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CUMULATIVE PM_{2.5} EXPOSURE AND TELOMERE LENGTH IN WORKERS EXPOSED TO WELDING FUMES

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Abstract

Telomeres are genomic structures that reflect both mitotic history and biochemical trauma to the genome. Metals inherent in fine particulate matter (PM_{2.5}) were shown to be genotoxic via oxidative damage. However, few studies investigated the induction time of cumulative PM_{2.5} exposure on telomere length in a longitudinal setting. Therefore, the purpose of this study was to assess the association between occupational PM_{2.5} exposure in various time windows and telomere length. The study population consisted of 48 boilermakers and the follow-up period was 8 yr. The main exposures were cumulative occupational PM_{2.5} in the month, year, and career prior to each blood draw, assessed via work history questionnaires and area air measures. Repeated telomere length measurements from leukocytes were assessed via real-time quantitative polymerase chain reaction (qPCR). Analysis was performed using linear mixed models controlling for confounders and white blood cell differentials. Cumulative PM_{2.5} exposure was treated continuously and categorized into quartiles, in separate analyses. At any follow-up time, for each milligram per cubic meter per hour increase in cumulative PM_{2.5} exposure in the prior month, there was a statistically significant decrease in relative telomere length of −0.04 units. When categorizing the exposure into quartiles, there was a significant negative association between telomere length and highest quartile of cumulative PM_{2.5} exposure in the prior month (−0.16). These findings suggest that genomic trauma to leukocyte telomeres was more consistent with recent occupational PM_{2.5} exposure, as opposed to cumulative exposure extending into the distant past.

Telomeres consist of thousands of hexameric (TTAGGG) n repeats at the distal ends of eukaryotic chromosomes. Telomeric sequences interact with various DNA binding proteins to form the protective telosome complex. This DNA–protein loop structure buffers chromosomal ends against induced nucleolytic decay and prevents chromosomal ends from being recognized by DNA repair machinery as double-stranded breaks (Blackburn, 1991). In somatic cells with only basal telomerase activity, telomeres truncate by approximately 30–100 bp after each mitotic division due to limitations in lagging strand DNA synthesis at chromosomal ends (Blackburn, 1991). As a result, telomere length may reflect the number of past cell divisions throughout the life span of tissues. In the event telomeres truncate to critically short lengths without activation of the apoptotic cascade, subsequent chromosomal fusions may result in adverse cellular events such as neoplasia (De Vivo et al., 2009). Indeed, telomere length has been established as a biomarker of numerous chronic diseases such as cancer and cardiovascular disease (Ma et al., 2011). Peripheral blood leukocytes (PBL) telomere length is primarily used as a surrogate for telomere length in their hematopoietic stem cell (HSC) progenitors and other tissues. Telomere length of PBL was found to be correlated across various target tissues such as skeletal muscle, skin, and subcutaneous fat (Daniali et al., 2013). The correspondence of telomere length between different somatic tissues in humans was also observed in other species (Youngren et al., 1998; Granick et al., 2011; Friedrich et al., 2000; Benetos et al., 2011). Further, Daniali et al. (2013) found that the rates of telomere shortening in the aforementioned tissues were similar, suggesting that differences in telomere length between proliferative and minimally proliferative tissues are established during early life, and that in adulthood, stem-cell progenitors of the different tissues replicate at similar rates (Daniali et al., 2013).

Reactive oxygen species (ROS) are prominent sources of oxidative stress to the genome. Formation of ROS such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals may be attributable to both intrinsic and extrinsic factors. In particular, fine airborne particulate matter (PM_{2.5}) was implicated as an environmental factor that induces the production of deleterious free radicals (Ghio et al., 2012; Torres-Ramos et al., 2011). The small aerodynamic diameter of PM_{2.5} imparts the ability for the particles to deeply deposit into alveoli (Figure 1). The associated metal agglomerates of lead (Pb), iron (Fe), nickel (Ni), cadmium (Cd), and arsenic (As) are formed from combustion processes, then diffuse into the systemic bloodstream where they produce oxidative stress on the DNA of circulating leukocytes, leading to epigenetic modifications, altered gene expression, and changes in telomere length (Nemery, 1990; Wu et al., 2012; Dioni et al., 2011; McCracken et al., 2010; Antonini et al., 2004). In addition, solubilized metals in the circulatory system were shown to induce apoptosis in PBL (Jimenez Del Rio et al., 2004). Homeostasis between white blood cell (WBC) counts and differentials, with their HSC progenitors, is tightly regulated (Yokomizo et al., 2008). Increased rate of leukocyte cell death is usually accompanied by feedback responses to replenish lost cells. Upon signaling of accelerated hematopoiesis, HSC proliferation and differentiation in bone marrow typically precede enhanced progenitor cell egress (Morrison et al., 1997). With increased cell division, egress, and differentiation, telomeres of HSC may undergo accelerated erosion because these cells only express low levels of telomerase (Chiu et al., 1996).

Solubilized metal particles were shown in previous studies to accumulate in hair, nails, and various organ systems (Anwar, 2005). In particular, the half-life of Pb deposited in bones ranges from 10 to 25 years, which is reflective of long-term cumulative exposure (Wilker et al., 2011). Since hematopoietic stem cells are located within bone marrow, the close vicinity to metal deposits may result in oxidative damage to their telomeres. Heavy metal particles are suspected carcinogens and were found to affect health outcomes through various mechanisms including lipid peroxidation of cellular membranes, formation of DNA adducts, oxidative damage, and both local and systemic inflammation (Nuernberg et al., 2008). Therefore, it stands to reason that exposure to PM_{2.5}-associated heavy metals may lead to adverse genomic events including alterations to telomeres (Sanders et al., 2009). In particular, Pb-induced toxicity arises from free radical damage through the generation of ROS and the exhaustion of glutathione (GSH) antioxidant reserves (Sanders et al., 2009). Excessive levels of free Fe iron catalyze Fenton reactions that produce toxic free radicals (Kodavanti et al., 1998; Toyokuni, 2002). Further, Ni particles were shown to be genotoxic by interacting with zinc fingers of DNA repair machinery and inhibiting base incision repair (Daset al., 2008). In addition to metal particles, polycyclic aromatic hydrocarbons (PAH), formed through incomplete combustion of carbon-based fuels, were found to induce ROS production, which may lead to genotoxic effects (Chung et al., 2007; Liu et al., 2006; Wilk et al., 2013). Indeed, a study of coke-oven workers demonstrated that those who were heavily exposed to PAH had shorter telomeres than controls, as well as higher levels of genetic and chromosomal alterations (Pavanello et al., 2010).

Certain occupational environments inherently contain elevated levels of PM_{2.5}, thus increasing the risk of ROS induced genomic damage and adverse health outcomes in workers. In particular, industrial boilermakers who perform arc welding using base metals of mild and stainless steel with electrodes of Fe and manganese (Mn) are highly exposed to fine particulate matter and their associated heavy metals (Santamaria et al., 2007). Previous panel studies found that welders in the Harvard Boilermakers Longitudinal Study had a median workday PM_{2.5} exposure of 649.8 µg/m³— levels far exceeding those experienced by the general population (Cavallari et al., 2008). PM_{2.5} from combustion processes were shown in several investigations to be associated with telomere length (Dioni et al., 2011; Hou et al., 2012). In a study of steel workers, investigators found a significant rise in leukocyte telomere length 3 d after PM exposure compared with baseline samples (Dioni et al., 2011). Further, a short-term study of truck drivers in Beijing, China, found an increase in telomere length in association with short-term personal PM_{2.5} exposure, whereas chronic exposure was associated with decreased telomere length (Hou et al., 2012).

Although PM_{2.5} constitutes a significant occupational hazard to high-exposure industrial professions, few if any studies examined the chronic effects of PM_{2.5} derived from welding fumes on telomere length. Since telomere length was found to be associated with numerous chronic diseases, assessing prolonged effects in a longitudinal setting may be informative in evaluating the viability of telomere length as a biomarker of adverse outcomes. Therefore, the primary aim of this study was to assess the longitudinal association between cumulative exposure to occupational PM_{2.5} in various time windows and telomere length, in workers highly exposed to welding fumes. Evaluating induction times in a longitudinal setting provides valuable biological and public health insights into the mechanism by which

environmental factors affect telomere length and risk of chronic diseases. Further understanding of occupational factors affecting telomere length may reinforce a basis for stricter PM_{2.5} permissible exposures to improve the occupational health and safety of exposed workers.

METHODS

Study Design

The Boilermakers Study is an ongoing prospective open cohort comprised of a series of periodic short-term panel studies. The source population consists of members of the International Brotherhood of Boilermakers Union, Local 29, in Quincy, MA. In total, 190 members were prospectively followed since the study inception in 1999. Their primary occupational activity is assembling and repairing boilers that provide high-pressure steam to drive electrical turbines in power plants. An overview of the study timeline and data collection procedure is illustrated in Figure 2. The current study population consisted of 48 subjects of the blood subcohort who had telomere length assessment and complete work history data on past welding hours in the last year, at baseline blood draw. Additional inclusion criteria for this study included being male union members who were apprentices or journeymen and over 18 years of age at the time of recruitment. The time scale of interest was months of follow-up. The follow-up period for the current longitudinal study was from February 1, 2003, to June 18, 2011. Repeated measures of telomere length for each subject were obtained at irregularly spaced time intervals from the baseline blood draw to the end of follow-up or until censored. A median of two and maximum of six repeated measures were sampled from the subjects.

Exposure Assessment—Cumulative PM_{2.5}

The main exposure of interest was time-varying cumulative PM_{2.5} from welding fumes. Three exposure windows were selected: career, 1 yr, and 1 mo prior to each blood draw. Data on occupational variables, work hours, and welding hours were collected via work history questionnaires at the study site. In total, 38 independent area PM_{2.5} measures were obtained using KTL Cyclones (BGI, Inc., Waltham, MA) from an oil-burning power plant located in eastern Massachusetts. The mean flow rate and sampling time were 4 L/min and 8 h, respectively. The estimated cumulative PM_{2.5} exposure in the month and year prior to blood draw was calculated by multiplying the median work area PM_{2.5} concentration by the estimated number of welding hours in the past month and year, respectively. The estimated career-long cumulative PM_{2.5} exposure prior to blood draw was calculated by multiplying the median work area PM_{2.5} concentration by the estimated number of welding hours in the past year, multiplied by the number of years employed as a boilermaker. The cumulative PM_{2.5} metrics were operationalized and analyzed in two ways. First, the exposure was retained as continuous to assess linear dose responses. Second, the exposure was categorized into quartiles and treated as indicator/dummy variables to assess nonlinear dose responses.

Outcome Assessment—Average Relative Telomere Length

Whole blood samples collected from the study site were extracted by a certified phlebotomist. Buffy coat was extracted from whole blood and stored in cell lysis solution at

–20°C until DNA extraction. Peripheral blood leukocyte DNA was extracted using the QIAmp DNA blood kits (QIAGEN, Venlo, Limburg). Average relative telomere length was assessed using real-time quantitative polymerase chain reaction (qPCR) (Cawthon, 2002). The metric is referred to as the telomere to single gene (T/S) ratio (–dCt) and its value should be proportional to the average telomere length across all chromosomes in a population of cells, for each subject. The T/S ratio (–dCt) for each sample was calculated by subtracting the average 36B4 Ct value from the average telomere Ct value. The T/S ratio value for all experimental samples was then compared to that of a calibrator sample, consisting of a pooled genomic DNA sample of de-identified volunteers from the general population recruited by the Brigham and Women's Hospital–Channing Division of Network Medicine Blood Laboratory. The calibrator sample was run on all qPCR plates and its purpose was to adjust for plate-to-plate variation in the assay. The relative T/S ratio (–ddCt) was determined by subtracting the T/S ratio of the calibrator sample from the T/S ratio of each unknown sample. The relative T/S ratio was then converted to the natural scale by exponentiation (2^{-ddCt}). A modified version of the real-time PCR telomere assay was performed in a 384-well format with a 7900HT real-time PCR system (Life Technologies, Carlsbad, CA). Briefly, 5 ng of buffy-coat-derived genomic DNA was dried down in a 384-well plate and resuspended in 10 µl of either the telomere or 36B4 PCR reaction mixture for 2 h at 4°C. The telomere reaction mixture consisted of 1× Qiagen Quantitect Sybr Green Master Mix, 2.5 mM of dithiothreitol (DTT), 270 nM of Tel-1 primer (GGTTTTTGAGGGTGAGGGTGAGGGTGAGG GTG AGGGT), and 900 nM of Tel-2 primer (TCCCGACTATCCCTATCCCTATCCCTATCCCT ATCCCTA). The reaction proceeded for 1 cycle at 95°C for 5 min, followed by 40 cycles at 95°C for 15 s, and 54°C for 2 min. The 36B4 reaction consisted of 1× Qiagen Quantitect Sybr Green Master Mix, 300 nM of 36B4U primer (CAGCAAGTGGGAAGGT GTAATCC), and 500 nM of 36B4D primer (CCCATTCTATCATCAACGGGTACAA). The 36B4 reaction proceeded for 1 cycle at 95°C for 5 min, followed by 40 cycles at 95°C for 15 s, and 58°C for 1 min 10 s. All samples for both the telomere and single-copy gene (36B4) reactions were performed in triplicate on different plates. Each 384-well plate contained a 6-point standard curve from 0.625 ng to 20 ng to assess PCR efficiency. The standard curve was generated with genomic DNA from the aforementioned pooled calibrator sample. The slope of the standard curve for both the telomere and 36B4 reactions was -3.4 ± 0.2 . Quality control samples were interspersed throughout the plates in order to assess interplate and intraplate variability of threshold cycle (Ct) values. The interplate percent coefficient of variation for both the telomere Ct and 36B4 Ct values was under 0.75%. Multiple telomere measurements for each subject within a 4-wk time frame were averaged to obtain a mean relative T/S ratio for that monthly unit of follow-up time.

White Blood Cell (WBC) Differentials

Complete blood counts including leukocyte differentials were assessed using a XE-2100 automated hematology system (Sysmex, Kobe, Japan) by Laboratory Corporation of America Holdings (LabCorp, Newton, MA). Proportions of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were obtained. Measurement of telomere length in blood represents an average telomere length across multiple subtypes of leukocytes. Previous studies suggested telomere lengths vary between these subtypes (Rufer et al., 1998;

Wong et al., 2011). Therefore, telomere length measurements might be affected if the proportion of one or more leukocyte subtype is altered. This augmentation in proportion may not represent true change in the telomere length in a given cell, but rather reflects change in the population of cells within the given sample. The change in proportion may be due to numerous factors including the presence of an infection during blood draw, seasonality of blood draw, and various immunologic or inflammatory conditions.

Covariate Assessment

Self-reported questionnaires were used to collect demographic data such as birth date, gender, race, past smoking status, current smoking status, and work history during the sampling period. Anthropometric data such as height and weight used to derive body mass index (BMI, kg/m²) were measured by research personnel at the study site.

ANALYSIS

Linear Mixed Models for Longitudinal Analysis

Linear mixed effects regression models with random intercept were used to estimate the associations between cumulative PM_{2.5} exposure in various time windows and repeated measures of relative telomere length. The use of multiple repeated measures on each subject substantially increases the power and statistical precision of the analysis beyond the apparent sample size, allowing statistical inference to be made with fewer subjects. Separate models were used in analyzing cumulative PM_{2.5} as a continuous variable and categorized into quartiles. The first quartile (lowest) of cumulative PM_{2.5} was used as the reference group. The linear mixed models also controlled for time-varying covariates such as current smoking intensity (cigarettes per day), log-transformed BMI (continuous), log-transformed number of years as a boilermaker (continuous), and time-invariant age at baseline blood draw (continuous), and leukocyte differentials of neutrophils, lymphocytes, monocytes, and eosinophils. Basophils were not controlled for due to miniscule proportions and lack of variability. Interaction terms between the exposure and follow-up time were included to assess effect modification of the rate of telomeric change. Analysis was implemented with SAS v9.3 using PROC MIXED. Restricted maximum likelihood (REML) was used for estimation. Linearity assumptions at baseline between the outcome and each continuous predictor were verified using simple linear regression. Normality and homoscedasticity assumptions were verified using Q–Q plots and residual plots, respectively. Multicollinearity between predictors was assessed using a correlation matrix with Pearson's rho statistic assuming a normal distribution; *p* values .05 were considered statistically significant.

Missing Data

Data collection occurred at irregularly spaced time intervals; therefore, data were unbalanced or missing across time points. Moreover, the study used an open cohort that was subject to staggered entry and loss to follow-up. Linear mixed models automatically accommodate unbalanced and/or missing data via maximum likelihood and REML estimation, assuming data are missing at random (MAR) and distributional assumptions are correct (Cnaan et al., 1997). However, verification of assumptions pertaining to missingness

at random or missingness not at random (MNAR) is rarely possible in epidemiological studies (Cnaan et al., 1997).

RESULTS

Baseline Study Population Characteristics

The study population was composed of 48 male subjects of the boilermakers' blood subcohort. The subjects were 87.5% self-identified whites, whereas the remaining 12.5% of subjects were identified as African-American, Hispanic, and Asian (Table 1). Race was not controlled for in the analysis because there was no statistical difference in telomere length between groups and majority of subjects were self-identified as white. Ever-smokers comprised 79.2% of the sample at baseline. A two sample Student's *t*-test indicated no statistical difference in telomere length between ever and never-smokers. Further, there was no statistical difference in telomere length between current smokers and nonsmokers. Current smoking intensity was included as a confounder in the analysis because of its established causal relationship with telomere length (Liu et al., 2013; Sun et al., 2012; Cassidy et al., 2010). The average age at baseline was 39.3 ± 12.8 (SD) years, having worked an average of 9.6 ± 10.1 (SD) years as a boilermaker. The average numbers of welding hours in the month and year prior to baseline blood draw were 37.4 ± 45.8 (SD) and 330.7 ± 345.2 (SD), respectively. The average body mass index was 28.6 ± 5.2 (SD) kg/m². The median work area PM_{2.5} concentration at the power generating station was 223.8 µg/m³ with an interquartile range of 262.3 µg/m³ from 1999–2000.

Linear Mixed Models: Longitudinal Analysis of the Association Between Cumulative PM_{2.5} and Telomere Length

A significant negative linear association was found between continuous cumulative PM_{2.5} exposure in the prior month and telomere length (Table 2). At any follow-up time, for each 1-mg/m³-h increase in cumulative PM_{2.5}, there was a decrease in relative telomere length of –0.04 units while controlling for covariates. When modeled categorically, the highest quartile of cumulative PM_{2.5} exposure in the past month was negatively associated with telomere length. The interaction terms between follow-up time and the two highest quartiles of cumulative PM_{2.5} were significant with estimates of 0.013. Neither cumulative PM_{2.5} exposure in the past year nor that over the career was associated with telomere length. There was no significant association between current smoking intensity and telomere length.

DISCUSSION

In this study, the association between cumulative PM_{2.5} in various exposure windows and telomere length was assessed over an 8-yr follow-up period. This study contributes to the overall knowledge of telomere length dynamics by exploring the time frame by which environmental factors affect telomeric attrition. Further, this study sheds light on the mechanism by which fine particulate matter (PM) affects biological and health outcomes in exposed populations. A significant negative linear association was found between telomere length and cumulative PM_{2.5} in the month prior to each blood draw. When modeled categorically, the highest quartile of cumulative PM_{2.5} in the previous month was associated

with decreased telomere length, compared to the lowest quartile. The second and third quartiles had comparable effect estimates as the highest quartile of cumulative PM_{2.5}, suggesting dose response may be nonlinear. No significant associations with telomere length were found for cumulative PM_{2.5} in the year and total career-years prior to blood draw. Interestingly, significant interaction terms were noted between follow-up time and the two highest quartiles of cumulative PM_{2.5} in the month prior to each blood draw. This finding suggests that greater recent exposure to PM_{2.5} may decrease the rate of telomere erosion. However, caution needs to be taken when interpreting this finding because the limited sample size and relatively short follow-up time of this occupational cohort. However, given the significance of the results even with the limited size of the study population, a larger sample size may increase the statistical efficiency and significance of the effect even further, assuming no sampling bias, measurement error, and confounding. The significant interactions may serve as hypothesis-generating findings for future studies with greater statistical power.

Taken together, these results suggest that telomeric shortening in PBL and/or their hematopoietic progenitors may respond more readily to recent short-term PM exposures, as opposed to extended exposures. There may be plausible biological explanations for this observation. Peripheral blood leukocytes have an average lifespan of approximately 5–13 d, depending on the subtype (Tough et al., 1995; Umeki et al., 1998). Given the rate of cellular turnover, PM exposure beyond 1 mo prior to blood draw may not markedly affect the current generation of PBL, which was harvested for outcome assessment. Previous studies showed that telomere length in PBL respond to short-term PM exposure, albeit with different study populations, exposure definitions, and exposure windows (Dioni et al., 2011; Hou et al., 2012). The findings from this study may seem to call into question the effectiveness of PBL as biological markers of deleterious PM exposure extending into the distant past. If genotoxic damage induced by PM_{2.5} results in changes that are then lost as PBL turnover, it appears that these changes might not exert a significant effect on future outcomes. The marginal effect modification observed over the extended follow-up time would be consistent with the hypothesis that in the long run, cumulative PM_{2.5} may not be substantially affecting health outcomes mediated through leukocyte telomere alterations. However, marked and permanent changes in leukocyte telomere length occur over decades of time through telomeric alterations in their hematopoietic progenitors. Leukocyte telomere length serves as a proxy for telomere length in hematopoietic stem cells and was shown to be correlated with other target tissues (Daniali et al., 2013). Although the definitive effect of PM on hematopoietic stem cell viability and telomere length has yet to be established, one may postulate that inhaled metals composing welding fumes readily diffuse from the alveoli to the bloodstream, where they eventually deposit and accumulate in bone (Figure 1) (Santamaria et al., 2007; Gordon 2004; Antonini et al., 2004). As indicated previously, half-life of Pb ranges from 10 to 25 yr in bone. Since HSC are situated within bone marrow, the close vicinity to metal deposits may result in chronic oxidative stress to HSC. The local HSC may respond to sufficient oxidative stress generated from metal cations by undergoing autophagy, apoptosis, or necrosis. HSC in the vicinity would be exposed not only to the metal cations but also to ROS produced by apoptotic and necrotic cells. Progenitors and adjacent cells in the vicinity of the apoptotic or necrotic processes may have to undergo

increased mitosis to replace their neighboring cells, which may contribute to accelerated telomere erosion. Indeed, previous studies demonstrated that airborne PM affects the differentiation and production of WBC from hematopoietic progenitors (Mukae et al., 2001). Mukae et al. (2001) found that repeated exposure to PM stimulated bone marrow to increase production of polymorphonuclear leukocytes (PMN) and accelerated release of immature cells into the circulation. The follow-up time and statistical power of the current study may not be sufficient to capture the chronic effects of PM_{2.5}-associated heavy metals on hematopoietic progenitors; therefore, the null findings may be considered noninformative.

Interestingly, there was no marked difference in telomere length between smokers and nonsmokers at baseline. In addition, no significant associations were found between current smoking intensity and telomere length in the longitudinal analyses. Smoking was found to be negatively associated with telomere length, albeit not consistently, in many epidemiological studies (Mirabello et al., 2009). The discrepancy might be due to the way smoking was assessed and operationalized in different studies. There are several accepted ways to assess smoking in epidemiological studies: (1) average number of cigarettes in a time period, (2) cumulative pack-years of smoking, (3) current, past, ever, and never smoking, and (4) cumulative average. Each method has different assumptions about the dose response and induction time in relation to telomere length. In addition, each method requires certain biological assumptions regarding the rate of deposition, repair capacity, and repair rate of the target tissue. From a biological perspective, there may not have been a measurable effect of smoking because the effect of exposure to welding fumes on leukocyte telomere length subsumes or overwhelms that of smoking. As a tool for evaluating health effects in populations, epidemiological studies often lack the power to tease apart biological effects of multiple similar exposures and their modalities, assuming effects actually exist.

The most notable strength of this study was its prospective nature, which allowed for the temporality between exposure, outcome, and covariates to be determined. In addition, the study design imparts robustness against recall bias of exposure and differential misclassification of both outcome and exposure. Further, examination of three different exposure windows enabled determination of induction times relevant to telomeric shortening in WBC and provided insights into the utility of telomeres as biological indicators of past environmental exposures. Moreover, the use of multiple repeated measures throughout the long-term follow-up substantially increased the statistical power of this study beyond its apparent sample size, allowing statistical inferences to be made with fewer subjects.

As with all scientific investigations, there were several limitations of note. One cannot exclude the possibility of bias due to unmeasured confounders. However, the most notable confounders related to both occupational exposures to PM_{2.5} and telomere length from prior studies were controlled for in the analysis. Further, there may be residual confounding if the surrogate confounders that were used in the analysis were not completely correlated with the confounders of interest. BMI may not have been perfectly correlated with adiposity in this relatively young and physically fit population. Although controlling for time-varying confounders in traditional models may result in selection bias (conditioning on a collider), the degree of bias depends on the “true” causal structure and associational strength of measured and unmeasured covariates (Hernan et al., 2006). As with most occupational

cohorts involving volunteers, sample size in this study was limited. However, the entire source population from which the subjects were recruited was only 400 union members; therefore, blood subcohort already encompasses a considerable proportion of the totality. In addition, the use of multiple repeated measures should compensate for this limitation by significantly increasing the statistical power to detect small effect sizes. There was the possibility of healthy worker (selection) bias in which boilermakers who were healthier, with lower cumulative $PM_{2.5}$ exposure, and longer telomeres tended to be recruited and/or remain in the study compared to others. Healthy worker recruitment bias would reduce generalizability of the results to the source population. Health worker selection bias or loss of follow-up would likely bias the estimates toward the null and underestimate the true effect. Finally, the linear mixed models may have been over-fitted because numerous confounders and WBC differentials needed to be controlled for in order to minimize bias in the estimates. There is a classical trade-off between bias and variance when considering the number of covariates to control (Briscoe et al., 2011). Although variance may be inflated from overfitting, statistically significant estimates were still obtained for the analysis involving cumulative $PM_{2.5}$ in the month prior to blood draw.

Accurate classification of the cumulative $PM_{2.5}$ metric rests upon several crucial assumptions. The cumulative metric was derived from area measures of $PM_{2.5}$, which assumes that all subjects were equally exposed. Further, variability in the cumulative $PM_{2.5}$ metric was primarily driven by estimated welding time. There may be misreporting of welding hours in work logs, which may increase the variance of the measure and underestimate the significance of the effects. In using area $PM_{2.5}$ measures, it was assumed that the composition of welding fumes, base metals, electrodes, and work environment did not substantially change throughout the follow-up period. Further, it was assumed that welding activities and $PM_{2.5}$ levels at other work sites throughout the follow-up were comparable to the oil-burning power plant. A notable caveat of this study was that exposure to PM from non welding activities such as cutting and grinding, in addition to nonoccupational sources such as traffic, cooking, and secondhand smoke, could not be accounted for in the exposure assessment. This caveat would underestimate the degree of PM exposure to the subjects. However, such exposure misclassification was unlikely to be differential in that it may be related to the outcome of telomere length. A cumulative exposure metric was chosen as opposed to a period average because it was more appropriate for continuous outcomes that respond in a proportional and irreversible direction (Smith, 1987). Using a cumulative metric, it was assumed that telomeres in hematopoietic progenitors that derive PBL are constantly shortening due to limited telomerase expression. In addition, it was assumed that any changes in the rates of attrition attributable to $PM_{2.5}$ were proportional to the amount of exposure.

CONCLUSIONS

In summary, findings from this study were consistent with the hypothesis that increased cumulative $PM_{2.5}$ exposure in the month prior to outcome assessment was associated with decreased telomere length. This result seemed to suggest that telomeric attrition in PBL was more attributable to acute environmental $PM_{2.5}$ exposure, as opposed to cumulative exposure extending further into the past. This observation may be due to periodic

replenishment of WBC. However, caution needs to be taken when interpreting these findings since practical limitations in the sample size, follow-up period, exposure assessment, and use of PBL as surrogates do not preclude the possibility of chronic effects of PM_{2.5} on the telomeres of HSC from which PBL are derived. Future prospective studies need to focus on employing larger sample sizes with a balanced study design. Further, extended follow-up periods with equally spaced repeated measures and consistent personal PM_{2.5} monitoring would be beneficial in eliciting the nuances of long-term telomere length dynamics in response to fine PM exposure.

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Figure 1.

Hypothesized mechanism of action for the effect of environmental PM_{2.5} on leukocyte telomere length. Metal particles associated with PM_{2.5} may affect leukocyte telomere length through several pathways: (1) direct oxidative damage to circulating leukocytes, (2) direct oxidative damage to circulating leukocytes followed by short-term accelerated HSC proliferation, differentiation, and egress, and (3) chronic oxidative damage to HSC progenitors of circulating leukocytes.

Figure 2.

Boilermakers longitudinal study timeline. Data collection on the outcome, covariates, and white blood cell differentials occurred at baseline and at irregularly spaced intervals throughout the follow-up for each subject. Occupational exposure to cumulative PM_{2.5} was assessed prior to each data collection. The variables n and m denote certain points in time.

TABLE 1

Characteristics of the Boilermakers Longitudinal Study Population at Baseline (2003–2011)

Variable	Total n = 48		Mean Rel.	
	Frequency	(%)	Telomere Length	Std. Dev.
Current Smoking Status				
Non-smoker	25	52.1	0.63	0.16
Smoker	22	45.8	0.65	0.16
Past Smoking				
Never	10	20.8	0.62	0.18
Ever	38	79.2	0.66	0.16
Self-Reported Race				
White	42	87.5	0.61	0.22
Others	6	12.5	0.65	0.16
	Median	IQR	Mean	Std. Dev.
Relative Telomere Length (2^{-ddCt})	0.65	0.53 – 0.74	0.65	0.16
Age	37.6	28.7 – 50.4	39.3	12.8
Current Smoking Status (cigarettes per day)	0	0 – 17.0	9.0	11.4
Number of Years Working as a Boilermaker	6.0	2.0 – 11.0	9.6	10.1
Welding Hours in the Month Prior to Blood Draw	18.0	10.0 – 40.0	37.9	45.8
Welding Hours in the Year Prior to Blood Draw	200.0	70.0 – 500.0	330.7	345.2
Body Mass Index (BMI), kg/m ²	27.8	25.0 – 32.9	28.6	5.2
Cumulative PM _{2.5} in the Month Prior to Blood Draw (mg/m ³ * hr)	4.0	2.2 – 9.0	8.5	10.2
Cumulative PM _{2.5} in the Year Prior to Blood Draw (mg/m ³ * hr)	44.8	15.7 – 111.9	74.0	77.3
Cumulative PM _{2.5} in the Years as a BM Prior to Blood Draw (mg/m ³ * hr)	223.8	67.1 – 671.3	713.7	1457.9

Note. Discrepancies in counts due to missing data. A two-sample Student's *t*-test was used to compare telomere length in covariates with two categories (*t*-statistic), whereas a one-way analysis of variance (ANOVA) was used to evaluate covariates with three or more categories (*F*-statistic).

Asterisk indicates *p* values < .05, considered statistically significant.

TABLE 2

Longitudinal Analysis of the Association between Cumulative PM_{2.5} Exposure in Various Time Windows and Telomere Length (2003–2011)

	Month Prior to Blood Draws			Year Prior to Blood Draws			Career-length Prior to Blood Draws		
	Coefficient	95%CI: Lower	95%CI: Upper	Coefficient	95%CI: Lower	95%CI: Upper	Coefficient	95%CI: Lower	95%CI: Upper
Main Effect: Continuous Cumulative PM _{2.5} ¥									
Continuous Cumulative PM _{2.5} (log mg/m ³ *hr)	-0.040	-0.080	0.001 *	-0.022	-0.053	0.009	-0.021	-0.048	0.006
Follow-up Time (months)	-0.004	-0.008	0.000	-0.002	-0.006	0.002	-0.004	-0.009	0.001
Cumulative PM _{2.5} × Time Interaction	0.0020	-0.0001	0.0042	0.0004	-0.0005	0.0014	0.0006	-0.0002	0.0014
Main Effect: Quartiles of Cumulative PM _{2.5} ¥									
Cumulative PM _{2.5} Quartile 4 vs. 1	-0.162	-0.299	-0.025 *	-0.123	-0.265	0.020	-0.118	-0.246	0.010
Cumulative PM _{2.5} Quartile 3 vs. 1	-0.175	-0.356	0.007	-0.018	-0.135	0.099	-0.001	-0.140	0.138
Cumulative PM _{2.5} Quartile 2 vs. 1	-0.159	-0.335	0.016	-0.0286	-0.177	0.121	-0.023	-0.131	0.084
Follow-up Time (months)	-0.012	-0.022	-0.002 *	-0.001	-0.003	0.001	-0.002	-0.004	0.001
Cumulative PM _{2.5} Quartile 4 × Time Interaction	0.013	0.002	0.023 *	0.002	-0.003	0.006	0.003	0.000	0.006
Cumulative PM _{2.5} Quartile 3 × Time Interaction	0.013	0.002	0.023 *	0.001	-0.002	0.003	0.0001	-0.0035	0.0036
Cumulative PM _{2.5} Quartile 2 × Time Interaction	0.009	-0.002	0.020	-0.0004	-0.0059	0.0052	0.001	-0.002	0.004

Note. Linear mixed effects models with random intercept. ¥. Models controlled for neutrophil %, lymphocyte %, monocyte %, eosinophil %, current smoking intensity (cigarettes per day), age at baseline blood draw (years), body mass index (BMI, log kg/m²), and years as a boilermaker (log years).

Asterisk indicates *p* values .05, considered statistically significant.