Biomarkers, 2009; 14(1): 17–25

informa
healthcare

RESEARCH ARTICLE

Ethane and *n*-pentane in exhaled breath are biomarkers of exposure not effect

Katrine A. Gorham[1], Mads P. Sulbaek Andersen[1], Simone Meinardi[1], Ralph J. Delfino[2], Norbert Staimer[2], Thomas Tjoa[2], F. Sherwood Rowland[1], and Donald R. Blake[1]

[1]*Department of Chemistry, University of California, Irvine, CA, USA, and* [2]*Department of Epidemiology, School of Medicine, University of California, Irvine, CA, USA*

Abstract

The relationship of exhaled ethane and *n*-pentane to exhaled NO, carbonylated proteins, and indoor/outdoor atmospheric pollutants were examined in order to evaluate ethane and *n*-pentane as potential markers of airway inflammation and/or oxidative stress. Exhaled NO and carbonylated proteins were found to have no significant associations with either ethane ($p = 0.96$ and $p = 0.81$, respectively) or *n*-pentane ($p = 0.44$ and 0.28, respectively) when outliers were included. In the case where outliers were removed *n*-pentane was found to be inversely associated with carbonylated proteins. Exhaled hydrocarbons adjusted for indoor hydrocarbon concentrations were instead found to be positively associated with air pollutants (NO, NO_2 and CO), suggesting pollutant exposure is driving exhaled hydrocarbon concentrations. Given these findings, ethane and *n*-pentane do not appear to be markers of airway inflammation or oxidative stress.

Keywords: *Ethane; pentane; nitric oxide; carbonylated proteins; oxidative stress; exhaled breath analysis*

Introduction

Oxidative stress is a physiological condition that occurs when there is an imbalance of oxidants and antioxidants, favouring the former, and can arise from oxidants produced within the body as well as exposure to atmospheric oxidants. Under normal conditions reactive oxygen species (ROS) are removed by a number of different antioxidant enzymes, including superoxide dismutase, catalase, or glutathione peroxidase (Sies 1985). When an imbalance of oxidants and antioxidants occurs these removal processes are no longer effective and oxidative damage can be inflicted on macromolecules such as lipids, proteins, carbohydrates, and nucleic acids.

The presence of the hydrocarbons ethane and *n*-pentane in exhaled breath as a result of lipid peroxidation is of particular interest to this study. Lipid peroxidation occurs by unspecific oxidation of polyunsaturated fatty acids (PUFA), via abstraction of a hydrogen from a methylene carbon on the fatty acid chain, and results in toxic lipid peroxides and hydroperoxides (Halliwell & Chirico 1993). In the presence of iron-containing compounds (e.g. haemoglobin, cysteine–$FeCl_3$ complex or EDTA–Fe^{3+}) lipid hydroperoxides decompose via complex decomposition reactions yielding a variety of fragmentation products including short chain alkanes such as ethane and *n*-pentane (Horvat et al. 1964, Sevanian & Hochstein 1985). It is widely thought that oxidation of ω-6-polyunsaturated fatty acids and ω-3-polyunsaturated fatty acids result in *n*-pentane and ethane cleavage products, respectively (Sies 1986). Consequently, numerous studies have examined short chain alkanes in exhaled breath in attempts to demonstrate their potential use as biomarkers of oxidative stress (Dillard et al. 1978, Drury et al. 1997, Pryor & Godber 1991, Paredi et al. 2000, Phillips et al. 2000, Aghdassi & Allard 2000, Cope 2005, Kanoh et al. 2005, Cope et al. 2006, Solga et al. 2006, Lärstad et al. 2007). While clinical studies have found between-subject differences in exhaled hydrocarbons, a methods development study

Address for Correspondence: Katrine A. Gorham, Department of Chemistry, University of California, Irvine, CA 92697, USA. Tel: 949.824.6029. E-mail: kgorham@uci.edu

(Received 28 August 2008; revised 9 December 2008; accepted 7 January 2009)

ISSN 1354-750X print/ISSN 1366-5804 online
DOI: 10.1080/13547500902730680

by Drury et al. (1997), is one of the few studies that examined within-subject changes.

Previous studies have primarily focused on oxidative stress caused by disease or clinical conditions (Drury et al. 1997, Paredi et al. 2000, Aghdassi & Allard 2000, Cope 2005, Kanoh et al. 2005, Cope et al. 2006, Solga et al. 2006). Exhaled hydrocarbons may be useful for distinguishing between diseased individuals, but their sensitivity as biomarkers of acute pro-oxidant effects is not well understood. Exposure to a highly oxidizing environment containing primary and secondary pollutants from anthropogenic sources can also be a source of oxidative stress to the human body. Ozone (O_3) and nitrogen oxides (NO and NO_2) are common air pollutants and are considered to be important atmospheric gases that initiate lipid peroxidation (Cross et al. 2001, Montuschi et al. 2002).

Highly polluted oxidizing environments can cause inflammation of the respiratory track, which has previously been correlated with oxidative stress (Sies 1986). However, there are few data evaluating the relationship of biomarkers of airway inflammation to oxidative stress in humans (Wood et al. 2003). There is some evidence that airway oxidative stress, in contrast to airway inflammation, is resistant to treatment with anti-inflammatory corticosteroids in people with asthma (Montuschi et al. 2002, Shahid et al. 2005). The goal of this study was to investigate possible associations between biomarkers of airway inflammation (exhaled NO) and systemic oxidative stress (carbonylated plasma proteins) with the putative exhaled lipid peroxidation products, ethane and *n*-pentane.

There is evidence that oxidative damage results from exposure to reactive oxygen species from the environment and normal metabolic processes. Oxidative damage accumulates over a lifetime and is partly responsible for aging, thus making elderly subjects more sensitive to an oxidizing environment and susceptible to oxidative stress (Packer 1995). For these reasons, we recruited a group of elderly residents in a retirement community located in Riverside, California. The air quality in this area is particularly poor. It is located downwind of Los Angeles and local levels of oxidizing atmospheric gases, such as ozone, often exceed national and state standards (CARB 2007). During 2006, Riverside had 45 days exceeding the state 1-h standard and 30 days exceeding the national 8-h standard for ozone (the 2006 maximum 8 h average was 0.117 ppm ozone, and the maximum 1 h average was 0.151 ppm ozone) (CARB 2007).

In this study, 16 elderly subjects were followed over 12 weeks and their exhaled breath was analyzed using canister sampling and gas chromatography analysis (Kamboures et al. 2005). Exhaled NO was chosen as a non-invasive measure of airway inflammation and was determined using procedures recommended by the American Thoracic Society and European Respiratory Society (ATS/ERS 2005). Oxidation of certain proteins results in the formation of carbonyl derivatives, which have known associations with oxidative stress (Berlett & Stadtman 1997). For this study, enzyme-linked immunosorbent assay (ELISA) techniques were used to measure carbonylated proteins as oxidative stress markers. Mixed model statistical analysis was performed to investigate possible associations between the inflammation and oxidative stress biomarkers with breath hydrocarbons. Furthermore, the relationship between exhaled ethane and *n*-pentane with various air pollutants (NO, NO_2, and CO) was evaluated.

Methods

Study design

Subjects participating in this study were volunteers residing in a retirement community, located in Riverside, California. All protocols were approved by the UCI Institutional Review Board. The group consisted of 16 subjects (13 male, three female) with a mean age of 83 ± 4 years. All subjects were diagnosed with coronary artery disease, a disease that is strongly influenced by inflammation (Ridker 2007) and oxidative stress (Espinola-Klein et al. 2007). For example, over 20 large studies have shown that risk of cardiovascular events increase with elevations in an inflammatory biomarker (C-reactive protein), with risks similar to that for elevated low-density lipoproteins and blood pressure (Ridker 2007).

This study was carried out in two 6-week phases with samples obtained once every week. The first phase was in September and October, 2006, during the warmer months, when there was more photochemical activity. The second phase was in January and February 2007, during the cooler months, when there was less photochemical activity. All subjects were sampled at the same centrally ventilated room during both phases of the study. Subjects were asked to refrain from exercise, and food or beverage intake 1 h before sample collection. It is possible that exercise and food and beverage intake before sample collection may have influenced exhaled gas concentrations, but we did not have sufficient data to test this.

Hydrocarbons and CO_2

Samples for hydrocarbon and CO_2 analysis were collected in electropolished 1.9 l stainless steel canisters that were fitted with Swagelok Nupro metal bellows valves (Figure 1). Prior to sampling, each canister and valve assembly was baked at 150°C for 24 h, flushed with ultra-high purity helium, and evacuated to 10^{-2} Torr. Each

canister was fitted with a disposable 12 inch (30.5 cm) long, 0.25 inch (0.6 cm) diameter Teflon tube through which the subjects exhaled. One breath sample and one simultaneous background room sample were obtained from each subject on each sampling date. Subjects were directed to take three tidal breaths, each time exhaling through the tube, before filling lungs to total capacity and exhaling into the evacuated canister. In order to avoid upper airway contamination of the sample, the initial 2 s of the exhaled breath was bypassed at the canister valve and not collected.

A persistent problem associated with the measurement of exhaled hydrocarbons is the accounting for hydrocarbons that are present in the inspired air. Often the differences in ambient air concentrations and exhaled breath concentrations are very small, which makes the detection of small endogenous produced hydrocarbons challenging. Different procedures have been used to account for background hydrocarbon concentrations (Cope 2005). In one of these techniques, the subject breathes 'hydrocarbon free' air to washout the lung before a breath sample is collected. However, the purity of synthetic 'hydrocarbon free' air is highly variable and can have total hydrocarbon concentrations as high as 1 ppm (Cope 2005). In the present study, the subjects were allowed to breathe ambient air before collection of the exhaled sample. When this approach is used, it is important that an ambient sample is collected simultaneously for each breath sample because ambient hydrocarbon concentrations can vary greatly over a relatively short period of time (Kamboures et al. 2005). Previous studies have found that the washout time needed to remove ambient ethane and *n*-pentane from the airway is 5–10 min (Kanoh et al. 2005, Drury et al. 1997, Lärstad et al. 2007). It cannot be ruled out that the strong associations between room and exhaled hydrocarbons would not have been as strong when using washout techniques.

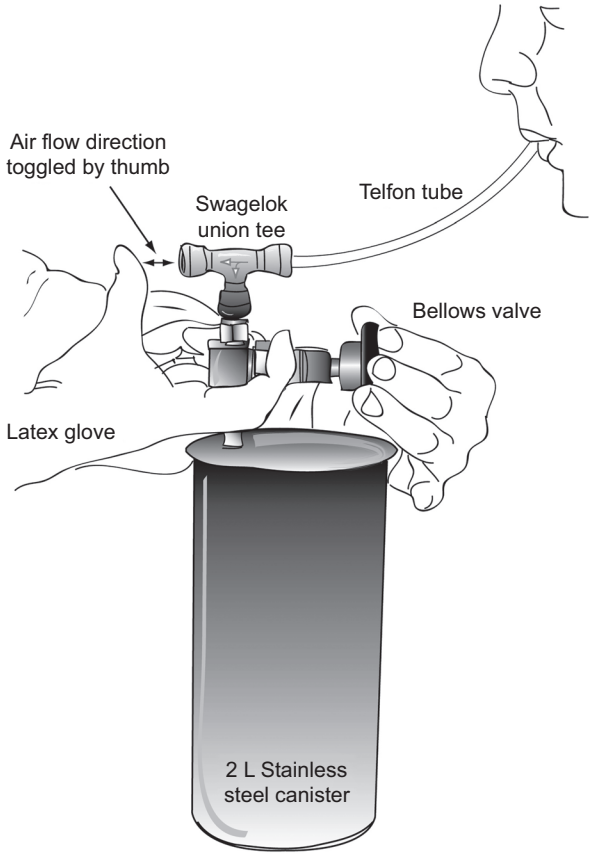

Figure 1. Diagram of a hydrocarbon/CO_2 breath sampling apparatus.

Breath and room samples were analyzed for hydrocarbons by cryogenic preconcentration and injection (264 cm^3 at STP) into a multi-column/detector chromatography system, which has been described in detail elsewhere (Colman et al. 2001). This system employed the use of two flame ionization detectors (FID), two electron capture detectors (ECD), and a quadropole mass spectrometer. Trace gases were quantified using five different column/detector combinations: DB-1/FID, PLOT+DB-1/FID, Restek1701/ECD, DB-5+Restek1701/ECD and DB-5 ms/MSD. The breath and room samples were also analyzed for CO_2 using a separate GC system. For analysis of CO_2, aliquots of sample were injected onto a Carbosphere 80/100 packed column output to a thermal conductivity detector (TCD). Alveolar gradient concentrations of exhaled gases were calculated by subtracting the background room concentration from the corresponding breath concentration. Quoted uncertainties for the linear least-squares analysis of the data are two standard deviations from the regression results.

Exhaled NO

Exhaled NO was measured using standardized ATS/ERS-procedures (ATS/ERS 2005), with modifications suggested by Linn & Gong (2004), involving collection of exhalate into a non-reactive 1.5 l Mylar reservoir bag (Ionics Inc., Boulder, CO, USA) for later chemical analysis. Subjects inhaled orally to total lung capacity and then immediately performed a slow vital capacity manoeuvre into an offline apparatus attached to a Mylar bag. During sampling a flow rate of 100 ml s^{-1} was maintained. Approximately 200 ml of dead-space air was vented over 2 s prior to collecting the bag sample to reduce contamination from the upper airways (Jöbsis et al. 2001). To control for inspired ambient NO, an NO/NO_2 chemisorbent filter was placed at the air intake of the apparatus, and subjects breathed through it for 15 s (≥ 2 tidal breaths) before sampling. Three breath samples were collected to assess reliability. Mylar bags were sealed, refrigerated at 6°C, and analyzed within 20 h for $F_{E_{NO}}$ concentration with a chemiluminescence NO

analyzer (NOA™ 280i Sievers, GE Analytical Instruments, Boulder, CO, USA). To address intravariability among the three samples per subject we first calculated the within-individual variability and then averaged these estimates across the population (Chinn 1991). Pairs that were within two standard deviations of this within-individual population average were retained and all such pairs were then averaged to obtain a single measurement.

Carbonylated proteins

Blood samples were collected from the subjects on each of the study dates. A protein carbonyl ELISA kit was used for the direct measurement of dinitrophenylhydrazine (DNPH)-derivatized carbonyl proteins (Cell Biolabs, San Diego, CA, USA). Total plasma protein content of each sample was determined by using the BCA Protein Assay kit (Pierce Biotechnology, Rockford, IL, USA) to express carbonyl concentrations in nmol mg^{-1} protein. As discussed in detail by Requena et al. (2000), the predominant carbonyl products resulting from protein oxidation are glutamic and aminoadipic semialdehydes.

Indoor and outdoor pollution

Indoor and outdoor home measurements of air pollutants and weather variables were made at the retirement community. These data were used to limit exposure error expected when using available regional air monitoring data. Air samplers were placed at an indoor location adjacent to areas of the retirement community where the majority of activities occur. Outdoor home samplers were placed in an air monitoring trailer supplied by the California Air Resource Board. Continuous (1-min) NO and NO_2 measurements were obtained both indoors and outdoors by using Thermo Environmental NOx Analyzers (Model 42, Thermo Environmental Instruments Inc, Franklin, MA, USA). Dasibi Carbon Monoxide Analyzers (Model 3008, Dasibi Environmental Corp, Glendale, CA, USA) were used to measure continuous (1-min integrated) indoor and outdoor CO levels.

Mixed model description and statistical analysis

Linear mixed effects models (Verbeke & Molenberghs 2001) were used to estimate the association of the dependent variables FE_{NO} and carbonylated proteins with the exhaled hydrocarbons, and between exhaled hydrocarbons as a dependent variable with the criteria pollutant gases. This model structure appropriately handles the correlation present when repeated measures are taken on individuals over time. We selected a two-stage hierarchical model with random effects at the subject level. An autoregressive covariance structure was used for the random effects, allowing the correlation between repeated measures to decrease with time. Residual diagnostics were conducted to investigate the presence of influential data points and deviations from assumed functional form. We found that outliers more than three standard deviations from the mean hydrocarbon distribution influenced many of the models. Therefore, we present the data with and without these outliers.

Results and discussion

Using the analytical method described above, 57 trace gases were identified and quantified in the exhaled breath samples. For the purpose of the present study, only concentrations of exhaled ethane and *n*-pentane are reported.

The measurements for ethane and *n*-pentane are shown in Figure 2. As seen in Figure 2, there is a strong correlation between the breath and room hydrocarbon concentrations ($R^2 = 0.93$ and 0.74, for ethane and *n*-pentane, respectively). Assuming a linear relationship between breath and room hydrocarbon concentrations, least-squares analysis of the data in Figure 2 give slopes of 1.06 ± 0.05 for ethane and 1.06 ± 0.10 for *n*-pentane. Slopes larger than 1 indicate that the rate of clearance from the lung is larger than the uptake within the lung. Both hydrocarbons are slightly elevated in the breath samples in comparison to the room samples, although within the measurement uncertainties, the

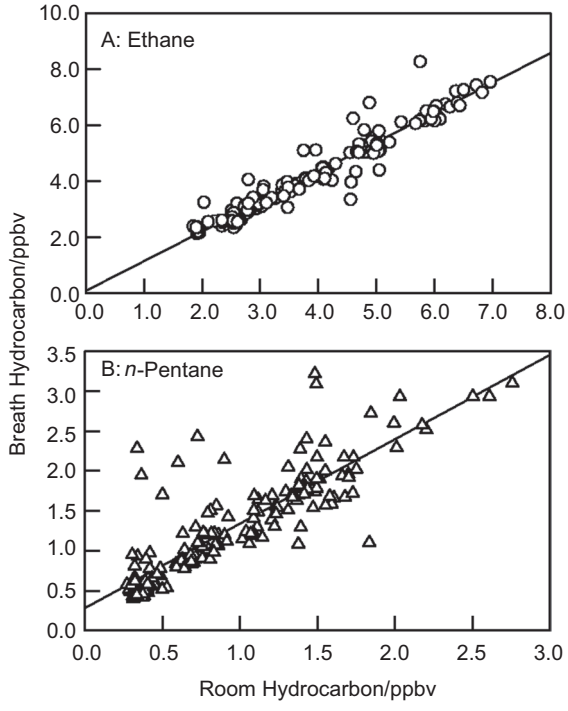

Figure 2. Exhaled breath concentrations versus room air concentrations for ethane (A) and *n*-pentane (B).

breath to room ratios are indistinguishable from each other and there is no statistically significant difference between breath and room for these two gases. Figure 2 shows that background room hydrocarbon concentrations varied by more than a factor of 3 during the study period, and illustrates the importance of obtaining accurate room air samples simultaneously with each breath sample. Hence, for the following analyses, room air was subtracted from breath air, and breath hydrocarbons were normalized to exhaled CO_2 to obtain an estimate of endogenously produced ethane and *n*-pentane. One of the limitations that must be considered when using this technique is the possibility that residual ethane and *n*-pentane in the airways is not fully accounted for by subtracting background room concentrations.

The relationship between ethane and *n*-pentane with exhaled NO was evaluated using bivariate and mixed model analysis. Figure 3 shows the alveolar gradients for ethane and *n*-pentane plotted versus exhaled NO. Exhaled hydrocarbon concentrations are normalized to exhaled CO_2, which had a mean concentration of $4.3 \pm 0.7\%$ (1σ). Assuming that exhaled NO is a marker for airway inflammation, a simple bivariate correlation could be indicative of an association between oxidative stress and airway inflammation. As seen from Figure 3, there are no significant correlations between exhaled ethane or *n*-pentane with exhaled NO ($R^2_{\text{ethane}} = 0.02$ and $R^2_{n\text{-pentane}} = 0.0005$). As seen from Table 1, a mixed linear regression analysis of the data, as described above, also failed to show any significant association between the exhaled hydrocarbons and exhaled NO. This suggests that the short chain alkanes in exhaled breath are not related to airway inflammatory processes and may not be biomarkers of oxidative stress.

A mixed model analysis was also used to evaluate the relationship between the exhaled hydrocarbons and carbonylated proteins. Carbonylated proteins are primarily associated with protein oxidation, but also reflect oxidative mediated lipid peroxidation via the formation of carbonyl-protein adducts. A positive association between hydrocarbons and carbonylated proteins would suggest that an increase in exhaled hydrocarbons could be indicative of oxidative stress. As shown in Table 2, there is no significant association between the hydrocarbons and the carbonylated

Table 1. Statistical evaluation of exhaled ethane and *n*-pentane as predictors of exhaled NO.

	DF	Mixed model estimate (95% confidence limits)	*p*-Value
Outliers[a] *retained*			
Ethane	147	1.7 (−60.3, 63.7)	0.96
n-Pentane	151	−62.8 (−222.2, 96.6)	0.44
Outliers[a] *removed*			
Ethane	145	−0.3 (−70.4, 69.8)	0.99
n-Pentane	147	−86.9 (−277.4, 103.6)	0.37

[a]Outliers are defined as observations 3 standard deviations beyond the mean of the predictor.

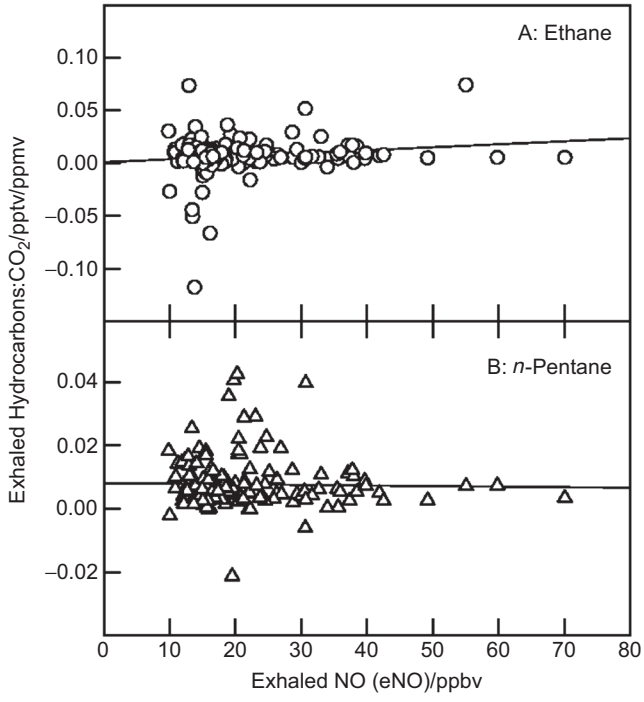

Figure 3. Correlation of exhaled ethane (A) and *n*-pentane (B) with exhaled NO. Exhaled hydrocarbon concentrations are normalized to exhaled CO_2.

proteins when outliers were included. Without the inclusion of outliers, still no significant association between ethane and the carbonylated proteins exists, while the association between *n*-pentane and the carbonylated proteins is significant, but negative. As the results show, no positive relationship was identified between exhaled hydrocarbons and carbonylated proteins.

Continuous measurements of outdoor and indoor gaseous air pollutants (NO, NO_2, and CO) were performed in order to look for relationships with exhaled concentrations of ethane and *n*-pentane. By examining the relationship between exhaled hydrocarbons and indoor/outdoor air pollutants it is possible to evaluate to what extent atmospheric pollutants influence concentrations of exhaled ethane and/or *n*-pentane. The indoor and outdoor criteria air pollutant measurements are summarized in Table 3. The change in hydrocarbon per interquartile range (IQR) increase in the air pollutant is listed in Table 4. The influence of exposure to criteria pollutants was investigated by using the average concentrations of criteria pollutants for the previous 1 h as well as the previous 8 h. Model results are reported in Table 4, with and without inclusion of outliers in high exhaled ethane and *n*-pentane concentrations (>3σ). Inclusion of the outliers significantly reduces the estimated hydrocarbon change per IQR pollutant gas toward zero and confidence limits widen. Figures 4 and 5 show the change in hydrocarbon per IQR increase in air pollutant with outliers removed for high exhaled ethane and *n*-pentane concentrations. In general, the criteria

Table 2. Statistical evaluation of exhaled ethane and *n*-pentane as predictors of carbonylated proteins.

	DF	Mixed model estimate (95% confidence limits)	*p*-Value
Outliers[a] retained			
Ethane	124	−0.0040 (−0.036, 0.028)	0.81
n-Pentane	128	−0.034 (−0.097, 0.028)	0.28
Outliers[a] removed			
Ethane	118	−0.018 (−0.087, 0.050)	0.59
n-Pentane	123	−0.11 (−0.21, −0.0096)	0.032

[a]Outliers are defined as observations 3 standard deviations beyond the mean of the predictor.

Table 3. Summary of indoor and outdoor air pollution measurements.

	Mean	Standard deviation
Indoor (8-h average)		
NO (ppbv)	6.63	4.24
NO_2 (ppbv)	11.41	2.54
CO (ppmv)	0.50	0.15
Outdoor (8-h average)		
NO (ppbv)	5.66	3.87
NO_2 (ppbv)	14.13	7.42
CO (ppmv)	0.20	0.13

Table 4. Statistical evaluation of ethane and *n*-pentane as markers for exposure to criteria air pollutants (1-h and 8-h averages). 95% confidence limits are listed in the parentheses.

Change in hydrocarbon per IQR (10^{-3} pptv/ppmv)	Conc. Avg.	NO	NO_2	CO
Hydrocarbon outliers[a] removed				
Indoor				
Ethane	1-h	12.66 (6.94, 18.4)	4.74 (0.43, 9.06)	4.64 (1.77, 7.52)
	8-h	6.55 (2.01, 11.1)	4.36 (−0.97, 9.69)	2.76 (−0.14, 5.65)
n-Pentane	1-h	4.52 (1.70, 7.34)	2.52 (0.56, 4.47)	1.16 (−0.20, 2.52)
	8-h	2.83 (0.70, 4.97)	1.87 (−0.52, 4.25)	0.05 (−1.24, 1.36)
Outdoor				
Ethane	1-h	7.94 (2.91, 12.97)	5.34 (1.35, 9.31)	−0.90 (−3.92, 2.13)
	8-h	1.62 (−0.99, 4.24)	1.93 (−1.91, 5.78)	−3.69 (−6.75, −0.63)
n-Pentane	1-h	3.92 (1.52, 6.32)	3.66 (1.88, 5.44)	2.13 (0.79, 3.46)
	8-h	1.49 (0.31, 2.67)	2.48 (0.77, 4.19)	1.46 (0.08, 2.84)
Hydrocarbon outliers[a] retained				
Indoor				
Ethane	1-h	12.39 (2.79, 22.00)	4.25 (−2.90, 11.40)	6.62 (1.85, 11.40)
	8-h	5.51 (−2.04, 13.06)	3.01 (−5.66, 11.68)	3.11 (−1.67, 7.89)
n-Pentane	1-h	3.97 (−0.03, 7.96)	1.59 (−1.30, 4.49)	0.96 (−1.02, 2.94)
	8-h	3.33 (0.33, 6.33)	1.55 (−1.96, 5.07)	0.76 (−1.18, 2.71)
Outdoor				
Ethane	1-h	0.41 (−3.85, 4.67)	0.94 (−5.29, 7.17)	−2.59 (−7.77, 2.59)
	8-h	7.34 (−1.12, 15.80)	4.91 (−1.62, 11.44)	0.64 (−4.44, 5.71)
n-Pentane	1-h	1.45 (−2.09, 4.99)	2.63 (−0.06, 5.31)	2.07 (−0.02, 4.15)
	8-h	1.31 (−0.46, 3.07)	2.54 (−0.04, 5.11)	0.98 (−1.18, 3.14)

[a]Outliers are defined as observations 3 standard deviations beyond the mean of the predictor.

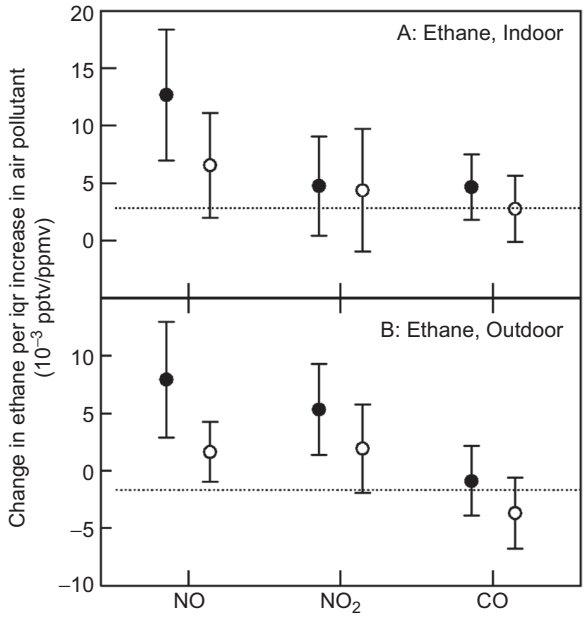

Figure 4. Relationship between change in exhaled ethane and indoor (A) and outdoor air (B) pollutants using the last 1-h concentration average (solid symbols) and the last 8-h concentration average (open symbols) for NO, NO_2 and CO, respectively.

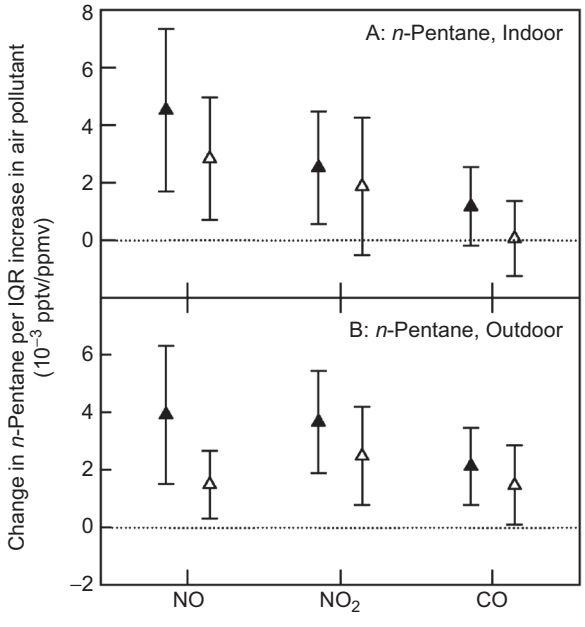

Figure 5. Relationship between change in *n*-pentane and indoor (A) and outdoor (B) air pollutants using the last 1-h concentration average (solid symbols) and the last 8-h concentration average (open symbols) for NO, NO_2 and CO, respectively.

pollutant gases were positively associated with the exhaled breath gases. The most significant change in hydrocarbon per change in IQR pollutant gas is for the 1-h NO average, although this estimate does not exceed 0.015 (pptv/ppmv). Finally, the results using 1-h pollutant averages are consistently higher relative to the 8-h averages.

Table 5. Statistical evaluation of the relationship between indoor hydrocarbons (ethane and *n*-pentane) and air pollutants (NO, NO_2 and CO). Air pollutant measurements are 24-h averages and hydrocarbon measurements are from background canister sampling.

	Correlation coefficients probability				
	Ethane	*n*-Pentane	NO	NO_2	CO
Ethane	1.00000				
n-Pentane	0.83046	1.00000			
	<0.0001				
NO	0.40769	0.28295	1.00000		
	<0.0001	0.0002			
NO_2	0.47359	0.33859	0.90244	1.00000	
	<0.0001	<0.0001	<0.0001		
CO	0.25706	0.07862	0.69156	0.79016	1.00000
	0.0006	0.3024	<0.0001	<0.0001	

The relationship between indoor hydrocarbons (ethane and *n*-pentane) with indoor air pollutants (NO, NO_2, and CO) was also evaluated. Table 5 summarizes the associations between each of these relationships. Significant associations were found between the hydrocarbons and NO_x, and to a lesser extent CO. This suggests that increased exhaled hydrocarbons could be a result of exposure to higher ambient levels of hydrocarbons during air pollution events. If this was the case, elevated levels of exhaled hydrocarbons may be predominantly a marker of exposure to a polluted environment, thus overwhelming any small signal of oxidative stress.

Conclusion

The present work investigates the associations between a biomarker of airway inflammation (exhaled NO), a biomarker of oxidative stress as measured by protein oxidation (carbonylated proteins), and two suspected biomarkers of oxidative stress (exhaled ethane and *n*-pentane). Sixteen subjects were studied each with up to 12 repeated measures. Subjects reside in a highly polluted and oxidizing environment and are at high risk for developing oxidative stress that could induce airway inflammation. Simultaneous background sampling showed hydrocarbon concentrations in breath being slightly, but not significantly, elevated over room air samples. Analysis of exhaled concentrations of ethane and *n*-pentane from elderly subjects did not show significant associations with exhaled NO, which is a widely employed marker of airway inflammation (Choi et al. 2006, Gustafsson 2005). Additionally, neither of the exhaled hydrocarbons had positive associations with carbonylated proteins measured in the blood, which are

indicative of oxidative stress. Finally, positive but weak associations between measurements of exhaled hydrocarbons (ethane and *n*-pentane) and air pollutants (NO_x and CO) were observed. It is conceivable that exposure to higher levels of gaseous air pollutants has a weak but significant impact on exhaled ethane and *n*-pentane. This may be secondary to residual ethane and *n*-pentane in the airways not adjusted for by subtracting room ethane and *n*-pentane from exhaled breath concentrations. Assuming that these hydrocarbon gases are positively correlated to the criteria gases in both outdoor and indoor environments that the subjects have been exposed to, it is possible that the association is not casual but instead a reflection of air pollutant levels.

This study suggests exhaled ethane and *n*-pentane are not sufficiently accurate markers of oxidative stress or airway inflammation, but instead are markers of previous exposure to air pollutants.

Acknowledgements

We thank members of the Rowland-Blake research group from the Department of Chemistry, University of California Irvine, who assisted in the laboratory and with data analysis. We also thank staff from the Department of Epidemiology, University of California Irvine, the General Clinical Research Center (MO1 RR00827), University of California Irvine, the California Air Resources Board, and the South Coast Air Quality Management District, who assisted in the collection of data. MPSA acknowledges the Comer foundation for a research fellowship. The project described was supported by grant number ES012243 from the National Institute of Environmental Health Sciences, U.S. National Institutes of Health, the U.S. Environmental Protection Agency grant number RD83241301, and the California Air Resources Board contract number 03-329. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the funding agencies. The authors declare they have no competing financial interests.

***Declaration of interest*:** The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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