors and models were also adjusted for smoking status. To address collinearity between PM_{2.5} and metal content, we used a two-step approach based on treating the residuals from linear regression models of each metal on PM_{2.5} as surrogates for metal exposures in epidemiologic models for night rMSSD.

Results

The median PM_{2.5} exposure was 650 µg/m³; median metal exposures for iron, manganese, aluminum, copper, zinc, chromium, lead, nickel and vanadium ranged from 226 µg/m³ to non-detectable. We found inverse linear associations in exposure-response models with increased metal exposures associated with decreased night rMSSD. A statistically significant association for manganese was observed, with a decline of 0.130 msec (95% CI: -0.162, -0.098) in night rMSSD for every 1 µg/m³ increase in manganese. However, even after

adjusting for individual metals, increases in total PM_{2.5} exposures were associated with declines in night rMSSD

Conclusions

These results support the cardiotoxicity of PM_{2.5} metal exposures, specifically manganese. However the metal component alone did not account for the observed declines in HRV. Therefore, results do not exclude the importance of other particulate matter elemental components.

Background

The consistent association between particulate matter (PM) exposures and cardiovascular health effects is well documented [1-3]. As we seek to better understand the mechanisms, research priorities have shifted to identify the PM characteristic(s) responsible for the observed cardiovascular health effects [1, 4, 5]. Evidence from epidemiological and toxicological studies suggest that composition may play a role in particle-associated cardiovascular responses [4, 5].

To date, the majority of epidemiological studies have characterized PM exposures by mass concentration. PM with an aerodynamic diameter $\leq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) is often measured due to its ability to penetrate deep into the alveolar regions of the lung, where it can initiate cardiovascular and other health effects. Some studies have focused on identifying air pollution sources responsible for the observed health effects, and there is growing evidence linking traffic-related particle exposures to cardiovascular responses [6]. However, information is lacking on the specific chemical or class of chemical components associated with adverse cardiovascular health outcomes [5, 7]. Through source-related studies and toxicological evidence, particulate components including soluble organic compounds and metals, specifically transition metals, have been linked to cardiovascular outcomes [4]. The role of metals is further implicated by a study of air pollution exposures among older men finding that metal processing genes modified cardiovascular autonomic responses to $\text{PM}_{2.5}$ [8]. However, only one epidemiological study [9] has examined the association between specific particulate matter metal components and cardiovascular outcomes in humans.

We sought to investigate the association between individual $\text{PM}_{2.5}$ metal components and heart rate variability (HRV), a measure of cardiovascular autonomic control. Our previous research among a cohort of boilermaker welders occupationally exposed to high-levels of metal-rich PM examined the association between total workday HRV and workday $\text{PM}_{2.5}$ metal exposures [9]. We found statistically significant increases in the 5-min SDNN (standard deviation of normal-to-normal intervals), averaged over the 8-10 hour workday with

personal lead and vanadium exposures. This is contrary to the majority of studies reporting an inverse association between HRV and total PM_{2.5} exposure [1]. In a subsequent investigation of this cohort, we observed that night may be a susceptible period for particle-related changes in cardiovascular autonomic response as there was an inverse exposure-response relationship between workday PM_{2.5} mass and long-duration night HRV, specifically rMSSD (square root of the mean squared differences of successive intervals) [10]. In the present study we sought to examine the association between the metal components of PM_{2.5} both independently and after adjustment for total PM_{2.5} and rMSSD during the susceptible night period in a panel study of welders.

Methods

Subject recruitment

The Institutional Review Board at the Harvard School of Public Health approved the study protocol, and informed written consent was obtained from each adult prior to participation. Between 2004 and 2006, 26 boilermaker welders were recruited at an apprentice welding school. These boilermakers were invited to participate in the study on multiple occasions over the two-year sampling period; 22 (85%) were monitored on one occasion, 3 (12%) were monitored twice, and one was monitored on three occasions for a total of 31 monitoring occasions. On each monitoring occasion, welders were continuously monitored over both a work and non-work day.

Data collection

Workday monitoring took place at a union welding school, where welders practiced welding, cutting, and grinding techniques. Welders primarily performed shielded metal arc (stick) and gas metal arc welding (TIG), 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. In addition to workday monitoring, participants were also continuously monitored over a non-workday when they were not welding, grinding or cutting,

to establish baseline night HRV. This occurred within 6 months of workday monitoring, but 81% of the observations occurred within the same week. A questionnaire was used to collect information on medical history, current cardiopulmonary symptoms, medication use, demographics, occupational history, and lifestyle factors including smoking history.

ECG monitoring and tape processing

Participants were fitted with a standard 5-lead ECG Holter monitor. To facilitate good lead contacts, the participant's skin was shaved, if necessary, cleansed with an alcohol wipe, and slightly abraded. Study staff checked leads at the workplace periodically. Each 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 the work and non-work periods, used standard criteria to accept or reject all normal or abnormal findings. Tapes were analyzed in the time domain and rMSSD was summarized over the 7-hr night period (00:00 to 07:00).

Particulate matter exposure assessment and analysis

Personal, integrated, gravimetric samples were collected over the duration of the work shift using a KTL cyclone (GK2.05SH, BGI Incorporated, Waltham, MA) with an aerodynamic diameter cutpoint of 2.5 μm used in line with a pump drawing 3.5 L/min of air. The cyclone was secured to the participant's shoulder in the breathing zone area, and the pump was placed in a padded pouch that was carried by the participant for the entire workday. Each cyclone was fitted with a cassette holding a 37 mm polytetrafluoroethylene membrane filter (Gelman Laboratories, Ann Arbor, MI). The filters were weighed in a temperature- and humidity-controlled room using a standard protocol before and after sampling on an MT5 micro-balance from Mettler-Toledo Incorporated (Columbus, OH). We divided the blank corrected mass of each sample by the sample air volume to calculate the $\text{PM}_{2.5}$ mass concentration.

The filters from the cyclone samples were analyzed for elemental components using x-ray fluorescence (XRF). Desert Research Institute (Reno, NV, USA) performed all XRF analyses on the PANanalytical Epsilon 5 Energy Dispersive XRF analyzer (Almelo, the Netherlands) and utilized standard operating procedures including quality control and assurance measures [11, 12]. For each element the corresponding limit of detection (LOD) was reported as μg of element per filter. Sample values below the LOD were utilized as reported. Metal exposures were calculated by dividing the metal mass by the sampled air volume.

Statistical methods

Paired t-tests were used to compare the mean rMSSD between work and non-work periods. Due to the skewed exposure distributions, exposure medians and interquartile ranges (Q25 – Q75) were calculated for total $\text{PM}_{2.5}$ as well as the following metals: aluminum, chromium, copper, iron, lead, manganese, nickel, vanadium, and zinc. Spearman correlations between PM mass and metal exposures were estimated.

To investigate the association between $\text{PM}_{2.5}$ metal exposures and night HRV, we used linear mixed-effects regression models with random intercept and unstructured covariance to account for correlated outcomes among subjects who participated on multiple occasions. Regression models included a continuous covariate for non-work, night rMSSD to control for subject specific risk factors for HRV such as age and health status that don't vary over the time frame of interest. In addition, we adjusted all models for cigarette smoking by including a dichotomous variable representing smoking status at the time of monitoring. Each metal was modeled separately and due to small sample size, multiple metal models were not considered simultaneously. Since metal and total PM mass exposures covaried, we also investigated the effect of each metal, independent of $\text{PM}_{2.5}$, by including total $\text{PM}_{2.5}$ in the model along with the metals.

As an alternative approach designed to address the collinearity amongst exposure variables, we also investigated the association between night HRV and $\text{PM}_{2.5}$ metal exposures

using a two-step residual model. In the first step, each metal was regressed on total PM_{2.5} in a separate linear model, and the residuals were computed for each observation. Residuals are, by definition, the portion of the outcome (metal content) that is uncorrelated with the independent variable, total PM_{2.5} concentration. We therefore considered the residuals to be measures of the independent contribution of each metal and in the second step, treated them as new exposure variables in the regression models for the health outcome. All analyses were performed using SAS version 9.1.

Results

The 26 male welders were monitored over a total of 31 measurement occasions. Their mean age was 45 years, 80% were white and 76% were non-smokers (Table 1). Five reported hypertension and five reported cardiac compromises, including two reports of myocardial infarction, one stent, one murmur and one arrhythmia. Two individuals reported both hypertension and cardiac conditions. Over the 31 measurement occasions, night rMSSD was lower on workdays as compared to non-workdays, although the difference was not statistically significant ($p = 0.33$).

The median PM_{2.5} exposure of the participants was 649.8 $\mu\text{g}/\text{m}^3$ (Table 2). For the 31 exposure measurements, elemental concentrations were below the LOD for 21 (68%) vanadium, 12 (39%) nickel and 1 (3%) chromium and lead samples. Welders had the highest exposure to iron, with a median concentration of 225.6 $\mu\text{g}/\text{m}^3$, followed by manganese at 27.22 $\mu\text{g}/\text{m}^3$. Aluminum, copper, and zinc median exposures ranged from 4.58 - 0.98 $\mu\text{g}/\text{m}^3$. Chromium, lead, nickel and vanadium were present in the lowest median exposures. In addition to the elements presented in Table 2, XRF analysis also revealed the presence of other elements with median exposures of 21.97 $\mu\text{g}/\text{m}^3$ for sodium, 20.92 $\mu\text{g}/\text{m}^3$ for potassium, 20.34 $\mu\text{g}/\text{m}^3$ for silica, 19.43 $\mu\text{g}/\text{m}^3$ for calcium, 4.58 $\mu\text{g}/\text{m}^3$ for aluminum, 3.44 $\mu\text{g}/\text{m}^3$ for sulfur, and 1.86 $\mu\text{g}/\text{m}^3$ for copper as well as other elements at quantities below a median exposure of 1 $\mu\text{g}/\text{m}^3$.

With the exception of vanadium, each metal exposure was strongly correlated with total PM_{2.5}, with Spearman correlation coefficients ranging from 0.53 for zinc to 0.97 for iron (Table 3). Iron, chromium, aluminum and manganese were most highly correlated with PM_{2.5} (0.97 – 0.91), followed by copper (0.84). The other statistically significant correlations with PM_{2.5} were for lead (0.70), nickel (0.63) and zinc (0.53). Vanadium, which had a large percentage of non-detectable samples, showed no correlation with total PM_{2.5} exposures, with a Spearman correlation coefficient of –0.07. The correlations amongst the metals were similarly positive and strong.

Mixed model regression analyses revealed, after adjusting for non-work night rMSSD, total PM_{2.5} exposure was associated with a statistically significant ($p < 0.05$) decline in night rMSSD; (–0.006 msec/ $\mu\text{g}/\text{m}^3$, 95% CI: –0.008, –0.003). In separate regression models, increases in each PM_{2.5} metal exposure were also associated with declines in night rMSSD (Table 4, Model 1). The largest decline in night rMSSD per 1 $\mu\text{g}/\text{m}^3$ increase in metal was for vanadium, followed by chromium and nickel, although the confidence intervals were broad for these associations. Smaller effects, yet statistically significant ($p < 0.05$) associations, were observed for aluminum, iron, and manganese (Table 4, Model 1). When we expressed exposure in interquartile ranges, the largest change in night rMSSD per increase in interquartile range of metal was observed for iron (–4.17 msec) followed by manganese (–3.69 msec) and aluminum (–2.36 msec). In order to try and apportion the association with PM_{2.5} to one or more of the metal components, we needed to adjust for PM_{2.5} in each of the models. Collinearity precludes any useful interpretation of the regression coefficients in a model that includes PM_{2.5} as well as a metal component (Table 4, Model 2).

To address this collinearity problem, we used residual models to further explore the association with PM_{2.5} by evaluating the contribution of each metal component. The residual model has the advantage of providing a measure of metal exposure that is independent of PM_{2.5}. Therefore, these models allowed us to adjust for PM_{2.5} in the models for each specific metal component. When each metal was regressed on PM_{2.5}, the residuals for each model had

a mean of zero with interquartile ranges as presented in Table 5. For the regression models using the residual metal exposures, an inverse linear association was seen between HRV and aluminum, chromium, manganese, lead and vanadium (Table 5, Model 3). Since the metal residual was no longer correlated with PM_{2.5}, we can interpret these results as the effect of increasing metal exposures while holding PM_{2.5} constant. After accounting for PM_{2.5} exposure, the associations between the individual metals and HRV weakened or changed in direction (Table 5, Model 4). However an inverse, linear exposure-response relationship remained for aluminum, manganese, lead and vanadium, as is consistent with Model 1, although the confidence intervals of these associations widened. Holding each metal exposure constant, we observed consistent declines in HRV with increasing total PM_{2.5} exposure (Table 5, Model 4).

Discussion

This study provides valuable information on how the metal content of PM contributes to cardiovascular responses, specifically to changes in cardiovascular autonomic control. By expanding our investigation of a cohort exposed to high levels of metal-rich PM to include the susceptible night period, we observed an association between increased individual PM_{2.5} metal exposures, specifically manganese and vanadium, and declines in night rMSSD. We observed heterogeneous associations across the different metals, and in terms of toxicity, the largest declines in HRV were observed per 1 µg/m³ increase in vanadium, although the confidence intervals were wide, as a number of the vanadium exposures were below the limit of detection. We observed smaller, yet statistically significant (p<0.05), declines in HRV per 1 µg/m³ increase aluminum, iron and manganese. Expressed per increase in interquartile range of exposure, the largest decline in HRV was seen for iron and manganese.

Since PM_{2.5} and metal exposures were correlated, we confirmed the results with residual models. The residual method of analysis confirmed the manganese effect: a -0.250 (95% CI: -0.331, -0.169) msec change in night rMSSD was observed with every 1 µg/m³ increase in manganese residual. An inverse exposure-response relationship was also

confirmed for aluminum, chromium, lead, and vanadium exposures. Since the metal residual was no longer correlated with $PM_{2.5}$, we were able to explore the effect of increasing metal exposures while holding $PM_{2.5}$ constant by adjusting for $PM_{2.5}$ in the metal residual models. We found that after adjusting for $PM_{2.5}$, an inverse relationship, similar in magnitude, persisted for residual manganese, although the confidence intervals were broad. The magnitude of the inverse association also persisted for vanadium, which in each model had the largest effect estimate although once again broad confidence intervals and a large number of non-detectable samples.

The association between total $PM_{2.5}$ exposures and night HRV observed in this study is consistent with our previous report among this population [10]. As we hypothesized, unlike our previous study that evaluated daytime HRV over work (Magari 2002), using the susceptible night period, we observed a negative association between night HRV and $PM_{2.5}$ metal exposures. However, a study of highway patrol troopers observed a positive association with increases in post-shift HRV with a source factor related to speed-changing traffic which was dominated by copper, aldehyde and sulfur content of $PM_{2.5}$ exposures [13]. In both our previous investigation and the patrol trooper study the timing of HRV response occurs during or immediately post-exposure; unlike the current study, where HRV was measured in the evening following workday exposure. These disparate results may be capturing the varying time-course of metal responses or the complex interplay between different metals, which is supported by toxicological studies.

In a study of rats, Campen *et al.* [14] found that intratracheal instillation of nickel and vanadium exposures produced immediate and delayed cardiovascular effects. In addition, vanadium effects were exacerbated by concurrent nickel exposures, yet attenuated by concurrent iron exposures [15]. While the exact mechanism is unknown, once 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 [16]. 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 [17], and an association between transition metal exposures and cardiovascular autonomic changes in mice [18] and rats [15] yet not dogs [19]. Alternately, other metals such as zinc has been shown to trigger effects by directly interacting with cellular proteins [20, 21].

Results from our current study signal the cardiotoxicity of vanadium and manganese. Both the standard and residual models presented an inverse exposure-response relationship for these metals and the manganese and vanadium effects persisted after adjustment for total PM_{2.5} exposure suggesting that these metal effects are independent of total fine particulate matter effects. The cardiovascular effects of vanadium have been observed in instillation studies of vanadium in rats [14]. While the cardiotoxicity of manganese has not been previously reported, manganese neurotoxicities are well recognized, and both epidemiological and toxicological studies suggest that manganese exposure may also lead to cardiovascular toxicities [22]. Abnormal ECG parameters, including sinus arrhythmia and ST-T changes, are increased in workers exposed to manganese oxide as compared to controls [22] and Barrington *et al.* [23] report decreased autonomic function and low 24-hr HRV, in manganese alloy workers, though this association has yet to be corroborated by *in vivo* studies, as no significant HRV changes in dogs were observed subsequent to short-term inhalation of aerosol manganese [19].

Yet, neither vanadium nor manganese can completely explain the declines in HRV observed within this cohort. An inverse association was observed with PM_{2.5} in the residual models adjusted for manganese. This inverse association was also observed in many of the other metal models. This suggests that when the metal content is held constant, there remains a total PM_{2.5} exposure effect. We were unable to determine which, if any, chemical component this is due to. However, the non-metal components may also play a role in the observed cardiotoxicity of PM_{2.5} exposures. In fact, we investigated the association between PM_{2.5} silicon exposures and night rMSSD, we observed a -0.17 (95% CI: -0.24, -0.10) msec change per 1 µg/m³ increase in silicon PM_{2.5} exposure after adjusting for non-work HRV and smoking status. Due to limitations in the XRF analysis technique, we were unable to measure

the silicon complex within the welding fume, however previous investigation of welding fume exposure suggests that silicon is likely in the silica state [24]. Decreases in HRV with silica exposures have been previously observed in a study of aged, ApoE knockout transgenic mice where both ambient particulate matter or silica produced decreases in HRV parameters [25]. The health effects of occupational silica exposure are well recognized; both acute and chronic silica exposures, in the form of crystalline silica, can lead to silicosis, a debilitating fibrotic disease of the lung. Silica exposure has been linked to inflammation and autoimmune diseases [26]. The cardio-toxicity of silica exposure warrants further research.

While we observed signals of cardiotoxicity for manganese and vanadium, we did not observe an effect for nickel, which has previously been implicated in changes in heart rate and HRV [18]. It is unlikely that one component of PM is responsible for all cardiovascular effects and it is unclear what role the composition mixtures play. One major limitation of this study is the exposure source, which differs substantially from ambient PM_{2.5} or other sources of PM_{2.5}. While welding fumes are enhanced in some metals, they may have lower than average proportions of other metals, which may be relevant for other exposure scenarios, and may explain differences in study results.

In addition to composition, the total PM_{2.5} effect may be related to other particle characteristics. While we determined the elemental composition of the welding fume, we did not fully characterize size fractions including coarse and ultrafine and we did not quantify exposures by surface area or number counts; all of which have been linked to particulate matter toxicity [4, 7]. Previous research suggests, for the welding methods used by this cohort, most particles generated are < 1 µm [27] and it is unclear what role the ultrafine particle size plays in the observed HRV declines.

This study is strengthened by detailed exposure assessment with personal PM_{2.5} exposure monitoring with elemental determination. In total, the elemental mass determined by XRF analysis accounted for 56% of the sample mass. However, one limitation of XRF analysis is that it cannot discern metal oxidation states or identify metals complexes. Thus

while we identified elemental concentrations, the solubility of each element, which may effect toxicity, is unknown.

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. Baseline measures controlled for potential confounding by time-invariant, individual factors such as smoking, health status or general physical activity levels. This study is also strengthened by having separated the exposure period (occurring during work) and the outcome period (occurring in the evening after work) by time, which 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 night 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 by these factors 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 confounding by smoking is low.

Nevertheless, while we observed statistically significant results for manganese exposures, the lack of statistical significance in the relationships with other metals may be due, in part, to the small sample size. In addition, for vanadium, while there is a signal of cardiotoxicity, the large number of samples below the limit of detection and wide confidence intervals suggests the need for additional evidence. Lastly, the mechanism of metal-related

cardiovascular toxicity may be complex and while we observed signals of toxicity in the night period, it is possible that we did not capture early or later effects.

Conclusions

In summary, this study supports the cardiotoxicity of the metal component of particulate matter exposures. There appears to be a difference in cardiac autonomic response among the metals with a consistent exposure-response relationship observed for manganese. Yet, results do not exclude the importance of other metal and non-metal particulate matter components in the observed cardiovascular autonomic effects.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

JMC contributed to study design, data collection, analysis and interpretation of the results, and manuscript preparation. EAE and JS contributed to study design, statistical analyses and critical review of the manuscript. SCF contributed to data collection, interpretation of the results, and critical review of manuscript. RFH and RH contributed to exposure assessment and critical review of the manuscript. DCC contributed to study design and analysis, interpretation of the results, and critical review of the manuscript. All authors read and approved the final manuscript.

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References

1. Pope CA, Dockery DW: **Health effects of fine particulate air pollution: lines that connect.** *J Air Waste Manag Assoc* 2006, **56**(6):709-742.
2. Peters A: **Particulate matter and heart disease: Evidence from epidemiological studies.** *Toxicol Appl Pharmacol* 2005, **207**(2 Suppl):477-482.
3. Schulz H, Harder V, Ibald-Mulli A, Khandoga A, Koenig W, Krombach F, Radykewicz R, Stampfl A, Thorand B, Peters A: **Cardiovascular effects of fine and ultrafine particles.** *J Aerosol Med* 2005, **18**(1):1-22.
4. Schwarze PE, Ovreivik J, Lag M, Refsnes M, Nafstad P, Hetland RB, Dybing E: **Particulate matter properties and health effects: consistency of epidemiological and toxicological studies.** *Hum Exp Toxicol* 2006, **25**(10):559-579.
5. Brook RD: **Is air pollution a cause of cardiovascular disease? Updated review and controversies.** *Rev Environ Health* 2007, **22**(2):115-137.
6. Adar SD, Kaufman JD: **Cardiovascular disease and air pollutants: evaluating and improving epidemiological data implicating traffic exposure.** *Inhal Toxicol* 2007, **19** Suppl 1:135-149.
7. Schlesinger RB, Kunzli N, Hidy GM, Gotschi T, Jerrett M: **The health relevance of ambient particulate matter characteristics: coherence of toxicological and epidemiological inferences.** *Inhal Toxicol* 2006, **18**(2):95-125.
8. Park SK, O'Neill MS, Wright RO, Hu H, Vokonas PS, Sparrow D, Suh H, Schwartz J: **HFE genotype, particulate air pollution, and heart rate variability: a gene-environment interaction.** *Circulation* 2006, **114**(25):2798-2805.
9. Magari SR, Hauser R, Schwartz J, Williams P, Smith TJ, Christiani DC: **The association of particulate air metal concentrations with heart rate variability.** *Environ Health Perspect* 2002, **110**(9):875-880.
10. Cavallari JM, Eisen EA, Chen JC, Fang SC, Dobson CB, Schwartz J, Christiani DC: **Night Heart Rate Variability and Particulate Exposures among Boilermaker Construction Workers.** *Environ Health Perspect* 2007, **115**(7):1046-1051.
11. Chow J, Watson J: **Guidelines on Speciated Particulate Monitoring, Third Draft Report.** In. Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Air Quality Planning and Standards; 1998.
12. Watson J, Chow J, Frazier C: **X-ray fluorescence analysis of ambient air samples.** In: *Elemental Analysis of Airborne Particles.* Edited by Landberger SaC, M. NJ: Gordon and Breach Publishers; 1999: 67-96.
13. Riediker M, Devlin RB, Griggs TR, Herbst MC, Bromberg PA, Williams RW, Cascio WE: **Cardiovascular effects in patrol officers are associated with fine particulate matter from brake wear and engine emissions.** *Part Fibre Toxicol* 2004, **1**(1):2.
14. Campen MJ, Nolan JP, Schladweiler MC, Kodavanti UP, Costa DL, Watkinson WP: **Cardiac and thermoregulatory effects of instilled particulate matter-associated transition metals in healthy and cardiopulmonary-compromised rats.** *J Toxicol Environ Health A* 2002, **65**(20):1615-1631.

15. Campen MJ, Nolan JP, Schladweiler MC, Kodavanti UP, Evansky PA, Costa DL, Watkinson WP: **Cardiovascular and thermoregulatory effects of inhaled PM-associated transition metals: a potential interaction between nickel and vanadium sulfate.** *Toxicol Sci* 2001, **64**(2):243-252.
16. Gonzalez-Flecha B: **Oxidant mechanisms in response to ambient air particles.** *Mol Aspects Med* 2004, **25**(1-2):169-182.
17. Maciejczyk P, Chen LC: **Effects of subchronic exposures to concentrated ambient particles (CAPs) in mice. VIII. Source-related daily variations in in vitro responses to CAPs.** *Inhal Toxicol* 2005, **17**(4-5):243-253.
18. Lippmann M, Ito K, Hwang JS, Maciejczyk P, Chen LC: **Cardiovascular effects of nickel in ambient air.** *Environ Health Perspect* 2006, **114**(11):1662-1669.
19. Muggenburg BA, Benson JM, Barr EB, Kubatko J, Tilley LP: **Short-term inhalation of particulate transition metals has little effect on the electrocardiograms of dogs having preexisting cardiac abnormalities.** *Inhal Toxicol* 2003, **15**(4):357-371.
20. Tal TL, Graves LM, Silbajoris R, Bromberg PA, Wu W, Samet JM: **Inhibition of protein tyrosine phosphatase activity mediates epidermal growth factor receptor signaling in human airway epithelial cells exposed to Zn²⁺.** *Toxicol Appl Pharmacol* 2006, **214**(1):16-23.
21. Haase H, Maret W: **Protein tyrosine phosphatases as targets of the combined insulinomimetic effects of zinc and oxidants.** *Biometals* 2005, **18**(4):333-338.
22. Jiang Y, Zheng W: **Cardiovascular toxicities upon manganese exposure.** *Cardiovasc Toxicol* 2005, **5**(4):345-354.
23. Barrington WW, Angle CR, Willcockson NK, Padula MA, Korn T: **Autonomic function in manganese alloy workers.** *Environ Res* 1998, **78**(1):50-58.
24. Antonini JM: **Health effects of welding.** *Crit Rev Toxicol* 2003, **33**(1):61-103.
25. Corey LM, Baker C, Luchtel DL: **Heart-rate variability in the apolipoprotein E knockout transgenic mouse following exposure to Seattle particulate matter.** *J Toxicol Environ Health A* 2006, **69**(10):953-965.
26. Parks CG, Conrad K, Cooper GS: **Occupational exposure to crystalline silica and autoimmune disease.** *Environ Health Perspect* 1999, **107 Suppl 5**:793-802.
27. Hewett P: **The particle size distribution, density, and specific surface area of welding fumes from SMAW and GMAW mild and stainless steel consumables.** *Am Ind Hyg Assoc J* 1995, **56**(2):128-135.

Tables

Table 1 - Study population characteristics for boilermaker welder participants (n = 26)

Characteristics	Mean \pm SD or n (%)
Male	26 (100)
Age (yrs) ^a	45 \pm 11
Range	29 – 64
Race	
White	20 (80)
Black	1 (4)
Hispanic	3 (12)
Asian	1(4)
Current Smoker	6 (24)
Hypertensive	5 (20)
Night ^b rMSSD (msec)	
Workday ^c	30.3 \pm 16.0
Non-workday ^c	32.0 \pm 16.0

^aAt study entry. ^b(00:00 to 07:00) ^cOver 31 measurement occasions.

Table 2 - Composition and characteristics for personal, workday PM_{2.5} measurements (n=31)

PM _{2.5} Component	Below LOD	Exposure (µg/m ³)		
		Mean	Median	Q25 – Q75
Total	0	799.0	649.8	337.0 – 1052.2
Al	0	5.07	4.58	2.67 – 6.28
Cr	1	0.19	0.16	0.08 – 0.24
Cu	0	3.16	1.86	0.79 – 4.67
Fe	0	319.3	225.6	132.2 – 453.0
Mn	0	27.33	27.22	10.23 – 38.62
Ni	12	0.11	0.04	0.003 – 0.15
Pb	1	0.16	0.14	0.08 – 0.22
V	21	0.01	ND ^a	ND – 0.02
Zn	0	2.31	0.98	0.37 – 4.50

^aND – non-detectable

Table 3 - Spearman correlation coefficients within and between PM_{2.5} metal exposures and total PM_{2.5} exposure

	Al	Cr	Cu	Fe	Mn	Ni	Pb	V	Zn
PM _{2.5}	0.91	0.91	0.84	0.97	0.95	0.63	0.70	-0.07	0.53
Al	1.00	0.91	0.67	0.82	0.89	0.40	0.66	0.05	0.50
Cr		1.00	0.74	0.85	0.85	0.52	0.73	-0.02	0.48
Cu			1.00	0.86	0.73	0.82	0.64	-0.18	0.56
Fe				1.00	0.91	0.69	0.65	-0.08	0.50
Mn					1.00	0.55	0.63	-0.16	0.46
Ni						1.00	0.49	-0.15	0.40
Pb							1.00	-0.03	0.50
V								1.00	0.20

Table 4 - Associations between night HRV and individual PM_{2.5} metal exposures

	Model 1		Model 2			
	Metal		Metal		Particulate	
	β_1	95% CI	β_1	95% CI	β_2	95% CI
Al	-0.642**	(-1.07, -0.209)	-0.138	(-2.22, 1.95)	-0.004	(-0.022, 0.013)
Cr	-12.54*	(-29.38, 4.30)	3.40	(-19.22, 26.02)	-0.006*	(-0.014, 0.007)
Cu	-0.294	(-1.38, 0.786)	0.093	(-0.613, 0.799)	-0.006**	(-0.010, -0.001)
Fe	-0.013**	(-0.023, -0.002)	-0.002	(-0.032, 0.028)	-0.005	(-0.016, 0.007)
Mn	-0.130**	(-0.162, -0.098)	-0.145*	(-0.348, 0.683)	0.001	(-0.009, 0.010)
Ni	-4.76	(-24.69, 15.16)	1.03	(-11.10, 13.16)	-0.006**	(-0.010, -0.001)
Pb	-11.90	(-38.72, 14.92)	-0.545	(-23.61, 22.53)	-0.005**	(-0.010, -0.0004)
V	-65.35	(-180.29, 49.59)	-30.17	(-91.50, 31.16)	-0.005**	(-0.008, -0.001)
Zn	-0.108	(-1.06, 0.849)	0.105	(-0.625, 0.834)	-0.006**	(-0.011, -0.001)

Model 1: mixed effects linear regression models for each individual metal, adjusted for baseline night rMSSD and smoking status. Model 2: mixed effects linear regression models with each individual metal and PM_{2.5}, adjusted for baseline night rMSSD and smoking status. Regression coefficients (β) are expressed as change in msec of night rMSSD per 1 $\mu\text{g}/\text{m}^3$ increase in exposure after adjusting for baseline HRV, smoking status and with or without adjustment for total PM_{2.5}. * $p < 0.10$

** $p < 0.05$

Table 5 - The associations between night rMSSD and residual metal exposures

	Model 3			Model 4			
	Metal Residual	Metal Residual		Metal Residual		PM _{2.5}	
	Q25 – Q75	β_1	(95% CI)	β_1	(95% CI)	B ₂	(95% CI)
Al	-0.57 – 0.59	-1.15	(-3.04, 0.74)	-0.138	(-2.22, 1.95)	-0.005*	(-0.012, 0.001)
Cr	-0.03 – 0.02	-6.59	(-49.78, 36.59)	3.40	(-19.22, 26.02)	-0.006**	(-0.010, -0.001)
Cu	-1.30 – 0.95	0.388	(-0.679, 1.46)	0.093	(-0.613, 0.799)	-0.005**	(-0.010, -0.000)
Fe	-41.89 – 34.08	0.014	(-0.022, 0.050)	-0.002	(-0.032, 0.028)	-0.006*	(-0.012, 0.000)
Mn	-6.69 – 7.55	-0.250**	(-0.331, -0.169)	-0.145*	(-0.348, 0.058)	-0.003	(-0.007, 0.002)
Ni	-0.07 – 0.05	3.62	(-15.41, 24.52)	1.03	(-11.10, 13.16)	-0.005**	(-0.010, -0.000)
Pb	-0.05 – 0.02	-1.95	(-37.33, 33.43)	-0.545	(-23.61, 22.52)	-0.005**	(-0.010, -0.001)
V	-0.01 – 0.01	-62.94	(-184.35, 58.46)	-30.17	(-91.50, 31.16)	-0.005**	(-0.008, -0.002)
Zn	-1.69 – 1.24	0.076	(-0.991, 1.14)	0.105	(-0.625, 0.834)	-0.006**	(-0.010, -0.001)

The metal residuals are from the regression of total PM_{2.5} and each metal component, and represent the variation in metal exposure not due to PM_{2.5}. Model 3: mixed effects linear regression models for each individual metal residual, adjusted for baseline night rMSSD and smoking status. Model 4: mixed effects linear regression models with each individual metal residual and PM_{2.5}, adjusted for baseline night rMSSD and smoking status. Regression coefficients (β) are expressed as change in msec of night rMSSD (msec) per 1 $\mu\text{g}/\text{m}^3$ increase in metal residual after adjusting for baseline HRV, smoking status and with or without adjustment for total PM_{2.5}. * $p < 0.10$

** $p < 0.05$.