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Are the Associations of Cardiac Acceleration and Deceleration Capacities with Fine Metal Particulate in Welders Mediated by Inflammation?

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Abstract

Objective—To investigate whether associations of Acceleration Capacity (AC) and Deceleration Capacity (DC) with metal-$PM_{2.5}$ are mediated by inflammation.

Methods—We obtained $PM_{2.5}$, CRP, IL-6, 8 and 10; and electrocardiograms to compute AC and DC, from 45 male welders. Mediation analyses were performed using linear mixed models to assess associations between $PM_{2.5}$ exposure, inflammatory mediator, and AC or DC; controlling for covariates.

Results—The proportion of total effect of $PM_{2.5}$ on AC or DC (indirect effect) mediated through IL-6 on AC was 4% at most. Controlling for IL-6 (direct effect), a 1 mg/m^3 increase of $PM_{2.5}$ 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.

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Conflicts of Interest: None

Conclusion—IL-6 may be mediating the effect of metal particulates on AC.

Keywords

Deceleration; Heart Rate; Welding; Electrocardiography; Occupational Exposure

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 [7–9].

The mechanism(s) attributed to the increased risk of cardiovascular diseases, especially ischemic heart disease in welders remains unclear; though several mechanisms describing alterations at cellular and tissue levels have been proposed to account for the effect of fine particulates in elevating the risk of cardiovascular events in welders. One hypothesized mechanism is through the autonomic nervous system, which has been measured traditionally using Heart Rate Variability (HRV)[10–14]. Air pollution studies show a decrease in HRV with exposure to $PM_{2.5}$ in both occupational groups and in the general population[7, 10, 12]. A decrease in HRV has also been linked to an increase in adverse cardiovascular outcomes[13–16]. 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.

Baeur et al 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 [17, 18]. These measures 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[17].

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 cardiac autonomic dysfunction indices (AC, DC) are mediated by inflammation measured using mediators such as interleukins, adhesion molecules, and cytokines.

Therefore, using sensitive AC and DC indices, this study aims to investigate the pathway linking metal $PM_{2.5}$ exposure and cardiac autonomic function, by assessing markers of inflammation as potential mediators. This study will investigate whether the associations between acute occupational $PM_{2.5}$ exposure and AC and DC capacity are mediated by C-Reactive Protein (CRP) or Interleukins (IL-6, IL-8, and IL-10). Additionally, we will assess the direct (nervous) and indirect (inflammatory) pathways linking metal $PM_{2.5}$ exposure and cardiac autonomic function. We hypothesize that CRP, IL-6, IL-8 and IL-10 are mediators

of the associations between increasing occupational metal-rich $PM_{2.5}$ exposure and decreasing cardiac AC and DC.

METHODS

Subject Recruitment

We recruited 45 male boilermakers during five sampling periods between January 2010 and June 2012 from the boilermaker union in Quincy, Massachusetts through an outreach basis by telephone. These boilermakers were part of an ongoing "Harvard Boilermaker Study" initiated in 1999 to study the cardio-pulmonary effects of particulates[21]. 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 written informed consent was obtained from each study participant.

Data Collection

We collected repeated measures of $PM_{2.5}$ exposure, blood samples and continuous ECG data from 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 modified American Thoracic Society questionnaire[22]. 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.

$PM_{2.5}$ Assessment

We measured $PM_{2.5}$ 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 $PM_{2.5}$ inlet impactor to measure continuously and record at 1-minute intervals average concentrations of fine particulates during the welding shifts. The continuous DustTrak™ readings of $PM_{2.5}$ had been validated compared to gravimetric methods in welders[23]. We calculated mean shift concentrations of $PM_{2.5}$ exposure during each sampling period for each participant. Participants also kept a work log of tasks and exposure to second hand smoke during each shift.

Potential Inflammatory Mediators

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 collection tubes containing EDTA. 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 T. H. Chan School of Public Health where they were stored in a freezer maintained at −80°C till when they were analyzed. Three to six months later, serum samples for each participant were thawed and analyzed for CRP, and IL-6, IL-8 and IL-10 levels in duplicate. Quantification of CRP marker levels were conducted at our laboratory using multiplex electrochemiluminescence using the MULTI-SPOT® 96-well Human Vascular Injury Panel II assay (Meso Scale Discovery, Rockville MD) as per manufacturer's protocol. Similarly, plasma concentrations of IL-6, IL-8, and IL-10, were assessed using the MULTI-ARRAY® 96-well custom cytokine/chemokine assay. For each duplicate sample, any sample with a coefficient of variation (CV) greater than 20% required that all samples from that participant were reanalyzed.

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 verified the automated scans as correct or changed them to the appropriate designation. The data were then exported for analysis using the Physionet toolkit[24, 25]. 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 AC and DC[17]. 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}).

AC and 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 $PM_{2.5}$ 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 statistics for the exposure, outcome, and covariates to assess their distributions. CRP, IL-6, IL-8, and IL-10 were log-transformed to approximate a normal distribution. 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)[11, 26, 27]. These were the relevant covariates considered in the study of the acute association of $PM_{2.5}$ exposure on AC and DC using lagged hourly models.

For our analyses, we first obtained the total effect of workshift $PM_{2.5}$ 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 $PM_{2.5}$ 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 assess the direct and indirect effects (via the mediator) of $PM_{2.5}$ on AC and DC. In the first step we ran linear mixed models to assess the associations between workshift $PM_{2.5}$ as a continuous measure and shift changes in AC or DC controlling for covariates above, to estimate the direct effect (Figure 1). Then, we ran separate linear mixed models for association between $PM_{2.5}$ exposure and each potential inflammatory 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).

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[28] 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: [Sqrt ($a^2s_b^2$ + $b^2s_a^2$)][28]. 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 α=0.10 level in two sided tests for our final model in order not to miss any potential association. All analyses were performed using PROC MIXED in SAS version 9.4 (Cary, NC).

RESULTS

We collected 83 person-shifts of weld day $PM_{2.5}$, 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 1).

One of these 45 participants reported a history of palpitations and one 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 $PM_{2.5}$ measurements taken in 1–3 typical work shifts of 4 – 6 hours.

The mean $PM_{2.5}$ for all participants were 0.04mg/m^3 (range 0.00 – 1.43 mg/m^3) prior to their work shift (personal ambient levels), and 0.35mg/m^3 (range 0.01 – 2.96 mg/m^3) 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 2).

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 (Table 2).

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 $PM_{2.5}$ exposure nor with the baseline outcome.

Our mediation analyses showed that the point estimates for the effects of $PM_{2.5}$ on AC independent of CRP, IL-6, IL-8, and IL-10 (direct effect) were significant negative decreases in AC of 2.66, 2.16, 2.55 and 2.61 msec per mg/m^3 increase in work shift $PM_{2.5}$, respectively (Table 3, Figure 1). These were not significantly different from the effect of $PM_{2.5}$ on AC (total effect) which was a decrease of 2.62 (95% CI: 0.06 to 5.18) msec per mg/m^3 increase in work shift $PM_{2.5}$. Similarly, the effects of $PM_{2.5}$ on DC (conditionally) independent of CRP, IL-6, IL-8, and IL-10 (direct effect) were decreases in DC of 2.78, 2.51, 2.75 and 2.90 msec per mg/m^3 increase in work shift $PM_{2.5}$ respectively. However, only the direct effect of $PM_{2.5}$ on DC independent of IL-10 was significant at $p<0.10$, while CRP, IL-6, and IL-8 were marginally significant. Furthermore, the direct effects of $PM_{2.5}$ on DC independent of CRP, IL-6, IL-8, and IL-10 were not significantly different from the total effect of $PM_{2.5}$ on DC.

The indirect effects - pathway of $PM_{2.5}$ 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 $PM_{2.5}$ 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 $PM_{2.5}$ on DC respectively (Table 3). We report details of the indirect

pathway in Tables 4 and 5. There were no significant associations between work shift $PM_{2.5}$ and CRP, IL-6, IL-8, and IL-10 (Table 4). There were significant associations between increasing cross-shift IL-6 and decreasing AC and DC (Table 5).

DISCUSSION

The goal of this study was to investigate whether the associations between acute occupational $PM_{2.5}$ exposures and AC and DC are mediated CRP, IL-6, IL-8, and IL-10. True to our hypothesis, there was a 4% mediation by IL-6 on the association between metal-rich $PM_{2.5}$ and AC. Contrary to our hypotheses, our data do not support that acute occupational $PM_{2.5}$ exposures associated with reductions in AC and DC are mediated by CRP, IL-8, and IL-10. We also found significant exposure–response relationships (total effect) between increasing acute work shift $PM_{2.5}$ exposure with decreasing AC and DC as we had earlier observed in this study population [8, 29]. This finding supports the documented reduction in the capacity of the heart to accelerate and decelerate over time with acute insults from fine metal particulate exposures[30]. Furthermore, the reductions in the capacities of the heart to speed up and slow down (AC and DC) from metal $PM_{2.5}$ 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 $PM_{2.5}$ on autonomic influences as documented in this study population[31].

To our knowledge, this is the first study to evaluate the potential inflammatory-mediated mechanism of autonomic function in an occupational setting with metal-rich exposures. Previous studies in similar study populations had shown significant associations between increasing $PM_{2.5}$ levels and decreasing HRV[7, 10, 29]. Additionally, there have been reported associations between increasing $PM_{2.5}$ levels and decreasing AC and DC both during the short-term (hours) and long-term (years) follow up[30].

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 $PM_{2.5}$ exposure and cardiac autonomic dysfunction. Our data suggest that there is a large significant contribution of the direct response of metal $PM_{2.5}$ on AC following workshift exposure. This finding 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 $PM_{2.5}$ exposure on DC, they were not significant except for the model independent of IL-10. We may therefore deduce that the AC of the heart may be more sensitive to effects of metal rich particulates. We had observed within this study population possible differential response of AC and DC to chronic exposure of metal $PM_{2.5}$. 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 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 finding is in contrast to what was previously independently documented by Zhang and Bruno in this study population[26, 27]. Whereas Zhang et al and Bruno et al 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. Contrary to expectations, we observed no differences between paired baseline and post-shift mediator levels. It is well documented in literature that inflammation results in increase in post-shift levels of these markers[32, 33]. 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[34, 35]. 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[26, 27].

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 $PM_{2.5}$ 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 $PM_{2.5}$ causes inflammation which can be measured using blood markers[32, 33]. Although, we did not find this path significant for any of our potential mediators, we think that collecting the post-shift sample immediately after the workshift may not have left enough time for synthesis of these inflammatory markers.

We therefore suggest that similar larger 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.

Notwithstanding, IL-6 showed much more potential than the others in mediating effects of $PM_{2.5}$ on AC and DC as only path b models for IL-6 were nonetheless significant. Therefore the results of this study should be interpreted with these limitations in mind. At best, the proportion mediated by the indirect pathway linking $PM_{2.5}$ to AC was 4%.

AC 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. DC 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 $PM_{2.5}$ with both AC but non-significant association with DC. However, neither the direct nor indirect pathways linking metal $PM_{2.5}$ to DC 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 interrelationships between these biomarkers during systemic

inflammation[28]. Our results may therefore suggest that both AC and DC may be under both influences (nervous and autonomic) as they are often antagonistic, and that AC may be more sensitive to changes in the $PM_{2.5}$ 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 potential confounding by age, smoking, season and baseline cardiac autonomic function. We used repeated measures of continuous shift $PM_{2.5}$ measured using DustTrak™ Aerosol Monitor. The use of repeated measurements increases our statistical power, and can better assess exposure dynamics rather than single point measurements. Each study participant served as their own control pre- and post-shift and this would reduce population level confounding. There is a potential for unmeasured confounding due to residual confounding by secondhand smoke which we adjusted for categorically. Residual confounding by secondhand smoke would bias our results away from the null and the true estimates may be less than we observed in this study.

In conclusion, our study results show that IL-6 showed potential for mediating the effect of metal particulates on AC, and there was no significant mediation by CRP, IL-8, and IL-10. The potential mediation by IL-6 on the association between occupational metal-rich $PM_{2.5}$ on AC 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.

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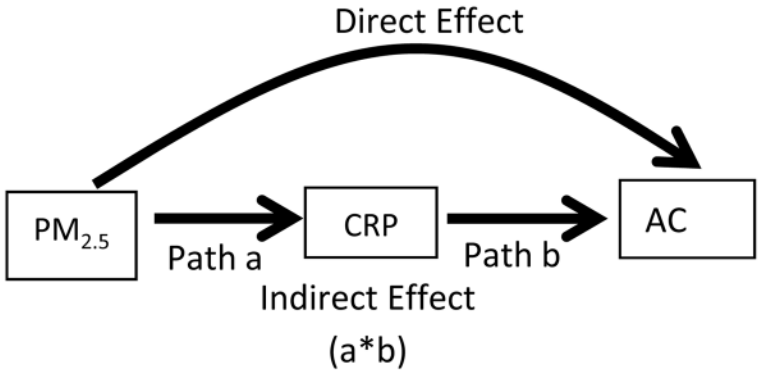

Figure 1.
Mediational Analyses showing associations between $PM_{2.5}$ exposure, mediator variable (CRP, IL-6, IL-8, or IL-10), and dependent variable (AC or DC).

Table 1

Demographics and characteristics for 45 study participants.

Individual Characteristics	**N**	**%**
Male	45	100
Caucasian	42	93
[a] Smokers	19	42
Other Characteristics		
[b] Time of ECG (AM)	18	40
[c] Season of ECG (winter)	33	73
[d] Heart problems	2	4
	Mean	**s.d.**
Age at start of study 2010 (years)	40	12
Last Weld Day before study (days)	44	51

[a] Current Smokers vs Non-smokers and Previous smokers

[b] Morning (AM) vs Afternoon(PM)

[c] Winter vs Summer

[d] Heart problems include reported previous history of arrhythmia and palpitations

Table 2

Baseline and Post-shift levels of metal $PM_{2.5}$, Acceleration (AC) and Deceleration (DC) Capacities of the heart, C-reactive protein, and Interleukins 6, 8 and 10.

	Baseline Mean (SD)	Postshift Mean (SD)	Postshift - Baseline Mean	p-value
Exposure				
[a] PM2.5 (mg/m^3)	0.04 (0.3)	0.35 (0.4)	0.31	[b] <0.01
Outcome				
[c] AC(msec)	−7.3 (4.0)	−5.5 (3.3)	1.8	[b] <0.01
DC(msec)	8.4 (3.7)	7.0 (4.0)	−1.4	[b] 0.02
Mediator				
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

[a] $PM_{2.5}$ ambient levels before shift and average levels during work shift are reported as baseline and post-shift respectively.

[b] Significant p-values ($p<0.05$)

[c] Acceleration Capacity is measured on a negative scale. N=45 participants

Table 3

Direct and Indirect Effects of Metal $PM_{2.5}$ on Acceleration (AC) and Deceleration (DC) Capacities of the Heart Mediated by Inflammatory Biomarkers. (N=45 participants)

Parameter	CRP models		IL-6 models		IL-8 models		IL-10 models	
	Effect Size	***b* PM%**	**Effect Size**	***b* PM%**	**Effect Size**	***b* PM%**	**Effect Size**	***b* PM%**
[a]*Acceleration Capacity(AC)*		0.02		4.00		2.67		0.38
Direct Effect	[c]2.66 (0.01, 5.32)		[c]2.16(−0.36, 4.69)		[c]2.55(−0.09, 5.19)		[c]2.61(−0.02, 5.25)	
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	[c]2.62 (0.06, 5.18)		[c]2.62(0.06, 5.18)		[c]2.62(0.06, 5.18)		[c]2.62(0.06, 5.18)	
Deceleration Capacity(DC)		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)		[c]−2.90(0.57, −6.37)	
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	[c]−2.81(0.58, −6.20)		[c]−2.81(0.58, −6.20)		[c]−2.81(0.58, −6.20)		[c]−2.81(0.58, −6.20)	

[a] Acceleration Capacity is measured on a negative scale, therefore positive estimates connote a decrease in AC on a negative scale.

[b] $PM_{2.5}$ (fine particulate matter), CRP (C-reactive protein), IL-6 (Interleukin 6), IL-8 (Interleukin 8), IL-10 (Interleukin 10), PM% (proportion mediated in percentage)

[c] Significant associations ($p<0.10$)

[d] 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.

Table 4

Main effect of $PM_{2.5}$ 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 $PM_{2.5}$	−0.18 (−0.89, 0.62)	2.15(−15.3, 16.7)	2.79(−5.84, 11.92)	0.54(−2.87, 5.01)

[a] Significant associations ($p<0.1$)

[b] 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).

Table 5

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

Parameter	[a]*Acceleration Capacity(AC)* (95% Confidence Interval)	*Deceleration Capacity(DC)* (95% Confidence Interval)
Models		
CRP	−0.003(−0.23, 0. 22)	0.05(0.35, −0.25)
IL-6	[b]0.04 (0.01, 0.07)	[b]−0.03 (0.01, −0.08)
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)

[a] Acceleration Capacity is measured on a negative scale, therefore positive estimates connote a decrease in AC on a negative scale.

[b] Significant correlations ($p<0.1$)

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