8-Isopostane as a Biomarker of Effect of Woodsmoke Exposure

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INTRODUCTION

In the past few decades, rising fossil fuel energy costs and the desire to use renewable energy resources have led to the increased residential use of biomass fuels, such as wood, in North America (Naeher et al., 2006). Biomass combustion is an important energy resource in both developed and developing countries, and these populations are thus exposed to biomass smoke. Common combustion applications include residential heating and cooking, and prescribed or controlled burns. Local forest fire activity is a potential exposure risk as well.

Biomass smoke is most commonly characterized by particulate matter (PM) concentration. The health effects of PM have been studied extensively. Woodsmoke consists mostly of fine particles, generally smaller than 1 micrometer in diameter (Kleenman et al., 1999). These small particles fall under the category of $PM_{2.5}$ (particulate matter less than or equal to 2.5 micrometers in diameter), which is heavily regulated by the Environmental Protection Agency (EPA) (Naeher et al., 2006). $PM_{2.5}$ is of particular concern to respiratory and cardiovascular health because such particles are more easily respirable to the alveolar sections of the lung (Proudfoot et al., 1999). For epidemiological studies of PM exposure and health risk it is important to look for a non-invasive way to measure the amount of stress these particles impose on the lungs. In this study, 8-isoprostane in urine and exhaled breath condensate (EBC) and the pH level of EBC, are being proposed as possible effect biomarkers of woodsmoke exposure.

8-isoprostane comes from a family of eicosanoids that are produced by the random oxidation of tissue phospholipids. They appear in plasma and urine samples under normal conditions but they are elevated under conditions of oxidative stress. 8-isoprostane has been shown to have biological activity, and elevated levels have been shown in heavy smokers (Morrow et al., 1995). The pH levels in EBC samples have been shown to have been lowered in subjects experiencing oxidative stress (Respiratory Research). This study aims to see if there is a relationship between heavy exposure to woodsmoke and elevated levels of 8-isoprostane in biological samples or changes in pH of EBC.

METHODS

Woodsmoke Exposure:
Two woodsmoke exposure studies were conducted. Four subjects in the first burn and five subjects in the second burn were exposed to woodsmoke from a non-EPA certified woodstove. The exposure duration was two hours and $PM_{2.5}$ was measured during the entire exposure using the Dust Trak Aerosol monitor (TSI, Minneapolis, MN).

Sample collection:
Biological samples were collected from all subjects in both studies. EBC samples were collected using an RTube EBC collection device. EBC samples were collected prior to exposure and immediately following exposure. Spot urine samples were collected in polypropylene collection containers before exposure and at various time points post-exposure. For the first burn post-exposure urine samples were collected at 0, 4, 12, and 24 hours. For the second burn, spot urine samples were collected for all urine voids in the 24 hours following exposure. EBC and urine samples were aliquoted and stored at -20°C for the first burn and at -80°C for the second burn.

Sample Analysis:
Analysis of the EBC and urine samples from both woodsmoke exposures was done using Cayman Chemical's 8-Isoprostane Enzyme Immunoassay (EIA) kit, which is based on the competition between 8-Isoprostane and an 8-Isoprostane-acetylcholinestrase conjugate for a limited number of specific binding sites. The plate is read at 405 nm after a two-hour incubation period. Data was analyzed using formulas provided by the Cayman Chemical Company. For the second burn, the urine samples were run through a purification process using Cayman Chemical's Affinity Sorbent to isolate the 8-Isoprostane.

EBC pH:
The pH of the EBC samples was determined by using a micro pH probe. The pH was measured before deaeration with argon and after the deaeration. The samples were bubbled with argon gas at a steady rate for an average time of fifteen minutes, which was sufficient enough to remove all carbon dioxide from the sample.

Data Analysis:
Concentrations of 8-isoprostane and pH levels in biological samples before and after exposure were compared using a paired t-test.

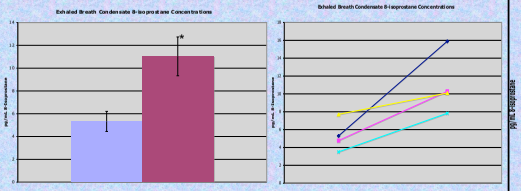
Human Subjects:
Informed consent and experimental procedures were approved by the University of Montana Institutional Review Board (IRB#161-07).

RESULTS

I. 8-Isoprostane

Exhaled Breath Condensate 8-isoprostane concentrations: First Burn Experiment

Fig. 1a and 1b

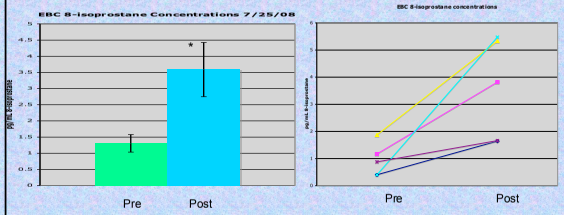


* p = 0.047376508 for paired t-test.

Note that concentrations were calculated for this exposure by using a corrected $\%B/B_0$, not a standard curve

Exhaled Breath Condensate 8-isoprostane concentrations: Second Burn Experiment

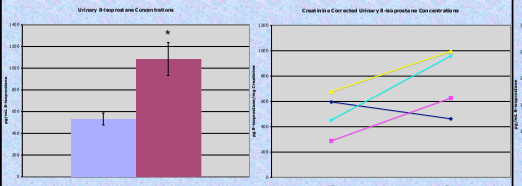
Fig. 3a and 3b



* p = 0.026974683 for paired t-test.

Urinary 8-isoprostane concentrations: First Burn Experiment

Fig. 2a and 2b

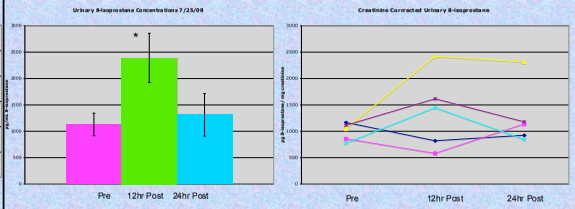


* p = 0.034635601 for paired t-test.

Note that concentrations were calculated for this exposure by using a corrected $\%B/B_0$, not a standard curve

Urinary 8-isoprostane concentrations: Second Burn Experiment

Fig. 4a and 4b

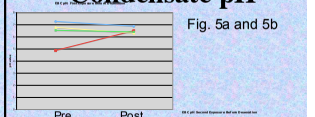


•p = 0.295667122 for paired t-test for pre v. 12 hr

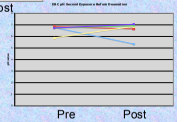
•p= 0.327501335 for paired t-test for pre v. 24 hr

II. Exhaled Breath Condensate pH

Fig. 5a and 5b

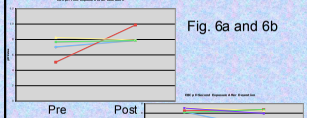


p= 0.779626715for paired t-test9

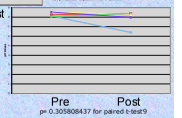


p= 0.9550106 for paired t-test9

Fig. 6a and 6b



p= 0.3238494for paired t-test9

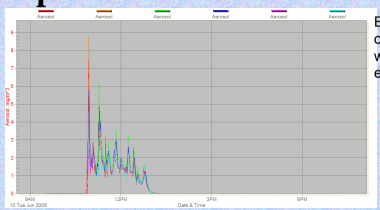


p= 0.305808437 for paired t-test9

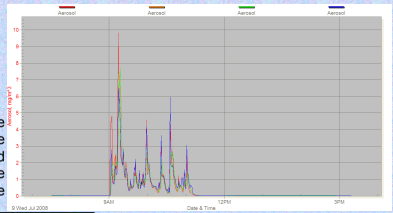
RESULTS

- EBC 8-isoprostane concentrations increased from pre exposure to post exposure in both exposure experiments (Fig. 1a,1b, 3a and 3b)
- Urinary 8-isoprostane concentrations increased post exposure in both exposure experiments. (Fig 2a, 2b, 4a and 4b)
- EBC pH data is inconclusive because the data did not show any significant increase or decrease (see p values); (Fig 5a, 5b, 6a, and 6b)
- Deaeration of EBC samples increased the pH values by approximately 20% in both the pre and post samples (data not shown)
- Urine and EBC purification put the concentration levels of samples purified within the confidence range of analysis. (data not shown)

Exposure Data



Exposure graph of the first woodsmoke exposure



Exposure graph of the second woodsmoke exposure

CONCLUSIONS

I. 8-isoprostane is a useful biomarker of systemic inflammation in healthy individuals following high woodsmoke exposure. Further evaluation of this biomarker in lower exposure residential settings is warranted.

II. The EBC pH findings were inconsistent as it showed neither an overall significant increase or decrease in pH. It is possible that this biomarker of effect is relevant only in susceptible populations.

III. According to our data, purification of EBC and urine samples was a necessary step in determining 8-isoprostane concentrations.

Literature Cited

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Acknowledgments

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