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Measured pulmonary and systemic markers of inflammation and oxidative stress following wildland firefighter simulations

Matthew D. Ferguson, PhD,

University of Montana, Center for Environmental Health Sciences

Erin O. Semmens, PhD,

University of Montana, School of Public and Community Health Sciences

Charles Dumke, PhD,

University of Montana, Department of Health and Human Performance

John C. Quindry, PhD, and

Auburn University, Department of Kinesiology

Tony J. Ward, PhD

University of Montana, School of Public and Community Health Sciences

Abstract

Objective—A controlled human exposure study was conducted to investigate the impact of inhalational exposures to wood smoke PM_{2.5} on measured concentrations of airway and systemic inflammatory biomarkers.

Methods—Mimicking wildland firefighter activities, 10 participants were exposed to three doses of wood smoke PM_{2.5} (filtered-air, 250 µg/m³, and 500 µg/m³) while exercising on a treadmill. Exhaled breath condensate (EBC) and blood plasma samples were obtained pre-, immediately post-, and 1-hour post-exposure. 8-isoprostane, pH, and myeloperoxidase were measured in EBC while H₂O₂, surfactant protein D, and pentraxin-3 (PTX3) were measured in both EBC and plasma.

Results—Only pH, 8-isoprostane, and PTX3 displayed significant changes when comparing pre- and post- exposures.

Conclusions—Markers of inflammation and oxidative stress, including PTX3, pH, and 8-isoprostane in EBC and/or plasma, are sensitive to wood smoke inhalation, with further investigations warranted.

INTRODUCTION

A common source of fine particulate matter (PM_{2.5}) in the western US during the summer months is wildland forest fires.¹ In 2014 alone, the US had 63,612 wildfires and 17,044

Corresponding Author: Tony Ward, University of Montana, 32 Campus Drive, Skaggs Building Room 176, Missoula, MT 59812, Phone: (406) 243-4092, Fax: (406) 243-4209, tony.ward@umontana.edu.

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prescribed burns with a combined burned area of about 6 million acres, leading the US Department of Interior and the US Forest Service to spend approximately \$1.5 billion on suppression efforts.² In response to wildland fires increasing in both frequency and intensity in recent years,^{3,4} the US Bureau of Labor Statistics estimates that the firefighter employment numbers will also continue to increase in the years to come.⁵

Wildfire smoke PM_{2.5} inhalational exposures are an occupational hazard for wildland firefighters. Firefighters can be exposed to PM_{2.5} concentrations ranging from 500–600 µg/m³ in a single work-day,^{6,7} with acute smoke exposures exceeding 6,000 µg/m³.⁷ Preliminary findings from field studies have shown that wood smoke PM_{2.5} exposures are associated with acutely diminished lung function,^{8–11} mucosa irritation,¹² pulmonary inflammation,¹³ systemic inflammation,^{14–16} and oxidative stress.¹⁷ However, there is much to be learned about the impact of wildfire smoke on human health, especially the impacts on wildland firefighters.

Field studies involving wildland firefighters are difficult to successfully carry out for a variety of reasons, including costs, logistics, and safety of research personnel. Therefore, controlled exposure studies within the laboratory are necessary because they provide an opportunity to study the effect of wood smoke PM_{2.5} on biomarkers of pulmonary and respiratory health outcomes with high precision. Biological samples may be useful in both laboratory- and field-based investigations to explore indicators of respiratory inflammation and oxidative stress. For example, exhaled breath condensate (EBC) can provide information on biomarkers such as pH, nitrite, H₂O₂, 8-isoprostane, and a variety of cytokines that are sensitive to acute wood smoke inhalation.^{18–24} Pentraxin-3 (PTX3), a plasma biomarker, is produced following inflammatory stimuli,^{25,26} likely due to PTX3's role in immune cell functioning and its ability to recognize danger-associated molecular patterns. Case in point, PTX3 is an emerging biomarker that, when elevated, is sensitive to acute changes in vascular function.²⁷ In other studies, measures of 8-isoprostane and H₂O₂ are altered in EBC or plasma as a function of oxidative stress.^{28–31} As such, elevation of these biomarkers in tissues associate with vascular dysfunction, including instances of passive smoke exposure.³²

The purpose of this study was to identify the human health risks of wood smoke inhalation in a PM_{2.5} dose response manner. By mimicking occupational exposures encountered by wildland firefighters in a controlled environment, we present the results of a study that investigated the airway and systemic inflammatory effects (in both EBC and plasma) induced by inhaling wood smoke PM_{2.5}. Based on the results of previous studies,^{18–32} we measured biomarkers including 8-isoprostane, myeloperoxidase and pH from EBC samples, and provide a direct comparison of H₂O₂, PTX3, and surfactant protein D (SP-D) concentrations from both EBC and plasma samples.

METHODS

The Center for Environmental Health Science's Inhalational and Pulmonary Physiology Core at the University of Montana was utilized for the controlled wood smoke human exposure studies. Ten healthy males were exposed to three different conditions, including [1]

filtered-air (control), [2] 250 $\mu\text{g}/\text{m}^3$ wood smoke, and [3] 500 $\mu\text{g}/\text{m}^3$. Each wood smoke exposure trial was 90 minutes, occurring every 7 days (allowing for a 1 week washout period between exposures) over three consecutive weeks. Also, each exposure (including challenges and control) occurred in the morning while fasted. All parameters for the filtered-air (control) exposures were identical to the 250 $\mu\text{g}/\text{m}^3$ and 500 $\mu\text{g}/\text{m}^3$ exposures, with the exception of $\text{PM}_{2.5}$ concentrations directed to the mask.

For each participant, the exposure trials were assigned using a randomized cross-over design. Participants exercised at an absolute intensity (3.5 mph at 5.7 %) designed to mimic wildland firefighting activities for the 90 minutes of exposure. The treadmill speed and incline associate to an average energy expenditure of 22.5 ml/kg/min (oxygen consumption), which is the general level at which wildland firefighters work for extended periods. Blood and EBC were collected pre-exposure, immediately post-exposure, and 1-hour post-exposure for each of the 10 participants.

Participants

Inclusion criteria included a VO_2 max ≥ 40 ml/kg/min, be a nonsmoker, and have no current wood smoke exposure within their homes (e.g., wood stoves). Prior to data collection this project was approved by the University of Montana's Institutional Review Board, and informed consent was obtained prior to participant testing. Before participating in the trials, all study participants completed a personal readiness questionnaire (PARQ) that ensured no pre-existing health conditions, and also underwent rigorous physical tests to ensure the needed level of physical fitness. Throughout the duration of each exposure trial, individuals were asked about their perceived stress, and were allowed to discontinue their participation at any time.

Exposure facility

Wood smoke was generated in a wood stove (Englander, England Stove Works, Inc., Monroe, VA). The wood species used in this study was western larch (*Larix occidentalis* Nutt.) with a moisture content of 15%. During each exposure trial, smoke was delivered through a mixing and dilution chamber prior to reaching a nasal and mouth breathing facemask (Hans Rudolph, Inc., 8900 Series, Shawnee, Kansas) worn by the participant and equipped with a two-way non-rebreathing T-valve (Hans Rudolph, Inc., T-Shape™ Valve, Shawnee, Kansas). All exposures occurred while exercising (3.5 mph at 5.7%) on a treadmill (Model Q65, Quinton Instrument Company, Bothell, WA). $\text{PM}_{2.5}$ concentrations were measured at the mask using a continuous $\text{PM}_{2.5}$ monitor (DustTrak, TSI, Model 8530, Shoreview, MN). Another continuous $\text{PM}_{2.5}$ monitor (DustTrak TSI, Model 8533, Shoreview, MN), located at the dilution chamber, simultaneously measured $\text{PM}_{2.5}$ and PM_{10} . Throughout exposures, carbon monoxide (CO) was also continuously monitored and recorded at the mask using a Q-Trak (TSI, Model 7565, Shoreview, MN). Across all trials, the temperature of the filtered air and smoke delivered to the mask was maintained at room temperature (22 °C).

Biological sampling

Blood samples and EBC were collected before the exposure trials (pre-exposure), immediate post-exposure, and 1-hour post-exposure. EBC was collected using an R-tube breath condensate collector (Respiratory Research Inc., Charlottesville, VA) consisting of a pre-cooled aluminum sleeve placed over the outside of a disposable vertical condensation/collection tube and connected to a two-way non-rebreathing valve and a mouthpiece. Participants breathed into the tube for 10 minutes while holding the tube in an upright position. Blood samples were collected simultaneous to EBC. Whole blood was obtained by venipuncture in the antecubital space and collected into a sodium heparin vacutainer (Becton Dickinson, Franklin Lakes, NJ). Whole blood samples were centrifuged at 6000g at 4°C for 10 minutes to yield plasma. Plasma and EBC samples were then aliquoted and stored at -30° and -80° C, respectively until analysis.

EBC measures

A micro pH electrode (Lazar Research Laboratories Inc., model PHR-146B, Los Angeles, CA) coupled to a digital pH meter (Jenco, Model 60, San Diego, CA) was used to measure pH. Prior to these measurements, ultrapure argon gas was bubbled (de-aeration) through each EBC sample for 90 seconds. All measurements took place within a 2-hour window while samples were stored on ice. Commercially made enzyme-linked immunosorbent assays (ELISAs) were used to measure 8-isoprostane (Cayman Chemical Company, Ann Arbor, MI) and myeloperoxidase (R&D Systems, CAT# DMYE00, Minneapolis, MN).

Plasma and EBC assays

H₂O₂ levels were assessed in colorimetric assays (Life Tech, Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit, Cat# A22188, Carlsbad, CA) and optical density (O.D.) values were divided up into tertiles. PTX3 was also measured in both EBC and plasma using commercially made ELISA kits (R&D Systems, CAT# DPTX30, Minneapolis, MN). For SP-D (R&D Systems, CAT# DSFPD0, Minneapolis, MN) experimental reactions, ELISA kit manufacturer instructions were optimized.

Statistical approach

We report mean (standard deviation: sd) pre-exposure, immediate post-exposure, and one-hour post-exposure biomarker concentrations for each exposure condition. The primary objective of our statistical analyses was to evaluate if the relative change in each biomarker concentration from pre- to post-exposure (or pre- to one-hour post-exposure) varied by wood smoke exposure. Relative change was calculated in the following manner: [(post-exposure concentration minus pre-exposure concentration) divided by pre-exposure concentration] multiplied by 100. Due to our small sample size, the presence of outliers, and the skewed distribution of most biomarkers, our analyses investigating the relationship between exposure condition and relative change in biomarker concentration utilized the Skillings-Mack test, a nonparametric analog to repeated measures analysis of variance. Dunnett's post-hoc comparisons were also used to observe potential immediate post- or 1-hour post-exposure differences from filtered-air conditions. We also estimated Spearman correlation coefficients between biomarker concentrations measured in both EBC and plasma (H₂O₂,

SF-D, and PTX3). Results were classified as statistically significant when $P \leq 0.05$. All analyses were performed using Prism (GraphPad, v.5.0a) or Stata (StataCorp, v.9) software.

RESULTS

Across all trials, the average CO concentrations from filtered-air, 250 $\mu\text{g}/\text{m}^3$, and 500 $\mu\text{g}/\text{m}^3$ exposures were $0.003 (\pm 0.007)$ ppm, $0.87 (\pm 0.28)$ ppm, and $1.87 (\pm 0.65)$ ppm, respectively, below the Occupational Safety & Health Administration's Permissible Exposure Limit (PEL) of 35 ppm. $\text{PM}_{2.5}$ concentrations from filtered-air, 250 $\mu\text{g}/\text{m}^3$, and 500 $\mu\text{g}/\text{m}^3$ exposures averaged $5.2 (\pm 4.9)$ $\mu\text{g}/\text{m}^3$, $253.9 (\pm 5.8)$ $\mu\text{g}/\text{m}^3$, and $506.2 (\pm 4.8)$ $\mu\text{g}/\text{m}^3$, respectively. Preliminary data showed that >99% of $\text{PM}_{2.5}$ mass measured in the dilution chamber resulted from the PM_1 size fraction (data not shown). Means and normalized means (relative change) for all EBC and plasma measures (8-isoprostane, PTX3, SP-D, MPO, pH, and H_2O_2) are outlined in Table 1.

The relative change in pre- to post-exposure (or pre- to one-hour post-exposure) EBC 8-isoprostane concentrations did not vary significantly by exposure condition (Table 1). Therefore Figure 1 presents the percent changes in EBC 8-isoprostane for filtered air compared to the combined wood smoke exposures (250 and 500 $\mu\text{g}/\text{m}^3$ exposures), for both the immediate post and 1-hour post time points. The relative change from pre- to immediate post-exposure 8-isoprostane levels in EBC following wood smoke exposure were significantly lower than the relative change following filter air exposure. Wood smoke resulted in significantly higher 8-isoprostane than filtered-air at 1-hour post-exposure (Figure 1).

No significant differences in relative change in EBC MPO (Figure 2) or EBC and plasma H_2O_2 (Figure 3) concentration by exposure condition were found, although there appeared to be a dose-response curve for the MPO. When investigating the pH measures in the EBC samples, the decrease in the 500 $\mu\text{g}/\text{m}^3$ wood smoke post-exposures was significantly lower (Dunnett's test; $p < 0.05$) than filtered-air post-exposures (Figure 4).

As seen in Table 1 and Figure 5, relative changes in PTX3 in EBC were greater than those observed in plasma. However, no significant effect of exposure concentration on relative change in EBC levels of PTX3 was observed (though there does appear to be a dose-response for the 1-hr post measures). In contrast, plasma levels of PTX3 were significantly different between all three levels of exposures at both immediate ($p = 0.048$) and 1-hour ($p = 0.012$) post-exposure (see Table 1). Also, multiple comparison analyses (Dunnett's Test) showed that 1-hour post-exposure plasma levels of PTX3 were significantly higher ($p < 0.05$) for both 250 $\mu\text{g}/\text{m}^3$ and 500 $\mu\text{g}/\text{m}^3$ wood smoke exposures, when compared to the same filtered-air time points.

As seen in Figure 6, relative changes in SP-D concentrations measured in EBC did not differ by exposure condition, even when 250 $\mu\text{g}/\text{m}^3$ and 500 $\mu\text{g}/\text{m}^3$ wood smoke exposures were combined (a similar strategy was used in presenting the results of the 8-isoprostane EBC findings in Figure 1). Relative changes in plasma levels at 1-hour post did result in significant differences between the three exposure conditions (Table 1), though overall

trends were not consistent with a normal dose-response relationship. Consistent with other markers, EBC changes were greater than those in plasma.

In analyses examining correlations between H₂O₂, SF-D, and PTX3 measured in EBC and the same biomarkers measured in plasma, we observed little evidence of correlations between markers in EBC and plasma (results not shown). We did see a highly significant relationship between pre-exposure PTX3 in EBC and PTX3 measured in plasma immediately post- ($r = 0.89$; $P = 0.001$) and one-hour post-exposure ($r = 0.84$; $P = 0.005$) when PM_{2.5} was extremely low. However, this relationship did not persist under medium or high exposures.

DISCUSSION

The current laboratory investigation was designed to mimic wildland firefighting scenarios and provide a controlled venue to quantify a comprehensive biomarker panel of oxidative stress and inflammatory markers from EBC and blood plasma. The assembled biomarker panel is of strategic importance to understanding workplace air pollution exposure in that the air-lung interface is the boundary between the biological environment and the ambient environment. Foreign substances deposited at this boundary can elicit natural responses that can lead to pulmonary^{33,34} and systemic^{35,36} measures of inflammation and oxidative stress. Specific markers can be linked to acute and chronic conditions, and offer useful insights into clinical risks. In this study, we report the findings from EBC and plasma measures following human exposures to filtered-air, 250 µg/m³ wood smoke PM_{2.5}, and 500 µg/m³ wood smoke PM_{2.5} concentrations while exercising on a treadmill for 90 minutes.

One of the key advantages of the currently described laboratory approach is the ability to replicate “real-world” exposures within a controlled environment. In this regard, measured CO and PM_{2.5} concentrations in these exposure trials were consistent with (and representative of) field studies where comparable CO and PM levels were recorded during wildland firefighting activities.⁶ Though within the expected physiologic range,³⁷ exposure to PM_{2.5} in our investigation resulted in marked elevations in many of the biomarkers within the panel (Table 1). Specifically, the main findings from this investigation are that markers of inflammation and oxidative stress, including PTX3, pH, and 8-isoprostane levels in EBC and/or plasma, are sensitive to wood smoke inhalation.

8-isoprostane concentrations (see Figure 1) in EBC did not increase immediately following wood smoke exposures, but were elevated 1-hour post exposure. In a similar controlled wood smoke exposure study, Riddervold et al.¹⁸ reported similar EBC results as our filtered-air samples. In contrast, 8-isoprostane levels decreased in all their wood smoke exposures with the exception of their high (400 µg/m³) wood smoke exposure. Importantly, the time points used in the Riddervold et al. study¹⁸ were 3.5 hours and 6 hours post-exposure, which are hours later than the time points used in the present study and may support a delayed onset response as observed currently.

In other studies, urinary 8-isoprostane were observed to be elevated following specific exposures, including occupational exposures to welding emissions,³⁸ chronic wood smoke

exposures from cookstoves,³⁹ and wood smoke exposures in wildland firefighters.⁴⁰ Collectively, these studies suggest that particulate exposure, including wood smoke, increase 8-isoprostane concentrations in body tissues including urine. The results in our study also suggest that 8-isoprostane levels in EBC are affected by wood smoke inhalation during physical activity that mimics wildland firefighting.

The levels of MPO observed in EBC following wood smoke exposures resulted in a dose-response relationship (see Figure 2) that was numerically compelling, but didn't achieve statistical significance. To our knowledge this is the first investigation of wood smoke exposure during exercise to include MPO as part of the biomarker panel. Much like SP-D, the peroxidase enzyme MPO contributes to impaired microbial activity. As applied to this investigation, demarginated neutrophils will phagocytose microorganisms followed by a burst of highly reactive oxygen species mediated by MPO.⁴¹ This enzyme is itself an antimicrobial system in which intercellular activity directs MPO and associated reactive oxygen species to the phagosome.⁴² Relative to these experiments, extracellular release of MPO also occurs during phagocytosis or when the cell is attempting to phagocytose particulate.⁴² Based on this well defined understanding of neutrophil function, our results suggest that immune cells in the lung were likely phagocytosing wood smoke particles, resulting in the release of MPO into the lining of the lung. Due to the apparent, but non-significant, dose-response relationship of MPO following wood smoke exposures, it is possible that these cells are actively releasing MPO into the lung resulting in increased levels in the EBC. Given the consistent, but non-significant findings observed currently, additional post exercise/exposure time points are recommended with the intent of observing the full time course of potential MPO-based responses.

In contrast to several other redox sensitive biomarkers investigated in this study, H₂O₂ was apparently unaltered by exercise/exposure in both EBC and plasma samples (see Figure 3). Studies measuring exhaled levels of H₂O₂ report increased concentrations in smokers,⁴³ increased levels in patients with COPD,^{44–46} and even higher H₂O₂ in exacerbated COPD when compared to stable COPD.⁴⁷ H₂O₂ levels in EBC did not increase in a cohort of school children exposed to ambient levels (>20 µg/m³) of wood smoke,⁴⁸ though these levels were much lower than those used in the present study. Plasma levels of H₂O₂ correlate with hypertension cases, including those who are predisposed to hypertension.⁴⁹ The above referenced studies showing increases of H₂O₂ report on chronic underlying conditions. The exposures used in this study may not have resulted in robust changes in H₂O₂ because of the acute nature of the study design and because the study participants were of relative good health and suggest that cumulative dose is important. In support, our results show the most pronounced, but non-significant, increase in H₂O₂ in the 500 µg/m³ exposure at 1-hour post exercise/exposure. It is possible that compensatory antioxidant activity in the lung was overwhelmed leading to release of H₂O₂, although this conclusion cannot be supported currently. Future studies should include expansion of the biomarker panel to antioxidant activity measures (e.g., superoxide dismutase, glutathione) to better resolve the matter.

Importantly, redox and inflammatory stress measures often associate with changes in pH. Findings from the current study indicate that there were significant decreases in pH following wood smoke exposures (see Figure 4). In support, previous studies report

comparable drops in EBC pH concomitant to airway inflammation. In studies evaluating impacts of acute lung injury or disease on acidity, decreases in pH were caused by, or associated with inflammation.^{50–52} In a study exposing humans to filtered-air, 200 $\mu\text{g}/\text{m}^3$, and 400 $\mu\text{g}/\text{m}^3$ wood smoke concentrations for 3.5 hours, “estimated change from baseline” pH levels in EBC increased in all measures except the elevated exposure 6-hours post-exposure.¹⁸ Importantly, the Riddervold et al.¹⁸ participants were at rest throughout the entire duration of exposure. The similarity of exposure levels between Riddervold et al. and the present study suggests that other factors besides exposure concentrations may influence these disparate outcomes. The most obvious differences between these two studies is duration of exposure (3.5 hours in the Riddervold et al. study and 1.5 hours in our study) and the exercise component of our study. Future studies should consider controlling for exertion to further determine how breathing rates and cardiovascular activity influence these biomarker outcomes in a dose response fashion.

As described above, markers of inflammation were among the key dependent variables examined currently. Examples of commonly measured pentraxins include C-reactive protein (CRP) and serum amyloid P (SAP). These short chain pentraxins recognize specific pathogen-associated molecular patterns (fungi, yeasts, bacteria, etc.) and play an important role in the innate immune system. Pentraxin elevation in body tissues are associated with elevated risk for cardiovascular and other diseases promoted by inflammation.^{53–54} The long chain pentraxin, PTX3, is emerging as an important acute phase protein as concentrations can increase rapidly in response to infections and inflammation.⁵⁵ For instance, PTX3 appears to be a sensitive marker of vascular damage,⁵⁶ sepsis,⁵⁷ and inflammation.^{58,59} Little is known how PTX3 responds to acute wood smoke exposures, prompting inclusion in the current study.

Results from this study showed that PTX3 increased in a concise dose-response manner (in both the EBC and plasma), with significant effects observed in plasma (see Figure 5). The similar trends between PTX3 and MPO (in EBC) might partly be explained by neutrophil release of PTX3 following inflammatory events.^{60,61} Findings from prior studies suggest that neutrophils release PTX3 into neutrophil extracellular traps further targeting pathogens.⁶² The resulting significant differences between filtered-air and both wood smoke 1-hour post-exposures supports previous studies suggesting PTX3 as an acute phase protein.⁵⁵ Taken together, these results suggest that PTX3 is an acute phase protein with sensitivity to the concentration of inhaled wood smoke.

Filtered-air and the wood smoke exposures resulted in a rise of SP-D in the EBC, though these results are not significant (see Figure 6). The synthesis of SP-D generally occurs in all mucosal tissues as part of the innate immune system and plays a significant role in host defense.^{63,64} SP-D protein has co-locates with IgA in the process of pathogenic clearance and impairment of microbial colonization.^{64,65} SP-D also binds to endotoxin,⁶⁶ reducing potential adverse effects from particle exposure as endotoxin contributes to pro-inflammatory outcomes.⁶⁷ Similar to our findings, Stockfelt et al.⁶⁸ measured SP-D levels in plasma following human exposures to wood smoke (i.e., 3-hour sedentary exposures to filtered-air, 146 $\mu\text{g}/\text{m}^3$, and 295 $\mu\text{g}/\text{m}^3$) with no significant increases observed.

Limitations of study

The present study was designed to specifically mimic wildland firefighting activities, including moderate exercise within a controlled setting. Although the CO and PM_{2.5} wood smoke exposures were consistent with field research studies where CO and PM concentrations were recorded during wildland firefighting activities,⁶ there were several limitations for which the reader should be made aware. Notably, the smoke generated in the Inhalation Facility resulted from a wood stove, and was not “real-world” forest fire smoke. In addition, the exposure times were relatively short (1.5 hours) compared to wildland fire fighters who are exposed to much longer durations. Also, the cumulative smoke dose may have been lower compared to an accumulation of a full day of work fighting wildfires or the acute exposures they may experience (upwards of 6,000 µg/m³).⁷ These limitations should be fully considered in follow up investigations.

CONCLUSIONS

As wildfires are predicted to increase across the western US in the foreseeable future, so will the potential for occupational wood smoke exposures to wildland firefighters. Importantly, carrying out wood smoke exposure studies in the field poses numerous logistical, financial, and safety challenges. Therefore, conducting controlled smoke exposure studies is critically important, as it provides the opportunity to investigate specific health endpoints with the goal of preventing acute and chronic health effects experienced during occupational firefighting activities.

The goal of this study was to investigate the airway and systemic inflammatory effects induced following wood smoke inhalation. The main findings of our study were that: (1) MPO levels in EBC showed a non-significant increase in a dose-response manner following wood smoke inhalation; (2) significant reductions in pH occurred following wood smoke exposures; (3) PTX3 increased in a dose-response manner in both EBC and plasma (note that significant effects were observed in plasma, suggesting its potential as a sensitive marker of inflammation following wood smoke exposure); and (4) SP-D levels in EBC and plasma tended to be higher following wood smoke exposures when compared to filtered-air samples, though no significance was observed.

This study is novel in that it incorporated low- to moderate- intensity exercise into the exposure trials to mimic occupational exertion by wildland firefighting. Exposures with an exercise component resulted in higher breathing and heart rates that increased particle deposition and blood flow through the lung. To our knowledge, this is also the first controlled human wood smoke study that examined H₂O₂, PTX3, and SP-D concentrations from both EBC and plasma samples. In general, these biomarkers resulted in a greater magnitude of changes in EBC, but similar pattern of responses were found in plasma. Future studies should investigate these EBC and plasma biomarkers measured during actual wildfire events to validate the use of controlled exposure studies to predict adverse health outcomes from “real world” occupational exposures. This would also include trying to better understand the additional influences of high intensity exercise and heat stress on systemic inflammation.

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Figure 1. Relative change of 8-isoprostane in EBC as a function of exposure concentration. Dunnett's comparisons were performed comparing the combined wood smoke exposures to their respective filtered-air timepoints (*, $p < 0.05$).

Figure 2.
Relative change of MPO in EBC as a function of exposure concentration. No significant comparisons (Dunnett's; $p < 0.05$) were observed.

Figure 3. Relative change of H₂O₂ in EBC (top) and plasma (bottom) as a function of exposure concentration. No significant comparisons (Dunnett's; $p < 0.05$) were observed.

Figure 4. Relative change of pH in EBC as a function of exposure concentration. Dunnett's comparisons were performed comparing wood smoke exposures to their respective filtered-air timepoints (*, $p < 0.05$).

Figure 5. Relative change of PTX3 in EBC (top) and plasma (bottom) as a function of exposure concentration. Dunnett's comparisons were performed comparing wood smoke exposures to their respective filtered-air timepoints (*, $p < 0.05$).

Figure 6. Wood smoke effects on SP-D levels in EBC (top) and plasma (bottom) as a function of exposure concentration. No significant comparisons (Dunnett's; $p < 0.05$) were observed.

Table 1

Average pre-exposure, post-exposure, and 1-hour post-exposure values, as well as relative change (%) for all measures, and their standard deviations.

Biomarker	Exp	n ^a	Relative Change ^b				
			pre mean (sd)	post mean (sd)	1-hr post mean (sd)	post-pre mean (sd), %	p-value ^c
8-isoprostane (EBC) pg/mL	A	9	0.917 (0.421)	1.512 (1.227)	0.701 (0.353)	88.9 (151.8)	–6.5 (67.2)
	B	10	0.651 (0.357)	0.588 (0.248)	0.894 (1.009)	–3.3 (36.1)	72.2 (221.7)
	C	10	0.654 (0.233)	0.689 (0.279)	0.838 (0.411)	4.6 (16.4)	31.5 (63.6)
PTX3 (EBC) ng/mL	A	9	0.051 (0.073)	0.069 (0.112)	0.032 (0.035)	279.9 (619.0)	261.9 (705.9)
	B	10	0.047 (0.050)	0.059 (0.065)	0.072 (0.100)	167.5 (556.7)	425.6 (949.6)
	C	10	0.033 (0.038)	0.048 (0.064)	0.079 (0.137)	135.6 (324.6)	1372.3 (4065.4)
SP-D (EBC) ng/mL	A	9	0.016 (0.012)	0.019 (0.017)	0.027 (0.030)	11.4 (31.8)	45.1 (69.4)
	B	10	0.030 (0.049)	0.044 (0.067)	0.019 (0.024)	56.8 (141.0)	5.6 (39.0)
	C	10	0.029 (0.042)	0.021 (0.019)	0.039 (0.044)	15.3 (81.4)	109.3 (183.4)
MPO (EBC) ng/mL	A	8	0.016 (0.015)	0.021 (0.026)	0.018 (0.019)	18.5 (66.8)	–5.3 (69.9)
	B	10	0.052 (0.111)	0.023 (0.024)	0.021 (0.029)	106.3 (297.8)	110.7 (238.5)
	C	10	0.058 (0.112)	0.027 (0.023)	0.030 (0.030)	303.2 (599.3)	430.3 (655.7)
H ₂ O ₂ (EBC) µM	A	9	0.729 (0.771)	0.542 (0.663)	0.537 (0.534)	607.0 (1398.6)	654.8 (1995.7)
	B	10	0.214 (0.206)	0.814 (1.397)	0.469 (0.465)	761.3 (1628.9)	350.4 (928.5)
	C	10	0.548 (0.561)	0.470 (0.543)	0.738 (0.983)	199.1 (466.6)	1603.5 (4054.2)
pH (EBC)	A	9	7.849 (0.342)	7.842 (0.372)	7.803 (0.359)	–0.1 (1.1)	–0.6 (1.3)
	B	10	7.968 (0.435)	7.878 (0.381)	7.828 (0.347)	–1.1 (1.8)	–0.9 (1.8)
	C	10	7.969 (0.329)	7.873 (0.287)	7.864 (0.304)	–1.2 (1.6)	–1.3 (1.4)
PTX3 (plasma) ng/mL	A	9	0.756 (0.472)	0.695 (0.392)	0.794 (0.388)	9.0 (74.8)	25.7 (76.7)
	B	10	0.590 (0.315)	0.704 (0.305)	0.807 (0.355)	33.2 (43.9)	49.5 (71.3)
	C	10	0.528 (0.328)	0.661 (0.331)	1.205 (1.643)	57.5 (92.4)	207.1 (498.5)
SP-D (plasma) ng/mL	A	9	4.777 (1.522)	4.487 (1.252)	4.610 (1.418)	–4.6 (8.8)	–3.1 (7.8)
						0.070	0.022

Biomarker	Exp	n ^a	Relative Change ^b					
			pre	post	1-hr post	post-pre	1-hr post-pre	p-value ^c
			mean (sd)	mean (sd)	mean (sd)	mean (sd), %	mean (sd), %	p-value ^c
H ₂ O ₂ (plasma) µM	B	10	4.343 (1.127)	4.875 (1.488)	5.068 (1.817)	12.2 (15.7)	14.5 (14.3)	
	C	10	4.668 (1.468)	4.410 (1.280)	4.616 (1.299)	-3.2 (15.5)	1.1 (16.6)	
	A	9	19.65 (8.24)	29.65 (31.50)	22.20 (14.22)	67.3 (161.7)	23.8 (75.6)	
	B	10	21.68 (14.78)	18.44 (11.35)	17.71 (7.80)	5.1 (88.5)	-11.4 (32.3)	0.73
	C	10	18.83 (4.67)	18.06 (18.06)	34.75 (60.36)	1.6 (52.7)	77.5 (299.9)	

Abbreviations: Exp, exposure (A, filtered-air; B, 250 µg/m³; C, 500 µg/m³); pre, sample obtained immediately prior to trial; post, sample obtained immediately after completion of trial; 1-hr post, sample obtained one hour after completion of trial; EBC, exhaled breath condensate; SD, ± standard deviation.

^aDue to one subject dropping out of the study, only 9 subjects participated in the filter-air exposures. Due to an analytical error, only 8 EBC samples were analyzed for MPO.

^b $((\text{Post-trial minus pre-trial})/\text{pre-trial}) \times 100$

^cSkellings-Mack tests to determine if relative change from pre-trial measurement varied significantly by exposure condition.