Identification of Persons Who Are Responsive To Wood Smoke Particle-Induced Airway Inflammation With Assessment of The Effect of GSTM1, Asthma Status And Sex On This Response.

David B. Peden (✉ david_peden@med.unc.edu)

University of North Carolina at Chapel Hill <https://orcid.org/0000-0003-4526-4627>

Laura Zhou

University of North Carolina at Chapel Hill

Allison J. Burbank

University of North Carolina at Chapel Hill

Martha Almond

University of North Carolina at Chapel Hill

Michelle L. Hernandez

University of North Carolina at Chapel Hill

Katherine H Mills

University of North Carolina at Chapel Hill

Terry L. Noah

University of North Carolina at Chapel Hill

Heather Wells

University of North Carolina at Chapel Hill

Haibo Zhou

University of North Carolina at Chapel Hill

Neil E. Alexis

University of North Carolina at Chapel Hill

Research

Keywords: Wood smoke particle exposure, airway neutrophil response, responder status

Posted Date: November 17th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1065589/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Identification of Persons Who Are Responsive to Wood Smoke Particle-induced Airway Inflammation with assessment of the effect of GSTM1, asthma status and sex on this response.

David B. Peden, MD^{1,2*}, Laura Zhou, MS^{1,4*}, Allison J. Burbank, MD^{2,4*}, Martha Almond, RRT¹, Michelle L. Hernandez, MD^{2,4}, Katherine H Mills, MS², Terry L. Noah, MD^{1,3}, Heather Wells, MS¹, Haibo Zhou, PhD^{1,4} and Neil E. Alexis, PhD^{1,2}. (*DBP, AB and LZ contributed equally to this work)

1. Center for Environmental Medicine, Asthma and Lung Biology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

2. Division of Allergy & Immunology, Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

3. Division of Pulmonology, Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

4. Childrens Research Institute, Department of Pediatrics, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

5. Department of Biostatistics, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC

Corresponding Author:

David B. Peden, MD, MS

Center for Environmental Medicine, Asthma and Lung Biology

104 Mason Farm Road, CB 7310, School of Medicine, University of North Carolina at Chapel Hill

Chapel Hill, NC 27599-7310

ABSTRACT:

Background: We are currently screening human volunteers to determine their sputum polymorphonuclear neutrophil (PMN) response 6 and 24 hours following initiation of exposure to wood smoke particles (WSP). Inflammatory responders ($\geq 10\%$ increase in %PMN) are identified for their subsequent participation in mitigation studies against WSP-induced airways inflammation. In this report we compared responder status (N=52) at both 6 and 24hr time points to refine/expand its classification, assessed the impact of the GSTM1 genotype, asthma status and sex on responder status, and explored whether sputum soluble phase markers of inflammation correlate with PMN responsiveness to WSP.

Results: In the entire cohort, we found a significant, but very small, decrease in FVC and systolic blood pressure immediately following WSP exposure and sputum %PMNs were significantly increased at 24 hours post exposure, the latter finding was also significantly correlated with sputum IL-1b, IL-6, IL-8, and PMN/mg; a similar response was not found at the 6 hour %PMN response. Blood endpoints in the entire cohort showed a significant increase in %PMN and PMN/mg at 6 but not 24 hours. Six-hour responders tended to be 24-hour responders and vice versa, but 24-hour responders also had significantly increased IL-1b, IL-6, IL-8 at 24 hours post WSP exposure. The GSTM1 null genotype significantly ($p < 0.05$) enhanced the %PMN response at 6 hours in the entire cohort, by 24% in the 24-hour responders and not at all in the 6 hours responders. Asthma status enhanced the 24 hour %PMN response in the entire cohort and in the 6- and 24-hour responders. Sex had no effect on %PMN response.

Conclusions: The 24 hour time point was more informative than the 6 hour time point in optimally defining airway inflammatory responsiveness to WSP exposure. GSTM1 and asthma status are significant effect modifiers of this response. These study design and subject parameters should be considered before enrolling volunteers for proof-of-concept WSP mitigation studies.

KEYWORDS:

Wood smoke particle exposure; airway neutrophil response; responder status

BACKGROUND:

Wood smoke particles (WSP) produced by combustion of biomass contribute to both household and ambient air PM_{2.5} air pollution, and are associated with exacerbations of asthma, pneumonia, chronic obstructive pulmonary disease and cardiovascular morbidity¹. Household WSP reach levels ≥ 1000 ug/m³. WSP from wildfires accounts for up to 29% of ambient fine size (2.5-micron diameter or less) particulate matter (PM)_{2.5} levels in the US airshed and often abruptly produce ambient air PM_{2.5} levels >250 ug/m³, with firefighters often exposed to levels >750 ug/m³.²

We are currently undertaking proof of concept clinical trials of interventions to mitigate the effect of WSP on airway inflammation using a 2-hour controlled exposure to 500ug/m³ WSP with exercise sufficient to evoke a minute ventilation of 20 L/min/m² body surface area. We are optimizing these studies by only recruiting volunteers with a $\geq 10\%$ increase in the polymorphonuclear neutrophil (PMN) content of the sputum differential count following an open-label screening challenge to WSP. This procedure eliminates non-informative volunteers and is similar to that described by Holz and colleagues^{3 4} who validated an ozone challenge protocol employed to screen proposed anti-inflammatory agents.

In Holz et al's ozone challenge protocol, only volunteers shown to be responsive to ozone as defined by a $\geq 10\%$ increase in PMN content of the sputum differential count following a screening challenge to ozone were recruited into the intervention study. Using this double-blinded, placebo-controlled optimized protocol, they found that both inhaled fluticasone and oral prednisone reduced ozone-induced neutrophilic inflammation. Our group led a second study using Holz et al's validation protocol and found similar results⁵. Subsequently, this protocol has

been employed to screen several agents for their action on acute ozone-induced inflammation. Additionally, we have used this definition of responsiveness to assess the effect of the GSTM1 genotype to low level ozone, ⁶ and to assess the differences in gene expression between people who are responsive (responders) or non-responsive (non-responders) to ozone ⁷.

This report documents response of 52 volunteers who have undergone our screening WSP protocol. It expands upon our recent preliminary report of 27 volunteers who had completed this screening protocol, where we observed that 67% of healthy volunteers were responders, and that the GSTM1 null genotype appeared to increase inflammatory response to WSP as observed 24 hours after challenge ⁸. This report expands on these results with a larger sample size which now includes persons with asthma and defines responder status at 2 post exposure time points, namely at 6 hours and 24 hours post challenge. In this report we compare responders and non-responders at both time points, assess the correlation between the 6 and 24 hour neutrophil response and determine the consistency of responsiveness between %PMNs and soluble phase markers of airway inflammation. In addition, we report on the effect of WSP exposure on lung function (forced vital capacity (FVC), forced expiratory volume at 1 second (FEV1), respiratory rate) and cardiovascular outcomes (heart rate, systolic and diastolic blood pressure), and evaluated these outcomes in persons defined as PMN responders at both 6 and 24 hours post WSP challenge. Finally, we assessed whether the GSTM1 null genotype, asthma status and sex are associated with early and late time point responder status to WSP.

RESULTS:

Following baseline (pre-exposure) assessments of lung function, cardiovascular outcomes and collection of induced sputum, volunteers underwent exposure to 500 $\mu\text{g}/\text{m}^3$ wood smoke particles for 2 hours, with alternating 15-minute periods of exercise (cycle ergometer) and rest to achieve 25 l/m^2 body surface/min minute ventilation. Induced sputum induction was performed 6 and 24 hours after initiation of the WSP challenge.

The primary endpoints for this study were identification of persons with at least a 10%-point increase in the percentage of sputum PMNs (%PMN) at 6 and 24 hours post WSP challenge compared to that at pre-exposure baseline, and the effect of the GSTM1 null genotype on responder status. Additional endpoints assessed the impact of asthma status and sex on 6 and 24 hour-responder statuses, the correlation between sputum soluble phase markers of inflammation (IL-1beta, IL-6, IL-8, TNFalpha) and 6 and 24 hour %PMN responses and the effect of WSP exposure on lung function (spirometry) and cardiovascular (systolic and diastolic blood pressure, heart rate) outcomes.

Historic baseline vs. Air Challenge control measures in sputum.

As the goal of this screening protocol is to identify persons who are responsive to the inflammatory effect of WSP for subsequent entry into an intervention study, we chose to compare post WSP exposure responses to a pre-exposure baseline, rather than undertake an additional clean air control challenge. In support of this approach, we pooled our own historical data from several air control chamber challenges previously undertaken that involved 2-3 hours of exercise like that employed for this WSP challenge. At pre air challenge baseline, the %PMN in sputum was 31.7 ± 3.0 % (mean, SEM, n=66) vs. 29.4 ± 2.9 % (n=68) at 6 hours post air challenge and 37.0 ± 8.0 % (n=10) at 24 hours post air challenge (p=0.7). The pre-WSP baseline value was 33.2 ± 3.2 %PMNs (n=50) and was not statistically different (p=0.72) from the baseline, 6h or 24h historic time point values. It has also been reported that FVC and FEV1 following an air control session are unchanged or increase slightly. These observations support using pre-exposure baseline measures without an air exposure control visit to lower subject burden and increase efficiency for screening responsive volunteers for enrollment into intervention studies.

Endpoints for the Entire Cohort:

As of April of 2020 (the date at which the protocol was paused due to the COVID-19 pandemic), 52 volunteers had completed this WSP screening protocol. **Table 1** below outlines the demographics of these volunteers.

Table 1

Total Subjects	N=52
Healthy	N=40
Asthmatic	N=12
Female	N=26 (50%)
Race	
Asian	N=2
Black	N=9
White	N=40
Other	N=1
BMI	25.6+0.7 (range 17.4-38)
Age (years)	27.4+0.8 (range 19.4-40.3)
GSTM1 null	N=20
GSTM1 sufficient	N=26
Refused GSTM1 testing	N=6

Figure 1 depicts spirometric and blood pressure endpoints at baseline and immediately after WSP challenge of these volunteers. There is a significant, but very small, decrease in FVC (1A), and systolic blood pressure (1B) immediately following WSP challenge. Of 52 subjects, 50 provided at least one sputum sample, with 47 (35 healthy volunteers and 12 with asthma) providing matched baseline and 6-hour timepoint sputum samples.

Forty-one (30 healthy volunteers and 11 with asthma) subjects were able to provide matched baseline and 24-hour timepoint samples. **Figure 2** depicts the sputum % PMN and soluble phase inflammatory mediators 6 and 24 hours post WSP challenge. Only the %PMNs in sputum (Figure 2A) are significantly increased by WSP 24 hours after exposure. PMN/mg sputum and sputum levels of IL-1beta, IL-6, IL-8 and TNFalpha (Figure 2, panels B-F respectively) were unchanged by WSP 6 or 24 hours post exposure.

We also assessed the correlation between the change from baseline of %PMNs at 6 and 24 hours with corresponding change from baseline (expressed as %change) of the PMN/mg sputum and sputum levels of IL-1beta, IL-6, IL-8 and TNFalpha using Spearman's Rank Correlation as presented in **Table 2** below. Only one significant correlation was found at 6 hours (PMN/mg), but 3 cytokine variables and absolute neutrophils were found to be significantly associated at 24 hours post WSP exposure (IL-1beta, IL-6, IL-8, PMN/mg).

Table 2:

	6 hour correlations % change in % PMNs vs % change in inflammatory markers			24 hour correlations % change in % PMNs vs % change in inflammatory marker		
	rho	P	N	rho	p	N
IL-1beta	0.06	0.70	42	0.39	0.02	36
IL-6	-0.25	0.10	43	0.36	0.04	34
IL-8	-0.08	0.60	43	0.37	0.02	37
TNFalpha	0.03	0.80	43	0.09	0.60	36
PMN/mg	0.35	0.02	47	0.60	0.01	41

We also examined the relationship between change from baseline in sputum %PMN and PMN/mg sputum at 6 and 24 hours with % change from baseline in FVC, FEV1, systolic and diastolic blood pressure. Of these relationships, the only significant finding ($p < 0.05$) was

between the %change in PMN/mg sputum at 24 hours and % change in FVC ($r=-0.36$); we did observe a trend ($p<0.1$) between PMN/mg sputum at 24 hours and % change in FEV1 ($r=-0.31$).

Table 3

Endpoint	Baseline			6hr post			24 hr post		
	Mean	SE	N	Mean	SE	N	Mean	SE	N
<i>%PMN blood</i>	54.90	1.08	53	61.65*	0.88	52	57.21	1.09	53
<i>PMN 10⁶/ul blood</i>	3.10	0.12	53	4.52*	0.14	52	3.50*	0.16	53
<i>IL-8 blood</i>	13.60	1.60	48	9.59	0.75	50	10.88	0.88	43
<i>IL-6 blood</i>	0.80	0.10	47	1.01	0.11	50	0.82	0.11	43
<i>IL-1 beta blood</i>	0.10	0.02	41	0.08	0.02	49	0.06	0.01	38
<i>TNFalpha blood</i>	2.36	0.21	48	2.09	0.17	50	2.15	0.15	43
<i>CRP blood</i>	2.60	0.40	45	2.60	0.42	44	2.67	0.46	45

We also measured blood endpoints from volunteers at 6 and 24 hours post WSP exposure (Table 3 above). These included circulating levels of PMNs (cells/ul), the PMN differential count (expressed as percent of total nucleated cells), and levels of IL-1beta, IL-6, IL-8, TNFalpha and C-reactive protein (CRP). The % PMN and PMN/mg levels were significantly ($p<0.05$) increased above baseline at 6 hours, but not 24 hours. All other measures were unchanged across all the cohorts.

Endpoints of Responders defined by 6-hour %PMN sputum response:

Forty-seven volunteers provided sputum samples at baseline and six hours after initiation of WSP challenge. Of these volunteers, 30 (64%) were responsive to WSP, as defined by a $\geq 10\%$ point increase in sputum %PMNs. **Figure 3** depicts spirometric and blood pressure endpoints at baseline and immediately after WSP challenge in responders and non-responders. There is a significant, but very small, decrease in FVC (3A), and systolic blood pressure (3B) immediately

following WSP challenge in responders but not in the non-responders. **Figure 4** depicts the airway inflammatory response (cells and soluble phase mediators) to woodsmoke particles of 6 hour responders (N=30) and non-responders (N=17). On average, 6-hour responders also had significantly ($p<0.05$) increased %PMN responses at 24 hours, but no other inflammatory endpoints demonstrated this effect. Systemic inflammatory endpoints were similar when stratified based on airway response at 6 hours (data not shown).

Endpoints of Responders defined by 24-hour %PMN sputum response:

Forty-one volunteers provided sputum samples at baseline and 24 hours after initiation of WSP challenge. Of these volunteers, 28 (68%) were responsive to WSP, as defined by a $\geq 10\%$ increase in sputum %PMNs. **Figure 5** depicts spirometric and blood pressure endpoints at baseline and immediately after WSP challenge of volunteers. There is a significant, but very small, decrease in FVC (3A), and systolic blood pressure (3B) immediately following WSP challenge in responders but not in non-responders **Figure 6** depicts the airway inflammatory response (cells and soluble phase mediators) to woodsmoke particles of 24-hour responders (N=28) and non-responders (N=13). On average, 24-hour responders had significantly increased %PMN and PMN/mg responses at both 24 and 6 hours. Interestingly, and unlike 6-hour responders, 24-hour responders showed significantly increased levels of several pro-inflammatory mediators at 24 hours post WSP exposure (IL-1b, IL-6, IL-8). Systemic endpoints were again similar when stratified based on airway response at 24 hours (data not shown).

Effect of GSTM1 genotype, asthma status and sex on the airway inflammatory response to WSP:

We assessed the role of GSTM1 genotype, asthma status and sex on the sputum %PMN, PMN/mg sputum and sputum IL-1beta, IL-6, IL-8 and TNFalpha response to WSP at 6 and 24 hours using the entire cohort, the 6-hour responder cohort and the 24-hour responder

cohort. We used linear regression modeling approaches in which a given response was expressed as % of baseline, where, $R_{outcome} = \frac{postbaselineoutcome}{baselineoutcome}$, as our main response variable, with a responder defined as $R \geq 1.1$ (equivalent to a 10% increase from baseline in %PMN). For instance, $R_{\%PMN24} = \frac{\%PMN_{at24hourspost}}{\%PMN_{atbaseline}}$. To determine if there is a difference in post baseline versus baseline, we first fit an intercept only model, where the response is defined as $R_{outcome} - 1$. Thus, the hypothesis test for WSP effect is equivalent to testing whether the ratio is statistically different from 1, or equivalently, a t-test to test if $\hat{\beta}_0 = 0$ in an intercept only model. We next expanded our regression model to examine if there are any differences in the ratio between the status of GSTM1, Asthmatics, and responder status (with beta1 in Table 4 indicates the slope for those variables). We also fit the regression model to allow for other covariates of interest that might influence the response variable, as described fully in Methods.

Using this technique, we showed the effect of GSTM1, Asthma and Sex on sputum %PMNs as seen in Table 4 below. We found that the GSTM1 null genotype significantly ($p < 0.05$) enhanced only the %PMN variable and none of the other inflammatory variables as follows: in the entire cohort (Table 4a) at 6 hours post WSP exposure; by 24% in the 24-hour responders (Table 4b); and not at all in the 6-hour responders (Table 4c). Likewise, asthma status enhanced just the %PMN variable and did so at 24 hours in each of the cohorts (entire cohort; 24-hour responders; 6-hour responders), with sex having no effect on this measure. These factors had no effect on any of the other inflammatory outcomes (data not shown).

Table 4a. GSTM1, Asthma status, and Sex effect on cytokine and neutrophil response at 24 hour and 6 hours in the Entire Cohort

	GSTM1			Asthma status			Sex		
	N	beta1/SE	p <0.05	n	beta1/SE	p <0.05	n	beta1/SE	p <0.05
%PMN @24hr	38	- 0.602/0.500	-	44	1.170/0.501	*	44	- 0.880/0.449	-
%PMN @6hr	42	- 0.514/0.311	*	49	0.755/0.646	-	49	- 0.388/0.576	-

Table 4b. GSTM1, Asthma status, and Sex effect on cytokine and neutrophil response at 24 hour and 6 hours in the 24 hour - Responders

	GSTM1			Asthma status			Sex		
	N	beta1/SE	P <0.05	n	beta1/SE	p <0.05	n	beta1/SE	p <0.05
%PMN @24hr	28	- 1.160/0.536	*	32	1.215/0.533	*	32	-0.69/0.532	-
%PMN @6hr	25	- 0.542/0.336	-	29	0.880/0.902	-	29	0.428/0.914	-

Table 4c. GSTM1, Asthma status, and Sex effect on cytokine and neutrophil response at 24 hour and 6 hours in the 6 hour - Responders

	N	beta1/SE	P <0.05	N	beta1/SE	p <0.05	N	beta1/SE	P <0.05
%PMN @24hr	23	- 0.715/0.703	-	28	1.587/0.636	*	28	- 0.900/0.685	-
%PMN @6hr	27	- 0.385/0.329	-	33	0.820/0.816	-	33	0.090/0.804	-

DISCUSSION:

This protocol was developed to screen and identify volunteers who are inflammatory responders to WSP for their entry into subsequent early phase mitigation studies against WSP-

induced airways inflammation. We used our previously published inflammatory responder classification that identified responders as having a greater than 10%-point increase from pre-exposure baseline in sputum %PMN at 6 hours, but here, extended our responder classification to examine the utility of the 24 hour %PMN time point. We further the effect of the GSTM1 genotype, asthma status and sex on responder status, and explored whether WSP exposure induced changes in spirometry and blood pressure outcomes and if sputum inflammatory mediators were associated with %PMN responder status.

Within the overall cohort, we found that sputum %PMN were significantly elevated at 24 hours after WSP exposure. Circulating PMN cell count was also increased at 6 and 24 hours, but no other sputum markers of inflammation, namely soluble phase mediators and PMN/mg, were increased after WSP exposure. When we stratified persons on the basis of neutrophilic airway responsiveness to WSP, we found that 64% were responders at 6 hours and 68% were responders at 24 hours. We also found that the FVC and systolic blood pressure were decreased in the overall cohort and in 6 and 24-hour responder cohorts.

As the goal of the screening protocol is to optimally identify inflammatory responders for intervention studies, we compared results of volunteers defined as responsive using the 6-hour sputum to those defined on the basis of the 24-hour sputum. While the % PMNs were observed to be significantly increased in the 6 hour and 24-hour responder cohorts, we also observed that all of the sputum cytokines except for TNFalpha were increased at 24 hours and not at 6 hours. When we examined the correlation between all the sputum inflammatory markers (cytokines and PMN/mg) with change in %PMNs at 6 and 24 hours, we found that while the %PMNs and PMN/mg sputum significantly correlated in both responder groups, the inflammatory cytokines were significantly correlated only in the 24-hour responder group. We also assessed the effect of the GSTM1 genotype, asthma status and sex on the airway inflammatory response to WSP and found that GSTM1 and Asthma status both significantly impacted %PMN responses in the overall cohort as well as those classified as responders at both 6 and 24 hours.

We further observed that the individual airway inflammatory markers had the highest degree of correlation with each other 24 hours rather than 6 hours after initiation of WSP exposure. In our estimation, 24 hours rather than 6 hours, is the more informative timepoint for establishing inflammatory responder status to WSP exposure in order to enter phase I/II proof of concept mitigation studies. We have conducted previous intervention studies with other air pollutants like ozone ⁵ where examining the impact of the GSTM1 genotype on the airway inflammatory response has revealed informative information in terms of responder status at 6 vs 24 hours post exposure.⁶ Like the results reported here with WSP, we have previously found that that the GSTM1 genotype is an important determinant of response to ozone, that significantly impacts the inflammatory responder status of that inhaled pollutant. Indeed, in a previous study of 59 volunteers assessed for the effect of 0.06 ppm ozone on lung function and inflammation, we did not see a significant effect of GSTM1 when %PMNs were expressed as a continuous outcome variable in GSTM1 null vs sufficient volunteers⁶. However, when we reanalyzed those data in a follow study, we observed that the likelihood of being an inflammatory responder to ozone, defined using similar criteria to what we employed in this study, was 13-fold higher in the GSTM1 null population than in the sufficient population ⁷. Our group have also performed studies that suggest that inflammatory responders to air pollutants like ozone possess unique genomic signatures and microRNA expression profiles that mediate important biological processes like immune cell trafficking, and immune and inflammatory cell function. ^{8,9} These studies, together with the data presented here on WSP, suggest that identification of risk factors or testing of interventions to mitigate airway responses to air pollutants like wood smoke particles, should focus on inflammatory responsive individuals, where effect modifiers like GSTM1 genotype and asthma status need to be considered when evaluating respiratory outcomes.

CONCLUSIONS:

Our data suggest that 24 hours rather than 6 hours post exposure, is the more informative time point for defining airway inflammatory responders to wood smoke particle exposure. Further, GSTM1 and asthma status should be considered important effect modifiers of responder status.

METHODS:

A total of 52 subjects (26 male, 40 healthy, 12 asthmatics), aged 18-45 years completed the WSP screening protocol (Table 1, subject demographics). Fifty subjects provided at least one sputum sample; 47 (35 healthy; 12 asthmatics) provided matched baseline and 6-hour timepoint sputum samples; and 41 (30 healthy; 11 asthmatics) provided matched baseline and 24-hour timepoint sputum samples. All subjects were non-smokers with no acute respiratory illness in the prior 4 weeks, and no current allergic rhinitis symptoms. Asthmatics had mild to moderate physician diagnosed asthma, a positive methacholine challenge test and were not on oral corticosteroid therapy. The GSTM1 genotype was determined by buccal swab analysis using methods previously described ⁶. Twenty subjects were GSTM1null, twenty-six were GSTM1 sufficient, with six refusing GSTM1 genotyping. Written consent was obtained from all participants, and the study was approved by the University of North Carolina Institutional Review Board.

Details of the WSP exposure protocol are described previously.¹⁰ In brief, baseline induced sputum samples were obtained prior to the WSP chamber visit and at 6 and 24 hours following WSP exposure. The WSP chamber used wood smoke generated by heating red oak wood on an electric heating element. Subjects were exposed to 500 µg/m³ WSP over a 2-hour period with alternating 15-minute periods of rest and exercise on a cycle ergometer at a level sufficient to produce a minute ventilation of 20 L/min/m² body surface area. Induced sputum was collected and processed according to previously published methods.¹⁰ The primary endpoints were sputum percent neutrophils (%PMNs) at 6 and 24 hours post- initiation of the WSP exposure

compared to baseline samples. Consistent with previous studies at our center,^{10 11} inflammatory responders were defined as those who experienced a ≥ 10 percentage point increase in sputum %PMN. Measures of lung function (spirometry), cardiovascular status (blood pressure, heart rate) sputum soluble markers and serum inflammatory markers were also recorded at baseline and 6 and 24 hour post WSP exposure time points.

Statistical Analyses:

We compared the effect of woodsmoke particle (WSP) exposure 6 and 24 hours after beginning the 2-hour exposure challenge to baseline lung function, sputum and blood measures. We analyzed these data by fitting a mixed model as implemented in GraphPad Prism 8.0. This mixed model uses a compound symmetry covariance matrix and is fit using Restricted Maximum Likelihood (REML). In the absence of missing values, this method gives the same P values and multiple comparisons tests as repeated measures ANOVA. In the presence of missing values (missing completely at random), the results can be interpreted like repeated measures ANOVA.

To assess the effect of GSTM1, Asthma and sex on sputum inflammatory measures, we assessed the sputum outcomes of interest measured at baseline, 6 hours after exposure commenced, and 24 hours after exposure commenced. To investigate if wood smoke (WSP)

had an effect on subjects, for endpoint X, we used the ratio, defined as $R_x = \frac{X_{post}}{X_{pre}}$. For instance,

$R_{\%PMN24} = \frac{\%PMN_{at24hours}}{\%PMN_{atbaseline}}$. For the form of the outcome variable, we also considered the

differences from the baseline, as well as various transformations, e.g., log transformation. For modeling, we selected the ratio form of our outcomes based on the QQ plots to assess the model assumptions.¹² The hypothesis test for WSP effect is to test whether the ratio is statistically different from 1. We fit the following regression model,

$$Y = \beta_0$$

where $Y = R_{PMN} - 1$ is the response variable, and β_0 is the intercept. The p-value for testing $\beta_0 = 0$ is equivalent of testing $R_{PMN} = 1$, i.e., if there is a significant change in outcome at post exposure from pre-exposure.

We then employed the regression model to examine whether GSTM1 status or Asthma status have a significant effect on the ratio of interest. We fit the following model,

$$R_{PMN} = \beta_0 + \beta_1 * x_{covar}$$

where x_{covar} is the covariate of interest. For GSTM1 status, we will use GSTM1-Null (GSTM1-) as the reference group ($x_{covar} = 1$ if GSTM1+, else $x_{covar} = 0$). For Asthma status, we will use Healthy as the reference group ($x_{covar} = 1$ if Asthmatic, else $x_{covar} = 0$). The p-value for β_1 tests whether $\hat{\beta}_1 = 0$, i.e., if there is a significant change in the outcome between the covariate levels. We fit the models using *lm()* in R 3.6.1¹².

LIST OF ABBREVIATIONS

WSP wood smoke particles; GSTM1 glutathione S transferase mu 1; PMN polymorphonuclear neutrophil; FVC forced vital capacity; FEV1 forced expiratory volume in 1 second; IL interleukin; TNFalpha tumor necrosis factor alpha; ug/m³ microgram per cubic meter; BMI body mass index; ANOVA analysis of variance; l/m² liters per square meter

DECLARATIONS

Ethics approval and consent to participate

This study (IRBIS 15-1775 titled "To identify persons who are susceptible to WSP-induced inflammation and examine the role of GSTM1 and other factors in this susceptibility") was reviewed and approved by the Office of Human Research Ethics of the University of North Carolina at Chapel Hill (the UNC-CH IRB). All volunteers provided informed consent prior to participation in this study.

Consent for publication

All data are mean and standard error of the mean with no individually identifiable personal data being reported

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Additionally, this study is listed in ClinicalTrials.gov (NCT02767973), and data will be posted in that database upon completion of the study. Where informed consent was given, all unused de-identified biospecimens will be stored and curated in the CEMALB Biorepository (IRB# 05-2528) for potential future use.

Competing interests

Authors

"The authors declare that they have no competing interests"

Funding

Research reported in this publication was primarily supported by the National Institute of Environmental Health Sciences of the National Institutes of Health under award number R01ES025124 and Assistance Agreement no. 83578501-0 awarded by the US Environmental Protection Agency (EPA) to the University of North Carolina. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the Environmental Protection Agency. The EPA does not endorse any products or commercial services mentioned in this publication. L.Z. is supported by grant 5T32ES007018. M.L.H and A.J.B. are supported by grant R01HL135235. H.Z. is supported by grant P30ES010126. The NIEHS, NHLBI, and EPA had no role in the design of the study, or collection, analysis, and interpretation of data or in writing the manuscript.

All sources of funding for the research reported should be declared. The role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript should be declared.

Authors' contributions

DBP conceived the study, oversaw regulatory and medical aspects of the study, and was involved in data collection, data analysis and manuscript preparation. LZ conducted statistical data analysis and contributed to manuscript preparation. AJB developed the IRB application and was involved in medical oversight of the study and data collection. MA was involved in IRB preparation and data collection. MLH was involved in medical oversight of the study and data collection in data collection. KHM was involved in IRB preparation and data collection. TLN was

contributed to medical oversight of the study and data collection. HW oversaw sample processing and contributed to data collection and analysis. HZ oversaw statistical analysis of the data and contributed to manuscript preparation. NEA was involved in manuscript preparation and all aspects of sample collection, laboratory processing and analysis.

Acknowledgements

Not applicable

Authors' information (optional)

Not applicable

REFERENCES

1. Oluwole, O., Arinola, G.O., Huo, D. et al. Household biomass fuel use, asthma symptoms severity, and asthma underdiagnosis in rural schoolchildren in Nigeria: a cross-sectional observational study. *BMC Pulm Med* 17, 3 (2017). <https://doi.org/10.1186/s12890-016-0352-8>
2. Aguilera, R., Corringham, T., Gershunov, A. et al. Wildfire smoke impacts respiratory health more than fine particles from other sources: observational evidence from Southern California. *Nat Commun* 12, 1493 (2021). <https://doi.org/10.1038/s41467-021-21708-0>
3. Holz, O., Biller, H., Mueller, M. et al. Efficacy and safety of inhaled calcium lactate PUR118 in the ozone challenge model - a clinical trial. *BMC Pharmacol Toxicol* 16, 21 (2015). <https://doi.org/10.1186/s40360-015-0021-1>
4. Olaf Holz, Ruth Tal-Singer, Frank Kannies, Kathy J. Simpson, Anthony Gibson, Rupert S. J. Vessey, Stanislaw Janicki, Helgo Magnussen, Rudolf A. Jörres and Kai Richter Validation of the Human Ozone Challenge Model as a Tool for Assessing Anti-Inflammatory Drugs in Early Development *J Clin Pharmacol* 2005 45: 498 DOI: 10.1177/0091270004273527
5. Neil E. Alexis, John C. Lay, Angela Haczku, Henry Gong, William Linn, Milan J. Hazucha, Brad Harris, Ruth Tal-Singer, David B. Peden Fluticasone Propionate Protects

- 445 against Ozone-Induced Airway Inflammation and Modified Immune Cell Activation Markers in
 446 Healthy Volunteers EHP June 1, 2008, <https://doi.org/10.1289/ehp.10981>
- 447 6. Neil E. Alexis, Haibo Zhou, John C Lay, Bradford Harris, Michelle L Hernandez, sui-
 448 Shan Lu, Philip A. Bromberg, David Diaz-Sanchez, Robert B. Devlin, Steven R. Kleeberger,
 449 David B. Peden The Glutathione-S-Transferase Mu 1 null genotype modulates ozone-induced
 450 airway inflammation in humans J Allergy Clin Immunol. 2009 Dec; 124(6): 1222–1228.e5. doi:
 451 10.1016/j.jaci.2009.07.036
- 452 7. Neil E. Alexis, John C. Lay, Haibo Zhou, Chong S. Kim, Michelle L. Hernandez, Howard
 453 Kehrl, Milan J. Hazucha, Robert B. Devlin, David Diaz-Sanchez, David B. Peden. The
 454 glutathione-S-transferase mu 1 (GSTM1) null genotype and increased neutrophil response to
 455 low-level ozone (0.06 ppm) JACI, 2013 Feb;131(2):610-2
- 456 8. Rebecca C. Fry, Julia E. Rager, Rebecca Bauer, Elizabeth Sebastian, David B. Peden,
 457 Ilona Jaspers, Neil E. Alexis Air toxics and epigenetic effects: ozone altered microRNAs in the
 458 sputum of human subjects Am J Physiol Lung Cell Mol Physiol. 2014 Jun 15; 306(12): L1129–
 459 L1137. doi: 10.1152/ajplung.00348.2013
- 460 9. Fry RC, Rager JE, Zhou H, Zou B, Brickey JW, Ting J, Lay JC, Peden DB, Alexis NE.
 461 Individuals with increased inflammatory response to ozone demonstrate muted signaling of
 462 immune cell trafficking pathways. Respir Res. 2012 Oct 3;13(1):89.
- 463 10. Allison J. Burbank, Michelle L. Hernandez, Katherine H. Mills, Ethan M. Alt, Haibo Zhou,
 464 David B. Peden Characterizing Airway Inflammatory Responses to Wood-Smoke Inhalation
 465 JACI 2019 Volume 143DOI: <https://doi.org/10.1016/j.jaci.2018.12.073>
- 466 11. Madeline A Dillon, Bradford Harris, Michelle L Hernandez, Baiming Zou, William Reed,
 467 Philip A Bromberg, Robert B Devlin, David Diaz-Sanchez, Steven Kleeberger, Haibo Zhou, John
 468 C Lay, Neil E Alexis, and David B Peden Enhancement of systemic and sputum granulocyte
 469 response to inhaled endotoxin in people with the GSTM1 null genotype. Occup Environ Med.
 470 2011 Oct; 68(10): 10.1136/oem.2010.061747. doi: 10.1136/oem.2010.061747

12. R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>To assess the effect of GSTM1 genotype, asthma status and sex on sputum

FIGURES, TABLES AND ADDITIONAL FILES

Table Titles and Legends

Table 1 Title: Subject Demographics.

Table 2 Title: Correlations between sputum %PMNs and other sputum inflammatory endpoints

Table 3 Title: Systemic Endpoints of Entire Cohort at 6 and 24 hours Post WSP Exposure

Table 3 Legend: Descriptive data (mean, standard error and n for available sample) for each systemic endpoint of the overall cohort at baseline, 6 and 24 hours after two-hour controlled exposure to 500ug/m³ WSP. Cytokines in blood are measured as pg/ml. Asterisk denotes p<0.05 vs. baseline values, which were present only for the circulating PMN differential count (%PMN) and absolute PMN levels (10⁶/ul blood)

Table 4 Title: Regression analyses for GSTM1, asthma and sex on %PMN:

Figure Titles and Legends

Figure 1 Title: Spirometric and Blood Pressure response to WSP in the overall cohort

Figure 1 Legend: Spirometric and blood pressure endpoints at baseline and immediately after WSP challenge. Fifty subjects provided at least one sputum sample, with 47 (35 healthy volunteers and 12 with asthma) providing matched baseline and 6-hour timepoint sputum samples

Figure 2 Title: Inflammatory Response to Woodsmoke Particles of the Entire Cohort

Figure 2 Legend: Sputum % PMN and soluble phase inflammatory mediators 6 and 24 hours post WSP challenge.

Figure 3 Title: Spirometric and blood pressure endpoints at baseline and immediately after WSP challenge of responsive volunteers as defined by %PMNs at 6 hours.

497 Figure 3 Legend: Spirometric and blood pressure endpoints at baseline and immediately after
498 WSP challenge in responders and non-responders

499 Figure 4 Title: Inflammatory Response to Woodsmoke Particles of responsive volunteers as
500 defined by %PMNs at 6 hours.

501 Figure 4 Legend: The airway inflammatory response (cells and soluble phase mediators) to
502 woodsmoke particles of 6-hour responders (N=30) and non-responders (N=17)

503 Figure 5 Title: Spirometric and blood pressure endpoints at baseline and immediately after WSP
504 challenge of responsive volunteers as defined by %PMNs at 24 hours

505 Figure 5 Legend: Spirometric and blood pressure endpoints at baseline and immediately after
506 WSP challenge of volunteers

507 Figure 6 Title: Inflammatory Response to Woodsmoke Particles of responsive volunteers as
508 defined by %PMNs at 24 hours

509 Figure 6 Legend: The airway inflammatory response (cells and soluble phase mediators) to
510 woodsmoke particles of 24-hour responders (N=28) and non-responders (N=13).

Figures

Figure 1

Spirometric and Blood Pressure response to WSP in the overall cohort Spirometric and blood pressure endpoints at baseline and immediately after WSP challenge. Fifty subjects provided at least one sputum sample, with 47 (35 healthy volunteers and 12 with asthma) providing matched baseline and 6-hour timepoint sputum samples

Figure 2

Inflammatory Response to Woodsmoke Particles of the Entire Cohort Sputum % PMN and soluble phase inflammatory mediators 6 and 24 hours post WSP challenge.

Figure 3

Spirometric and blood pressure endpoints at baseline and immediately after WSP challenge of responsive volunteers as defined by %PMNs at 6 hours. Spirometric and blood pressure endpoints at baseline and immediately after WSP challenge in responders and non-responders

Figure 4

Inflammatory Response to Woodsmoke Particles of responsive volunteers as defined by %PMNs at 6 hours. The airway inflammatory response (cells and soluble phase mediators) to woodsmoke particles of 6-hour responders (N=30) and non-responders (N=17)

Figure 5

Spirometric and blood pressure endpoints at baseline and immediately after WSP challenge of responsive volunteers as defined by %PMNs at 24 hours Spirometric and blood pressure endpoints at baseline and immediately after WSP challenge of volunteers

Figure 6

Inflammatory Response to Woodsmoke Particles of responsive volunteers as defined by %PMNs at 24 hours. The airway inflammatory response (cells and soluble phase mediators) to woodsmoke particles of 24-hour responders (N=28) and non-responders (N=13).