Arterial Stiffness, Oxidative Stress, and Smoke Exposure in Wildland Firefighters

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Objectives *To assess the association between exposure, oxidative stress, symptoms, and cardiorespiratory function in wildland firefighters.*

Methods *We studied two Interagency Hotshot Crews with questionnaires, pulse wave analysis for arterial stiffness, spirometry, urinary 8-iso-prostaglandin F2α (8-isoprostane) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), and the smoke exposure marker (urinary levoglucosan). Arterial stiffness was assessed by examining levels of the aortic augmentation index, expressed as a percentage. An oxidative stress score comprising the average of z-scores created for 8-OHdG and 8-isoprostane was calculated.*

Results *Mean augmentation index % was higher for participants with higher oxidative stress scores after adjusting for smoking status. Specifically for every one unit increase in oxidative stress score the augmentation index % increased 10.5% (95% CI: 2.5, 18.5%). Higher mean lower respiratory symptom score was associated with lower percent predicted forced expiratory volume in one second/forced vital capacity.*

Conclusions *Biomarkers of oxidative stress may serve as indicators of arterial stiffness in wildland firefighters.* Am. J. Ind. Med. 57:748–756, 2014. © 2014 Wiley Periodicals, Inc.

KEY WORDS: *vascular stiffness; 8-iso-prostaglandin F2α; 8-hydroxy-2'-deoxyguanosine; spirometry; levoglucosan*

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BACKGROUND

Firefighters, both structural and wildland, are known to have cardiovascular and respiratory problems [Musk et al., 1979; Sardinas et al., 1986; Chia et al., 1990; Rothman et al., 1991; Guidotti and Clough, 1992; Liu et al., 1992; Materna et al., 1992; Scannell and Balmes, 1995; Betchley et al., 1997; Austin et al., 2001; Burgess et al., 2001; Kales et al., 2003; Slaughter et al., 2004; CDC, 2006; Gaughan et al., 2008; Yoo and Franke, 2009]. Cardiovascular disease (CVD) events are the leading cause of on-duty and lifetime mortality among structural (career and volunteer) firefighters [Sardinas et al., 1986; Kales et al., 2003; Yoo and Franke, 2009]. The deleterious effects of smoke exposure to structural firefighters have been extensively researched [Musk et al., 1979; Sardinas et al., 1986; Chia et al., 1990; Guidotti and Clough, 1992; Liu et al., 1992; Scannell and Balmes, 1995; Burgess et al., 2001; Kales et al., 2003;

CDC, 2006; Yoo and Franke, 2009]. Exposure to particulates and other contaminants, heavy physical exertion and cardiovascular strain have been found to be among the chief health hazards associated with structural firefighting [Gledhill and Jamnik, 1992; Takeyama et al., 2005; Delfino et al., 2009]. Those findings, however, may not be generalizable to wildland firefighters for a number of reasons, including the difference in smoke composition, comparative younger age of wildland firefighters, often shorter career tenure, and the longer duration of respiratory exposures for wildland firefighters. Additionally, structural firefighters routinely wear respiratory protection when responding to fires while wildland firefighters do not.

Fine particulate exposure has been associated with acute changes in cardiovascular and pulmonary function [Vallyathan et al., 1995; Mott et al., 2005; Dominici et al., 2006; Cavallari et al., 2008; Fang et al., 2008, 2009]. Free radical mechanisms have been implicated as a contributing factor in general toxicity, inflammation, asthma, fibrogenesis, bronchopulmonary carcinogenesis, and atherosclerotic plaque formation [Jarjour and Calhoun, 1994; Leonard et al., 2007; LeBlanc et al., 2010].

Wood fires produce smoke with abundant particles in the inhalable range ($<100 \, \mu m$) and contain both carbon radicals and precursors. The latter are able to react with H_2O_2 after exposure to cells and generate the highly reactive hydroxyl radical (OH) from a Fenton-like reaction [Leonard et al., 2007]. The authors additionally observed that carbon radicals were stronger (per unit mass) in larger (coarse) sized particles while OH and other ROS were stronger (per unit mass) in the smaller (ultrafine) sized particles. Finally, the authors noted that fine and ultrafine woodsmoke particles also significantly increased H_2O_2, DNA strand breaks and lipid peroxidation in exposed RAW 264.7 cells.

Pyrolysis of organic components may increase the potency and or toxicity of particulate by producing ROS. Measurement of levoglucosan, a sugar anhydride by-product of incomplete combustion of cellulose, may be used to indicate relative exposure to products of pyrolysis from burning biomass [Simoneit et al., 1999]. A recent study examined personable exposure to airborne respirable levoglucosan using a Dorr-Oliver cyclone and air sampling pump, and cross-shift changes in lung function in a population of 17 wildland firefighters for 4 days at a large wildland fire [Gaughan et al., 2014]. The authors reported that levoglucosan was found mainly in the respirable fraction, defined as under $2.5 \, \mu m$, with higher concentrations during fireline construction than in mop-up operations. Furthermore, larger cross-shift declines in forced expiratory volume in one second (FEV_1) were associated with exposure to higher concentrations of respirable levoglucosan (*P*-value <0.05).

Urinary levoglucosan has also been investigated as a biomarker for smoke exposure. Bergauff et al. examined cross-shift changes in urinary levoglucosan in nine firefighters exposed to wood smoke in a controlled setting. They observed elevated urinary levoglucosan following smoke exposure in some but not all firefighters [Bergauff et al., 2010]. Moreover, the authors noted the contribution of dietary intake to urinary levoglucosan levels.

Urinary 8-iso-prostaglandin F2α (8-isoprostane), a biomarker of oxidative stress generated by lipid peroxidation, may serve as a biomarker for atherosclerosis [Cipollone et al., 2000]. Oxidative deoxyribonucleic acid (DNA) damage and repair has also been linked to atherosclerosis. Martinet et al. examined five human carotid endarterectomy specimens and five mammary artery specimens for 8-hydroxy-2'-deoxyguanosine (8-OHdG), an oxidized nucleoside of DNA. The authors observed an increased amount of 8-OHdG in plaques compared to the underlying media or non-atherosclerotic mammary arteries [Martinet et al., 2002]. Urinary 8-OHdG is excreted upon DNA repair and may serve as a non-invasive biomarker of global oxidative DNA damage. For instance, acute changes in urinary 8-OHdG concentrations have been reported in occupational studies examining welders [Kim et al., 2004; Nuernberg et al., 2008].

Elevated arterial stiffness is a characteristic of large artery pathology, a major contributor to CVD, and may serve as an indicator of pre-clinical atherosclerosis and/or hypertension [Blankenhorn and Kramsch, 1989; Duprez and Cohn, 2007]. Assessment of arterial stiffness is done through ultrasound or measurement of pulse wave velocity. The aortic augmentation index, is an indirect measure of systemic arterial stiffness based on pulse wave velocity and is calculated as a percentage. A recent meta-analysis demonstrated that a 10% increase in augmentation index % was associated with a 31.8% increased risk of cardiovascular events and a 34.8% increased risk of total mortality [Vlachopoulos et al., 2010]. The augmentation index % has been successfully implemented in occupational research settings [Nürnberger et al., 2002; Fang et al., 2008].

There are four types of wildland firefighter suppression crews: engine crew, hand crew, helicopter crew, and smoke jumpers. Type 1 Interagency Hotshot Crews are an elite type of hand crew, comprising up to 20 firefighters who construct fire lines using hand tools during the most dangerous phases of fire suppression.

The question addressed by the present study was whether wildland firefighting exposures are associated with oxidative stress concentrations and with pulmonary and vascular function. To answer these questions, we assessed spirometry, vascular function, symptoms, and systemic biomarkers of exposure, inflammation and oxidative stress in members of two Type 1 wildland firefighter Interagency Hotshot Crews.

METHODS

The National Interagency Fire Center (NIFC) Risk Management Committee arranged for two crews to

participate in this study: the Alpine Interagency Hotshot Crew, Rocky Mountain National Park, Estes Park, CO and the Pike Interagency Hotshot Crews, Pike and San Isabel National Forests, Monument, CO. The Interagency Hotshot Crews participated in our study by completing questionnaires, pulse wave analysis and spirometry in May 2011. Serum cholesterol, and biomarkers of systemic inflammation (high sensitivity c-reactive protein (hsCRP) and fibrinogen), oxidative stress (8-isoprostane), oxidative DNA damage (8-OHdG), and smoke exposure (urinary levoglucosan) were measured. The study protocol was approved by the Harvard School of Public Health (HSPH) Institutional Review Board and the National Institute for Occupational Safety and Health (NIOSH) Human Subjects Review Board. Informed consent was obtained from each research participant.

Medical Testing Methods

Pulse wave analysis

Vascular function was measured using a pulse wave analysis system according to the manufacturer's instructions [SphygmoCor CP, Atcor Medical Pty Ltd., Sydney, Australia]. Briefly, participants were seated with the dominant arm extended onto a flat surface so that the antecubital fossa was at heart level. Following 5 min of rest, a high-fidelity micro-nanometer was used to flatten the radial artery with gentle pressure. Ten seconds of sequential pulse pressure waveforms were recorded at each reading. The waveforms were then transformed into a corresponding central aortic waveform via a validated transfer function where the systolic part of the central aortic waveform is characterized by a first peak caused by left ventricular ejection and a second peak caused by wave reflection. The difference between the two peaks reflects the degree to which the central aortic pressure is augmented by wave reflection. We calculated each participant's aortic augmentation index %, defined as the ratio of augmented pressure to pulse pressure (i.e., augmentation index % = augmented pressure/pulse pressure × 100) [Nürnberger et al., 2002] and heart rate corrected to 75 beats per minute. Larger augmentation index % values denote increased wave reflection. A minimum of three within-session recordings were obtained from each participant.

Spirometry

Pulmonary function was determined on each participant using an ultrasonic flow spirometer [EasyOne™ Diagnostic Spirometry System 2001, ndd Medical Technologies, Zurich, Switzerland]. Technicians completed a NIOSH-approved spirometry course followed American Thoracic Society (ATS) guidelines [Miller et al., 2005]. Test results were interpreted using reference values generated from the Third National Health and Nutrition Examination Survey (NHANES III) [Hankinson et al., 1999]. Airways obstruction was defined as a forced expiratory volume in the first second (FEV_1) to forced vital capacity (FVC) ratio below the lower limit of normal according to published reference equations [Pellegrino et al., 2005]. We examined percent predicted FEV_1 (FEV_1%-predicted), percent predicted FVC (FVC %-predicted), and percent predicted FEV_1/FVC (FEV_1/FVC %-predicted). We followed ATS procedure by inquiring about current medications but did not ask participants to abstain from using their medications prior to participating in this study for safety purposes. Reports were reviewed for quality by a respiratory physiologist (C.R.O) experienced in clinical pulmonary function laboratory administration.

Blood

Whole non-fasting serum samples (30 ml/sample) were collected by venous phlebotomy in EDTA tubes, and buffy coat was extracted and stored in cell lyses solution at $-20°C$ for analyses of typical cardiovascular-related biomarkers, specifically, hsCRP and fibrinogen. We additionally examined total cholesterol, high-density lipoprotein cholesterol (HDL-cholesterol), low-density lipoprotein cholesterol (LDL-cholesterol), and triglycerides. These analyses were conducted by Quest Diagnostics Inc., Denver, CO.

Urine

Urine samples were analyzed for 8-OHDG and 8-isoprostane using competitive enzyme-linked immunoassays (EKS-350, Assay Designs, Inc., Ann Arbor, MI; 8-Isoprostane EIA Kit, Cayman Chemical Company, Ann Arbor, MI) as well as for creatinine (picric acid colorimetric assay; Oxford Biomedical Research, Oxford, MI). We also examined urine for levoglucosan concentration. Two hundred microliter of urine or levoglucosan standards (Blank, 6.25–100 μg/ml in saline) were added to 1.5 ml low retention microcentrifuge tubes. To this 30U of urease (Sigma, St. Louis, MO) was added and incubated for 1.0 hr at 37°C. After incubation, 600 μl of cold (4°C) ethanol was added to precipitate the protein. Approximately 400 mg of sodium sulfate was added volumetrically and allowed to sit for 2.0 min. Samples and standards were then centrifuged for 2.0 min at 14,000g. After centrifugation, 600 μl of each were transferred to clean low retention microcentrifuge tubes and evaporated to dryness using a vacuum centrifugation and gentle heat. Two hundred microliter of n-methyl-n-(trimethylsilyl) trifluoroacet (MSTFA) (Sigma Chemical Co.,) was then added to each tube, vortexed and incubated at 72°C for 1.0 hr. After derivatization samples and standards were analyzed on an Agilent 6890 gas chromatograph coupled to an Agilent 5975C mass spectrometer using a 30-m HP5-MS column (Agilent Technologies, Santa Clara, CA). Samples were injected (1 μl) in splitless mode into a 250°C inlet with a

6.0-min solvent delay. Analytes were eluted from the column using 1.0 ml/min helium and an oven temperature program as follows: 70°C for 3.0 min and then ramped at 25.0°C/min to a final temperature of 275°C. The MS source temperature was maintained at 230°C, and the quadrupole temperature was maintained at 150°C. Ions were scanned between 50 and 400 m/z. Levoglucosan from each sample was identified by the MS spectra and retention time (against the known standards) and quantified using the 204 m/z ion. The standard plot from which the samples were extrapolated used a polynomial curve fit of 204 m/z area under the curve ion count versus standard concentration. Specimens were analyzed by NIOSH's Health Effects Laboratory Division, Allergy and Clinical Immunology Branch, Morgantown, WV.

Questionnaires

The questionnaire was based on two standardized questionnaires, the American Thoracic Society-Division of Lung Disease-78 (ATS-DLD-78) supplemented with questions from NHANES III [Ferris, 1978; Wasserfallen et al., 1997]. This modified questionnaire was designed to acquire information concerning chronic cardiovascular and respiratory conditions; lifetime diagnoses; tobacco history; symptom history; dietary intake; sleep patterns; medication use; and occupational history. A validated symptom scale, with Likert scoring where $0 = $ none, $1 = $ trivial, $2 = $ mild, $3 = $ moderate, and $4 = $ severe for upper and lower airways symptoms, was used to derive overall symptom scores by summing the responses to questions about 19 symptoms. Symptoms ascertained included cough, wheeze, sputum production, shortness of breath or chest tightness, and shortness of breath while walking, as well as various eye, nose, and throat symptoms. Subjects additionally completed a semi-quantitative food frequency questionnaire, adult version, 2007. Subjects were asked to report the average daily consumption of various foods in the preceding year. Responses ranged from "never" to "six or more servings per day." The food frequency questionnaire also assessed the frequency of multivitamin and mineral supplement usage. Frequency factors of related foods items were then summed to calculate the daily servings for each food group. The nutrient value of the food item was multiplied by the frequency of consumption in order to obtain macro and micro nutrient intake. Scoring was done by the Nutrition Department, Harvard School of Public Health (https://regepi.bwh. harvard.edu/health/nutrition.html) [Willett et al., 1985].

Statistical Methods

Descriptive statistics were calculated for demographic and clinical variables. Mean values among subgroups were compared using Student's *t*-test techniques. Ordinary least squares regression techniques were used to examine associations between augmentation index %, FEV_1%-predicted, FVC %-predicted, FEV_1/FVC %-predicted, 8-OHdG concentration, and 8-isoprostane concentration using the following predictor variables: levoglucosan concentration, serum lipid levels, hsCRP concentration, fibrinogen concentration, LDL-cholesterol, HDL-cholesterol, Interagency Hotshot Crew, volunteer firefighter (yes or no), cumulative time spent fighting fires (seasons), medical diagnoses, allergies, upper and lower respiratory symptom scores, and history of tobacco use. Levoglucosan, 8-isoprostane, 8-OHdG, and hsCRP values were not normally distributed and thus the data were log_{10}-transformed to achieve an approximate normal distribution. We additionally calculated an "oxidative stress score" comprising z-score's of 8-isoprostane and 8-OHdG, where z-value's for each variable were calculated and then averaged to yield a score for each participant. The motivation for calculating the z-score was prompted by the possibility that while 8-isoprostane is considered a marker of lipid peroxidation and 8-OHdG has been linked to DNA damage and repair, they may both be measuring the overall level of oxidative stress in the urine. Thus, we standardized the two biomarkers of oxidative stress on one scale. These values were also examined in multivariable analysis. The z-value was calculated by subtracting the mean value from each subject's value and then dividing by the standard deviation of the values. Multivariable models were chosen based on an initial evaluation using stepwise selection techniques, followed by examining univariate associations and sequentially adjusting for other predictors. Associations were considered statistically significant if *P*-values were <0.05 and borderline significant at ≥ 0.05 and <0.10. All analyses were conducted using SAS statistical software (version 9.3).

RESULTS

Thirty-eight of the 39 current members of both crews participated in the surveys in May 2011 during their training sessions at their respective home base parks (97%). One member was training off-site the day of our survey and as a result, could not participate. Members of the Pike Interagency Hotshot Crew had been exposed to smoke for 2 days at the Sand Gulch fire 4 days before the testing. The Sand Gulch fire in Wetmore, CO, was a 495-acre wildland fire at 7,000 feet elevation in high difficulty terrain. Fire behavior activity was described by crew members as high/extreme on the first day and low/smoldering on the second. It was reported that the average shift duration each day was 16 hr and it was estimated the crew members were on the fire line performing firefighting activities for 12.5 hr each day.

Participant demographic and clinical characteristics, overall and stratified by crew are shown in Tables I and II. The two crews were very similar. They were all male with a median age of 28 years and had spent a median of 3 years (seasons) working as an Interagency Hotshot Crew member.

TABLE I. Demographic Characteristics of Interagency Hotshot Crew Members

Variable	All (N = 38)	Alpine interagency hotshot crew (n = 18)	Pike interagency hotshot crew (n = 20)
Age (years)[a]	29 (4.34)	29.9 (5.44)	28.2 (2.97)
Time spent as a firefighter (years)[a]	3.65 (3.38)	4.12 (3.89)	3.25 (2.92)
Male %	100	100	100
White, non-Hispanic n, %	34 (90)	15 (83)	19 (95)
Current smoker n, %	2 (5)	1 (6)	1(5)
Former smoker n, %	10 (27)	2 (11)	9 (45)
Current chewing tobacco (yes vs. no) n, %	16 (42)	7 (39)	9 (45)
Volunteer firefighter n, %	6 (16)	5 (28)	1 (5)
Permanent employee n, %	11 (29)	5 (28)	6 (30)

[a]Mean value (standard deviation).

Approximately 5% of the participants were current smokers and nearly half (42%) reported current chewing tobacco use. Approximately one-third (29%) of the participants were classified as "permanent" employees, the remainder were "seasonal" hires. Additionally, six of the participants (16%) reported working as a volunteer structural firefighter off-season. Values for clinical characteristics were also comparable when examined by crew and within the normal range.

Seven participants reported a history of physician-diagnosed asthma (18%). The median age at asthma

TABLE II. Clinical Characteristics of Interagency Hotshot Crew Members

Variable	All (N = 38)	Alpine interagency hotshot crew (n = 18)	Pike interagency hotshot crew (n = 20)
Augmentation index % (adjusted for heart rate to 75 BPM)[a]	5 (13)	10 (11)	1.0 (13)[b]
Hypertension (ever) n, %	3 (8)	2 (11)	1 (5)
Elevated cholesterol (ever) n, %	4 (10)	3 (17)	1 (5)
Total cholesterol (mg/dl)[a]	170 (35.8)	175 (40.1)	164 (28.4)
HDL-cholesterol (mg/dl)[a]	55.3 (10.6)	54.6 (11.4)	56.25 (9.81)
LDL-cholesterol (mg/dl)[a]	93.8 (28.1)	101 (32.1)	82.9 (16.5)
Triglycerides (mg/dl)[a]	109 (61.4)	97.6 (39.1)	125.8 (84.0)
HsCRP (mg/L)[a]	1.25 (1.95)	1.23 (2.11)	1.28 (1.78)
Fibrinogen (mg/dl)[a]	270.2 (60.2)	295 (41.2)	221 (64.3)
Log_{10} 8-isoprostane (ng/ml)[a]	−0.14 (0.42)	−0.20 (0.41)	−0.08 (0.42)
Log_{10} 8-OHdG (ng/ml)[a,c]	0.75 (0.36)	0.61 (0.39)	0.88 (0.29)[b]
Oxidative stress score[a,d]	0.41 (0.45)	0.48 (0.45)	0.34 (0.45)
Log_{10} levoglucosan (μg/ml)[a]	1.17 (0.31)	1.14 (0.25)	1.20 (0.37)
Asthma (ever) n, %	7 (18)	6 (33)	1 (5)
Allergies (ever) n, %	17 (45)	11 (61)	6 (30)
Upper respiratory symptom score[a,e]	7.63 (7.1)	7.28 (7.11)	7.95 (7.21)
Lower respiratory symptom score[a,f]	2.34 (3.87)	1.56 (2.52)	3.05 (4.73)
Pulmonary function[a]			
FEV_1%-predicted	103 (10.2)	101 (10.1)	104 (10.5)
FVC%-predicted	107 (13.0)	104 (11.4)	111 (13.7)
FEV_1/FVC	95.6 (9.14)	97.1 (9.80)	94.3 (8.53)

[a]Mean value and standard deviation.
[b]Significantly different from Alpine Interagency Hotshot Crew at P-value <0.05.
[c]8-hydroxy-2'-deoxyguanosine (8-OHdG).
[d]Derived from the average of z-scores for 8-isoprostane and 8-hydroxy-2'-deoxyguanosine(8-OHdG).
[e]Range 0–24; higher score denotes more frequent symptoms.
[f]Range 0–19; higher score denotes more frequent symptoms.

TABLE III. Predictors of Arterial Stiffness (Augmentation Index %), Linear Regression Estimates and 95% CIs

Variable[a]	Unadjusted	Adjusted[b]
Smoking history (ever)	11.2 (2.81, 19.7)	11.5 (3.73, 19.3)
Oxidative stress score[c]	10.2 (1.35, 19.0)	10.5 (2.51, 18.5)

[a]Age, chewing tobacco status, HDL-cholesterol, LDL-cholesterol, triglycerides, high sensitivity CRP, fibrinogen, allergy history, lower respiratory symptom score, upper respiratory symptom score, Interagency Hotshot Crew, FEV_1%-predicted, FVC%-predicted, and FEV_1/FVC%-predicted were not significantly associated with mean augmentation index % values.
[b]Estimates controlling for all other specified variables in the Table III.
[c]Average z-scores of log_{10} values of 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-isoprostane.

diagnosis was 7 years and the median time spent as a firefighter for these seven participants was 2 years (seasons). Among these individuals, the median FEV_1%-predicted was 105%, the median FVC %-predicted was 109%, and the median FEV_1/FVC %-predicted was 96%. Among the four individuals with current asthma, three were currently taking medication for their asthma. One of the seven participants with a history of asthma also reported a smoking history.

Four values for levoglucosan were below the limit of detection and were not included in the analysis. Detectable levels were observed for all values of 8-OHdG, 8-isoprostane, hsCRP, and fibrinogen.

In multivariable analysis, mean augmentation index % was higher for participants with higher oxidative stress scores. Specifically, for every one unit increase in oxidative stress score, mean augmentation index % increased 10.2% (10.2%, 95% CI: 1.35, 19.0%) (Table III). This association remained significant after adjusting for smoking status ($P = 0.01$). No other variables were associated with augmentation index %, including our exposure variable, levoglucosan. Table III details these results.

However, higher levoglucosan concentration was positively associated with oxidative stress scores (Table IV). Specifically, for every twofold increase in log_{10} levoglucosan concentration, mean 8-OHdG increased by log_{10} 0.14 μg/ml (95% CI: 0.02, 0.25) (regression estimate 0.41 (95% CI: 0.04, 0.79)) and 8-isoprostane increased by log_{10} 0.16 ng/ml (95% CI: 0.02, 0.29) (regression estimate 0.52 (95% CI: 0.06,

0.97)). Additionally, 8-OHdG values for participants who had recently been exposed to smoke particulate as measured by Interagency Hotshot Crew were higher than those with no recent exposure ($P = 0.01$). This association became borderline significant after adjusting for levoglucosan ($P = 0.07$) (Table IV).

Finally, higher lower respiratory symptom score (LRSS) was associated with lower FEV_1/FVC %-predicted after adjusting for smoking status and history of asthma. Specifically, for every twofold increase in mean LRSS, the mean FEV_1/FVC %-predicted dropped on average by 1.66% (95% CI: 0.10%, 3.22%) (regression estimate: −0.83 (95% CI: −1.61, −0.05)). No other variables were associated with FEV_1/FVC %-predicted, most notably, neither oxidative stress score nor levoglucosan.

DISCUSSION

Particulate exposure has been associated with increased arterial stiffness in occupational cohorts. Fang et al. examined changes in augmentation index % in 26 welders over 24 hr on a welding day and non-welding day. Following welding fume exposure, the authors observed an increase in afternoon augmentation index % and a decrease in next morning augmentation index % [Fang et al., 2008]. The results suggested that exposure to welding fume particulate is associated with acute adverse vascular responses. In our homogenous group of healthy workers, we observed higher

TABLE IV. Predictors of Oxidative DNA Damage and Repair (8-OHdG[a]), Linear Regression Estimates and 95% CIs

Log_{10} 8-OHdG[a,b]	Unadjusted	Adjusted[c]
Log_{10} levoglucosan[d] concentration	0.45 (0.07, 0.84)	0.41 (0.04, 0.79)
Interagency Hotshot Crew	0.27 (0.05, 0.50)	0.21 (−0.02, 0.45)

[a]8-hydroxy-2'-deoxyguanosine (8-OHdG).
[b]Smoking/chewing tobacco status, hsCRP, HDL-cholesterol, LDL-C, triglycerides, fibrinogen, asthma history, allergy history, lower respiratory symptom score, upper respiratory symptom score, FEV_1%-predicted FVC%-predicted, and FEV_1/FVC%-predicted, and age were not significantly associated with mean log_{10} 8-hydroxy-2'-deoxyguanosine (8-OHdG) values.
[c]Estimates controlling for all other specified variables in the table.
[d]Levoglucosan (LG).

augmentation index % values for participants with higher oxidative stress values which were also associated with larger levoglucosan values. A possible explanation for this finding could be that our population was younger by comparison (28 vs. 41 years) and/or our assessment occurred 4 days post-exposure to smoke.

Similar to other studies, we observed an association between cross-shift differences in oxidative DNA damage and recent occupational exposure to particulate matter. Kim et al. obtained 5 days of cross-shift urinary 8-OHdG measurements from 20 welders exposed to metal fumes. The authors reported that urinary 8-OHdG levels were significantly elevated in post-shift samples compared to those collected pre-shift [Kim et al., 2004]. Nuernberg et al. [2008] observed similar 8-OHdG elevation post-shift in 63 welders. Moreover, the authors found an unexpected inverse relationship between post-shift and 8 hr post-shift values of 8-isoprostane and $PM_{2.5}$ ($P < 0.05$). We additionally observed a difference in 8-isoprostane by levoglucosan concentration. We did not observe a difference in mean values of 8-OHdG or 8-isoprostane between participants who identified themselves as having a history of smoking and those who did not ($P = 0.51$ and 0.64, respectively).

Levoglucosan was positively associated with both biomarkers of oxidative stress, 8-OHdG and 8-isoprostane. Urinary levoglucosan has been shown to increase in mice after exposure to wood smoke [Migliaccio et al., 2009]. However, when examining urinary levoglucosan concentration, it is important to control for dietary confounders such as smoked or fried foods and caramel. Based on responses to the Food Frequency Questionnaire, 32 (84%) reported consuming two slices of bacon or less once per week; 34 (89%) reported eating one candy bar or less per week. All participants reported eating fried foods at home or away four to six times per week or less, although 22 (58%) reported consuming fried foods less than once per week. Inhaled levoglucosan is probably completely eliminated from the body within 24 hr post-exposure. Thus, it is possible that the elevated levels of levoglucosan we observed were caused by diet, rather than exposure.

There is evidence that particulate matter [PM] produced by woodsmoke fires may be more toxic than PM from ambient air due to the chemical components found in the smoke [Wegesser et al., 2009]. Leonard et al. [2007] examined aerodynamically size-selected aerosol samples at a large wildland fire. The authors found smoke particles in all size fractions [from smaller than $0.056\,\mu m$ to greater than $10\,\mu m$] and a bell-shaped distribution with highest overall mass fraction on filters in the fine range. The authors additionally observed that radical signals were size dependent and resulted in reactive oxygen species (ROS) generation.

Recent animal studies suggest that exposure to ultra-fine particles may additionally augment cardiac dysfunction through an ROS mechanism [LeBlanc et al., 2010]. LeBlanc et al. recently demonstrated that local ROS generation can influence vascular reactivity in coronary arterioles. It follows that excessive coronary ROS generation following pulmonary woodsmoke exposure could impair endothelium-dependent arteriolar reactivity.

Seven participants reported a history of asthma; four with current asthma. Similar asthma prevalences were previously observed in this population [Gaughan et al., 2008]. We did not see a difference in %-predicted lung function values in these subjects from the rest nor did we observe an association between oxidative stress score or levoglucosan and a history of asthma. However, we did observe that higher LRSS were associated with lower FEV_1/FVC %-predicted after adjusting for asthma history and smoking status ($P < 0.05$).

We recognize several limitations to our study. Our sample size was small and the resultant lack of statistical power may have hindered our ability to observe associations if they did exist. While we did observe a significant association between higher oxidative stress scores with recent firefighting activities, the generally qualitative and self-reported nature of exposure characterization in our study may have limited our ability to have identified statistically significant cardiopulmonary effects related to firefighting exposures. Additionally, this was a cross-sectional study with essentially no control group. This design limited examination of acute changes. Our study also lacked cross-shift data during periods in which the crews were working but not fighting a fire. Future studies should examine these individuals cross-shift at a wildland fire and when they are not being exposed to smoke aerosol to ascertain whether any observed changes are part of their normal variation. However, a recent study did not find significant changes in cross-shift lung function on burn days compared to non-burn days [Adetona et al., 2011]. Also, urinary levoglucosan may be more representative of diet than as an indicator of wood-smoke exposure. Finally, all spirometric values were obtained pre-bronchodilator. Post-bronchodilator values would have allowed us to examine reversibility and may have been particularly informative for participants reporting a history of asthma.

This is the first study to examine the association between systemic oxidative stress and arterial stiffness in wildland firefighters. Future studies should additionally examine endothelial function which is governed by smooth muscle tone, collage/elastin, calcification, and other factors that affect the entire arterial wall. The two together could provide different but complementary information on vascular health.

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