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## Citation

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# **FINE PARTICULATE EXPOSURE AND CARDIAC AUTONOMIC EFFECTS**

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A Dissertation Submitted to the Faculty of  
The Harvard T. H. Chan School of Public Health  
in Partial Fulfillment of the Requirements  
for the Degree of Doctor of Science  
in the Department of Environmental Health  
Harvard University  
Boston, Massachusetts.

May, 2015

## **FINE PARTICULATE EXPOSURE AND CARDIAC AUTONOMIC EFFECTS**

### **Abstract**

**Background:** Heart Rate Variability (HRV) as a research outcome has the potential for misclassification due to its inability to account for changes in the heart rate. HRV can be parsed into Acceleration Capacity (AC) and Deceleration Capacity (DC) which address these limitations.

**Objectives:** To investigate the associations between AC and DC with short-term and long-term metal PM<sub>2.5</sub> exposures; and examine if these associations are mediated by inflammation.

**Methods:** A panel of 45-50 male welders, mean age 39-40 years, had continuous PM<sub>2.5</sub> exposure during typical welding work shifts for 4-6 hours repeated 2-5 times over sampling periods in 2010-2012. We also obtained continuous recordings of digital electrocardiograms (ECG) over their work shift using Holter monitors during the same time; and analyzed blood samples before and after each welding shift for potential mediators of inflammation.

In our first analysis, we used linear mixed models to assess the association between hourly PM<sub>2.5</sub> exposure and each of simultaneously measured hourly AC and DC, controlling for covariates.

Then, mediation analysis was done using linear mixed models to assess the associations between shift PM<sub>2.5</sub> exposure, potential mediator levels, and AC and DC, controlling for relevant covariates in order to deduce the direct and indirect effects (via the mediator) of PM<sub>2.5</sub> on AC and DC.

In our final analysis, we used linear regression to assess the association between CEI PM<sub>2.5</sub> exposure and each of current AC and DC, controlling for confounders.

**Results:** Negative exposure-response associations were found for AC and DC with increased PM<sub>2.5</sub> both in the short-term and long-term exposure after adjusting for covariates. In our mediation models, the proportion of the total effect of PM<sub>2.5</sub> on AC or DC (indirect effect) mediated through IL-6 on AC was at best 4%.

**Conclusions:** There are sustained acute and chronic effects of metal particulates on AC and DC even after exposure has ceased. These findings suggest that there may be more pathways that sustain response following exposure other than a direct effect of metal particulates on AC and DC. Furthermore, there may be complex mediating pathways involving interleukin 6.

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## **Acknowledgements**

I am thankful to God to be able to present this dissertation, which addresses the cardiac autonomic health effects of occupational exposure to particulate matter among a group of boilermaker construction workers. The work presented in this dissertation expand upon work that has been ongoing for more than a decade and a half; Dr. David Christiani with Harvard T.H. Chan School of Public Health in collaboration with the Boilermakers Union, Local 29 located in Quincy, MA, to understand the cardiopulmonary health effects of particulate exposures. Prior work on this project had documented short and intermediate cardiac health effects, and we were able to uncover long term cardiac health effects of occupational particulate exposures. In addition, we were able to tease out the specific autonomic components (accelerations and decelerations) that are affected by work exposures on different time scales. It is my hope that this research would inform further research on mechanisms of these effects to provide a platform for targeted interventions.

This research would not have been possible without the help of many people. First, I would like to thank Drs. David Christiani, Xihong Lin, and Alex Lu for serving on my research committee. Their feedback and guidance during this work was tremendous. I would also like to express my sincere appreciation to my advisor, Dr. David Christiani, for giving me the opportunity to work on this project for my doctoral degree, and for his continued support throughout this work. I also want to appreciate Dr. Murray Mittleman and the staff of Cardiovascular Epidemiology Research Unit of Beth Israel Deaconess Hospital for their dedication in processing the ECG data. My special thanks go to Prof. Georg Schmidt of Munich

University of Technology, Munich, Germany for his professional input. I owe my gratitude to all the doctoral students who had spent their time and energy working on this project including Drs. Shannon Magari, Jee Young Kim, JC Chen, Paul Boyce, Jennifer Cavallari, Shona Fang, Jason Wang, and Angela Tiateng. I especially want to thank Dr. Angela Tiateng for her time and priceless research inputs, and Jinming Zhang who I had the pleasure of working closely with.

My experience of producing this dissertation gave me the opportunity to have field experience at the union hall with the study participants along with our study team, to gather the information needed for this dissertation. I am thankful to Drs. David Christiani, Murray Mittleman, and Jennifer Cavallari for their roles in obtaining the parent grant award from NIOSH for this project that fueled the study. This dissertation would also not have been possible without the work of the previous doctoral students, who established relationships with the workers and the union, and the dedicated study team members including Christine Dobson, Ema Rodrigues, Li Su, and Mike Wang for all their hard work and continuous support.

I am also deeply grateful for the support and editorial comments that I received from Donald Halstead and the members of the Dissertation Writing Group. I am also indebted to Adanna Chukwuma for her help in proofreading this work.

I would not have been able to accomplish this work without the individuals mentioned above as well as financial support provided by the Harvard-NIOSH Education and Research Center, and Leventis Foundation Fellowship for which I am grateful to have received. I also wish to acknowledge all my professors and students at the Harvard T. H. Chan School of Public

Health for teaching me epidemiology, biostatistics, and their applications to environmental and occupational health. I specially want to thank Drs. Paul Catalano, Mary Kay Smith-Fawzi and Murray Mittleman. I am indebted to Dr. Jack Dennerlein, Dr. Oscar Arias, and the team at Harvard Center for Work, Health and Well-being; and Drs. Laura Kubzansky and Julia Boehm for giving me the privilege of having hands-on experience with research data. I want to thank Dr. Promise Abuwa for inspiring me to enter into the field of public health and for encouraging me to pursue a doctoral degree in environmental and occupational epidemiology. I hope this dissertation is able to further our understanding of the mechanisms by which exposure to particulate matter affects cardiovascular health, and will in the future benefit the health of workers occupationally exposed to particulate matter.

Finally, I am forever grateful to my wife Nelly for her love and support, my daughter Sharon, my parents Tony and Martha, my brothers Joseph, Emmanuel, John and Andrew, my in-laws, my spiritual fathers especially Bishop David Oyedepo, and my friends for all their encouragements.

Peter Umukoro

May 2015

## **CHAPTER 1. INTRODUCTION**

Cardiovascular disease (CVD) is the leading cause of non-communicable deaths under the age of 70 in the world accounting for up to 30% of deaths(1). Lifestyle and environmental factors have been largely implicated in the epidemic rise of CVD which has happened concurrently with industrialization over the few past decades(2, 3). Fine particulate ( $PM_{2.5}$ ) exposure has been documented as a major environmental factor for the increased cardiovascular morbidity and mortality(4, 5).  $PM_{2.5}$  is air-borne particulate matter with a mass median aerodynamic diameter less than 2.5micrometers ( $\leq 2.5\mu m$ ), and could be easily inhaled into the alveoli and deeper lung tissues. In occupational settings, fine particulates have also been shown to have cardio-pulmonary effects, especially among workers with high exposures at work(6, 7).

One occupational group that daily has high exposures to fine particulates at work is the boilermakers. Boilermaker construction workers (boilermakers) are welders who work in power plants and are exposed to large amounts of particulate fumes. Occupational exposure to welding fumes occurs during the metal joining process. Boilermakers are also exposed to the residual fly ash left following the combustion of the fuel in the power plant. Based on 2012 estimates from the Bureau of Labor Statistics (BLS), 357,400 workers in the United States are employed as welders, cutters, solderers, and brazers(8). While  $PM_{2.5}$  is highly ubiquitous in the environment, exposures in these work settings where there is increased generation of particulates may be even more substantial. For example, epidemiologic studies have shown that boilermakers involved in welding activities are exposed to high levels of particulate matter in weld fumes(6, 7). In addition, several other workers such as construction workers and related fields are also exposed to high levels of particulate matter(9, 10).

Several studies have linked ambient PM<sub>2.5</sub> exposure with cardiovascular disease(4, 5, 11, 12), but there are fewer studies that have examined this association in an occupational setting with much higher airborne exposures from work processes. Whereas the exposure distribution and composition of fine particulate matter in the ambient environment has been studied in most parts of the world, comparably fewer studies describe PM<sub>2.5</sub> exposure distribution in occupational settings that even have greater exposures. While the effects of ambient PM<sub>2.5</sub> may be similar to occupational PM<sub>2.5</sub> exposure, differences in industrial composition may modify effects.

The evidence suggests that boilermakers have increased risk of cardiac autonomic dysfunction from acute exposures to metal-rich particulates(6, 7, 13-17). However, previous research has used Heart Rate Variability (HRV) as a marker for autonomic function/dysfunction. HRV as a dependent variable in cardiovascular research has been critiqued due to its inability to account for changes in the heart rate and its potential of misclassification. Whereas HRV measures the variability of the heart rate during a time period including when heart rate is static, accelerating or decelerating; newer indices of cardiac autonomic function: Acceleration Capacity (AC) and Deceleration Capacity (DC) measure the variability during speeding up and slowing down of the heart rate respectively. Previously, research has shown negative associations between short-term particulate exposures (PM<sub>2.5</sub>) and indices of autonomic dysfunction (HRV), both in the general population and among occupational groups. But it is not quite clear, if this results in a decrease in the heart's ability to speed up (AC), or to slow down (DC) its rate.

In 2006, Baeur described the phase-rectified signal averaging (PRSA) method for calculating the heart's acceleration capacity (AC) and deceleration capacity (DC), which are measures of the responsiveness of the heart like HRV but which account for heart rate . These have been demonstrated to be more predictive of morbidity and mortality among post-ischemic coronary

artery disease patients than traditional HRV. Therefore, using sensitive indices - Acceleration Capacity and Deceleration Capacity - the studies presented in this thesis aim to investigate the potential for cardiac autonomic dysfunction from PM<sub>2.5</sub> exposure.

Various theories have been suggested to account for the increased risk of cardiovascular diseases especially ischemic heart disease among welders. Several mechanisms describing alterations at cellular and tissue levels have been proposed to account for the effect of fine particulates in increasing the risk of cardiovascular events in welders. These include pulmonary inflammation(16) that spreads to secondarily involve the heart(15, 18), alterations of the autonomic nervous system that lead to changes in heart rate and heart rate variability indices(18, 19), and direct affectation of the heart and nerves from translocation of inhaled particles either in their whole or soluble forms(16). Toxicology studies have summarized three plausible pathophysiological pathways in which fine particulates may affect the heart and blood vessels: inflammation, direct effect on the automaticity of the heart muscle, and through neural responses in the airways.

There are few studies that have documented a potential for an inflammation-mediated pathway for effect of particulate on HRV(5, 16, 20). However, it is unclear if exposure to fine particulate resulting in a decrease in both accelerations and decelerations of the heart rate is mediated by inflammation. A thorough understanding of underlying mechanism will be essential in estimating exposure-disease burden, and recommending preventive strategies to reduce exposure.

Furthermore, very few studies have examined these exposures over the course of the work-life among workers who have been in these occupational settings for long periods(17), and have the potential for a cumulative multiplier effect from PM<sub>2.5</sub> exposure over the years. The effect of these exposures in the long term remains unclear, and resulting cardiac autonomic function in terms of acceleration and deceleration capacities is unknown.

In the studies presented within this thesis, we sought to explore further the association between PM<sub>2.5</sub> and cardiac autonomic dysfunction among the boilermakers population. Our research differs from prior research in this field in a number of ways. First, we use more sensitive markers of autonomic dysfunction, Acceleration Capacity (AC) and Deceleration Capacity (DC) rather than the previously used Heart Rate Variability. Second, we considered only PM<sub>2.5</sub> from welding day exposure since we were interested in the effect of welding fume on the heart. We however adjusted for baseline levels of our outcomes (AC or DC) in all our analyses to reduce confounding. Third, we used two different analytic approaches to investigate the acute effect of exposure of PM<sub>2.5</sub> on the heart, and the results were consistent. Finally, we evaluated for chronic effects of PM<sub>2.5</sub> using participants' previous work history to create a chronic exposure index.

This thesis expands the current knowledge and understanding of the cardiovascular autonomic responses to fine particulate matter. All three analyses are distinct and use a subset of the total study population comprised of panels of boilermakers sampled over three to five sampling periods (summer or winter) between January 2010 and June 2012 from the boilermaker union in Quincy, Massachusetts. We recruited 72 male boilermakers as part of an ongoing "Harvard Boilermaker Cohort" initiated in 1999 by my advisor, Dr. David Christiani to study the cardio-pulmonary effects of particulates(21). Although we had recruited 72 boilermakers in total, we restricted our analyses for each study to those participants who were monitored on welding days and had

complete data for all the variables in that study. Participants had to have continuous simultaneous PM<sub>2.5</sub> and digital ECG on welding days and had blood samples taken and analyzed for inflammatory markers. The details and the results of each analysis are in chapters 2-4.

Chapter 2 examines the association between short term effects of PM<sub>2.5</sub> arising from welding fumes and acceleration (AC) and deceleration capacities (DC) of the heart summarized by the hour. The aim of this analysis was to examine if the effects of PM<sub>2.5</sub> on heart rate variability resulted in either changes in AC, DC, or both. First, we examine the associations without any lag between exposure and outcome. Then, we examine the associations using hourly lagged models.

Following the results of chapter 2, we went on to examine in chapter 3 the potential of the short term effects of PM<sub>2.5</sub> being mediated by inflammation. First, we re-examined the association of acute PM<sub>2.5</sub> (work shift averages) on changes in AC and DC(post shift minus baseline). Then, we carried out mediation analyses using each potential inflammatory marker.

Chapter 4 investigates the association between long term effects of PM<sub>2.5</sub> using a chronic exposure index on acceleration (AC) and deceleration capacities (DC) of the heart. The goal of this analysis was to check if continuous exposure to PM<sub>2.5</sub> in the long term affects the heart's response to speed up or slow down. We computed a Chronic Exposure Index (CEI) by summing average PM<sub>2.5</sub> exposures over the workdays of each participant using previous work history, which we used in our analyses.

The underlying goal of this thesis is to inform and drive further research on particulate exposures and cardiovascular health especially in occupational health settings. It is my hope that the results of this research will create a framework for future regulation in this arena.

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## **CHAPTER 2**

# **SHORT-TERM METAL PARTICULATE EXPOSURES DECREASE CARDIAC ACCELERATION AND DECELERATION CAPACITIES IN WELDERS: A REPEATED-MEASURES PANEL STUDY**

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**Keywords:** Deceleration, Heart Rate, Welding, Electrocardiography, Occupational Exposure

**Word Count:** 3292

## ABSTRACT

**Background:** Studies have shown negative association between short-term particulate exposures ( $PM_{2.5}$ ) and Heart Rate Variability. Acceleration and Deceleration Capacities – different metrics of cardiac autonomic function – measure the variability of the heart rate during speeding up and slowing down of the heart respectively.

**Objective:** To investigate the associations between AC and DC with occupational short-term metal  $PM_{2.5}$  exposures.

**Methods:** A panel of 48 male welders had  $PM_{2.5}$  exposure measurements over 4-6 hours repeated over five sampling periods between January 2010 and June 2012. We simultaneously obtained continuous recordings of digital electrocardiograms (ECG) using a Holter monitor. We analyzed ECG data in the time domain to obtain the hourly AC and DC. Linear mixed models were used to assess the associations between hourly  $PM_{2.5}$  exposure and each of hourly AC and DC, controlling for age, smoking status, active smoking, exposure to second hand smoke, season/time of day when ECG reading was obtained, and baseline AC or DC. We also ran lagged exposure response models for each successive hour up to 3 hours after onset of exposure.

**Results:** Mean (SD) shift  $PM_{2.5}$  exposure during welding was 0.47 (0.43)  $mg/m^3$ . Negative exposure-response associations were found for AC and DC with increased  $PM_{2.5}$  exposure. In our adjusted models without any lag between exposure and response, a  $1mg/m^3$  increase of  $PM_{2.5}$  was associated with a decrease of 1.46 (95% CI: 1.00, 1.92) msec in AC and a decrease of 1.00 (95% CI: 0.53, 1.46) msec in DC. The effect of  $PM_{2.5}$  on AC and DC was maximal immediately post exposure and lasted 1 hour following exposure.

**Conclusion:** There are short-term effects of metal particulates on AC and DC.

## INTRODUCTION

Short and long term particulate exposures have been shown to have adverse effect on cardiovascular outcomes<sup>1-4</sup>. One of the mechanisms involved is through affectation of the autonomic nervous system, which has been measured traditionally using Heart Rate Variability (HRV)<sup>5-7</sup>. Air pollution studies have consistently shown a decrease in HRV with exposure to PM<sub>2.5</sub><sup>5, 8, 9</sup>. A decrease in HRV has also been linked to an increase in adverse cardiovascular outcomes<sup>10-13</sup>.

Whereas there is ubiquitous exposure to particulates in ambient air, occupational exposure to particulates is usually greater. For example, welders have been shown to have been exposed to about twenty four times the level of ambient PM<sub>2.5</sub> exposure levels<sup>14, 15</sup>. Furthermore, an exposure-related decrease in HRV has been demonstrated among these welders<sup>16</sup>.

Although previous research suggests that there is a negative exposure-response relationship between exposures to metal PM<sub>2.5</sub> and cardiac autonomic dysfunction, this was evaluated using HRV as the index of autonomic dysfunction, which has limitations. HRV fails to account for the heart rate, and is therefore prone to misclassification<sup>17</sup>. Current mechanistic research using HRV cannot clarify if a decrease in HRV means a decrease in accelerations and/or decrease in decelerations of the heart rate.

In 2006, Baeur described the phase-rectified signal averaging (PRSA) method for calculating the heart's acceleration capacity (AC) and deceleration capacity (DC), which are measures of the responsiveness of the heart, like HRV<sup>17, 18</sup>. These have the advantage over HRV of parsing the Holter data into accelerations and decelerations while also accounting for the heart rate; and have been demonstrated to be more predictive of morbidity and mortality among post-ischemic

coronary artery disease patients than traditional HRV<sup>17</sup>. Therefore, using sensitive indices - Acceleration Capacity and Deceleration Capacity - this study aims to investigate the potential for cardiac autonomic dysfunction from metal PM<sub>2.5</sub> exposure.

## **METHODS**

### **Subject Recruitment**

We recruited 72 male boilermakers during five sampling periods between January 2010 and June 2012 from the boilermaker union in Quincy, Massachusetts. These boilermakers were part of an ongoing “Harvard Boilermaker Cohort” initiated in 1999 to study the cardio-pulmonary effects of particulates<sup>19</sup>. The “Harvard Boilermakers’ Cohort” was a coalition of different smaller panel studies that were conducted at different sampling periods to answer specific research questions including the effect of secondhand smoke and metal particulates on cardio-pulmonary effects. Participants were mostly monitored on consecutive non-welding and welding days during each sampling period. Although we had recruited 72 boilermakers, we restricted our study to 52 participants who were only monitored on welding days. We were able to analyze data from 48 boilermakers for our study. They constituted 67% of the participants in the existing cohort whom we were able to obtain PM<sub>2.5</sub> exposure from during welding shift, as well as record continuous digital ECG recording during welding shifts recruited within sampling periods between 2010 and 2012. We were only able to record simultaneous PM<sub>2.5</sub> and digital ECG on non-welding days for 20 participants, poor quality ECG data in 2 participants, and failed ECG data retrieval in 2 other participants. We conducted our study during winter or summer when 75% of the study participants had not actively welded two weeks prior to our data collection. The Institutional Review Board at the Harvard T. H. Chan School of Public Health approved the study protocol, and informed consent was obtained from each study participant.

## **Data Collection**

We collected continuous PM<sub>2.5</sub> exposure and continuous ECG data of study participants at a union welding school. The welding school was designed for training apprentices and had booths where boilermakers practiced welding (mainly stick- and gas- metal arc welding), cutting and grinding.

We also collected medical history and medication use information, demographics, lifestyle information including smoking, typical diet, and occupational history using self-administered questionnaire. Participants were asked to report any of the following heart and blood vessel problems, diagnosed by a physician: hypertension, use of blood pressure medications such as beta blockers or ACE inhibitors, congestive heart failure, myocardial infarction, angina, arrhythmia, heart/chest surgery, or otherwise non-classified heart problems - not diagnosed by a physician.

## **PM<sub>2.5</sub> Assessment**

We measured PM<sub>2.5</sub> concentrations during welding shifts of study participants using personal DustTrak<sup>TM</sup> Aerosol Monitor (TSI, Inc., St. Paul, MN). The DustTrak<sup>TM</sup> monitor was strapped to the participant's shoulder close to their breathing zone. DustTrak<sup>TM</sup> has a PM<sub>2.5</sub> inlet impactor to measure continuously and record at 1-minute intervals average concentrations of fine particulates during the welding shifts. The continuous DustTrak<sup>TM</sup> readings of PM<sub>2.5</sub> had been validated compared to gravimetric methods in welders<sup>20</sup>. We calculated mean hourly concentrations of PM<sub>2.5</sub> exposure during each work shift for each participant.

## **ECG recording and processing**

Study participants wore a standard five lead ECG Holter monitor after a thirty minute rest period in the morning on arrival at the union hall. The rest period was allowed for so that we could record their unbiased resting ECG free from acute changes resulting from commuting to the study site. To ensure that the leads of the ECG were well secured and remained secured on the chest of participants, we shaved their skin if necessary, cleaned with an alcohol wipe after slightly abrading the skin, and research staff checked them intermittently<sup>5</sup>. The participants had this monitor worn throughout the welding shift. The digital recordings were then downloaded and sent to the Cardiovascular Epidemiology Research Unit (CVERU) of Beth Israel Deaconess Medical Center (Boston, MA) for processing and analysis. Holter recordings were uploaded into the GE MARS ECG analysis system, which automatically scans recordings for areas of noise and groups heartbeats as normal or arrhythmic. Trained technicians blinded to the exposure status of the participant from whom the ECG reading was obtained verified the automated scans as correct or changed them to the appropriate designation. The data were then exported for analysis using the Physionet toolkit<sup>21</sup>. To remove artifacts from the data, they used only beats with an RR interval within 5% difference of adjacent beats. They used an automated process described by Bauer<sup>17</sup> to create 5-minute segments with anchors for the Phase-Rectified Signal Averaging (PRSA) method of computing the acceleration capacity (AC) and deceleration capacity (DC). In brief, to compute the DC, this involves identifying heartbeat intervals longer than the preceding interval as anchors (for AC, beats shorter than preceding beats were anchors). Overlapping segments of interval data were then automatically generated from the ECG such that all segments are aligned at the anchors in the center and averaged. The PRSA method then quantifies the signals within aligned segments using the Haar wavelet analysis with a scale of 2

by a computer processing of the ECG with visual and digital outputs. Thus, AC and DC were calculated separately as a quarter of the difference between two sums, that is, the sum of the averaged anchor points RR intervals ( $X_0$ ) with the succeeding RR intervals ( $X_1$ ) and the sum of the two averaged RR intervals preceding anchor points ( $X_{-2}$ ,  $X_{-1}$ )<sup>18</sup>.

### **Acceleration Capacity (AC) and Deceleration Capacity (DC)**

Using the digital ECG data in the time domain, we computed the average AC and average DC for each simultaneous hour of PM<sub>2.5</sub> exposure and monitoring by taking the mean of the twelve adjacent 5-minute segments of the ECG within each hour of the day using the automated output.

### **Data Analysis**

We calculated summary measures of potential covariates, and percentiles of our exposure and outcome to further understand their distribution. Potential covariates that we considered include: age, race, smoking status, actively smoking during work and second hand smoke exposure, time of day when ECG was obtained, season of study, previous weld exposure (last weld day), chronic effects of welding (years of boilermaker), presence of heart problems, and baseline cardiac autonomic function (baseline AC or DC for AC and DC models respectively). We then explored the inter-relationships between them by using spearman's correlations for continuous variables and t-tests for binary variables. In order not to have missed any inter-relationships between potential covariates with our exposure/outcome, we used  $\alpha=0.10$  level for these correlations and t-tests between covariates and outcome/exposure.

For our model without any lag between exposure and response, we used linear mixed models to assess the associations between hourly PM<sub>2.5</sub> as a continuous measure and simultaneously measured hourly AC, and a separate model for PM<sub>2.5</sub> and DC. Furthermore we used a backward method of model selection using a p-value of 0.2 each for staying in the model to select our final model. We compared models using the log likelihood ratio test. We considered controlling for age, smoking status, last weld day (unmeasured acute weld exposure), number of years as a boilermaker (chronic effects), baseline AC or DC (for AC or DC models respectively), and time of day and season when ECG reading was obtained. Our final model included age, baseline AC or DC, smoking status, active smoking, second hand smoke exposure, time of day and season of ECG. For the lagged models, we used linear mixed models to assess the associations between hourly PM<sub>2.5</sub> and lagged hourly AC or DC. Statistical significance was assessed at  $\alpha=0.10$  level in two sided tests for our final model. All analyses were performed using PROC MIXED in SAS version 9.4 (Cary, NC).

## RESULTS

We successfully collected 892 person-hours of weld day PM<sub>2.5</sub> and 1392 person-hours of weld day ECG from 48 participants, all males with a mean age of 40 years and had been boilermakers for a median of 4 years (range 0.25 – 21 years). The study population included 19 (40%) smokers (Table 2.1). Five (5) of these 48 participants reported heart problems and possible heart-related problems. Of these 5, 1 reported arrhythmia, 1 reported sinus (sinoatrial node) problems, 1 reported angina in the past year, 1 reported mitral valve prolapse with murmur, and 1 reported hypertension for which he was taking a beta-blocker. No other participant reported the use of beta-blockers or ACE inhibitor drug use. Less than half (38%) of the baseline ECG were

taken in the morning, and each participant had PM<sub>2.5</sub> measurements taken in 2-5 typical work shifts of 4 – 6 hours.

Table 2.1: Demographics and characteristics for the 48 study participants.

<i><b>Individual Characteristics</b></i>	<u><i><b>N</b></i></u>	<u><i><b>%</b></i></u>
Male	48	100
Caucasian	42	88
<sup>1</sup> Smoking status	19	40
<i><b>Other Characteristics</b></i>		
<sup>2</sup> Time of ECG	18	38
<sup>3</sup> Season of ECG	33	69
<sup>4</sup> Heart problems	5	10
	<u>Mean</u>	<u>s.d.</u>
Age at start of study 2010 (years)	40	12
No. of years as a BM (years)	9	9
Last Weld Day before study (days)	44	51

<sup>1</sup>Current Smokers vs Non-smokers and Previous smokers

<sup>2</sup> Morning (AM) vs Afternoon(PM)

<sup>3</sup>Winter vs Summer

<sup>4</sup> Heart problems include reported previous history of arrhythmia, cardiac sinus problems, or palpitations

In contrast to the age at the start of study (2010), the mean number of years as a boilermaker was 9 years (range 1 – 35 years). The mean PM<sub>2.5</sub> during the work shift for participants measured was 0.47mg/m<sup>3</sup> (range 0.01 – 1.40 mg/m<sup>3</sup>). The mean (range) baseline AC was -7.1msec (-2.1 to -

22.6) on the negative scale with the middle half of the participants ranging between -4.5 to -9.1msec (Table 2.2).

Table 2.2 – Baseline and Hourly levels of metal PM<sub>2.5</sub> (mg/m<sup>3</sup>), Acceleration (AC) and Deceleration (DC) Capacities of the heart (msec).

	Baseline Mean (SD)	<sup>1</sup> Hourly Average Mean (SD)	Hourly Average minus Baseline Mean	p-value
<b><i>Exposure</i></b>				
<sup>2</sup> PM <sub>2.5</sub> (mg/m <sup>3</sup> )	0.04 (0.3)	0.50 (0.4)	0.46	<b>&lt;0.01</b>
<b><i>Outcome</i></b>				
<sup>3</sup> AC(msec)	-7.1 (3.8)	-6.3 (3.1)	0.8	0.25
DC(msec)	8.8 (3.4)	7.8 (3.8)	-1.0	0.17

Bold values indicate significant associations (p<0.05)

<sup>1</sup> This represents the mean of the hourly averages (hour 1, 2, and 3) from the onset of welding.

<sup>2</sup> PM<sub>2.5</sub> ambient levels before shift and average hourly levels during work shift are reported as baseline and hourly average respectively.

<sup>3</sup> Acceleration Capacity is measured on a negative scale. N=48 participants

The mean (range) baseline DC was 8.8msec (3.3 to 22.4) on the positive scale with the middle half of the participants ranging 6.6 to 10.5msec.

Spearman's correlations coefficients (and p-value) for correlation between potential covariates and PM<sub>2.5</sub> or baseline measures of AC and DC were mostly statistically non-significant.

However, season of the year and active smoking were correlated with AC and/or DC, but not with PM<sub>2.5</sub>. Among the other covariates considered, age was neither correlated with PM<sub>2.5</sub>

exposure nor with the baseline AC or DC. The number of years as a boilermaker and last weld day were only moderately correlated with PM<sub>2.5</sub> exposure but not with baseline AC or DC. There were no differences in categories of other covariates in terms of measures of shift PM<sub>2.5</sub>, baseline AC and DC.

The linear mixed models analyses for our model without any lag revealed that PM<sub>2.5</sub> levels were associated with a decrease in AC and DC after adjusting for age, smoking status, active smoking, second hand smoke exposure, and baseline AC or DC (Tables 2.3 and 2.4).

Table 2.3: Main effect of PM<sub>2.5</sub> (mg/m<sup>3</sup>) on acceleration capacity (msec) and deceleration capacity (msec) without adjustment for hourly pre-exposures from the linear mixed effect response-lagged models with 48 participants.

Parameter	<sup>1</sup> <i>Acceleration Capacity(AC)</i> (95% Confidence Interval)	<i>Deceleration Capacity(DC)</i> (95% Confidence Interval)
<i>Models</i>		
No lag	<b>1.46 (1.00, 1.92)</b>	<b>-1.00 (-0.53, -1.46)</b>
1 hour lag	<b>0.73 (0.26, 1.20)</b>	<b>-0.40 (0.08, -0.87)</b>
2 hour lag	-0.29 (-0.83, 0.24)	0.65 (1.22, 0.07)
3 hour lag	-0.34 (-0.92, 0.25)	0.52 (1.15, -0.12)

<sup>1</sup>Acceleration Capacity is measured on a negative scale, therefore positive estimates connote a decrease in AC on a negative scale.

Bold values indicate significant associations (**p<0.1**). Models are adjusted for age, smoker status, actively smoking ,second hand smoke exposure, time of day and season of ECG reading, and baseline AC or DC.

Table 2.4: Main effect of PM<sub>2.5</sub> (mg/m<sup>3</sup>) on acceleration capacity (msec) and deceleration capacity (msec) with adjustment for hourly pre-exposures from the linear mixed effect response-lagged models with 48 participants.

Parameter	<sup>1</sup> <i>Acceleration Capacity(AC)</i> (95% Confidence Interval)	<i>Deceleration Capacity(DC)</i> (95% Confidence Interval)
<i>Models</i>		
No lag	<b>1.46 (1.00, 1.92)</b>	<b>-1.00 (-0.53, -1.46)</b>
1 hour lag	<b>0.81 (0.30, 1.32)</b>	<b>-0.70 (-0.14, -1.26)</b>
2 hour lag	<b>1.00 (0.13, 1.87)</b>	-0.45 (0.52, -1.42)
3 hour lag	0.41 (-1.65, 2.46)	-0.12 (2.48, -2.71)

<sup>1</sup> Acceleration Capacity is measured on a negative scale, therefore positive estimates connote a decrease in AC on a negative scale.

Bold values indicate significant associations (p<0.1). Models are adjusted for age, smoking status, actively smoking ,second hand smoke exposure, time of day and season of ECG reading, and baseline AC or DC.

When we introduced lags by the hour between PM<sub>2.5</sub> exposure and AC or DC, there were associations between metal PM<sub>2.5</sub> exposure and lagged responses in AC and DC at 1 hour post exposure. These were consistent with or without adjustment for PM<sub>2.5</sub> exposure in the previous hour(s).

## DISCUSSION

The goal of this study was to investigate the associations between acceleration capacity and deceleration capacity with acute occupational PM<sub>2.5</sub> exposures. Consistent with our hypothesis, occupational PM<sub>2.5</sub> exposure was associated with acceleration capacity and deceleration capacity. We found significant negative exposure–response relationships between short-term PM<sub>2.5</sub> exposure with acceleration capacity and deceleration capacity. These associations were qualitatively consistent with or without adjustment for PM<sub>2.5</sub> exposure in the previous hour(s). There may therefore be a reduction in the capacity of the heart to accelerate and decelerate over time with acute insults from these exposures. These data imply that there may be changes in parasympathetic control, sympathetic modulation and/or non-autonomic control of the heart with acute exposure to particulates. Few studies have documented associations between particulate exposures and heart rate variability in the short term<sup>2, 5, 7, 9, 22</sup>. Yet fewer studies have observed associations between short-term fine particulate exposure and deceleration capacity<sup>23</sup>. This study is the first to our knowledge to demonstrate declines in acceleration capacity following acute occupational particulate exposures among healthy welders.

We cannot directly compare our study results with those of prior studies due to the differences in exposure characteristics and study population. We did find declines in deceleration capacity slightly greater than declines reported following short-term PM<sub>2.5</sub> exposures among post ischemic patients<sup>23</sup>. In addition, we observed declines in acceleration capacity among non-ischemic welders which has never been reported. This result highlights the possibility of differences in effect among our study population (active healthy welders) compared to other studies (post myocardial infarction patients) that were mostly conducted in clinical settings. Furthermore, the different constituents of the particulate exposures may have varying effects on

the electrical activity of the heart. While organic and elemental carbons have been implicated as suspects, metals have also been shown to play a role<sup>6, 14, 23, 24</sup>. Welders are exposed to metal-rich fumes, and this may be responsible for the effects on acceleration capacity we found in this study.

Only five of the study participants reported known or possible heart problems, with one reporting angina, myocardial infarction or other symptoms of ischemia. There was no difference in quality of results in the sensitivity analyses that excluded these five participants. This suggests that the effects of particulates in ischemic and non-ischemic conditions are qualitatively similar.

Acceleration capacity is a measure of the responsiveness of the heart to speed up when stimulated, and it is known to be under both autonomic (sympathetic) and non-autonomic control<sup>11, 12</sup>. Deceleration capacity on the other hand, describes the behavior of the heart when the heart rate is slowing, and it reflects a measure of parasympathetic modulation of the heart<sup>23</sup>. We found significant effects with both the acceleration capacity and deceleration capacity, as well as lagged responses between PM<sub>2.5</sub> and deceleration capacity. We would therefore hypothesize that during acute exposure to particulates, there is a blunting of the autonomic response of the heart to the activation of both parasympathetic and sympathetic nervous system influences to regulate the response of the heart, as well as the interplay of non-autonomic control (cytokines and inflammatory mediators) which may be playing a major role in the lagged responses of AC and DC to exposure of metal particulates. We found the effects on AC and DC lasted up to one to two hours post-exposure. We doubt the sustenance of autonomic effects up to this time, and we would hypothesize that non-autonomic influences may begin to play a role at this time. If this hypothesis is correct, then our results are not completely consistent with previous knowledge that AC is influenced by both autonomic and inflammatory control, and that

DC which is mainly under autonomic control<sup>17</sup>, since we found that sustained effects of PM<sub>2.5</sub> exposure affect both AC and DC. This discrepancy may be explained by AC and DC being antagonistically opposed to each other. There may be greater sensitivity on AC by acute insults as the effect on AC remains until two hours following the insult.

Deceleration capacity has been shown to be predictor of mortality following acute myocardial infarction, even among patients with preserved acceleration capacity, as there is a rapid decline in the parasympathetic innervation of the heart following ischemia<sup>17, 25</sup>. We did observe declines in the DC among these healthy welders as only one of them had had an ischemic event within the last year of the study. However, the clinical usefulness of a decline in the deceleration capacity of the heart of healthy persons is unknown. Even more so, the significance of declines in acceleration capacity in both ischemic/non-ischemic hearts is uncertain. This may be due to the multi-factorial influences on the acceleration capacity. Therefore, whereas deceleration capacity specifically measures vagal output to the heart, sole sympathetic innervation to the heart cannot be captured by acceleration capacity directly.

Short-term metal particulates have effect on the AC and DC lasting one hour post exposure. This is similar to the early phase of the multiphasic response of HRV to particulates<sup>8</sup>. However, this result was not sustained up to three hours post exposure. This may be as a result of misclassification of HRV with respect to accelerations and decelerations. Furthermore, there was no difference between mean hourly AC and DC compared to baseline. This may be the result of averaging over few hours after PM<sub>2.5</sub> exposure ceases to have effect on AC and DC. Although, there is still a difference between mean hourly PM<sub>2.5</sub> exposure levels and baseline, there is no concurrent significant difference in AC and DC. This result suggests that the heart may be adapting its response to the continuous exposure of fine particulate. This is further

supported by the intermediate decline in AC at the ‘one hour lag’ compared to the ‘no lag’ and ‘two hour lag’ indicating some attempt of the heart at recovery (Table 2.4).

Based on our study results, reducing exposure would be a major goal of health promotion for these workers. The use of personal protective equipment such as a respirator has been shown to reduce exposures to particulates and improve heart rate variability<sup>26</sup>. Our study has its strengths and limitations. Importantly, we captured ECG tracings digitally that were parsed into phases of accelerations and decelerations accounting for differences in heart rate. Our results are therefore not confounded by heart rate. We also adjusted for confounding by potential confounders. We used repeated continuous shift PM<sub>2.5</sub> measured using DustTrak<sup>TM</sup> Aerosol Monitor. We were also able to account for previous welding exposure by restricting most of our study population to those who had not welded two weeks prior to our study, and considered adjusting for prior uncaptured PM<sub>2.5</sub> exposure using ‘last weld day’ in our models. PM<sub>2.5</sub> exposure measurements were obtained in comparable work settings at the union hall and may reflect what their typical exposures would have been if they were followed up at their work places, which would be challenging to do. There is a potential for selection bias in our study as we were only able to retrieve weld day data for 67% of our participants. However, there were no differences in baseline characteristics of the welders whom we were not able to retrieve their records and those in our study. We also followed up study participants for only 3 hours following onset of exposure. In conclusion, we found that short-term particulate exposure resulted in an immediate decrease in acceleration capacity and deceleration capacity of the heart lasting up to one hour post exposure in an occupational population.

These results suggest that there may be more pathways that sustain the acute cardiac response following metallic PM<sub>2.5</sub> exposure other than a direct autonomic effect of particulates on AC and DC<sup>15, 27</sup>.

## **ACKNOWLEDGEMENTS**

This research was supported by the National Institute of Environmental Health Sciences (NIEHS) grant numbers R01ES009860, P50ES00002, ES009860 and ES00002. We thank the participants and the leadership of Local 29 of the International Brotherhood of Boilermakers, Iron Ship Builders, Blacksmiths, Forgers and Helpers in Quincy, MA. We also thank Georg Schmidt for his expert contribution.

## **COMPETING INTERESTS**

The authors declare they have no actual or potential competing financial interests.

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## **CHAPTER 3**

# ARE THE ASSOCIATIONS OF CARDIAC ACCELERATION AND DECELERATION CAPACITIES WITH FINE METAL PARTICULATE IN WELDERS MEDIATED BY INFLAMMATION?

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**Keywords:** Deceleration, Heart Rate, Welding, Electrocardiography, Occupational Exposure  
**Word Count:** 3945

## ABSTRACT

**Background:** Whereas Heart Rate Variability (HRV) measures the variability of the heart rate during a time period including when heart rate is static, accelerating or decelerating; Acceleration Capacity (AC) and Deceleration Capacity (DC) measure the variability during speeding up and slowing down of the heart rate respectively. Heart Rate Variability (HRV) as a dependent variable in cardiovascular research has been critiqued due to its inability to account for changes in the heart rate and its potential of misclassification. Previous research has shown negative associations between short-term particulate exposures ( $PM_{2.5}$ ) and indices of autonomic dysfunction (HRV and DC), both in the general population and among occupational groups. Fewer studies have documented a potential for an inflammation-mediated pathway for effect of particulate on HRV. However, it is unclear if exposure to particulate resulting in a decrease in both accelerations and decelerations of the heart rate is mediated by inflammation.

**Objective:** To investigate whether the associations between AC and DC with occupational short-term metal  $PM_{2.5}$  exposures are mediated by inflammation using CRP, IL-6, IL-8 and IL-10.

**Methods:** A panel of forty five (45) male boilermaker welders, mean age 39 years, had measurements of continuous  $PM_{2.5}$  exposure measured during typical welding work shifts for 4-6 hours repeated over 1-3 sampling periods between January 2010 and June 2012. We also simultaneously obtained paired (baseline and post-shift) blood samples for measurements of CRP, IL-6, 8 and 10, and digital electrocardiograms (ECG) using a Holter monitor, repeated over the same sampling periods. We analyzed ECG data in the time domain to obtain the baseline and post-shift AC and DC. Mediation analysis was done using linear mixed models to assess the

associations between shift PM<sub>2.5</sub> exposure, potential mediator post shift levels, and post-shift AC and DC, controlling for baseline mediator levels, AC and DC, age, active smoking, exposure to secondhand smoke, season and time of day when ECG reading was obtained, in order to deduce the direct and indirect effects (via the mediator) of PM<sub>2.5</sub> on AC and DC.

**Results:** Mean (SD) shift PM<sub>2.5</sub> exposure during welding was 0.35 (0.4) mg/m<sup>3</sup> and ranged from 0.01 – 2.96 mg/m<sup>3</sup>. Negative exposure-response association was found for AC and DC (total effect) with increased shift PM<sub>2.5</sub> exposure after adjusting for age, active smoking, exposure to secondhand smoke, season and time of day when ECG reading was obtained, and baseline AC and DC. In our mediation models, the proportion of the total effect of PM<sub>2.5</sub> on AC or DC (indirect effect) mediated through IL-6 on AC was at best 4%. Controlling for IL-6 (direct effect), a 1mg/m<sup>3</sup> increase of PM<sub>2.5</sub> was associated with a decrease of 2.16 (95% CI: -0.36, 4.69) msec in AC and a decrease of 2.51 (95% CI: -0.90, 5.93) msec in DC. There was however no effect of PM<sub>2.5</sub> on any of the CRP, IL-6, IL-8 or IL-10 post-shift changes.

**Conclusion:** Although IL-6 showed a potential of mediating the effect of metal particulates on AC, we cannot conclude that the effect of metal particulates are mediated by any of CRP, IL-6, IL-8 or IL-10. This finding suggests the need to evaluate more complex pathways in a larger study for the potential for inflammation-mediated pathway of metal particulates on AC and DC.

## INTRODUCTION

Welders have increased risk of adverse cardiovascular outcomes from short and long term particulate exposures(1-6). There is evidence that welders have an increased risk of morbidity and mortality from ischemic heart disease among welders(7-9).

The mechanism(s) accounting for increased risk of cardiovascular diseases especially ischemic heart disease among welders remains unclear. Several mechanisms describing alterations at cellular and tissue levels have been proposed to account for the effect of fine particulates in increasing the risk of cardiovascular events in welders. These include pulmonary inflammation(5) that spreads to secondarily involve the heart(10, 11) , alterations of the autonomic nervous system that lead to changes in heart rate and heart rate variability indices(11, 12) , and direct affectation of the heart and nerves from translocation of inhaled particles either in their whole or soluble forms.

One of the key mechanisms proposed in these adverse cardiovascular system effects is through affectation of the autonomic nervous system, which has been measured traditionally using Heart Rate Variability (HRV)(10, 13-16). Air pollution studies have consistently shown a decrease in HRV with exposure to PM<sub>2.5</sub> in both occupational groups and in the general population(7, 13, 14). A decrease in HRV has also been linked to an increase in adverse cardiovascular outcomes(15-18). However, studies using HRV as an index of the autonomic activity of the heart have limitations arising from the heterogeneity of HRV during accelerations, static period of heart rate, and decelerations.

In 2006, Baeur described the phase-rectified signal averaging (PRSA) method for calculating the heart's acceleration capacity (AC) and deceleration capacity (DC), which are measures of the

responsiveness of the heart, like HRV(11, 12). These have the advantage over HRV of parsing the Holter data into accelerations and decelerations while also accounting for the heart rate; and have been demonstrated to be more predictive of morbidity and mortality among post-ischemic coronary artery disease patients than traditional HRV(11).

Importantly, local and systemic inflammation has been investigated as a possible intermediate step from exposure to fine particulate. Studies have mixed conclusions about the impact of the resulting systemic inflammation on adverse cardiovascular outcomes, and the inter-relationships between particulate exposure, inflammation and alteration in autonomic signals to the heart(5, 19, 20). Thus, it is unclear if the association between fine particulate matter and recent cardiac autonomic dysfunction indices (AC, DC) are mediated by inflammation measured using mediators such as interleukins, adhesion molecules, and cytokines.

Therefore, using sensitive indices - Acceleration Capacity and Deceleration Capacity - this study aims to investigate the pathway linking metal PM<sub>2.5</sub> exposure and cardiac autonomic dysfunction, by exploring markers of inflammation as potential mediators. That is, this study will investigate whether the associations between acute occupational PM<sub>2.5</sub> exposures and each of acceleration capacity and deceleration capacity are mediated by any of CRP, IL-6, IL-8, and IL-10. In addition, we will explore the strengths of the direct (nervous) and indirect (inflammatory) pathways linking metal PM<sub>2.5</sub> exposure and cardiac autonomic dysfunction.

## **METHODS**

### ***Subject Recruitment***

We recruited 72 male boilermakers during sampling periods between January 2010 and June 2012 from the boilermaker union in Quincy, Massachusetts. These boilermakers were part of an ongoing “Harvard Boilermaker Cohort” initiated in 1999 to study the cardio-pulmonary effects

of particulates(21). We were able to analyze data from 45 boilermakers for our study who had complete data. They constituted 63% of the participants in the existing cohort whom we were able to obtain PM<sub>2.5</sub> exposure from during welding shift, as well as record baseline and post-shift blood samples for markers and digital ECG on welding days between 2010 and 2012. Although we had recruited 72 boilermakers in total, we restricted our study to the 52 participants who were monitored on welding days. We were only able to record simultaneous PM<sub>2.5</sub> and digital ECG on non-welding days for 20 participants, and we had failed initialization and data retrieval in 2 participants. We failed to obtain paired blood samples or lost results due to error in analysis from 5 participants; therefore our final study had 45 participants. We conducted our study at off-peak seasons for the boilermakers, which are during winter or summer when 85% of the study participants had not actively welded two weeks prior to our data collection. The Institutional Review Board at the Harvard T. H. Chan School of Public Health approved the study protocol, and informed consent was obtained from each study participant.

### ***Data Collection***

We collected PM<sub>2.5</sub> exposure, blood samples and continuous ECG data of study participants at a union welding school. The welding school was designed for training apprentices and had booths where boilermakers practiced welding (mainly stick- and gas- metal arc welding), cutting and grinding.

We also collected medical history and medication use information, demographics, lifestyle information including smoking, typical diet, and occupational history using self-administered questionnaire. Participants were asked to report any of the following heart and blood vessel problems, diagnosed by a physician: hypertension, use of blood pressure medications such as

beta blockers or ACE inhibitors, congestive heart failure, myocardial infarction, angina, arrhythmia, heart/chest surgery, or otherwise non-classified heart problems.

### ***PM<sub>2.5</sub> Assessment***

We measured PM<sub>2.5</sub> concentrations during welding shifts of study participants using personal DustTrak<sup>TM</sup> Aerosol Monitor (TSI, Inc., St. Paul, MN). The DustTrak<sup>TM</sup> monitor was strapped to the participant's shoulder close to their breathing zone. DustTrak<sup>TM</sup> has a PM<sub>2.5</sub> inlet impactor to measure continuously and record at 1-minute intervals average concentrations of fine particulates during the welding shifts. The continuous DustTrak<sup>TM</sup> readings of PM<sub>2.5</sub> had been validated compared to gravimetric methods in welders(22). We calculated mean shift concentrations of PM<sub>2.5</sub> exposure during each sampling period for each participant. We also kept a work log of tasks and exposure to second hand smoke for each participant during each shift.

### ***Potential Mediators' (C-Reactive Protein, and Interleukins 6, 8 and 10) Assay***

We obtained blood samples from each participant by venous puncture. We collected morning baseline samples after overnight fast. We collected blood samples from each draw into serum separator tubes. We then centrifuged the tubes containing the blood samples immediately, and aliquoted their plasma into cryogenic storage tubes. These storage tubes with plasma were kept on dry ice until transport back to the laboratory at the Harvard School of Public Health where they were stored in a freezer maintained at  $-80^{\circ}\text{C}$  till when they were analyzed. Three to six months later, serum samples for each participant were thawed and analyzed in duplicates. Quantification of marker levels were conducted at our laboratory using the enzyme-linked immunosorbent assay (ELISA) method with commercially available kits (R&D Systems Incorporated Minneapolis, Minnesota, USA). For each duplicate sample, any sample with a

coefficient of variation (CV) greater than 20% required that all samples from that participant were reanalyzed.

The performance of the assays was monitored with internal control standards supplied by the manufacturers of the ELISA kit. In addition, plasma was pooled to form a quality control (QC) sample that was assayed with each batch of samples. Ten percent (10%) of each batch of study samples was comprised of blinded pooled QC samples(5). The values from the pooled QC samples were used to determine the intra- and inter-assay CV. The intra- and inter-assay CV's were all less than 20%, indicating satisfactory reproducibility of the assays.

### ***ECG recording and processing***

Study participants wore a standard five lead ECG Holter monitor after a thirty minute rest period in the morning on arrival at the union hall. The rest period allowed us record their unbiased baseline ECG for ten minutes free from acute changes resulting from commuting to the study site. To ensure that the leads of the ECG were well secured and remained secured on the chest of participants, we shaved their skin if necessary, cleaned with an alcohol wipe after slightly abrading the skin, and research staff checked them intermittently. The participants had this monitor worn throughout the welding shift and up to 1-2 hours after the shift. However, we used the ten-minute recording immediately after the work shift as their post-shift ECG. The digital recordings were then downloaded and sent to the Cardiovascular Epidemiology Research Unit (CVERU) of Beth Israel Deaconess Medical Center (Boston, MA) for processing and analysis. Holter recordings were uploaded into the GE MARS ECG analysis system, which automatically scans recordings for areas of noise and groups heartbeats as normal or arrhythmic. Trained technicians blinded to the exposure status of the participant from whom the ECG reading was obtained verified the automated scans as correct or changed them to the appropriate designation.

The data were then exported for analysis using the Physionet toolkit(23, 24). To remove artifacts from the data, they used only beats with an RR interval within 5% difference of adjacent beats. They used an automated process described by Bauer to create 5-minute segments with anchors for the Phase-Rectified Signal Averaging (PRSA) method of computing the acceleration capacity (AC) and deceleration capacity (DC)(11). In brief, to compute the DC, this involves identifying heartbeat intervals longer than the preceding interval as anchors (for AC, beats shorter than preceding beats were anchors). Overlapping segments of interval data were then automatically generated from the ECG such that all segments are aligned at the anchors in the center and averaged. The PRSA method then quantifies the signals within aligned segments using the Haar wavelet analysis with a scale of 2 by a computer processing of the ECG with visual and digital outputs. Thus, AC and DC were calculated separately as a quarter of the difference between two sums, that is, the sum of the averaged anchor points RR intervals ( $X_0$ ) with the succeeding RR intervals ( $X_1$ ) and the sum of the two averaged RR intervals preceding anchor points ( $X_{-2}$ ,  $X_{-1}$ ) .

### ***Acceleration Capacity (AC) and Deceleration Capacity (DC)***

Using the digital ECG data in the time domain, we computed the baseline and post-shift AC and DC for the each workshift of PM<sub>2.5</sub> exposure by taking the mean of the adjacent 5-minute segments of the ECG just before and immediately after the workshift respectively using the automated output.

### ***Data Analysis***

We calculated summary measures of potential covariates, and percentiles of our exposure and outcome to further understand their distribution. We observed that the distribution of our mediators CRP, IL-6, IL-8, and IL-10 were lognormal. Therefore, we transformed their values by

using the natural logarithm in all our analyses. Based on our previous work using this study population, we had planned *a priori* to adjust for age, active smoking status, secondhand smoke exposure, time of day when ECG was obtained, season of study, previous weld exposure (last weld day), presence of heart problems, and baseline cardiac autonomic function (baseline AC or DC for AC and DC models respectively). These were the relevant covariates considered in the study of the acute association of PM<sub>2.5</sub> exposure on AC and DC using lagged hourly models.

For our analyses, we first obtained the total effect of workshift PM<sub>2.5</sub> exposure on AC or DC by running linear mixed models controlling for baseline AC or DC, age, active smoking, secondhand smoke exposure, season and time of day when ECG reading was obtained. Next, mediation analyses were carried out in three stages using linear mixed models to assess the associations between shift PM<sub>2.5</sub> exposure, potential mediator post shift levels, and post-shift AC and DC, controlling for baseline mediator levels, AC and DC, age, active smoking, secondhand smoke exposure, season and time of day when ECG reading was obtained, to deduce the direct and indirect effects (via the mediator) of PM<sub>2.5</sub> on AC and DC. First, we ran linear mixed models to assess the associations between workshift PM<sub>2.5</sub> as a continuous measure and shift changes in AC or DC controlling for baseline mediator levels, AC or DC, age, active smoking, secondhand smoke exposure, season and time of day when ECG reading was obtained to estimate the direct effect (Figure 3.1). Then, we ran separate linear mixed models for association between PM<sub>2.5</sub> exposure and each mediator adjusting for baseline mediator levels (path a), and separate models for associations between mediators (CRP, IL-6, IL-8, and IL-10) and either of AC or DC (path b).

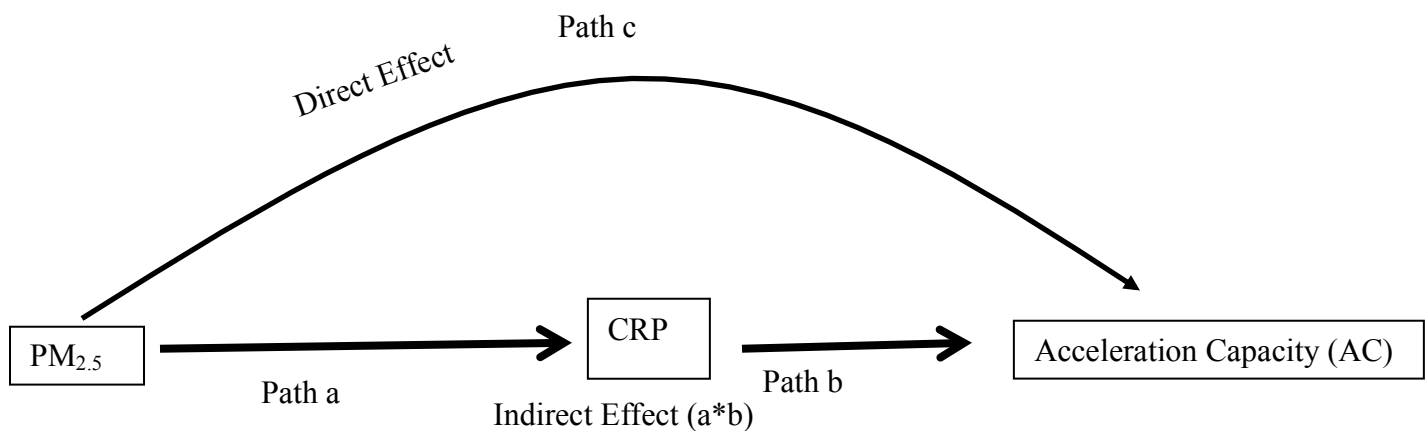


Figure 3.1: Mediational Analyses showing associations between PM<sub>2.5</sub> exposure, mediator variable (CRP, IL-6, IL-8, or IL-10), and dependent variable (AC or DC).

Finally, to estimate the indirect effect (path  $a*b$ ), we estimated point estimates by multiplying point estimates from models for path a and path b. Standard errors for the indirect path were calculated using the Sobel's criteria(25) which involved calculating the standard error of the indirect path by using the square root of the sum of the product of the squares of the “crossed” point estimates and standard error for paths a and b:  $[\text{Sqrt}(a^2 s_b^2 + b^2 s_a^2)](25)$ . Confidence intervals of the indirect effect were now calculated from the estimated standard error. We calculated the proportion mediated by dividing the strength (point estimates) of indirect effect by the sum of the direct and indirect effects. Statistical significance was assessed at  $\alpha=0.10$  level in two sided tests for our final model. All analyses were performed using PROC MIXED in SAS version 9.4 (Cary, NC).

## RESULTS

We successfully collected 83 person-shifts of weld day PM<sub>2.5</sub>, 83 paired (baseline and post-shift) person-shifts of weld day ECG, and 133 person-shifts of paired (baseline and post-shift) blood assay for CRP, IL-6, IL-8 and IL-10 from 45 participants over the three sampling periods, who were all males with a mean age of 40 years. The study population included 42 (93%) Caucasians and 19 (42%) smokers (Table 3.1).

Table 3.1: Demographics and characteristics for 45 study participants.

<b><i>Individual Characteristics</i></b>	<b><i>N</i></b>	<b><i>%</i></b>
Male	45	100
Caucasian	42	93
<sup>1</sup> Smokers	19	42
<b><i>Other Characteristics</i></b>		
<sup>2</sup> Time of ECG (AM)	18	40
<sup>3</sup> Season of ECG (winter)	33	73
<sup>4</sup> Heart problems	2	4
	<b><u>Mean</u></b>	<b><u>s.d.</u></b>
Age at start of study 2010 (years)	40	12
Last Weld Day before study (days)	44	51

<sup>1</sup>Current Smokers vs Non-smokers and Previous smokers

<sup>2</sup>Morning (AM) vs Afternoon(PM)

<sup>3</sup>Winter vs Summer

<sup>4</sup>Heart problems include reported previous history of arrhythmia, cardiac sinus problems, or palpitations

One (1) of these 45 participants reported a history of palpitations and one (1) reported prior angina. No participant reported the use of beta-blockers or ACE inhibitor drug use. Less than

half (40%) of the baseline ECG and blood samples for mediator assay were taken in the morning, and each participant had PM<sub>2.5</sub> measurements taken in 1-3 typical work shifts of 4 – 6 hours.

The mean PM<sub>2.5</sub> for all participants were 0.04mg/ m<sup>3</sup> (range 0.00 – 1.43 mg/m<sup>3</sup>) prior to their work shift (personal ambient levels), and 0.35mg/m<sup>3</sup> (range 0.01 – 2.96 mg/m<sup>3</sup>) during the work shift. The mean (range) AC was -7.3msec (-0.8 to -18.6) at baseline whereas it was -5.5msec (0.1 to -15.6) on the negative scale (Table 3.2).

Table 3.2 – Baseline and Post-shift levels of metal PM<sub>2.5</sub> (mg/m<sup>3</sup>), Acceleration (AC) and Deceleration (DC) Capacities of the heart (msec), C-reactive protein (mg/L), and Interleukins 6, 8 and 10 (pg/mL).

	Baseline Mean (SD)	Postshift Mean (SD)	Postshift - Baseline Mean	p-value
<i>Exposure</i>				
*PM <sub>2.5</sub> (mg/m <sup>3</sup> )	0.04 (0.3)	0.35 (0.4)	0.31	<b>&lt;0.01</b>
<i>Outcome</i>				
+AC(msec)	-7.3 (4.0)	-5.5 (3.3)	1.8	<b>&lt;0.01</b>
DC(msec)	8.4 (3.7)	7.0 (4.0)	-1.4	<b>0.02</b>
<i>Mediator</i>				
CRP(mg/L)	3.58 (4.5)	3.64 (4.9)	0.06	0.91
IL-6(pg/mL)	75.6(591.5)	80.0(594.8)	4.4	0.95
IL-8(pg/mL)	18.6 (20.1)	19.9 (21.3)	1.3	0.60

**Bold values** indicate significant p-values (p<0.05)

\*PM<sub>2.5</sub> ambient levels before shift and average levels during work shift are reported as baseline and post-shift respectively.

+Acceleration Capacity is measured on a negative scale. N=45 participants

The mean (range) baseline DC was 8.4msec (-2.7 to 17.2) whereas it was 7.0msec (-12.2 to 17.6) on the positive scale. Median values of CRP (mg/L), IL-6 (pg/mL), IL-8 (pg/mL) and IL-10

(pg/mL) at baseline were 1.87, 21.9, 15.0, and 6.5 respectively, and after the work shift (post-shift) were 1.76, 24.1, 16.2, and 6.0 respectively.

Correlations between potential covariates and baseline measures of AC, DC, CRP, IL-6, IL-8, and IL-10 were not statistically significant. However, season of the year and active smoking were correlated with AC and/or DC. Among the other covariates considered, age was neither correlated with PM<sub>2.5</sub> exposure nor with the baseline outcome.

Our mediational analyses showed that the point estimates for the effects of PM<sub>2.5</sub> on AC independent of CRP, IL-6, IL-8, and IL-10 (direct effect) were significant decreases (on the negative scale) in AC of 2.66, 2.16, 2.55 and 2.61msec per mg/m<sup>3</sup> increase in work shift PM<sub>2.5</sub> respectively (Table 3.3, Figure 3.1). These were not significantly different from the effect of PM<sub>2.5</sub> on AC (total effect) which was a decrease of 2.62(95% CI: 0.06 to 5.18)msec per mg/m<sup>3</sup> increase in work shift PM<sub>2.5</sub>. Similarly, the effects of PM<sub>2.5</sub> on DC independent of CRP, IL-6, IL-8, and IL-10 (direct effect) were decreases in DC of 2.78, 2.51, 2.75 and 2.90msec per mg/m<sup>3</sup> increase in work shift PM<sub>2.5</sub> respectively. However, only the direct effect of PM<sub>2.5</sub> on DC independent of IL-10 was significant at p<0.1, others (CRP, IL-6, and IL-8) were marginally significant. Furthermore, the direct effects of PM<sub>2.5</sub> on DC independent of CRP, IL-6, IL-8, and IL-10 were not significantly different from the total effect of PM<sub>2.5</sub> on DC.

Table 3.3: Direct and Indirect Effects of metal PM<sub>2.5</sub> (mg/m<sup>3</sup>) on Acceleration (AC) and Deceleration (DC) Capacities of the heart (msec) mediated by C-reactive protein (mg/L), Interleukins 6, 8 or 10 (pg/mL).

Parameter	CRP models		IL-6 models		IL-8 models		IL-10 models	
<sup>1</sup> <i>Acceleration Capacity(AC)</i>	Effect Size	PM%	Effect Size	PM%	Effect Size	PM%	Effect Size	PM%
		0.02		4.00		2.67		0.38
Direct Effect	<b>2.66 (0.01, 5.32)</b>		<b>2.16(-0.36, 4.69)</b>		<b>2.55(-0.09, 5.19)</b>		<b>2.61(-0.02, 5.25)</b>	
Indirect Effect	0.001(-0.02, 0.04)		0.09(-0.58, 0.65)		0.07(-0.20, 0.35)		0.01(-0.04, 0.10)	
Total Effect	<b>2.62 (0.06, 5.18)</b>		<b>2.62(0.06, 5.18)</b>		<b>2.62(0.06, 5.18)</b>		<b>2.62(0.06, 5.18)</b>	
<i>Deceleration Capacity(DC)</i>		0.32		2.71		2.48		1.40
Direct Effect	-2.78(0.74, -6.29)		-2.51(0.90, -5.93)		-2.75(0.76, -6.26)		<b>-2.90(0.57, -6.37)</b>	
Indirect Effect	-0.01(0.04, -0.06)		-0.07(0.50, -0.63)		-0.07(0.18, -0.33)		0.04(0.31, -0.23)	
Total Effect	<b>-2.81(0.58, -6.20)</b>		<b>-2.81(0.58, -6.20)</b>		<b>-2.81(0.58, -6.20)</b>		<b>-2.81(0.58, -6.20)</b>	

<sup>1</sup>Acceleration Capacity is measured on a negative scale, therefore positive estimates connote a decrease in AC on a negative scale.

PM<sub>2.5</sub>(fine particulate matter), CRP (C-reactive protein), IL-6 (Interleukin 6), IL-8 (Interleukin 8), IL-10 (Interleukin 10), PM% (proportion mediated in percentage)

**Bold values** indicate significant associations (p<0.1)

All models are adjusted for age, active smoking (smoker/non-smoker), time of blood sample/ECG collection (AM/PM), season (winter/summer), baseline levels of potential mediator (CRP, IL-6, IL-8 or IL-10), and baseline AC or DC. N=45 participants

The indirect effects - pathway of PM<sub>2.5</sub> on AC mediated by CRP, IL-6, IL-8, and IL-10 – constituted 0.02%, 4%, 2.67% and 0.38% of the total effect (proportion mediated, PM%) respectively, whereas the proportion mediated by the indirect effects - pathway of PM<sub>2.5</sub> on DC

mediated by CRP, IL-6, IL-8, and IL-10 – constituted 0.32%, 2.71%, 2.48% and 1.4% of the total effect of PM<sub>2.5</sub> on DC respectively (Table 3.3).

## DISCUSSION

The goal of this study was to investigate whether the associations between acute occupational PM<sub>2.5</sub> exposures and each of acceleration capacity and deceleration capacity are mediated by any of CRP, IL-6, IL-8, and IL-10. Contrary to our hypotheses, our data do not suggest that acute occupational PM<sub>2.5</sub> exposures associated with reductions in acceleration capacity and deceleration capacity are mediated by any of CRP, IL-6, IL-8, and IL-10. We however found significant negative exposure–response relationships (total effect) between acute work shift PM<sub>2.5</sub> exposure with acceleration capacity and deceleration capacity as we had earlier observed in this study population (8, 26). This creates further evidence to the documented reduction in the capacity of the heart to accelerate and decelerate over time with acute insults from fine metal particulate exposures. Furthermore, the reductions in the capacities of the heart to speed up and slow down (AC and DC) from metal PM<sub>2.5</sub> over the work shift lasting 4-6 hours were about three times the magnitude of the effect when exposure-response was modeled hourly (lagged hourly models) in our previous study. There may be therefore a cumulative multiplier effect of sustained exposure of metal PM<sub>2.5</sub> on autonomic influences as documented in this study population(27).

Teasing apart the total effect into direct and indirect effects will explore the strengths of the direct effect(nervous) and indirect effect(inflammatory) pathways linking metal PM<sub>2.5</sub> exposure and cardiac autonomic dysfunction. Our data suggest that there is a large significant contribution

of the direct response of metal PM<sub>2.5</sub> on acceleration capacity following workshift exposure. This provides further evidence to the importance of the autonomic influence on the heart rate during speeding up of the heart. Although we found similar contributions through the direct pathway on the association between acute workshift metal PM<sub>2.5</sub> exposure on deceleration capacity, they were not significant except for the model independent of IL-10. We may therefore deduce that the acceleration capacity of the heart may be more sensitive to effects of metal rich particulates. We had observed within this study population possible differential response of acceleration capacity and deceleration capacity to chronic exposure of metal PM<sub>2.5</sub>. In this study, we had set out to explore the potential for inflammatory-mediated pathway by considering several mediators. We analyzed blood samples for interleukins, adhesion molecules, cytokines and other inflammatory markers like C-reactive protein. To reduce the rate of random error (chance) in our results, we chose to investigate *a priori* only four of our strongest suspects (CRP, IL-6, IL-8, and IL-10) well documented to be involved in the pathophysiology of tissue inflammation(19, 20). We did not find significant differences between baseline and post-shift levels in the levels of any of CRP, IL-6, IL-8, and IL-10. This is in contrast to what was previously independently documented by Zhang and Bruno in this study population(28, 29). Whereas Zhang and Bruno had used paired samples from these participants on a non-weld day at baseline and next morning as post-exposure samples in assessing the effect of secondhand smoke exposure, we restricted our samples to the weld day because we were interested in determining the effect of weld fume exposure. We were surprised that there were no differences between paired baseline and post-shift mediator levels, because it is well documented in literature that inflammation results in increase in post-shift levels of these markers(30, 31). The onset of production of CRP *in vivo* following acute insult and injury resulting in inflammation is within six hours and continues to

rise to a peak at 48 hours with a half-life of 48 hours(32, 33). We may therefore be missing the right time window to capture observable differences between baseline and post-exposure mediator levels. Next-morning samples have been shown to capture better changes in inflammatory markers following injurious stimuli(28, 29).

A cursory look at our results shows that the indirect (inflammatory) pathway is of no significance and may be inconsequential. However, a more detailed analysis of the indirect pathway during the step by step mediation may suggest otherwise. The indirect pathway [path a\*b] is a product of the association between metal PM<sub>2.5</sub> exposure and the mediator (CRP for instance) [path a] and the association between the mediator and the outcome (AC for instance) [path b]. The former [path a] is well documented in literature that PM<sub>2.5</sub> causes inflammation which can be measured using blood markers(30, 31). Although, we did not find this path significant for any of our potential mediators (Appendix 3.A), we think that collecting the post-shift sample immediately after the workshift may not have left enough time for synthesis of these inflammatory markers.

Appendix 3.A : Main effect of PM<sub>2.5</sub> on C-reactive protein (mg/L), Interleukins 6, 8 or 10 (pg/mL) (path a)

Parameter	CRP(95% CI)	IL-6(95%CI)	IL-8(95%CI)	IL-10(95%CI)
Shift PM <sub>2.5</sub>	-0.18 (-0.89, 0.62)	2.15(-15.3, 16.7)	2.79(-5.84, 11.92)	0.54(-2.87, 5.01)

**Bold values** indicate significant correlations (p<0.1)

All models are adjusted for age, active smoking (smoker/non-smoker), time of blood sample/ECG collection (AM/PM), season (winter/summer), and baseline levels of potential mediator (CRP, IL-6, IL-8 or IL-10).

We therefore suggest that similar research in the future take this exposure-response window into cognizance. This in fact may be responsible for the weak strength of the indirect path (path a\*b), and same argument applies to path b (Appendix 3.B).

Appendix 3.B: Associations of C-reactive protein (mg/L), Interleukins 6, 8 or 10 (pg/mL) on AC and DC (path b).

Parameter	<sup>1</sup> <i>Acceleration Capacity(AC)</i> (Confidence Interval)	<i>Deceleration Capacity(DC)</i> (Confidence Interval)
<i>Models</i>		
CRP	-0.003(-0.23, 0.22)	0.05(0.35, -0.25)
IL-6	<b>0.04 (0.01, 0.07)</b>	<b>-0.03 (0.01, -0.08)</b>
IL-8	0.02 (-0.05, 0.10)	-0.02 (0.07, -0.12)
IL-10	0.02 (-0.14, 0.18)	0.07 (0.28, -0.14)

<sup>1</sup>Acceleration Capacity is measured on a negative scale, therefore positive estimates connote a decrease in AC on a negative scale.

**Bold values** indicate significant correlations ( $p < 0.1$ )

All models adjusted for age, active smoking, time of blood sample/ECG collection, season, baseline levels of potential mediator, and baseline AC or DC.

Notwithstanding, IL-6 showed much more potential than the others in mediating effects of PM<sub>2.5</sub> on AC and DC as only path b models for IL-6 were nonetheless significant (Appendix 3.B). Therefore the results of this study should be interpreted with these limitations in mind. At best, the proportion mediated by the indirect pathway linking PM<sub>2.5</sub> to AC was 4%.

Acceleration capacity is a measure of the responsiveness of the heart to speed up when stimulated, and it is believed to be under both autonomic (sympathetic) and non-autonomic control. Deceleration capacity on the other hand, describes the behavior of the heart when the heart rate is slowing, and it reflects a measure of parasympathetic modulation of the heart. We found significant associations (direct effect) of metal PM<sub>2.5</sub> with both acceleration capacity but non-significant association with deceleration capacity. However, neither the direct nor indirect pathways linking metal PM<sub>2.5</sub> to deceleration capacity account for the significant total effect for models with CRP, IL-6 and IL-8. We would therefore hypothesize that either there may be a significant direct effect, which we fail to detect because of lack of power, or perhaps a significant indirect effect, which we fail to detect because it is not a simple pathway involving only one mediator as our mediation models have assumed. In fact there are often complex inter-relationships between these biomarkers during systemic inflammation(25). Our results may therefore suggest that both acceleration and deceleration capacities may be under both influences (nervous and autonomic) as they are often antagonistic, and that acceleration capacity may be more sensitive to changes in the PM<sub>2.5</sub> levels.

Our study has its strengths and limitations. Importantly, we captured ECG tracings digitally that were parsed into phases of accelerations and decelerations accounting for differences in heart rate. Our results are therefore not confounded by heart rate. We also adjusted for confounding by potential confounders. We used repeated measures of continuous shift PM<sub>2.5</sub> measured using DustTrak<sup>TM</sup> Aerosol Monitor. There is a potential for selection bias in our study as we were only able to retrieve weld day data for 63% of our participants. However, there were no differences in baseline characteristics of the welders whom we were not able to retrieve their records and those in our study. In addition, our study may have missed the right exposure time window to detect

differences in mediator levels following PM<sub>2.5</sub> exposure, and may be underpowered to detect significant indirect effect. In conclusion, although our results show no significant mediation by any of the evaluated mediators, IL-6 showed a potential of mediating the effect of metal particulates on AC. We however cannot conclude that the effects of metal particulates on AC or DC are mediated by any of CRP, IL-6, IL-8 or IL-10. This finding suggests the need to evaluate more complex pathways in a larger study for the potential for inflammation-mediated pathway of metal particulates on AC and DC.

#### **ACKNOWLEDGEMENTS**

This research was supported by the National Institute of Environmental Health Sciences (NIEHS) grant numbers R01ES009860, P50ES00002, ES009860 and ES00002. We thank the participants and the leadership of Local 29 of the International Brotherhood of Boilermakers, Iron Ship Builders, Blacksmiths, Forgers and Helpers in Quincy, MA. We also thank Georg Schmidt for his expert contribution.

#### **COMPETING INTERESTS**

The authors declare they have no actual or potential competing financial interests.

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## CHAPTER 4

# LONG-TERM METAL PM<sub>2.5</sub> EXPOSURES DECREASE CARDIAC ACCELERATION AND DECELERATION CAPACITIES IN WELDERS.

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**Keywords:** Deceleration, Heart Rate, Welding, Electrocardiography, Occupational Exposure

**Word Count:** 3548

## ABSTRACT

**Background:** Short-term particulate exposures (PM<sub>2.5</sub>) decrease heart rate variability (HRV). The effect of long-term metal particulate exposures on cardiac autonomic function is unknown. To clarify if long-term metal particulates affect cardiac accelerations, decelerations or both, we used Acceleration Capacity (AC) and Deceleration Capacity (DC), new indices of cardiac autonomic function which measure the HRV during speeding up and slowing down of the heart rate respectively.

**Methods:** We calculated Chronic Exposure Index (CEI) for a panel of 50 boilermaker welders using their average typical shift PM<sub>2.5</sub> exposure, obtained in 3-6 typical welding shifts within sampling periods between January 2010 and June 2012, cumulated in retrospect over the work life of the study participants to time of study. We also obtained recent resting electrocardiograms (ECG) over ten minutes to obtain the AC and DC. We used linear regression to assess the association between CEI PM<sub>2.5</sub> exposure and each of AC and DC, controlling for age, acute effects of welding exposure, and time of day when ECG reading was obtained.

**Results:** Mean (SD) CEI for PM<sub>2.5</sub> exposure was 1.25 (1.77) mg/m<sup>3</sup>-years and ranged from 0.01 – 10.4 mg/m<sup>3</sup>-years. Negative crude exposure-response associations were found for both AC and DC with increased chronic PM<sub>2.5</sub> exposure. In our fully adjusted models, a 1 mg/m<sup>3</sup>-year increase in CEI for PM<sub>2.5</sub> was associated with a decrease of 1.31 (95% CI: 0.14, 2.75) msec resting AC, and a decrease of 1.15 (95% CI: -0.20, 2.09) msec resting DC.

**Conclusion:** Long-term metal particulate exposures decrease cardiac accelerations and decelerations.

## INTRODUCTION

Fine particulate (PM<sub>2.5</sub>) exposure has been documented as a major environmental factor for the increased cardiovascular morbidity and mortality, because of its ability to become absorbed following inhalation(1, 2). Fine particulates have also been shown to have cardio-pulmonary effects, especially among welders with large occupational exposures(3, 4).

Boilermaker construction workers (boilermakers) are welders who work in power plants and are exposed to large amounts of particulate fumes. Occupational exposure to welding fumes occurs during the metal joining process. Boilermakers are also exposed to the residual fly ash left following the combustion of the fuel in the power plant. Based on 2012 estimates from the Bureau of Labor Statistics (BLS), 357, 400 workers in the United States are employed as welders, cutters, solderers, and brazers (5). While PM<sub>2.5</sub> is highly ubiquitous in the environment, exposures in these work settings where there is increased generation of particulates may be even more substantial. For example, epidemiologic studies have shown that boilermakers involved in welding activities are exposed to over ten times ambient levels of particulate matter in weld fumes(3).

Several studies have linked ambient PM<sub>2.5</sub> exposure with cardiovascular disease(6, 7), but there are few studies that have examined this association in an occupational setting with much higher airborne exposures from work processes. According to our knowledge, no study has examined fine particulate exposures over the course of the work-life among workers who have been in these occupational settings for long periods, and have the potential for a cumulative multiplier effect from PM<sub>2.5</sub> exposure over the years(8). The effect of these exposures in the long term on cardiac autonomic function remains unclear.

Autonomic changes in the heart from exposures to particulates have traditionally been measured using heart rate variability (HRV) indices(9), which have an inherent potential for misclassification because of their inability to account for accelerations and decelerations. Baeur described the phase-rectified signal averaging (PRSA) method for calculating the heart's acceleration capacity (AC) and deceleration capacity (DC), which are measures of the responsiveness of the heart like HRV during speeding up and slowing down of the heart respectively (10). These have been demonstrated to be more predictive of morbidity and mortality among post-ischemic coronary artery disease patients than traditional HRV(10). Therefore, using sensitive indices - Acceleration Capacity and Deceleration Capacity - this study aims to investigate the potential for cardiac autonomic dysfunction from long-term metal PM<sub>2.5</sub> exposure. We will also characterize and describe PM<sub>2.5</sub> exposures during the work-life of boilermakers using a chronic exposure index; this will be measured by obtaining typical work shift PM<sub>2.5</sub> exposure in these workers, and using their previous detailed work shift schedules over the years to compute their long-term PM<sub>2.5</sub> exposure distribution patterns during their work life(11, 12).

## **METHODS**

### ***Subject Recruitment***

The participants were among 72 boilermakers who were part of an ongoing “Harvard Boilermaker Cohort” initiated in 1999 to study the cardio-pulmonary effects of particulates(13). We recruited 52 of the 72 male boilermakers sampled between January 2010 and June 2012 from the boilermaker union in Quincy, Massachusetts for our study. We were able to analyze data from 50 boilermakers for our study. They constituted 70% of the participants in the existing

cohort whom we were able to retrieve detailed work histories from when they became boilermakers, obtain PM<sub>2.5</sub> exposure measures, as well as record resting digital ECG in sampling periods between 2010 and 2012. Although we had recruited 72 boilermakers in total, we restricted our study to the 52 participants who were monitored at least once on a welding day to estimate their shift PM<sub>2.5</sub> exposure. Of these 52, we had failed initialization of Holter and poor data retrieval of ECG data in 2 participants. We recorded their most recent ECG during winter or summer when 85% of the study participants had not actively welded two weeks prior to our data collection. The Institutional Review Board at the Harvard T. H. Chan School of Public Health approved the study protocol. We obtained informed consent from all study participants.

### ***Data Collection***

We collected PM<sub>2.5</sub> exposure and resting ECG data of study participants at the boilermaker union welding school in Quincy, Massachusetts. Boilermakers practiced welding in booths designed for training apprentices at the welding school. We obtained from the boilermakers' union a detailed previous work schedule over their work life since they became boilermakers after we had obtained consent from the participants.

Participants completed a self-administered questionnaire on demographics, medical history and medication use information, lifestyle information including smoking, typical diet, and occupational history. We collected information on heart and blood vessel problems: myocardial infarction, angina, arrhythmia, hypertension, use of blood pressure medications such as beta blockers or ACE inhibitors, congestive heart failure, heart/chest surgery, or other heart problems diagnosed by a physician.

### ***PM<sub>2.5</sub> Assessment***

We measured PM<sub>2.5</sub> concentrations during welding shifts of study participants using personal DustTrak™ Aerosol Monitor (TSI, Inc., St. Paul, MN). The DustTrak™ monitor was strapped to the participant's shoulder close to their breathing zone. DustTrak™ has a PM<sub>2.5</sub> inlet impactor to measure continuously and record at 1-minute intervals average concentrations of fine particulates during the welding shifts. Kim had validated DustTrak™ continuous readings of PM<sub>2.5</sub> with gravimetric methods in welders(14). We calculated mean concentrations of PM<sub>2.5</sub> exposure during each work shift for each participant. Subsequently, we calculated the typical shift exposure for each participant by taking the average of PM<sub>2.5</sub> exposures for all work shifts for that participant.

### ***Chronic (PM<sub>2.5</sub>) Exposure Index***

We computed a Chronic Exposure Index (CEI) in mg/m<sup>3</sup>-years by multiplying the average PM<sub>2.5</sub> (in mg/m<sup>3</sup>) during a typical shift and the work life – summation of all the days (expressed in years) they had ever worked as a boilermaker. We used the sum of all days worked over the years because most boilermakers have variable off-work periods between workdays in a year. To express CEI in mg/m<sup>3</sup>-years consistent with other chronic exposure measures, we converted days to years by dividing by 365.

Work Life (years) =  $\Sigma$  workdays ever since boilermaker / 365

CEI (mg/m<sup>3</sup>-years) = PM<sub>2.5</sub> (mg/m<sup>3</sup>) \* work life (years)

### ***ECG recording and processing***

Study participants wore a standard five lead ECG Holter monitor after a thirty minute rest period in the morning on arrival at the union hall. The rest period was to allow us record their unbiased resting ECG free from acute changes resulting from commuting to the study site. To ensure the leads of the ECG were well secured and remained secured on the chest of participants, we shaved their skin if necessary, cleaned with an alcohol wipe after slightly abrading the skin, and research staff checked them intermittently(3) . The digital recordings were then downloaded and sent to the Cardiovascular Epidemiology Research Unit (CVERU) of Beth Israel Deaconess Medical Center (Boston, MA) for processing and analysis. Holter recordings were uploaded into the GE MARS ECG analysis system, which automatically scans recordings for areas of noise and groups heartbeats as normal or arrhythmic. Trained technicians blinded to the exposure status of the participant from whom the ECG reading was obtained verified the automated scans as correct or changed them to the appropriate designation. The data were then exported for analysis using the Physionet toolkit(15). To remove artifacts from the data, they used only beats with an RR interval within 5% difference of adjacent beats. They used an automated process described by Bauer to create 5-minute segments with anchors for the Phase-Rectified Signal Averaging (PRSA) method of computing the acceleration capacity (AC) and deceleration capacity (DC) (10). In brief, to compute the DC, this involves identifying heartbeat intervals longer than the preceding interval as anchors (for AC, beats shorter than preceding beats were anchors). Overlapping segments of interval data were then automatically generated from the ECG such that all segments are aligned at the anchors in the center and averaged. The PRSA method then quantifies the signals within aligned segments using the Haar wavelet analysis with a scale of 2 by a computer processing of the ECG with visual and digital outputs. Thus, AC and

DC were calculated separately as a quarter of the difference between two sums, that is, the sum of the averaged anchor points RR intervals ( $X_0$ ) with the succeeding RR intervals ( $X_1$ ) and the sum of the two averaged RR intervals preceding anchor points ( $X_{-2}$ ,  $X_{-1}$ ) (16).

### ***Acceleration Capacity (AC) and Deceleration Capacity (DC)***

Using the digitized data in the time domain, we computed the average AC and average DC for the resting 10-minute period by taking the mean of the first two 5-minute segments of the ECG using the automated output.

### ***Data Analysis***

We calculated summary measures of potential covariates, and percentiles of our exposure and outcome to further understand their distribution. Potential covariates that we considered include: age, race, smoking status, time of day when ECG was obtained, season of study, acute effects of welding, and presence of heart problems. We then explored the inter-relationships between them by using spearman's correlations for continuous variables and t-tests for binary variables. In order not to have missed any inter-relationships between potential covariates with our exposure/outcome, we used  $\alpha=0.10$  level for these correlations and t-tests between covariates and outcome/exposure.

We ran linear regression model to assess the crude association between  $PM_{2.5}$  Chronic Exposure Index (CEI) as a continuous measure and AC, and a separate model for CEI and DC. Because age was correlated with CEI, we first ran a linear regression model of age versus CEI, and used the residuals (variation in age not accounted for by CEI) to adjust for the age. This represents the biological age of the heart independent of exposure. We then ran age-adjusted models to control for age – using the biological heart age independent of CEI. Furthermore we used forward,

backward and stepwise methods of model building using a p-value of 0.1 each for entry and exit to select our final model. We considered controlling for age, smoking status, and time of day and season when ECG reading was obtained. Our final model was consistent irrespective of model building approach used, and had only age in the model when we constrained CEI to remain in the model. We also ran a model that included age and time of day ECG was taken, and another model that included age, time of day ECG was taken, and last work day to control for acute effects of particulates. Statistical significance was assessed at  $\alpha=0.05$  level in two sided tests for our final model. All analyses were performed using PROC REG in SAS version 9.4 (Cary, NC).

## RESULTS

There were 50 participants, all males with a mean age of 39 years and were retrospectively followed for a median of 4 years (range 0.25 – 21 years). The study population consisted of 44 (88%) whites and 18 (36%) smokers (Table 4.1). Ten (10) of these 50 participants reported heart problems and possible heart-related problems. Of these 10, 3 reported arrhythmias, 3 reported sinus (sinoatrial node) problems, 2 reported palpitations, and 2 reported complicated diabetes (with palpitations) that may affect autonomic control of the heart, for which they were taking insulin. No participant reported the use of beta-blockers or ACE inhibitor drug use. Most (72%) of the resting ECG were taken in the morning, and each participant had PM<sub>2.5</sub> measurements taken in 3-6 typical work shifts of 4 – 6 hours.

In contrast to the years of follow up, the mean work life was 2.9 years (range 0.2 – 10.6 years). The mean PM<sub>2.5</sub> during a typical work shift was 0.4mg/m<sup>3</sup> (range 0.01 – 1.40 mg/m<sup>3</sup>), and the mean CEI was 1.16 mg/m<sup>3</sup>-years (range 0.001 – 10.37 mg/m<sup>3</sup>-years).

Table 4.1: Demographics and characteristics for the 50 study participants with successful measurements and data retrieval.

<b><i>Individual Characteristics</i></b>	<b><u>N</u></b>	<b><u>%</u></b>
Male	50	100
Whites	44	88
<sup>1</sup> Smokers	18	36
<b><i>Other Characteristics</i></b>		
<sup>2</sup> Time of ECG (AM)	36	72
<sup>3</sup> Season of ECG (winter)	21	42
<sup>4</sup> Heart problems	10	20
	<b><u>Mean</u></b>	<b><u>s.d.</u></b>
Age at ECG measurement (years)	39	11
Age at becoming a boilermaker (years)	32	9
Length of follow up (years)	7	6
<sup>5</sup> Last Weld Day before study (days)	36	37

<sup>1</sup>Current Smokers vs Non-smokers and Previous smokers

<sup>2</sup>Morning (AM) vs Afternoon(PM)

<sup>3</sup>Winter vs Summer

<sup>4</sup>Heart problems include reported previous history of arrhythmia, cardiac sinus problems, or palpitations

<sup>5</sup>One participant had not welded for 3 years and was not included in this statistic

The mean (range) AC was -7.1msec (-0.7 to -16.9) on the negative scale with the middle half of the participants ranging between -4.2 to -9.2msec (Table 4.2). The mean (range) DC was 8.7msec (1.8 to 18.5) on the positive scale with the middle half of the participants ranging 6.4 to 10.3.

Spearman's correlations coefficients (and p-value) for correlation between CEI and AC were 0.26 (0.07), and -0.30 (0.03) for that between CEI and DC. Among the covariates considered, only age was correlated with both exposure and outcome. Coefficients (and p-value) for

correlations between age and CEI, age and AC, and age and DC, were 0.44 ( $<0.01$ ), 0.30 (0.03), and -0.28 (0.05) respectively.

Table 4.2 – Across subject distribution of PM<sub>2.5</sub> Chronic Exposure Index (CEI) in mg/m<sup>3</sup>-years and acceleration (AC) and deceleration (DC) capacities in msec for 50 participants.

	Percentiles			
	Mean (SD)	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>
<b>Exposure</b>				
<sup>1</sup> CEI	1.2 (1.7)	0.4	0.6	1.2
<sup>2</sup> Shift PM <sub>2.5</sub>	0.4 (0.3)	0.3	0.4	0.5
<sup>3</sup> Work Life	2.9 (2.6)	1.0	1.4	4.4
<b>Outcome</b>				
<sup>4</sup> AC(msec)	-7.1 (3.9)	-4.2	-6.4	-9.2
DC(msec)	8.7 (3.4)	6.4	8.4	10.3

<sup>1</sup>Cumulative Exposure Index (mg/m<sup>3</sup>-years) = PM<sub>2.5</sub>(mg/m<sup>3</sup>) x Work Life (years)

<sup>2</sup>This is the across subject average for typical shifts sampled

<sup>3</sup>Work Life (years) =  $\Sigma$  Work Days ever since boilermaker/365

<sup>4</sup>Acceleration Capacity is measured on a negative scale.

There was a marginal statistically significant difference between the exposure (CEI) indices of participants whose ECG were taken in the morning (0.90mg/m<sup>3</sup>-years) versus afternoon (1.84 mg/m<sup>3</sup>-years) ( $p=0.08$ ), but not with the outcomes (AC and DC). There were no differences in categories of other covariates in terms of measures of CEI, AC and DC.

The linear regression analyses revealed that elevated CEI PM<sub>2.5</sub> levels were associated with AC and marginally associated with DC after adjusting for age (Table 4.3). A 1mg/m<sup>3</sup>-year increase in CEI resulted in a decrease of 1.46msec (95% CI: 0.23, 2.69;  $p=0.02$ ) resting AC and a decrease of 0.99msec (95% CI: -0.08, 2.06;  $p=0.07$ ) resting DC. When we further controlled for the time of day ECG was taken and last day welded, the negative age-adjusted association between CEI and AC remained consistent. However, there was declining statistical significance of the age-adjusted model in the association between increased CEI and DC upon adjusting for

the time of day ECG was taken, and there was no association when acute effects of particulates were controlled for (using last weld day).

Table 4.3: Linear regression coefficients (b1) of main effect of Cumulative Exposure Index (mg/m<sup>3</sup>-years) on acceleration capacity (msec) and deceleration capacity(msec).

Parameter	<sup>a</sup> Acceleration Capacity(AC)		Deceleration Capacity(DC)	
	(95% C.I.)	p-value	(95% C. I.)	p-value
<i>Models</i>				
Model 1	0.65 (-0.05, 1.25)	0.07	-0.56 (-1.10, 0.02)	0.06
Model 2	<b>1.23 ( 0.23, 2.69)</b>	<b>0.02</b>	-1.07 (-2.06, 0.08)	0.07
Model 3	<b>1.26 ( 0.17, 2.69)</b>	<b>0.03</b>	-1.09 (-2.06, 0.12)	0.08
Model 4	<b>1.31 ( 0.14, 2.75)</b>	<b>0.03</b>	-1.15 (-2.09, 0.20)	0.10

<sup>a</sup>Acceleration Capacity is measured on a negative scale, therefore positive estimates connote a decrease in AC on a negative scale.

**Bold values** indicate significant correlations (p<0.05)

Model 1: AC or DC = b0 + b1\*CEI + e

Model 2: AC or DC = b0 + b1\*CEI + b2\*Age + e

Model 3: AC or DC = b0 + b1\*CEI + b2\*Age + b3\*TOD + e

Model 4: AC or DC = b0 + b1\*CEI + b2\*Age + b3\*TOD + b4\*LWD + e

CEI=Cumulative Exposure Index (mg/m<sup>3</sup>-years)

Age=The residual of age on CEI was used to represent the heart age (variation in age not explained by the exposure)

TOD=Time of Day ECG was taken (AM vs PM)

LWD=Last Weld Day

We evaluated distributional assumptions of our linear regression model by examining the residual versus predictor plots and using the Cook's distance. We identified a potential outlier using Cooks' distance (criterion>0.5). We therefore evaluated these associations with and without this observation. Without this outlying observation, these associations were consistent.

We further explored the data by adding a quadratic term for CEI in our models. The associations remained the same, but the quadratic term for CEI was not significant. Therefore, our final model for all 50 participants was the linear regression model that was adjusted for age which was the variable retained in our model selection process when CEI was constrained in the model.

## **DISCUSSION**

The goal of this study was to investigate the associations between acceleration capacity and deceleration capacity with chronic occupational PM<sub>2.5</sub> exposures. Consistent with our hypotheses, chronic occupational PM<sub>2.5</sub> exposure was associated with acceleration capacity and to a lesser extent deceleration capacity. We found a significant negative exposure–response relationship between chronic PM<sub>2.5</sub> exposure index and acceleration capacity. There may therefore be a reduction in the capacity of the heart to accelerate over time with repeated injury from these exposures. These data imply that there may be changes in sympathetic modulation and/or non-autonomic control of the heart with repeated exposure to particulates in the long term. Few studies have documented associations between particulate exposures and heart rate variability in the short term(1, 3, 17-19). Yet fewer studies have observed associations between fine particulate exposure and deceleration capacity, also in the short term(20). This study is the

first to demonstrate declines in acceleration capacity following occupational particulate exposure over a long term period.

Due to the differences in the length of follow up period, exposure characteristics, and study population, we cannot directly compare our study results with those of prior studies. Although, we did find a trend towards a decline in the deceleration capacity similar to declines reported following acute PM<sub>2.5</sub> exposures, we also did observe declines in acceleration capacity which has never been reported even in studies in the short term. This result highlights the possibility of differences in effect among our study population (active welders) compared to other studies (post myocardial infarction patients) that were mostly conducted in clinical settings. Ten of our study participants reported known or possible heart problems, but none reported angina, myocardial infarction or other symptoms of ischemia. This suggests that there may be modification of effects of particulates in ischemic versus non-ischemic conditions.

Furthermore, the different constituents of the particulate exposures may have varying effects on the electrical activity of the heart. While organic and elemental carbons have been implicated as suspects, metals have also been shown to play a role(4, 20-22). Welders are exposed to metal-rich fumes, and metals may be responsible for the effects on acceleration capacity we found in this study.

Acceleration capacity is a measure of the responsiveness of the heart to speed up when stimulated, and it is under both autonomic (sympathetic) and non-autonomic control. Deceleration capacity on the other hand, describes the behavior of the heart when the heart is slowing down, and it reflects a measure of parasympathetic modulation of the heart(20). While we found a significant effect with the acceleration capacity, the effect of particulates on the

deceleration capacity was only marginally significant. We would therefore hypothesize that over a work life of exposure to particulates, the non-autonomic control may be playing a more prominent role in the response of the heart. We also cannot conclude if there is a significant change in the nervous control from exposure to particulates in the long term from our results.

Deceleration capacity has been shown to be predictor of mortality following acute myocardial infarction even among patients with preserved acceleration capacity. This is because there is a rapid decline in the parasympathetic innervation of the heart following ischemia(10, 23). However, the clinical usefulness of the acceleration capacity is still unknown. This may be explained by the multi-factorial influences on the acceleration capacity. Therefore, whereas deceleration capacity specifically measures vagal output to the heart, sole sympathetic innervation to the heart cannot be captured by acceleration capacity directly.

Our results show that increasing age is a significant predictor of both acceleration and deceleration capacities. Ageing results in declines in both the ability of the heart to speed up and slow down. In our models, age was a more significant predictor than our exposure index. This may suggest that declines in biological functions with aging may be more important in determining autonomic responses of the heart than exposure to fine particulates. However, there are studies that have demonstrated that there are changes in the automaticity of the heart (using heart rate variability indices) even in the young on exposure to fine particulates(17, 24).

The exposure distribution of our chronic PM<sub>2.5</sub> index in this study was log-normal, like most occupational exposures, with the mean about twice the median across all subjects. We used repeated shift PM<sub>2.5</sub> real time dust track measures to derive average exposures of each participant. The PM<sub>2.5</sub> surprisingly followed a near-normal distribution and we would have

missed the accurate exposure distribution pattern if we used only shift PM<sub>2.5</sub> as our exposure measure. Work-life days (years) in our data also mirrored our chronic exposure index distribution pattern, and may be a fair exposure assessment in situations where shift PM<sub>2.5</sub> is unavailable to compute the chronic exposure index(8).

Based on our study results, reducing exposure and encouraging recovery activities following exposure would be a major goal of health promotion for these workers. Even with the potential for a healthy worker effect, we still found an association with long term exposure to particulates. The use of personal protective equipment such as a respirator has been shown to reduce exposures to particulates and improve heart rate variability(25). Furthermore, activities such as exercise, adequate sleep and good nutrition may allow for recovery of the heart after exposure to weld fumes during the day(26).

We recognize limitations in our study. Although, we were able to capture exposure to particulates in the long-term using a chronic index measure, this may have resulted in non-differential misclassification of the exposure. This would bias the results towards the null, and our results may therefore be slightly attenuated and the true effects may even be greater. There is a potential for selection bias in our study as we were only able to retrieve work records for 70% of our participants. However, there were no differences in characteristics of the welders whom we were not able to retrieve their records and those in our study. We are also not able to evaluate the impact of left censoring and healthy worker effect on our study results. In spite of the retrospective nature of our study, we used recent repeated shift PM<sub>2.5</sub> measured between 2010 and 2012, and retrieved work records to estimate their average exposure patterns over their work life. This would have reduced the potential for recall bias in our exposure. These PM<sub>2.5</sub>

measurements were obtained in comparable work settings at the union hall and may reflect what their typical exposures would have been if they were followed up prospectively.

Importantly, we captured ECG tracings digitally that were parsed into phases of accelerations and decelerations accounting for differences in heart rate. Our results are therefore not confounded by heart rate. We also attempted to adjust for confounding by potential confounders, but most were not retained in our final model. We were able to account for short-term effects of welding by restricting our study population to those who had not welded two weeks prior to our study, and adjusted for ‘last weld day’ in our models. Our results are robust and consistent with and without outlying observations.

In conclusion, we found that increasing long-term particulate exposure resulted in a decrease in acceleration capacity, and deceleration capacity to a lesser extent. This shows that acceleration capacity was more sensitive, and suggests that there may be potential differences in pathophysiological mechanisms of the effect of long term fine particulate exposure on the regulatory pathways for these indices.

## **ACKNOWLEDGEMENTS**

This research was supported by the National Institute of Environmental Health Sciences (NIEHS) grant numbers R01ES009860, P50ES00002, ES009860 and ES00002. We thank the participants and the leadership of Local 29 of the International Brotherhood of Boilermakers, Iron Ship Builders, Blacksmiths, Forgers and Helpers in Quincy, MA. We also thank Georg Schmidt for his expert contribution.

## **COMPETING INTERESTS**

The authors declare they have no actual or potential competing financial interests.

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## **CHAPTER 5. CONCLUSIONS**

## SUMMARY AND CONCLUSIONS

This dissertation adds to our knowledge and understanding of the cardiovascular health effects of metal-rich PM<sub>2.5</sub>. The studies are the first to explore the effects of PM<sub>2.5</sub> on cardiac acceleration and deceleration capacities within an occupational context. We provide evidence that metal-rich PM<sub>2.5</sub> exposure elicits an acute effect on both autonomic responses of accelerations and decelerations, which are immediate and last up to one hour post exposure. This dissertation also demonstrates that even among relatively healthy welders, chronic effects of PM<sub>2.5</sub> exposure exist with declines in both acceleration and deceleration capacities.

The exact mechanisms whereby PM<sub>2.5</sub> exposure alters cardiovascular function remain to be fully understood; however it is likely that multiple mechanisms occur concurrently. For example, in Chapter 2 we observed an immediate decline in both acceleration and deceleration capacities on PM<sub>2.5</sub> exposure as well as lagged declines lasting up to four hours for deceleration capacity. This suggests that there may be multiple responses occurring under different mechanisms. We propose that the more immediate response may be due to autonomic responses and the lagged responses may occur subsequent to systemic inflammation. Furthermore, in Chapter 3 we similarly observed immediate declines in both acceleration and deceleration capacities following exposure to work shift PM<sub>2.5</sub>, and that these declines may be mediated through complex mechanisms involving interleukin 6. This may be as a result of activation of inflammatory cells (T cells and macrophages) subsequent to the release of pro-inflammatory cytokines. Although we did not observe changes in the levels of any of our suspected mediators immediately after the shift, this was probably due to a mistiming of measurements, such that we missed the right exposure-response window, as there had not been enough time for the synthesis of these markers of inflammation, when we assayed blood samples for post-shift changes.

However, this association between PM<sub>2.5</sub> exposure and blood markers of inflammation is well established previously in literature especially with next morning samples after exposure(1, 2). In Chapter 4 we observed a long term effect of chronic PM<sub>2.5</sub> exposure in the past on both cardiac acceleration and deceleration capacities, indicating a decline in response by the autonomic nervous system to accelerate and decelerate over time with work exposures to PM<sub>2.5</sub>.

For each of the three investigations within this dissertation, particle exposure was characterized by PM<sub>2.5</sub> mean concentrations. However, certain particle characteristics of welding fume exposure, namely particle size and composition, may be responsible for the observed responses.

The studies in this dissertation had the advantages of combining personal exposure monitoring during a discrete occupational exposure period with a panel study design. The exposure patterns experienced in the work setting provided a relatively wide exposure range, which provided the opportunity to detect differential responses. While the focus of these studies was on occupational PM<sub>2.5</sub> exposures and autonomic cardiac health effects among workers, the knowledge gained also informs environmental health, more generally, as particulate matter studied in this setting is also found in ambient particulate air pollution, albeit at lower concentrations and different composition. Furthermore, though occupational and environmental PM exposures differ in composition, intensity and duration, the effects of PM on the cardiovascular system, whether from ambient or occupational sources, are likely to be similar.

In summary, this dissertation has demonstrated that metal-rich PM exposure elicits both acute and chronic effects on the autonomic responses of the heart. While much remains to be understood about the role of PM<sub>2.5</sub> on workers' cardiovascular health, especially the biological mechanisms, limiting exposure to PM<sub>2.5</sub> in the workplace is pertinent given the weight of

evidence in the air pollution literature, evidence from mortality studies among welders, and evidence that have been observed with respect to PM in previous studies and in this dissertation. Whereas the clinical significance of a decrease in deceleration capacity is an increased mortality risk among post-myocardial ischemic patients, even among those with preserved acceleration capacity(3), the implications of a decrease in both acceleration and deceleration capacity in response to short-term and long-term PM<sub>2.5</sub> exposure among relatively young and healthy workers are unclear. We however hypothesize that these may be poor prognostic risk factors and may increase the biologic aging of the heart. Although, the effect measured appears moderate, however, with a large number of individuals occupationally exposed to PM<sub>2.5</sub>, even relatively small sub-clinical health effects can potentially have a large impact on the public's health. Further studies should be designed to investigate the time course of acute responses over longer durations, responses under different working conditions, and the role of specific PM components such as metal constituents and ultrafine particles. Ultimately, identifying the agents and mechanisms responsible for PM-associated cardiovascular events are important for developing focused intervention efforts.

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