Journal of Breath Research

PAPER

Characterization of exhaled breath condensate (EBC) non-exchangeable hydrogen functional types and lung function of wildland firefighters

To cite this article: Jordan Nelson *et al* 2020 *J. Breath Res.* **14** 046010

Journal of Breath Research

CrossMark

RECEIVED
1 May 2020

REVISED
25 August 2020

ACCEPTED FOR PUBLICATION
10 September 2020

PUBLISHED
24 September 2020

PAPER

Characterization of exhaled breath condensate (EBC) non-exchangeable hydrogen functional types and lung function of wildland firefighters

Jordan Nelson[1], **Marie-Cecile G Chalbot**[1,2], **Zoran Pavicevic**[3] **and Ilias G Kavouras**[1,4,5]

[1] Department of Environmental Health Sciences, Ryals School of Public Health, University of Alabama at Birmingham, Birmingham, AL 35219, United States of America
[2] Department of Biology, College of Technology, City University of New York, Brooklyn 12222, United States of America
[3] Nursing Science Program, College of Graduate Science, University of Tennessee Health Sciences Center, Memphis, TN 38163, United States of America
[4] Department of Environmental, Occupational and Geospatial Health Sciences, CUNY Graduate School of Public Health and Health Policy, New York, NY 10027, United States of America

E-mail: ilias.kavouras@sph.cuny.edu

Keywords: NMR spectroscopy, metabolic profile, smoke inhalation, lung function, inflammation biomarkers

Supplementary material for this article is available online

Abstract

Inhalation of smoke is shown to be associated with adverse respiratory outcomes in firefighters. Due to invasiveness of procedures to obtain airways lining fluid, the immediate responses of the target organ (i.e. lung) are secondarily assessed through biomarkers in blood and urine. The objective of this study was to identify changes in metabolic profile of exhaled breath condensate (EBC) and lung function of firefighters exposed to wildfires smoke. A total of 29 subjects were studied over 16 events; 14 of these subjects provided cross-shift EBC samples. The predominant types of non-exchangeable hydrogen in EBC were saturated oxygenated hydrogen, aliphatic alkyl and allylic. Non-exchangeable allylic and oxygenated hydrogen concentrations decreased in post-exposure EBC samples. Longer exposures were correlated with increased abundance of oxidized carbon in ketones, acids and esters. Post-exposure lung function declines (forced expiratory volume in 1 s (FEV_1): 0.08 l, forced vital capacity (FVC): 0.07 l, FEV_1/FVC: 0.03 l, peak expiratory flow (PEF): 0.39 l s^{-1}) indicated airways inflammation. They were related to exposure intensity (FEV_1 and FVC) and exposure duration (PEF). This study showed that EBC characterization of non-exchangeable hydrogen types by NMR may provide insights on EBC molecular compositions in response to smoke inhalation and facilitate targeted analysis to identify specific biomarkers.

1. Introduction

Wildland firefighters work to suppress thousands of wildfires each year within the United States [1]. During these events, firefighters are regularly exposed to biomass smoke containing hundreds of potentially health hazardous air pollutants including gas and particle-phase air contaminants such as benzene, carbon dioxide, carbon monoxide, formaldehyde, polycyclic aromatic hydrocarbons, and particulate matter [2,3]. Conversely, during wildfire events, firefighters rarely utilize respiratory protection. Rather, a towel or bandana over firefighter's mouth is often the only form of personal protection utilized [3–6]. Smoke inhalation is considered the most important risk factor for acute and chronic disease development [7]. Acute health studies monitoring lung function across the work shift and season have been conducted. Among these studies, wildland firefighters are observed having respiratory health declines as a result of exposure to occupational biomass smoke. Gaughan *et al* reported cross-shift declines in lung function associated with elevated levels of levoglucosan, a trace marker of vegetation combustion (biomass smoke) utilized as a marker

[5] Author to whom any correspondence should be addressed.

of exposure [8]. Further, this effect was observed to last throughout the duration of the season. In particular, cross-season declines in lung function and increases in airway responsiveness post-season were observed among California wildland firefighters [9]. Over a career, these occupational exposures to wildfire smoke are estimated to increase the risk of developing cancer. A recent study estimated that wildland firefighters' exposure to respirable wildfire smoke may result in a 8%–43% increased risk for lung cancer that increases with career length [10]. This has similarly been observed among structural firefighters, where the relationship between lung cancer and cumulative exposure is nearly linear [11].

In firefighters, lung inflammation has been secondarily assessed by eosinophils and cytokines in sputum and blood (systemic inflammation), respectively [12–15]. These studies defined the significance of smoke inhalation for firefighters, but limits the comprehension of the physiological responses of the lung and more important, the dose–response relationship between exposure (smoke and its components) and airways inflammation. Exhaled breath condensate (EBC) has been used in several studies to investigate the role of ambient particulate pollution on asthmatic patients including children [16, 17]. It is non-invasive method where sufficient quantities can be collected within 5–10 min of normal tidal breathing. EBC, collected by cooling the exhaled breath, is a highly concentrated aqueous mixture of many exo- and endogenous chemical species. Analysis of EBC non-exchangeable hydrogen types by NMR spectrometry allows for the characterization of chemical groups abundance that is representative of the molecular composition. It can be used to identify the most important compound groups for subsequent target analysis of specific biomarkers. These biomarkers are released into the airway lining fluid (ALF) and enter EBC through gas/aqueous partitioning or aerosolization. Biomarkers obtained from EBC are associated with lung impairment and have been used to differentiate pathological conditions, detect genetic modifications, and uniquely identify environmental stimuli [16–28]. Distinctive NMR spectral fingerprints of EBC were observed for patients with airways diseases [16, 19, 20, 22]. Recently, EBC has been used to assess the effect of occupational exposures in industrial facilities [29–31]. In firefighters, changes in respiratory and systemic biomarkers of inflammation and oxidative stress increased exhaled CO, oxidative stress and pro-inflammatory biomarkers but decreased exhaled NO [32–34].

In this study, we determined changes in the metabolic profile and non-exchangeable hydrogen function types of EBC and lung function following smoke inhalation of firefighters in prescribed fires (R_x). R_x events are intentionally ignited fires that are managed at low-intensity flaming for land management purposes. Firefighters in R_x events spend a large amount of time within smoke plumes to actively manage the fire front and fire-line progression. As a result, they are exposed to elevated levels of $PM_{2.5}$ (particulate matter with aerodynamic diameter less than 2.5 μm), along with other biomass smoke constituents, that are equal or at higher concentrations than those experienced during natural wildfire events [35]. EBC analysis involved the detection of non-exchangeable hydrogen types by NMR spectroscopy that was indicative of the molecular composition. The NMR spectral fingerprint can be used to detect changes and identify the most important compound types for further analysis.

2. Materials and methods

2.1. Study participants

Wildland firefighters were recruited from The Nature Conservancy R_x fire crews. They performed activities such as ignition, holding, mop-up, monitoring and patrol during R_x fires. They were required to complete the firefighter type 2 or the prescribed fire crew member training that includes physical fitness testing (either moderate (3.2 km hike with 11.5 kg pack in 30 min) or rigorous (4.8 km hike with 20.5-pound pack in 45 min)). They were involved in multiple R_x fires in central Alabama. The exposure characterization during the R_x fires are described in Nelson *et al* (2020) [36]. Table 1 shows the characteristics of R_x events and the type of respiratory measurements. The R_x plots were located in properties within (or adjacent) to Cahaba River National Wildlife Refuge (#1, 3–8, 11 and 13), Camp Tukabatchee (#2, 9, 14 and 16) and Roberta Case Pine Hills Preserve (#10, 12 and 15) in central Alabama. All subjects received verbal and written information prior to enrollment and provided informed consent. A baseline questionnaire and lung function measurements were obtained from all study participants. A subset of them also provided EBC samples. Lung function measurements and EBC collection took place within 15 min prior to and after the completion of fire activities [36]. The study protocol was approved by the Institutional Review Board of the University of Alabama at Birmingham.

2.2. EBC collection and ^{1}H-NMR analysis

EBC was collected using an RTube™ sampling device (Respiratory Research, Inc. Austin, TX, USA) [37, 38]. Firefighters breathed tidally through a mouthpiece (using a nose clip) into a two-way nonrebreathing valve for 10–15 min, yielding approximately 1 ml of EBC. EBC tubes were sealed with caps to reduce losses of volatile species, wrap in aluminum foil to eliminate photochemical losses and stored in a cooler until laboratory processing after the completion of the field sampling. In the lab, EBC samples were immediately (between 1–2 h from the completion of field sampling) processed

Table 1. Biomass type, burnt area and duration of R_x fire, number of participants and respiratory type measurements.

R_x	Biomass load description	Burnt area (km^2)	Duration (h)	Fire crew	Respiratory type measurement
1	Longleaf/loblolly pine; pine needles, hardwoods; underbrush/litter	0.40	6.5	6	Spirometry and EBC
2		0.24	4.0	6	
3		0.22	3.5	6	
4		0.55	8.0	6	
5		1.60	6.0	10	
6		0.46	7.0	5	
7		0.65	8.0	6	
8		0.24	6.5	6	
9	Mixed pasture/grass and longleaf pine; head fire	0.64	5.0	4	
10	Longleaf/loblolly pine; pine needles, hardwoods; underbrush/litter	0.36	5.5	6	
11	0.56	6.5	6		
12	Grass	0.48	6.2	6	
13	Long leaf pine plantation	2.66	5.0	8	Spirometry
14	Longleaf/loblolly pine; pine needles, hardwoods; underbrush/litter	0.30	5.0	4	
15	Grass	0.52	6.8	6	
16	Longleaf/loblolly pine; pine needles, hardwoods; underbrush/litter; sand acer plane; some old structures within burn unit	0.46	6.0	8	

in a cold room, divided in aliquots of 500 μl and stored at -80 °C until further analysis. For the NMR analysis, EBC samples were rapidly thawed on ice in the cold room and transferred into 5.0-mm Norell NMR probe with 100 μl of a buffer solution of disodium phosphate/monosodium phosphate (0.2 M Na_2HPO_4/0.2 M NaH_2PO_4, pH 7.4) containing 30 mg of 3-(trimethylsilyl)propionic acid-d4 sodium salt (TSP-d_4) (as the internal standard, set at δ_H of 0.0 ppm) and 100 μl of sodium azide (NaN_3) (1% w/w). The solvent system was selected to maintain the same experimental conditions for all EBC samples, minimize changes in the chemical shift due to pH and facilitate compatibility with previous studies on the composition of non-exchangeable hydrogen types [39, 40]. All samples were analyzed on a 600.17 MHz Bruker Advance III NMR spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a proton-optimized triple resonance 'inverse' 5 mm cryogenic probe (CP TCI600S3 H-C/N-D-05 Z fitted with an actively shielded single axis z-gradient) at 298 K. A gradient water suppression pulse sequence with 1D excitation sculpting using 180 water-selective pulses (90° high power pulse duration of 7 μs, 180° high power pulse duration of 2 ms, 180° shaped pulse duration of 2 ms, gradient duration of 1 ms, recovery delay after gradient of 200 μs, relaxation delay 1 s and total acquisition time 1.70 s) in 1024 scans into 32 000 data points over a spectral width of 16.02 ppm (9615 Hz) was applied. The resultant ^{1}H-NMR spectra were processed with a 0.3 Hz exponential line broadening factor, and phase and baseline corrected using Advanced Chemistry Development NMR Spectrum Processor software (version 12.01; ACDLabs, Toronto, Canada). The ^{1}H-NMR spectra were integrated into six regions representing different types of non-exchangeable hydrogen as follows: (i) alkyl [H-C] δ_H: 0.70–1.80 ppm; (ii) allylic [H-C-C=] δ_H: 1.80–3.20 ppm; (iii) saturated oxygenated [H-C-O] δ_H: 3.20–4.70 ppm; (iv) vinylic [H-C = C] and acetalic [O-CH-O] δ_H: 5.00–6.50 ppm; (v) aryl [H-Ar] δ_H: 6.50–9.00 ppm; and (vi) carbonyl [H-C = O] δ_H: 9.00–12.00 ppm [39, 40]. The resonances assigned to water, acetate and propanodiol were excluded due to impurities in EBC R-Tubes. The same methodological approach has been used to characterize the chemical content of exposures in order to facilitate direct comparison of personal exposures to EBC [36]. Non-exchangeable hydrogen types concentrations are presented in mol per ml of EBC. Normalization of non-volatile metabolites concentrations to breath urea has been previously suggested to account for the effect of dilution [41]. Breath urea may vary from 390 ng ml^{-1} to 4.19 mg ml^{-1} using sensitive liquid chromatography techniques [42]. The limit of detection using authenticated standards by ^{1}H-NMR spectroscopy was 120 μg ml^{-1}, thus, urea may not be adequately identified in all samples. Urea's resonance (δ_H: 5.78 ppm) was included in vinylic and acetalic region. No significant differences were observed

2.3. Lung function

Pre- and post-exposure forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC) and peak expiratory flow (PEF) were measured with a handheld electronic spirometer that meets American Thoracic Society (ATS) requirements (Micro, UK) [15, 43, 44]. The measurements were executed with the subject standing and breathing through the mouthpiece using a nose clip. Lung function measurements were measured at body temperature and pressure saturated through the digital volume transduced in the spirometer. The performance of spirometers was assessed by biological calibration of spirometric maneuvers by the same investigator. Field personnel's FEV_1 and FVC was within 10% and PEF within 15% of the mean value. Height and age were used to calculate predicted values for FEV_1, FVC and PEF [43].

2.4. Statistical analysis

The mean and standard deviation (SD) of lung function parameters (FEV_1, FVC, %FEV1/FVC, PEF) and EBC non-exchangeable organic hydrogen type concentrations were reported. Taking into account that R_x fire crews were small in size (4–10 firefighters), sample size was estimated for a repeated events of correlated observations based on preliminary spirometry and EBC measurements [45]. To obtain sufficient power (power: 80%, $R = 0.95$) to detect a 15% decline in cross-shift lung function effects, a minimum of five events for FEV_1 ($n = 6$ subjects), FVC ($n = 7$ subjects), PEF ($n = 5$ subjects) was required. However, measurements were irregularly obtained due to sporadic occurrence of the R_x fires due to weather variability and crew availability resulting in the collection of 99 paired pre- and post-lung function measurements from 29 firefighters over 16 R_x fires and 51 paired pre- and post-EBC samples from 14 firefighters over 8 R_x fires (3 samples per firefighter and 6 samples per R_x fire). The intermittent field monitoring frequency allowed for the collection of samples from a larger number of individuals in multiple R_x fires to better describe the variation of exposures and resultant health outcomes in real conditions. As a result, individual observations were nested into groups by R_x fire. To determine the association of post-exposure measurements to pre-exposure measurements for each R_x fire a fixed-effects linear regression model for both lung function and EBC non-exchangeable hydrogen types [46, 47]. A dummy variable for each R_x fire was used to assess effect associated with each event. For this analysis, the statistical significance of the independent variables is presented (p-value < 0.01) to assess the effect of change of pre-exposure measurements and/or R_x fire on post-exposure measurements.

3. Results

3.1. R_x fires and participants

Table 1 shows R_x fires characteristics, the number of firefighters and type of collected respiratory assessments. The majority of R_x plots were composed of longleaf pine (*Pinus palustris*) growth and low-growing blueberries, huckleberries, various bluestems, Indian grass and other herbaceous plants. The R_x fires started in the morning (between 8:00 and 10:00 a.m.) and completed within 8 h (from 3.5 to 8 h). More crew members were engaged for larger plots; however, there was an association between the R_x plot size and duration of the event for R_x crews with less than six members (rate of 0.07 $km^2\ h^{-1}$).

Table 2 shows the demographic information and respiratory health (mean (standard deviation (SD) in parentheses) percent baseline to predicted lung function) of the firefighters. Lung function was measured in 29 participants and 14 of them provided pre- and post-exposure EBC samples. Most of them were non-smokers. About half of participants (14 for lung function and 7 for EBC samples) reported an annual visit to primary healthcare. Four participants (two for EBC samples) reported upper respiratory symptoms over the past 12 months. Only two of the participants have been previously diagnosed with chronic respiratory disease such as asthma and bronchitis. Pre-exposure lung function measurements were comparable to those computed based on anthropometric measurements with no differences between smokers and non-smokers, or those with and without self-reported respiratory symptoms or previously diagnosed diseases.

3.2. EBC non-exchangeable hydrogen functional types and lung function

Figure 1 shows characteristic ^{1}H-NMR spectra of pre- and post-exposure EBC including the boundaries of the six non-exchangeable hydrogen types. Table 3 shows the mean (standard deviation in parentheses) of pre- and post-exposure non-exchangeable hydrogen types (in molH/L) and lung function parameters. The mean (SD) of non-exchangeable hydrogen types and lung function for each R_x fire are presented in tables S1 and S2 in supplemental information (available online at stacks.iop.org/JBR/14/046010/mmedia).

For both pre- and post-EBC samples, non-exchangeable oxygenated hydrogen associated with alcohols, esters and ethers was the predominant type followed by alkyl and allylic hydrogen, typically present in aliphatic chains. Trace quantities of acetalic, vinylic and carbonyl hydrogen were detected. On average, post-exposure alkyl hydrogen concentrations were somewhat higher than pre-exposure levels (2.4 (2.1) molH/L vs. 2.6 (0.3) molH/L). Post-exposure allylic (0.8 (0.9) molH/L vs. 0.7 (0.5) molH/L) and oxygenated hydrogen (3.7 (3.0) molH/L vs.

Table 2. Firefighter characteristics. Demographic and physiological data, and respiratory function.

	Lung function	EBC
Number of firefighters	29	14
Age, *yrs*	26.8 (7.0)	26.5 (8.0)
Female, *n [%]*	10 [32]	6 [41]
Height, *m*	1.8 (0.1)	1.7 (0.1)
Weight, *kg*	77.1 (17.2)	75.5 (15.5)
Non-smoker, *n [%]*	21 [72]	12 [86]
Doctor's visit, *n [%]*	14 [45]	7 [47]
Chronic health conditions, *n [%]*	2 [5]	2 [13]
Self-reported respiratory symptoms past 12 months, *n [%]*	4 [13]	2 [13]
Lung function		
%predicted FEV_1, *mean (SD)*	96.5 (12.6)	-
%predicted FVC, *mean (SD)*	101.2 (13.0)	-
%predicted PEF, *mean (SD)*	87.3 (19.9)	-

SD: Standard deviation

Table 3. The mean (standard deviation in parentheses) of pre- and post-exposure non-exchangeable hydrogen types and lung function parameters.

	Post-exposure	Pre-exposure	R_x fire
EBC non-exchangeable hydrogen (mol/L)			
Alkyl [H-C][a]	2.6 (3.0)	2.4 (2.1)	a
Allylic [H-C-C=]	0.7 (0.5)	0.8 (0.9)	
Oxygenated [H-C-O][a]	2.8 (1.3)	3.7 (3.0)	a
Acetalic + Vinylic [O-CH-O] +[H-C=][a]	0.2 (0.2)	0.1 (0.1)	a
Aryl [H-Ar][a]	0.2 (0.2)	0.2 (0.2)	a
Carbonyl [H-C-O]	0.2 (0.3)	0.2 (0.2)	
Lung function			
FEV_1	3.92 (0.79)	4.00 (0.75)	
FVC[a]	4.94 (0.98)	5.01 (0.93)	a
FEV_1/FVC[a]	79.9 (8.0)	80.2 (7.9)	a
PEF[b]	7.86 (1.96)	8.25 (1.96)a	a

Data presented as mean (SD).

[a]Significant association ($p < 0.01$) ±

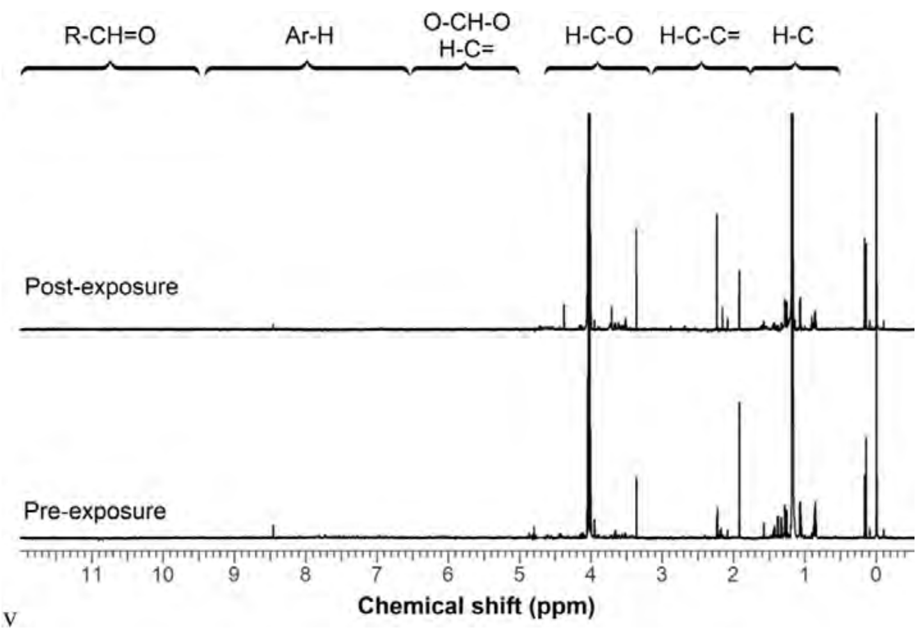

Figure 1. Representative 800 MHz ^{1}H-NMR of pre- and post-exposure EBC in a R_x fire (#3).

2.8 (1.3) molH/L) were lower than those measured pre-exposure. Post-exposure alkyl, oxygenated, acetalic + vinylic and aryl hydrogen concentrations were associated with the R_x fires ($p < 0.01$).

Figure 2 depicts the post-pre (Δ) differences on the relative (to the total aliphatic concentration) abundance of non-oxidized (in the form of H-C-O) and oxidized (in the form of H-C(=O)-OH) carbon for each paired EBC sample. The oxidized carbon was computed as the difference between allylic and aryl carbon abundance. The concentrations of alkyl, allylic, oxygenated and aryl carbon were calculated as the ratio of the corresponding non-exchangeable organic hydrogen to the H/C molar ratio (2 for alkyl and allylic hydrogen, 1.1 for oxygenated hydrogen and 0.4 for aryl factions) [48]. Two regimes were identified reflecting a strong proportional dependence between non-oxidized and oxidized fractions. The first regime (oxidation regime: upper left quadrant in figure 2(a)) was characterized by an increase of oxidized/aliphatic and decrease of non-oxidized/aliphatic ratio values post-exposure. As a result, the mean oxidized carbon concentration increased by 25% (0.4 molC l^{-1} to 0.7 molC l^{-1}) and non-oxidized carbon concentration decreased by approximately 48% (from 2.8 molC l^{-1} to 2.3 molC l^{-1}) (figure 2(b)). On the other hand, the abundance of the oxidized carbon for the second regime declined to 0.3 molC l^{-1} (from 0.9 molC l^{-1},−64%) while the abundance of non-oxidized carbon increased by 121% (2.7 molC l^{-1} to 4.0 molC l^{-1}).

The mean post-exposure FEV_1 (4.00 (0.75) vs. 3.92 (0.79)), FVC (5.01 (0.93) vs. 4.94 (0.98)), $\%FEV_1/FVC$ (80.2 (7.9) vs. 79.9 (8.0)) was reduced following exposure to smoke for the majority of R_x events. Post-exposure FVC and %FEV1/FVC were associated with R_x fires ($p < 0.01$). Post-exposure PEF measurements also declined compared to those measured pre-exposure reduced and associated with pre-exposure measurements for each firefighter and R_x fire ($p < 0.01$).

3.3. EBC composition, lung function and R_x characteristics

Because of size of the R_x plots and the number of crew members (more firefighters were deployed for larger R_x plots) aiming to complete the event within 6–7 h, two ratios were constructed to describe the exposure conditions based on R_x fire characteristics. The area-to-duration ($km^2\ h^{-1}$) was computed to determine the intensity of R_x fire conditions, with increasing ratio values being suggestive of a rapidly moving R_x fire event due to favorable topography, meteorological conditions or fire type/load (i.e. grass). Under these conditions, smoke exposures may be reduced due to effective smoke dispersion and dilution. The duration-to-firefighters (h/person) ratio was used as an indicator of exposure magnitude related to either exposure levels or the number of firefighters exposed. Increasing duration-to-firefighter ratio values were indicative of longer exposures periods for each firefighter.

Figure 3 shows the mean (SD) absolute post-pre (Δ) difference of FEV_1 and FVC and the area/duration ratio for each R_x fire. The mean (SD) relative post-pre difference (Δ) (normalized to pre-exposure PEF measurement) and duration/firefighter ratio is shown in figure 4. With the exception of R_x#13, higher absolute ΔFEV_1 and ΔFVC values were computed for increasing area/duration ratio. The PEF relative difference ($\Delta(PEF)/PEF_{pre}$) also increased as the duration/firefighter increased (figure 4). The R_x fire #13 was the largest plot with high challenging topography (steep hills) requiring the deployment of more crew members. The event lasted for 5 h due to rapidly changing wind conditions facilitating intense and fast moving fire front. Figure 5 shows the mean (SD) absolute post-pre (Δ) difference of non-oxidized and oxidized carbon concentration and the duration/firefighter ratio for each R_x fire. The absolute difference of the oxidized fraction was associated with changes of the duration/firefighter ratio. No association was observed for the non-oxidized fraction.

4. Discussion

The composition of non-exchangeable hydrogen types of EBC of firefighters exposed to smoke was determined for the first time. Previous studies have examined specific EBC biomarkers following smoke exposure in real or simulated conditions [32–34]. We found that post-exposure EBC spectral profile was characterized by elevated non-exchangeable aliphatic saturated hydrogen and lower aliphatic unsaturated and oxygenated hydrogen concentrations as compared to that observed prior to exposure. This indicated that smoke inhalation may trigger changes in EBC composition and the abundance of aliphatic and oxygenated chemical species such as long-chains eicosanoids and small molecules such as alcohols, saturated acids and esters. These may include degradation of glucose in alveolar epithelial can be degraded to pyruvate and lactate under hypoxic conditions; a process that would be consistent with the increase of the alkyl non-exchangeable hydrogen and decline of oxygenated hydrogen [49]. Methanol, formate and acetoin, associated with physiological responses to fight inflammation in alveolar and bronchial cells caused by inhaled contaminants, could also trigger changes in EBC non-exchangeable hydrogen composition [50]. Other molecular pathways include the increase in the abundance of small fatty (saturated) acids due to their migration to the inflammation foci, acetone, acetate and succinate formation (indicators of inflammation in the ALF [24, 51, 52]. As a result, both unoxidized (e.g. methanol, propanol, acetoin) and oxidized (e.g. carboxylic acids, acetone) may be related to inflammation

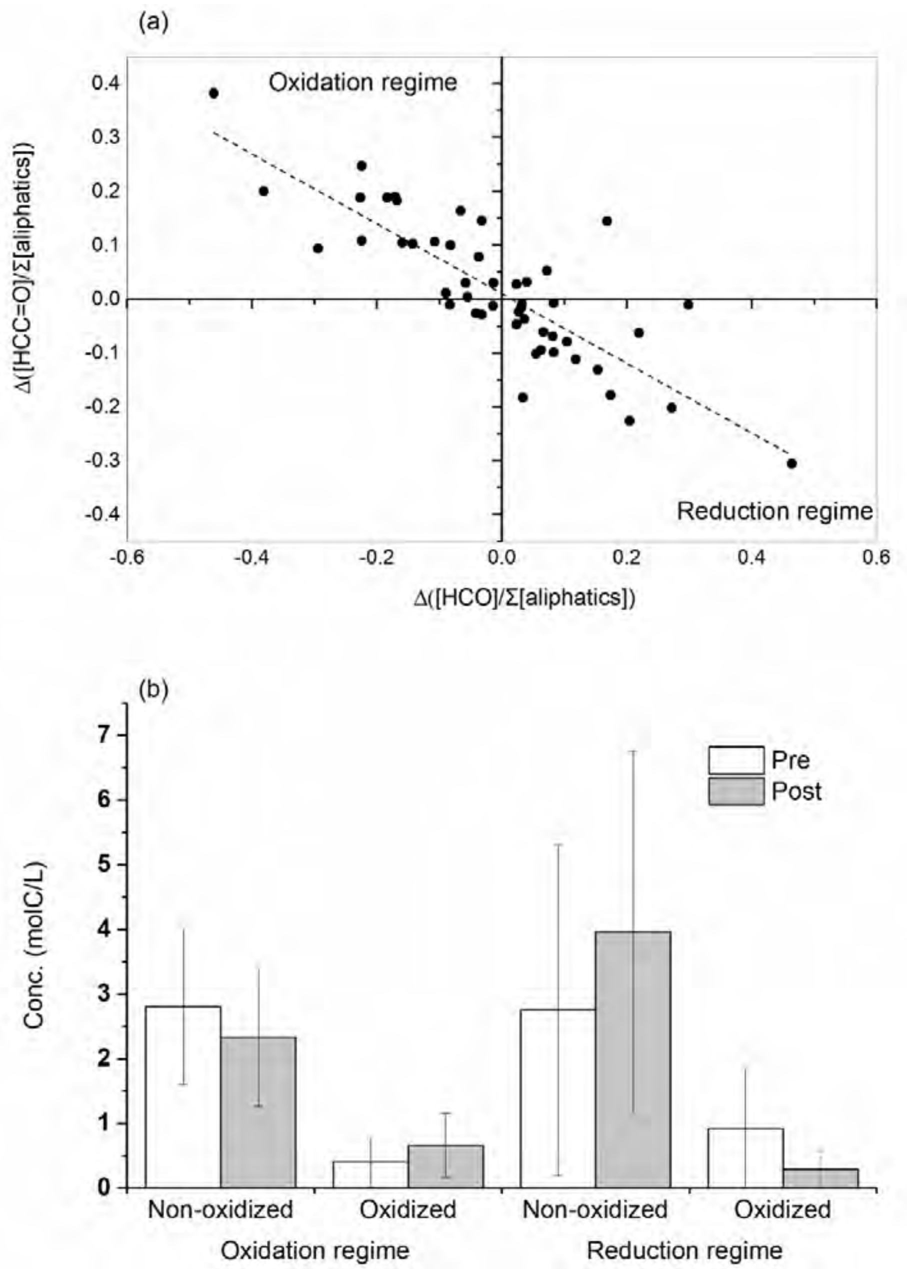

Figure 2. (a) Post-pre differences on the oxidized (HCC=O) and non-oxidized (H-C-O) ratios and (b) pre- and post-exposure carbon concentrations of the non-oxidized and oxidized fractions for each regime.

and can trigger changes in the relative abundance of non-exchangeable hydrogen functional types in EBC.

The type and the intensity of biological and physiological responses to smoke inhalation, and therefore EBC composition, may be dependent on both individual and smoke composition [53]. The comparison of the relative abundance of the non-oxidized to oxidized fraction (by carbon) showed two distinct regimes. For a subset of cases (i.e. oxidation regime), the difference between post-exposure and pre-exposure EBC composition was indicative of pathways leading to the formation of oxidized species, such as aldehydes, ketones and acids (species with H/R-C=O or C(O)=O groups). For the remaining cases (i.e. reduction regime), molecular responses led to increased abundance of non-oxidized species such as alcohols. The trends of oxidized carbon and the exposure duration indicator (duration/firefighter ratio) indicated that longer smoke exposures may induce molecular responses associated with the increased abundance of oxidized species in EBC.

Changes in the lung function due to smoke inhalation were also assessed. We found that post-exposure declined (for PEF and to a lesser extend for FEV_1 and FVC) as compared to pre-exposure measurements indicating the acute effect of smoke inhalation. This was consistent with the reduced lung function in forest firefighters and hot-spot wildland fire crews [].

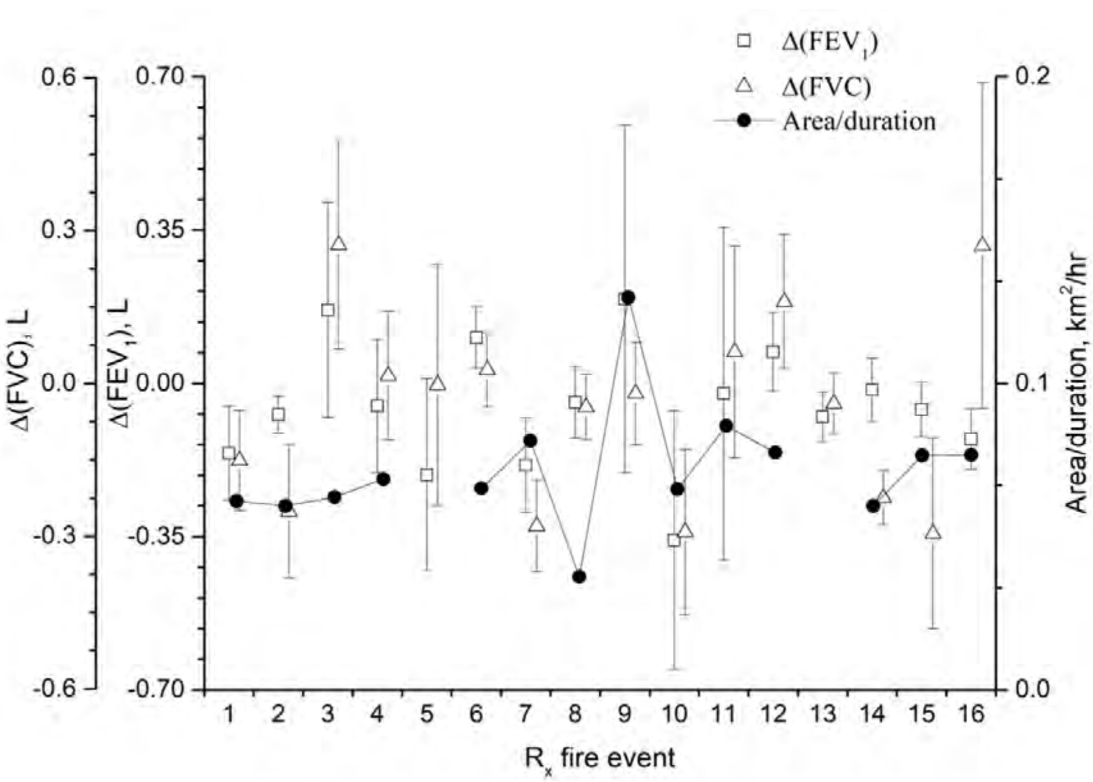

Figure 3. Distribution of absolute post-pre FEV1 and FVC difference ($\Delta(FEV_1)$ and $\Delta(FVC)$), and area/duration ratio for R_x fires #1–16. The square (or triangle) is the mean and the error bars are the SD.

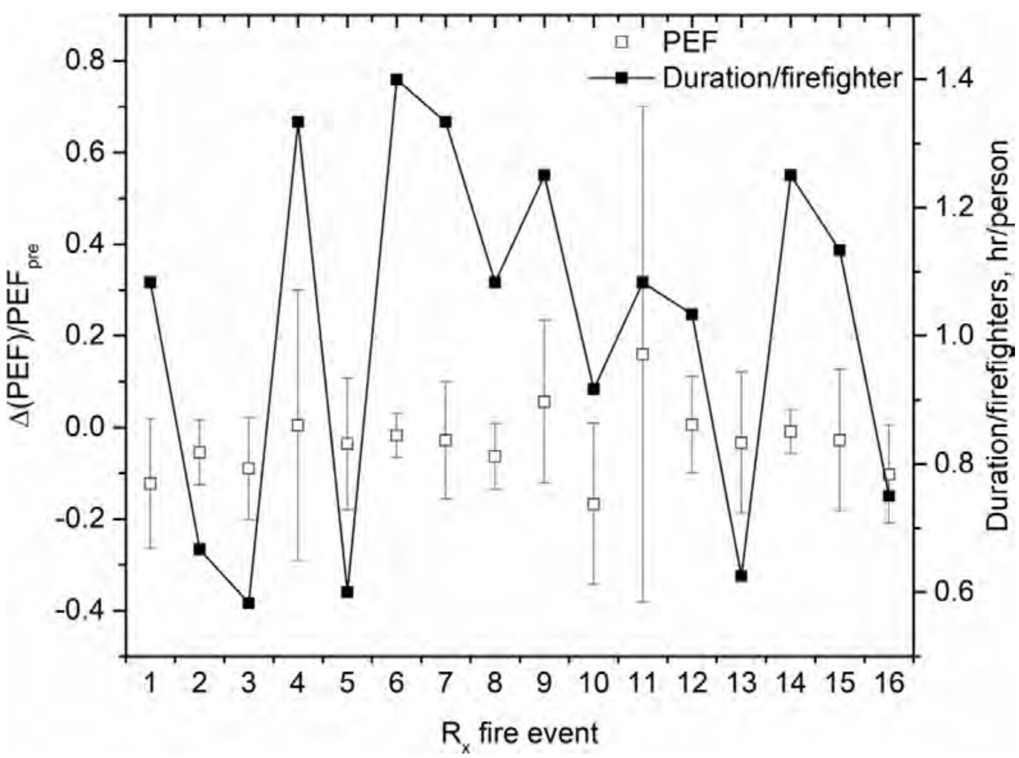

Figure 4. Distribution of the relative post-pre PEF difference ($\Delta(PEF)/PEF_{pre}$), and duration/firefighter ratio for R_x fires #1–16. The square is the mean and the error bars are the SD.

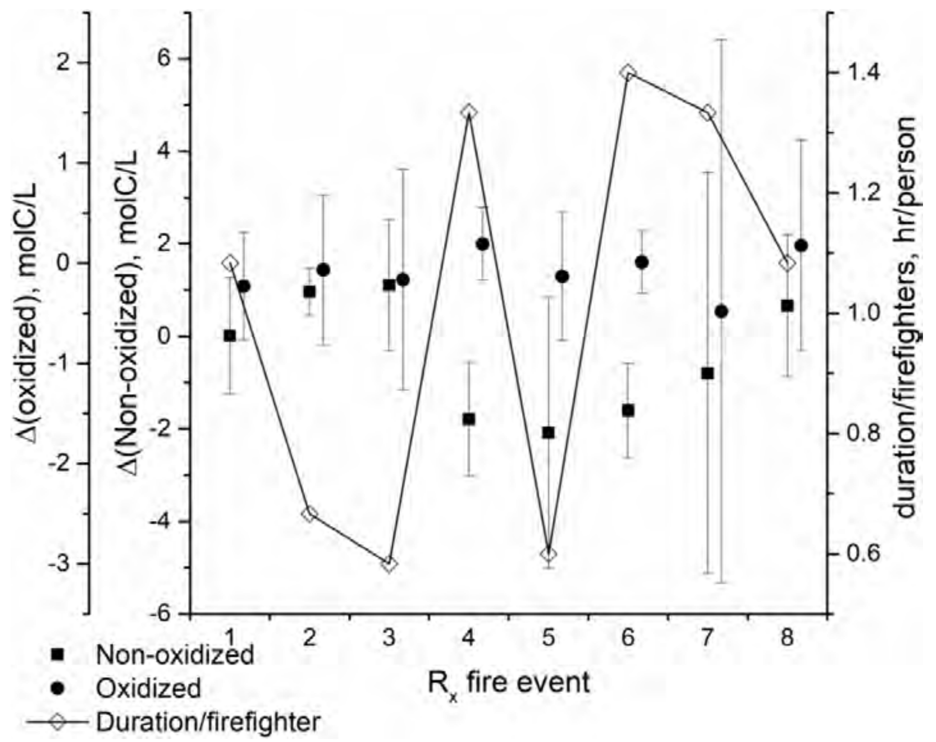

Figure 5. Distribution of absolute post-pre EBC non-oxidized and oxidized concentration difference (Δ(non-oxidized) and Δ(oxidized)), and area/duration ratio for R_x fires #1–8. The square is the mean and the error bars are the SD.

Adetona *et al* [54] also found a reduction of 24 ml in pre-exposure FVC and 24 ml in pre-exposure FEV_1 ($p < 0.01$) in firefighters following smoke inhalation in prescribed burns. FEV_1 and FVC are indicators of airflow limitation caused by bronchoconstriction and airway inflammation. PEF is an indicator of ventilatory impairment and is used as an indicator of airway caliber [55]. Previous studies have shown PEF and FEV_1 changes are poorly related during mild bronchoconstriction induced by different stimuli [56–58]. However, a reduction in airway caliber would result in a greater increase in airway resistance and consequently greater airflow limitation [58, 59]. Several previous studies have shown that airflow limitation can be present with normal PEF variability [60–63].

Lung function is reduced due to obstruction in the lungs, primarily caused by smoke-induced inflammation which may resolve over time [64]. In firefighters, airflow limitation may be caused by smoke-induced inflammation while changes in airway caliber may also be associated with longer exposures at low levels. Post-pre lung function declines were associated with R_x fires indicating a possible relationship between exposure conditions and lung function. The trends of FEV_1 and FVC and area/duration ratio suggested a possible association with exposure magnitude. On the other hand, the trends of PEF with the duration/firefighter ratio indicated that duration of exposure may be critical.

The vast majority of firefighters were healthy and non-smokers but it is possible smokers and those with pre-existing conditions may be more susceptible to smoke inhalation. Smoking is known to cause effects on EBC biomarkers and lung function. Considering the magnitude of smoke inhalation during R_x fires, it is less likely that smoking confounded post-exposure measurements. However, this factor was considered in interpreting our results. Nonetheless, the participants were representative of career and volunteer firefighter cohorts. Furthermore, exposure conditions in R_x fires have been previously shown to be comparable to wildfires [35].

In conclusion, our study has provided initial evidence of using EBC to evaluate the respiratory responses to smoke inhalation in firefighters. To date, there were only measurements of specific biomarkers in EBC. The potential use of EBC functional content lies with its ease of collection in field conditions and over time and the inherent ability of NMR spectrometry to uniquely chemically characterize samples with limited preparation in a reproducible and robust way.

Acknowledgments

We would like to thank the R_x crews of The Nature Conservancy in Alabama for their participation in the study. The study was supported by the NIOSH funded Deep South Center for Occupational Health and Safety Education and Research Center Pilot Project Grant (Award #: T42OH008436) and the North

Carolina Occupational Safety and Health Education and Research Center Pilot Project Grant (Award #: T42OH008673) of the National Institute of Occupational Safety and Health.

ORCID iD

Ilias G Kavouras https://orcid.org/0000-0003-0436-3784

References

[1] NICC 2017 National report of wildland fires and acres burned by state https://predictiveservices.nifc.gov/intelligence/2017_statssumm/fires_acres17.pdf
[2] IARC 2010 Working group on the evaluation of carcinogenic risks to humans *Painting, firefighting, and shiftwork* 98 (Lyon: IARC)
[3] Austin C C, Wang D, Ecobichon D J and Dussault G 2001 Characterization of volatile organic compounds in smoke at municipal structural fires *J. Toxicol. Environ. Health* A **63** 437–58
[4] Austin C C, Dussault G and Ecobichon D J 2001 Municipal firefighter exposure groups, time spent at fires and use of self-contained-breathing-apparatus *Am. J. Ind. Med.* **40** 683–92
[5] Reisen F and Brown S K 2009 Australian firefighters' exposure to air toxics during bushfire burns of autumn 2005 and 2006 *Environ. Int.* **35** 342–52
[6] Reisen F, Hansen D, Meyer C(Mick) 2011 Exposure to bushfire smoke during prescribed burns and wildfires: Firefighters' exposure risks and options. Environment International. **37** 314–21
[7] Naeher L P *et al* 2007 Woodsmoke health effects: a review *Inhalation Toxicol.* **19** 67–106
[8] Gaughan D M *et al* 2014 Exposures and cross-shift lung function declines in wildland firefighters *J. Occup. Environ. Hyg.*. **11** 6013
[9] Liu D, Tager I B, Balmes J R and Harrison R J 1992 The effect of smoke inhalation on lung function and airway responsiveness in wildland fire fighters *Am. Rev. Respir. Dis.* **146** 1469–73
[10] Navarro K M *et al* 2019 Wildland firefighter smoke exposure and risk of lung cancer and cardiovascular disease mortality *Environ. Res.* **173** 462–8
[11] Daniels R D *et al* 2014 Mortality and cancer incidence in a pooled cohort of US firefighters from San Francisco, Chicago and Philadelphia (1950-2009) *Occup. Environ. Med.* **71** 388–97
[12] Greven F E *et al* 2012 Acute respiratory effects in firefighters *Am. J. Ind. Med.* **55** 54–62
[13] Burgess J L *et al* 2002 Rapid decline in sputum IL-10 concentration following occupational smoke exposure *Inhalation Toxicol.* **14** 133–40
[14] Gaughan D M *et al* 2014 Arterial stiffness, oxidative stress, and smoke exposure in wildland firefighters *Am. J. Ind. Med.* **57** 748–56
[15] Gaughan D M *et al* 2008 Acute upper and lower respiratory effects in wildland firefighters *J. Occup. Environ. Med.* **50** 1019–28
[16] Bodini A *et al* 2017 Serum and exhaled breath condensate leptin levels in asthmatic and obesity children: a pilot study *J. Breath Res.* **11** 046005
[17] Linares S B *et al* 2014 Parameters of lung inflammation in asthmatic as compared to healthy children in a contaminated city *BMC Pulmon. Med.* **14** 111
[18] Paris D *et al* 2015 Inflammatory metabolites in exhaled breath condensate characterize the obese respiratory phenotype *Metabolomics* **11** 1934–9
[19] Lopes S P, Matos J T V, Silva A M S, Duarte A C and Duarte R M B O 2015 1H NMR studies of water- and alkaline-soluble organic matter from fine urban atmospheric aerosols *Atmos. Environ.* **119** 374–80
[20] Motta A *et al* 2014 NMR metabolomic analysis of exhaled breath condensate of asthmatic patients at two different temperatures *J. Proteome Res.* **13** 6107–20
[21] Bertini I, Luchinat C, Miniati M, Monti S and Tenori L 2013 Phenotyping COPD by 1H NMR metabolomics of exhaled breath condensate *Metabolomics* **10** 1–10
[22] De Laurentiis G *et al* 2013 Separating smoking-related diseases using NMR-based metabolomics of exhaled breath condensate *J. Proteome Res.* **12** 1502–11
[23] Matteo S *et al* 2011 Exploring airway diseases by nmr-based metabonomics: A review of application to exhaled breath condensate *J. Biomed. Biotechnol.* **2011** 403260
[24] De Laurentiis G *et al* 2008 Metabonomic analysis of exhaled breath condensate in adults by nuclear magnetic resonance spectroscopy *Eur. Respir. J.* **32** 1175–83
[25] Carraro S, Giordano G, Stocchero M and Baraldi E 2010 Exhaled breath condensate: metabolomics *Eur. Respir. Mon.* **49** 231–6
[26] Radulovic M *et al* 2015 Biomarkers of inflammation in persons with chronic tetraplegia *J. Breath Res.* **9** 036001
[27] Stiegel M A, Pleil J D, Sobus J R, Morgan M K and Madden M C 2015 Analysis of inflammatory cytokines in human blood, breath condensate, and urine using a multiplex immunoassay platform *Biomarkers* **20** 35–46
[28] Zheng Y *et al* 2014 Change in airway inflammatory markers in Danish energy plant workers during a working week *Ann. Agric. Environ. Med.* **21** 534–40
[29] Carraro S *et al* 2013 Asthma severity in childhood and metabolomic profiling of breath condensate *Allergy* **68** 110–7
[30] Fumagalli M *et al* 2012 Profiling the proteome of exhaled breath condensate in healthy smokers and COPD patients by LC-MS/MS *Int. J. Mol. Sci.* **13** 13894–910
[31] Maniscalco M *et al* 2018 Biomonitoring of workers using nuclear magnetic resonance-based metabolomics of exhaled breath condensate: A pilot study *Toxicol. Lett.* **298** 4–12
[32] Miranda A I *et al* 2012 Wildland smoke exposure values and exhaled breath indicators in firefighters *J. Toxicol. Environ. Health* A **75** 831–43
[33] Wu C M, Adetona A, Song C, Naeher L and Adetona O 2020 Measuring acute pulmonary responses to occupational wildland fire smoke exposure using exhaled breath condensate *Arch. Environ. Occup. Health* **75** 65–69
[34] Ferguson M D, Semmens E O, Dumke C, Quindry J C and Ward T J 2016 Measured pulmonary and systemic markers of inflammation and oxidative stress following wildland firefighter simulations *J. Occup. Environ. Med.* **58** 407–13
[35] Reinhardt T E and Ottmar R D 2004 Baseline measurements of smoke exposure among wildland firefighters *J. Occup. Environ. Hyg.* **1** 593–606
[36] Nelson J, Chalbot M-C G, Tsiodra I, Mihalopoulos N and Kavouras I G 2020 Physicochemical characterization of personal exposures to smoke aerosol and PAHs of wildland firefighters in prescribed fires *Expos. Health* (https://doi.org/10.1007/s12403-020-00366-5)
[37] Koczulla R *et al* 2009 Comparison of exhaled breath condensate pH using two commercially available devices in healthy controls, asthma and COPD patients *Respir. Res.* **10** 78
[38] Fox J R, Spannhake E, Macri K K, Torrey C M, Mihalic J N, Eftim S E, Lees P S and Geyh A S 2013 Characterization of a portable method for the collection of exhaled breath condesnate and subsequent analysis of metal content *Environ. Sci. Process. Impacts* **15** 721–9
[39] Chalbot M-C G, Chitranshi P, da Costa G G, Pollock E and Kavouras I G 2016 Characterization of water-soluble organic matter in urban aerosol by (1)H-NMR spectroscopy *Atmos. Environ.* **128** 235–45
[40] Chalbot M-C G and Kavouras I G 2019 Nuclear magnetic resonance characterization of water soluble organic carbon of atmospheric aerosol types *Nat. Prod. Commun.* **14**

[41] Horváth I *et al* 2017 A european respiratory society technical standard: exhaled biomarkers in lung disease *Eur. Respir. J.* **49** 1600965
[42] Pitiranggon M, Perzanowski M S, Kinney P L, Xu D, Chillrud S N and Yan B 2014 Determining urea levels in exhaled breath condensate with minimal preparation steps and classic LC-MS. *J. Chromatogr. Sci.* **52** 1026–103
[43] Hankinson J O and Fedan K 1999 Spirometric reference values from a sample of the general U.S. population *Am. J. Respir. Crit. Care Med.* **159** 179–87
[44] de Hartog J J *et al* 2010 Lung function and indicators of exposure to indoor and outdoor particulate matter among asthma and COPD patients *Occup. Environ. Med.* **67** 2–10
[45] Liu G and Liang K 1997 Sample size calculation for studies with correlated observations *Biometrics* **53** 937–47
[46] Gunasekara F I, Richardson K, Carter K and Blakely T 2014 Fixed effects analysis of repeated measures data *Int. J. Epidemiol.* **43** 264–9
[47] Braun L M *et al* 2016 Changes in walking, body mass index, and cardiometabolic risk factors following residential relocation: longitudinal results from the CARDIA study *J. Transp. Health* **3** 426–39
[48] Fuzzi S *et al* 2001 A simplified model of the water soluble organic component of atmospheric aerosols *Geophys. Res. Lett.* **28** 4079–82
[49] Lottes R G, Newton D A, Spyropoulos D D and Baatz J E 2015 Lactate as substrate for mitochondrial respiration in alveolar epithelial type II cells *Am. J. Physiol. Lung Cell Mol. Physiol.* **308** L953–L961
[50] Luster A D, Alon R and von Andrian U H 2005 Immune cell migration in inflammation: present and future therapeutic targets *Nat. Immunol.* **6** 1182–90
[51] Tannahill G M *et al* 2013 Succinate is an inflammatory signal that induces IL-1β through HIF-1α *Nature* **496** 238–42
[52] Wolak J E, Esther C Jr and O'Connell T M 2009 Metabolomic analysis of bronchoalveolar lavage fluid from cystic fibrosis patients *Biomarkers* **14** 55–60
[53] Kasurinen S *et al* 2017 Particulate emissions from the combustion of birch, beech, and spruce logs cause different cytotoxic responses in A549 cells *Environ. Toxicol.* **32** 1487–99
[54] Adetona O, Hall D B and Naeher L P 2011 Lung function changes in wildland firefighters working at prescribed burns *Inhalation Toxicol.* **23** 835–41
[55] Ichinose M Takahashi T, Sugiura H, Endoh N, Miura M, Mashito Y and Shirato K 2000 Baseline airway hyperresponsiveness and its reversible component: role of airway inflammation and airway calibr *Eur. Respir. J.* **15** 248–53
[56] Reddel H K, Salome C, Peat J K and Woolcock A J 1995 Which Index of peak expiratory flow is most useful in the management of stable asthma? *Am J Respir Crit Care Med.* **151** 1320–5
[57] Giannini D, Paggiaro. P, Moscato G, Gherson G, Bacci E, Bancalari L, Dente F L, Di Franco A, Vagaggini B and Giuntini C 1997 Comparison between peak expiratory flow and forced expiratory volume in one second (FEV1) during bronchoconstriction induced by different stimuli *J. Asthma* **34** 105–11
[58] Eid N, Yandell B, Howell L, Eddy M and Sheikh S 2000 Can peak expiratory flow predict airflow obstruction in children with asthma? *Pediatrics* **105** 354–8
[59] Britton J, Pavord I, Richards K, Knox A, Wisniewski A, Wahedna I, Kinnear W, Tattersfield A and Weiss S 1994 Factors influencing the occurence of airway hyperreactivity in the general population: the importance of atopy and airway calibre *Eru. Respir. J.* **7** 881–7
[60] Ferguson A C 1987 Persisting airway obstruction in asymptomatic children with asthma with normal peak expiratoru flow rates *J. Allergy Clin. Immunol.* **82** 19–22
[61] Paggiaro P M L *et al* 1993 The italian working group on the use of peak expiratory flow rate (PEFR) in asthma *Eur. Respir. Rev.* **14** 438–43
[62] Saetta M, Thiene G, Crescioli S and Fabbri L M 1989 Fatal asthma in a young patient with severe broncial hyperresponsiveness but stable peak flow records *Eur. Respir. J.* **2** 1008–12
[63] Bumbacea D *et al* 2004 Parameters associated with persistent airflow obstruction in chronic severe asthma *Eur. Respir. J.* **24** 122–8
[64] Betchley C, Koenig J Q, van Belle G, Checkoway H and Reinhardt T 1997 Pulmonary function and respiratory symptoms in forest firefighters *Am. J. Ind. Med.* **31** 503–9