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Plasma fluorescent oxidation products and short-term occupational particulate exposures

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Abstract

Background—Evidence suggests that fine particulate air pollution results in oxidative induced tissue damage.

Methods—A global fluorescent oxidation products (FLOx) assay (fluorescent intensity (FI) units per milliliter of plasma) was measured in blood samples collected from 236 nonsmoking, Caucasian, male trucking industry workers either prior to, during, or after their work shifts. Occupational exposures to PM_{2.5} were based on job-specific area-level sampling. Generalized linear models were used to determine associations between FLOx levels and PM_{2.5}, adjusted for age, time since last meal, alcohol consumption, aspirin, and cholesterol medications.

Results—The mean (standard deviation) level of FLOx was 265.9 FI/ml (96.0). Levels of FLOx were higher among older individuals and lower among those who had consumed alcohol in the past 24 hours. However, no associations were observed between FLOx and PM_{2.5}.

Conclusions—Our results indicate no association between occupational PM_{2.5} exposure and this marker of global oxidative stress.

Keywords

vehicle emissions; biomarkers; oxidative stress; trucking industry; diesel

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Introduction

There is growing evidence that both short and long-term exposure to fine particulate air pollution (PM_{2.5}, particulate matter with a mean aerodynamic diameter of 2.5 microns) results in increased morbidity and mortality from cardiovascular disease. [Pope and Dockery 2006, Zanobetti and Schwartz 2005] The responsible constituents in air pollution are unknown but attention has focused on the importance of particles from diesel and other motor vehicle exhausts. [Hoek, et al. 2002, Janssen, et al. 2002, Laden, et al. 2000, Nafstad, et al. 2004, Peters, et al. 2000, Peters, et al. 2004, Zmirou, et al. 1998] Although the mechanisms and pathways of disease have not been fully elucidated, experimental evidence in animals and humans suggests that exposure to traffic-related particulate matter (PM) results in reactive oxygen species [Gonzalez-Flecha 2004, Laumbach and Kipen, Li, et al. 2003, Li, et al. 2003, Risom, et al. 2005] and systemic inflammation, possibly as a result of oxidative related damage to cells and cellular processes.[Bennet, et al. 2003, Cesari, et al. 2003, Peters, et al. 2001, Pope, et al. 2004, Pope, et al. 2004, Ruckerl, et al. 2007, van Eeden, et al. 2001, Yamagami, et al. 2004]

Though there are many proposed oxidation markers, we elected to utilize the fluorescent oxidation products (FLOx) assay. This assay has been demonstrated to be a stable and sensitive measure of oxidation products reflective of a variety of oxidative processes (e.g. DNA damage, lipid peroxidation).[Dillard and Tappel 1971, Frankel 1998, Iio and Yoden 1988, Liang 2000, Liang and Lin 2000, Mohanakumar, et al. 1994, Pryor 1980, Shimasaki 1994, Sziraki, et al. 1998, Wu, et al. 2004] As the multiple constituents found in occupational PM_{2.5} likely generate oxidative stress through multiple pathways, we hypothesized that FLOx, with its measurement of global oxidative stress may better reflect and/or have stronger detectable associations with occupational exposures than other more pathway-specific makers of oxidative stress.

In one Italian study of individuals 80 years of age or older, those participants in the highest quartile of FLOx had a multivariable adjusted relative risk of subsequent myocardial infarction, congestive heart failure, or ischemic stroke of 7.25 (95% CI: 2.40–21.89), compared to those in the lowest quartile. There was also a significant trend (p-value <0.001) for a greater risk of a cardiovascular event with increasing levels of FLOx. [Mezzetti, et al. 2001] In the Health Professionals Follow-up Study, baseline levels of FLOx were associated with subsequent coronary heart disease. Men in the highest quartile of FLOx had a multivariable adjusted relative risk of coronary heart disease of 1.84 (95% CI: 1.07–3.16) compared to men in the lowest quartile. [Wu, et al. 2007] Higher levels of FLOx in this study were also associated with traditional cardiovascular risk factors such as smoking and high blood pressure among individuals without coronary heart disease. [Wu, et al. 2007] To date, however, no studies have examined the association of FLOx with occupational exposures. We have the unique opportunity to examine the effects of occupational exposures on FLOx levels in a population of trucking industry employees with a variety of exposure levels.

Methods

Study Population

A total of 357 blood samples were collected from trucking industry employees at two large trucking industry workplaces, one in Carlisle, PA (March, 2007) and one in Sauk Village, IL (June, 2007). Phlebotomy was provided onsite 24 hours a day over 5 days, and workers were able to have blood drawn either prior to, during, or after their work shift. Each worker who provided a blood sample also completed a health questionnaire providing information on demographics, cigarette and pipe smoking, exercise habits, medication use, medical history,

and timing and details of the most recent meal and alcohol consumption. After each blood sample was collected, they were kept refrigerated at 4°C. At the end of each day of blood collection, all samples were packed in a cooler with ice packs and express shipped to the processing blood laboratory in Boston, MA. All samples were received and processed within 24 hours of collection and reached no more than 6°C during the shipping process. These methods have been previously used in studies such as the Nurses' Health Studies and Health Professionals' Follow-Up Study, and have been shown to be a robust method of obtaining blood samples [Hankinson, et al. 1989, Hankinson, et al. 1998]. Upon arrival at the blood processing laboratory the chilled blood samples were centrifuged in a refrigerated unit and blood components aliquoted into cryotubes. The cryotubes were then stored in the vapor phase of liquid nitrogen freezers at -130°C.

For this analysis, we restricted the population to the 236 nonsmoking, Caucasian male workers with sufficient blood volume for analysis of FLOx. The protocol was approved by the human subjects committees at the Brigham and Women's Hospital, the Harvard School of Public Health, and the Veterans Affairs Boston Healthcare System, and each participant provided informed consent before participating and received \$20 for their efforts.

Measurement of fluorescent oxidation products

Measurement of FLOx was developed originally by Dillard and Tappel in the 1970s [Dillard and Tappel 1971] and was later modified by Shimasaki.[Shimasaki 1994] These methods have been described in detail previously. [Wu, et al. 2004] Briefly, plasma was extracted with ethanol-ether (3/1,v/v) and was measured by a spectrofluorometer at a wave length of 360/430 (excitation/emission wavelength). The fluorescence was determined as relative fluorescent intensity (FI) units per milliliter of plasma.

Occupational Exposures

Each worker provided information on the job he was performing on the day of the blood draw, the time that his work shift began, and if he had worked on the previous day. Workers were from one of six job groups - long-haul drivers, city pick-up and delivery drivers, dockworkers, combination workers (who do the duties of pick-up and delivery drivers or dockworkers as needed), hostlers, and office workers. [Laden, et al. 2007, Smith, et al. 2006] Long-haul drivers deliver freight from one large trucking terminal to another using large diesel-powered tractor trailers, and are often away from home for up to three days. Pick-up and delivery drivers use diesel-powered tractor trailers to bring freight to and from customers in the surrounding area and the loading dock. Dockworkers move freight from one truck to another on the loading dock, primarily using propane-powered forklifts, while hostlers move trucks and trailers around in the terminal yards. Office workers (mostly clerks, managers, and salespeople) spend the majority of their work time in an office environment and have little occupational exposure to vehicle exhaust.

Onsite 24 hours a day during each terminal visit, we collected 12-hour integrated area-level occupational PM_{2.5} samples in the terminal yard, loading dock, office, and selected truck cabs. PM_{2.5} was measured by a method that gives results consistent with the EPA PQ200 Federal Reference Method.[Tainio, et al. 2005, Yanosky and MacIntosh 2001] Particles were collected at a flow rate of 3.1 L/min on a 37 mm Teflon filter, 0.2 µm diameter pore size, after passing through a precision machined cyclone separator (GK2.05 SH (KTL), BGI, Inc., Waltham, MA) to remove particles greater than 2.5 µm aerodynamic diameter. Mass collected on the filter was determined by gravimetric analysis using an analytical balance (Mettler Micro-Gravimetric No. M5; Mettler Instruments Corporation, Hightstown, NJ). The filters were pre-weighted after humidity equilibrium (temperature within 20–23°C and relative humidity 40±5% for at least 48 h) in a chamber. At the end of sampling, the filter

was taken back to the laboratory and reweighed after humidity equilibrium to determine weight gain.

We first determined if each worker had his blood drawn before, during, or after his work shift. Individuals providing a blood sample prior to starting work (n=40) were assigned an occupational PM_{2.5} exposure of 0. To determine the occupational PM_{2.5} exposure for each worker, we identified the area-specific PM_{2.5} sample(s) sampling during the time between the workers' shift start time and the time of blood draw. If only one PM_{2.5} sample was identified, then that was assumed to be the average level for the whole time the worker was on shift. In the case that the worker's shift was associated with two samples, we created a weighted average based on the total time exposed to each sample. For truck drivers (long-haul and pick-up and delivery), we did not have representative samples available for all of the necessary time periods. We previously found that driver exposures are directly proportional to exposures in the terminal yard and that the proportions are a function of specific driver type and ambient temperature (as a proxy for open cab windows in pick-up and delivery (P&D) drivers who drive trucks with no air conditioning). [Davis, et al. 2011, Davis, et al. 2007] Specifically, for long-haul drivers and hostlers we used a factor of 2.1, 1.7 for P&D drivers when the ambient temperature >50° Fahrenheit (>10° Celsius) and 1.6 for P&D drivers when the ambient temperature ≤ 50° Fahrenheit (≤ 10° Celsius). In addition to the estimated work-shift PM_{2.5} level, we examined other metrics of occupational exposure including hours worked prior to blood draw, total hours worked in the last seven days, work on the previous day (yes/no), and the average PM_{2.5} exposure level times the total minutes of exposure.

Statistical Methods

We used linear regression models to examine the association of occupational PM_{2.5} exposures with FLOx. As the distribution of FLOx is right-skewed, we also considered models with a log-transformation, and assessed the normality of the model residuals to determine if the log-transformation was appropriate. The residuals for the untransformed models were not statistically significantly different from linear, therefore we present the results from these models. We report regression coefficients and standard errors based on an 1 unit increase in occupational PM_{2.5} exposure.

We considered age, body mass index (BMI), hours of exercise outside of work in the past week, medication, alcohol use, and secondhand smoke exposure in the past 24 hours, educational level, former cigarette smoking status, work shift, and illnesses in the past month as potential confounders. Each potential confounder was first included in a univariate model to determine if it was a predictor of FLOx. All predictors that were statistically significant (p<0.05) were included in multivariable models (referred to as the "parsimonious models"). However, we also considered multivariable models including all potential confounders ("fully adjusted models").

Based on results from the Health Professionals Follow-Up Study, where effects were strongest in those men who had not eaten in more than eight hours, we considered potential effect modification by time since last meal and included it in all models as a potential confounder. We also considered effect modification by medication use, former smoking status, work shift (days versus all other shifts), and if the participant was a driver or not. As mentioned above, due to few representative samples in vehicle cabs, exposures for drivers were based on a ratio of the levels in the terminal yard. To examine effect modification, we created models stratified by each of the potential effect modifiers and also created interaction terms to determine statistical significance. All analyses were performed in SAS 9.2.

Results

The personal characteristics of the 236 Caucasian male nonsmoking workers are presented in Table I. Participants were 51.0 (SD=8.8) years old on average, had mean FLOx levels of 265.9 FI/ml (SD=96.0), and spent an average of 5 hours (range 0 to 17 hours) working prior to blood draw. Occupational PM_{2.5} levels varied from 0 to 87.0 µg/m³ with a median of 13.0 µg/m³ and an interquartile range of 21.5 µg/m³. Histograms of FLOx and PM_{2.5} are provided in the Supplemental Appendix. Most of the subject characteristics did not vary across the quartiles of FLOx, with the exception of a statistically significant positive association with age and use of aspirin or cholesterol drugs and a negative association with consumption of alcohol in the last 24 hours. These covariates were included in our “parsimonious multivariable models” in addition to time since last meal.

Occupational exposures to PM_{2.5} were not associated with changes in FLOx levels (Table II) in the full sample, but non-statistically significant elevations were observed. Other metrics of occupational exposures, including hours worked prior to blood draw, total hours worked in the last seven days, work on the previous day (yes/no), and the average PM_{2.5} exposure level times the total minutes of exposure were also not predictors of FLOx (Table II). Lower levels of FLOx were observed for workers who had worked more than 30 minutes prior to blood draw. There also was an indication that individuals who worked the day before had lower levels. There was no evidence of effect modification of the association of FLOx and occupational PM_{2.5} on the day of blood draw (Table III).

Discussion

Among this population of U.S. trucking industry employees, differences in occupational exposures to PM_{2.5} were not associated with FLOx. Although ours is the first study to examine the effects of short-term occupational PM_{2.5} exposures on FLOx, other studies have demonstrated associations of occupational and environmental pollution with a wide variety of other markers of oxidative stress, attributable to various oxidative pathways including F2-isoprostanes and malondialdehyde (MDA) (lipid oxidation), nitrotyrosine (protein oxidation), and 8-hydroxydeoxyguanosine (8-OHdG) (DNA oxidation). [Allen, et al. 2009, Bae, et al., Harri, et al. 2005, Kim, et al. 2004, Lai, et al. 2005, Loft, et al. 1999, Romieu, et al. 2008, Rossner, et al. 2007, Rossner, et al. 2008, Rossner, et al. 2008, Sorensen, et al. 2003, Vinzents, et al. 2005, Wei, et al. 2009]

Statistically significant increases in urinary MDA levels were observed with ambient PM concentrations from the current day to the 2 previous days in a study of school children in Seoul [Bae, et al.] and with same day levels of PM_{2.5} and ozone in Mexican school children with asthma [Romieu, et al. 2008]. In a controlled exposure study of ten subjects with metabolic syndrome, 2-hour exposures to diesel exhaust (200 µg/m³) were not associated with higher levels of urinary F2-isoprostanes compared to periods of exposure to filtered air. [Allen, et al. 2009] In a study of 50 bus drivers and 50 controls in the Czech Republic, levels of PM_{2.5} and PM₁₀ were associated with statistically significant increases in F2-isoprostane, 8-OHdG, and nitrotyrosine. [Rossner, et al. 2007] In further analyses, they determined that these associations varied seasonally and that the markers of oxidative stress were not associated with volatile organic compounds (VOCs) and only with polycyclic aromatic hydrocarbons (PAHs) during the summer period. [Rossner, et al. 2008, Rossner, et al. 2008] In 57 non-smoking bus drivers in Copenhagen, 8-OHdG excretion in urine was significantly greater in drivers from central areas compared to suburban areas. [Loft, et al. 1999] Toll collectors (n=47) in Taiwan had greater 8-OHdG levels collected post shift compared to unexposed workers. [Lai, et al. 2005] whereas in 29 garage and waste collection workers in Helsinki, levels were not elevated compared to office workers. [Harri, et al. 2005] In

contrast, in boilermakers, boiler overhaul exposure (measured as PM_{2.5}) was associated with an increase in pre-and post shift urinary 8-OHdG.[Kim, et al. 2004] Personal PM_{2.5} exposures were significantly related to 8-OHdG measured in lymphocyte DNA in 50 students living in Copenhagen.[Sorensen, et al. 2003] Ultrafine particle exposures in 15 individuals bicycling through central Copenhagen for five days were related to oxidized purines (mainly 8-OHdG) in mononuclear cells isolated from venous samples.[Vincentz, et al. 2005] In a study of security gate workers exposed to traffic, post-workshift 8-OHdG concentrations were significantly and positively associated with PM_{2.5} mass, PAHs, and metals.[Wei, et al. 2009]

One potential explanation for our null findings is that our predicted occupational PM_{2.5} was not based on personal sampling of each individual, and may be a crude proxy for actual occupational PM_{2.5} exposures. In previous exposure assessments in these companies, we have shown that there is a high correlation between area and personal levels of exposure, and that area levels are an important predictor of personal levels. [Davis, et al. 2007, Davis, et al. 2006, Smith, et al. 2006] However, nondifferential misclassification is still likely. Another explanation may be that there are likely differences in the mixture of components and particle size fractions between terminal locations and job groups and that these various components may have different effects on levels of oxidative stress assessed globally. Interestingly, FLOx was also not associated with exposures to secondhand smoke in the past 24 hours. Previously in this population we have observed that these questions on secondhand smoke were associated with personal exposure measurements of nicotine, [Chiu, et al. 2010] and also were suggestively associated with C-reactive protein, a marker of systematic inflammation in these same samples. [Chiu, et al. 2011]

This study has several other important limitations. Given our sample size of 236, we only had 10% power to detect an association as small as the one observed for occupational PM_{2.5} and FLOx, therefore it is not surprising that our findings were not statistically significant. [Dupont and Plummer 1997] Since FLOx is a global marker of oxidative stress from a variety of processes it may not be highly correlated with those processes most affected by exposures to pollution. Another limitation is that although recent consumption of foods high in antioxidants or oxidants, may be an important determinant of levels of FLOx, we did not have sufficient information on recent food intake to adjust for these factors. This limitation may partially explain why our results in the small sample of individuals who had not eaten in the last eight hours were stronger than those who had recently eaten. We were able to adjust for a variety of other potential predictors of FLOx levels, and few of these confounded our associations. In conclusion, we did not observe an association between occupational exposure to PM_{2.5} in the unionized trucking industry and a marker of global oxidative stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Characteristics of the study population (N=236) by quartile of fluorescent oxidation product. All members of the population were Caucasian, male, current nonsmokers.

Table 1

Characteristic	All	Q1 (101.4-192.1)	Q2 (192.2-252.6)	Q3 (252.7-318.2)	Q4 (318.3-654.3)	p-value
Mean ± SD						
Age (years)	51.0 ± 8.8	48.2 ± 8.3	50.5 ± 8.8	52.1 ± 10.7	53.3 ± 6.4	0.002
Body mass index (kg/m ²)	30.3 ± 5.2	31.1 ± 6.7	29.5 ± 4.1	29.9 ± 4.7	30.6 ± 4.8	0.97
Hours of exercise outside of work	3.3 ± 4.4	3.3 ± 3.5	4.2 ± 6.0	2.8 ± 4.5	3.0 ± 2.8	0.44
Total hours worked in last 7 days	44.4 ± 13.9	46.2 ± 12.7	44.1 ± 14.1	41.6 ± 14.8	45.6 ± 13.8	0.99
Hours worked prior to blood draw	5.0 ± 4.7	4.8 ± 4.2	5.6 ± 4.5	5.0 ± 5.3	4.7 ± 4.8	0.65
Occupational PM _{2.5} exposure (µg/m ³)	15.8 ± 17.4	12.9 ± 11.8	17.8 ± 16.1	16.9 ± 19.6	15.8 ± 20.7	0.61
Number (%)						
Provided blood prior to work shift	40 (17.0)	6 (10.2)	7 (11.9)	12 (20.3)	15 (25.4)	0.02
Worked the previous day	129 (54.7)	36 (61.0)	34 (57.6)	29 (49.2)	30 (50.9)	0.25
Work shift						
Day	70 (29.7)	17 (28.8)	21 (35.6)	20 (33.9)	12 (20.3)	
Evening	46 (19.5)	13 (22.0)	11 (18.6)	11 (18.6)	11 (18.6)	
Night	57 (24.2)	13 (22.0)	10 (17.0)	15 (32.2)	19 (32.2)	0.16
Rotating	55 (23.3)	15 (25.4)	15 (25.4)	13 (22.0)	12 (20.3)	
Other	8 (3.4)	1 (1.7)	2 (3.4)	0 (0.0)	5 (8.5)	
Education						
High school	151 (64.0)	37 (62.7)	40 (67.8)	36 (61.0)	38 (64.4)	0.99
Greater than high school	85 (36.0)	22 (37.3)	19 (32.2)	23 (39.0)	21 (35.6)	
Former smoker						
Secondhand smoke exposure in the last 24 hours	111 (47.0)	29 (49.2)	23 (39.0)	30 (50.9)	29 (49.2)	0.70
Consumed alcohol in the last 24 hours	82 (34.8)	25 (42.4)	19 (32.2)	20 (33.9)	18 (30.5)	0.27
Illness in the last month?	39 (16.5)	14 (23.7)	11 (18.6)	9 (15.3)	5 (8.5)	0.02
Medication use	50 (21.2)	10 (17.0)	13 (22.0)	16 (27.1)	11 (18.6)	0.99
Aspirin or aspirin products						
Cholesterol drugs	54 (22.9)	8 (13.6)	13 (22.0)	15 (25.4)	18 (30.5)	0.04
High blood pressure drugs	60 (25.4)	12 (20.3)	12 (20.3)	15 (25.4)	21 (35.6)	0.03
Hours between last meal and blood draw	56 (23.7)	13 (22.0)	13 (22.0)	12 (20.3)	18 (30.5)	0.22

Characteristic	All	Q1 (101.4–192.1)	Q2 (192.2–252.6)	Q3 (252.7–318.2)	Q4 (318.3–654.3)	p-value
<2	93 (39.4)	25 (42.4)	20 (33.9)	21 (35.6)	27 (45.8)	
2–4	84 (35.6)	19 (32.2)	27 (45.8)	20 (33.9)	18 (30.5)	
5–7	40 (17.0)	9 (15.3)	8 (13.6)	12 (20.3)	11 (18.6)	0.42
8–11	11 (4.7)	4 (6.8)	2 (3.4)	2 (3.4)	3 (5.1)	
12+	8 (3.4)	2 (3.4)	2 (3.4)	4 (6.8)	0 (0.0)	

Note: All p-values are from linear regression models of each factor with quantiles of FLOx

Percents are based on the number of participants in each quartile

Table II
Associations of the fluorescent oxidation products assay (FI/ml) with selected measures of occupational exposure

	Age Adjusted		Parsimoniously Adjusted ^a		Fully Adjusted ^b	
	β (SE)	p-value	β (SE)	p-value	β (SE)	p-value
Occupational PM _{2.5} (per $\mu\text{g}/\text{m}^3$)	-0.18 (0.35)	0.61	-0.18 (0.36)	0.63	-0.21 (0.38)	0.58
Occupational PM _{2.5} (per $\mu\text{g}/\text{m}^3$) ^c	0.54 (0.40)	0.18	0.50 (0.42)	0.24	0.53 (0.42)	0.21
More than 30 minutes of work before blood draw (yes/no)	-31.07 (13.16)	0.02	-27.32 (13.30)	0.04	-31.78 (14.17)	0.03
Hours of work prior to blood draw	-1.36 (1.31)	0.30	-0.02 (0.02)	0.36	-0.02 (0.02)	0.30
Average PM _{2.5} exposure $\times$ work duration ($\mu\text{g}/\text{m}^3$ –minutes)	2.18×10^{-5} (5.46×10^{-4})	0.97	3.25×10^{-5} (5.63×10^{-4})	0.95	-2.68×10^{-5} (5.83×10^{-4})	0.96
Total hours worked in the last week	-0.07 (0.44)	0.87	-0.11 (0.46)	0.81	-0.16 (0.49)	0.74
Worked on the day before blood draw	-21.92 (13.76)	0.11	-19.29 (13.81)	0.16	-20.52 (14.41)	0.16

^a Adjusted for age, use of aspirin or cholesterol drugs, consumption of alcohol in the last 24 hours, and hours since last meal

^b Additionally adjusted for BMI, work shift, secondhand smoke exposure in the last 24 hours, and hours of exercise outside work

^c Models only in workers with at least 30 minutes of work before blood draw

Table III

Potential effect modification of the association of the fluorescent oxidation products assay (FI/ml) with occupational PM_{2.5} exposures on the day of blood draw

Potential Effect Modifier	N	β (SE)	p-value for interaction
Aspirin			
Yes	54	-0.24 (0.71)	0.93
No	182	-0.16 (0.43)	
Cholesterol drug			
Yes	60	-0.42 (0.71)	0.66
No	176	-0.09 (0.44)	
HBP drug			
Yes	56	0.15 (0.78)	0.95
No	180	-0.43 (0.41)	
Former Smoker			
Yes	111	-0.18 (0.53)	0.83
No	125	-0.15 (0.52)	
Time of last meal			
0-7 hours	217	-0.21 (0.37)	0.32
8+ hours	19	-1.46 (1.49)	
Work as a driver			
Yes	132	-0.50 (0.59)	0.51
No	104	0.23 (0.48)	

Each model adjusted for age, use of aspirin or cholesterol drugs, consumption of alcohol in the last 24 hours, and hours since last meal as appropriate