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RESEARCH ARTICLE

Effects on airways of short-term exposure to two kinds of wood smoke in a chamber study of healthy humans

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Abstract

Introduction: Air pollution causes respiratory symptoms and pulmonary disease. Airway inflammation may be involved in the mechanism also for cardiovascular disease. Wood smoke is a significant contributor to air pollution, with complex and varying composition. We examined airway effects of two kinds of wood smoke in a chamber study.

Materials and Methods: Thirteen subjects were exposed to filtered air and to wood smoke from the start-up phase and the burn-out phase of the wood-burning cycle. Levels of $PM_{2.5}$ were 295 µg/m^3 and 146 µg/m^3, number concentrations 140 000/cm^3 and 100 000/cm^3. Biomarkers in blood, breath and urine were measured before and on several occasions after exposure. Effects of wood smoke exposure were assessed adjusting for results with filtered air.

Results: After exposure to wood smoke from the start-up, but not the burn-out session, Clara cell protein 16 (CC16) increased in serum after 4 hours, and in urine the next morning. CC16 showed a clear diurnal variation. Fraction of exhaled nitric oxide (FENO) increased after wood smoke exposure from the burn-out phase, but partly due to a decrease after exposure to filtered air. No other airway markers increased.

Conclusions: The results indicate that relatively low levels of wood smoke exposure induce effects on airways. Effects on airway epithelial permeability was shown for the start-up phase of wood burning, while FENO increased after the burn-out session. CC16 seems to be a sensitive marker of effects of air pollution both in serum and urine, but its function and the significance need to be clarified.

Keywords: Controlled exposure, wood smoke, particles, biomarkers, humans, health effects, airway effects

Introduction

Exposure to particulate air pollution increases both long-term and short-term cardiopulmonary morbidity and mortality (Pope and Dockery, 2006). The mechanisms are not fully elucidated, but some main pathways have been suggested based on experimental and epidemiological evidence. One such pathway is that inhaled particulate matter (PM) induces local airway oxidative stress and inflammation. This leads to systemic proinflammatory and procoagulatory changes which cause cardiovascular events and in the long term increase atherosclerosis (Brook et al., 2004, Brook, 2008, Mills et al., 2009). The health effects of PM are determined not only by weight or number, but also by size, surface area and chemical composition (Donaldson and Tran, 2002, Schwarze et al., 2006). Specific studies of a given particle type are necessary to determine their toxicity. Most experimental evidence of the health effects of PM come from studies on diesel exhaust or concentrated ambient PM (Mills et al., 2009), and most information on health impacts from studies of urban PM (Naeher et al., 2007).

Wood combustion for residential heating is a major source of PM emissions in the developed world, and increasing as policy shifts energy production from fossil fuels towards renewable energy. In the EU-15 (15 EU-countries 2004) the contribution of domestic wood

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stoves was estimated to 25% in the year 2000, with an increase to 38% expected by 2020 (Amann, 2005). In the developing world biomass smoke exposure is often much higher (Naeher et al., 2007).

The relative toxicity of PM from combustion of wood compared to other PM is unclear. Two recent reviews did not find any evidence for less respiratory health effects by wood smoke-derived PM compared to other PM (Boman et al., 2003, Naeher et al., 2007). The latter called for further controlled experimental studies of acute effects and physiological responses to major types of biomass smoke.

In the first experimental chamber study of wood smoke exposure of healthy humans, we found increases in markers of airway effects (CC16, $FENO_{270}$, malondialdehyde (MDA)) as well as biomarkers of systemic inflammation and coagulation (Barregard et al., 2006, Barregard et al., 2008). Since then a small number of experimental studies of wood smoke have been published, all reporting only minor effects (Sehlstedt et al., 2010, Riddervold et al., 2011, Ghio et al., 2011), but also two real-world exposure studies reporting marked effects on biomarkers (Swiston et al., 2008, Allen et al., 2011).

Emissions from wood burning vary considerably depending on wood type, combustion appliance, combustion conditions and the phase of the combustion cycle (Mcdonald et al., 2000, Naeher et al., 2007, Kocbach Bolling et al., 2009). There is limited evidence on which emission characteristics are important for the health effects, or if there are threshold levels. When combustion is less complete the particles contain more organic compounds, which seems to influence toxicity (Kocbach Bolling et al., 2009). Recent animal and *in vitro* studies indicate that the effects might be different for particles from different combustion conditions (Danielsen et al., 2010, Danielsen et al., 2011). No human studies of wood smoke exposure have previously tested the effects of different phases of the combustion cycle.

Therefore, the aim of this study was to test the effects on symptoms and airways by two kinds of wood smoke exposure, one from the start-up phase and another from the burn-out phase of the wood burning cycle. We also wanted to test the effects of lower doses compared to our previous wood smoke exposure study, to determine whether the same effects on biomarkers could be observed at these levels.

Methods

Subjects

We recruited 16 healthy never-smoking subjects (8 men and 8 women) aged 20–57 (mean 31) from the staff of our department and among students. They all had normal spirometry values and none had symptomatic allergy. The subjects were not allowed to take any medication in the 2 days preceding each exposure session, and had to be free from any known infections for at least 1 week prior to the session. Three subjects were excluded before data analysis because respiratory symptoms emerged just before or between the sessions. The study was approved by the regional ethics committee of Gothenburg and the subjects gave their written informed consent.

Experimental design

The study design was similar to the one used previously (Sallsten et al., 2006, Barregard et al., 2006, Barregard et al., 2008), but included two different phases of the combustion cycle and aimed at lower inhaled doses. The volume of inhaled air was only about half that in our previous study since we decreased exposure time to 3 hours instead of four and did not have any periods of exercise, compared to 50 minutes in the previous study (see Table 4).

Subjects were exposed to filtered indoor air for 3 hours on the first occasion as control exposure, to wood smoke from the start-up phase of the wood burning cycle one week later and to wood smoke from the burn-out phase of the wood burning cycle two weeks later. Because the experiments had to be performed during a limited time period, a randomized design was not possible. Apart from the exposure, the sessions were identical. Sessions started with blood, urine and breath condensate sampling, and then the subjects entered the exposure chamber. Samples were also taken several times after exposure (Table 1). A new subject started the schedule every 10 minutes, which means that the first subject left the chamber soon after the last entered, and one whole session lasted 5 hours 40 minutes. In the chamber the subjects read or chatted. In the middle of the exposure the subjects had a small snack (sandwich), and they had free intake of soft drinks and water. The subjects were not allowed to eat closer than 1 hour before the first blood samples or to eat green salad, spinach, sausage, ham or >4 potatoes before NO-measurements (Olin et al., 2001a).

Table 1. Timing of breath, blood, and urine sampling in the first subject. A new subject started the schedule every 10 minutes.

Day of exposure	
Samples #1	
Urine[a]	At home
Breath and blood	07.00
Enter exposure chamber	08.30
Leave exposure chamber	11.30
Samples #2	
Urine[b]	11.35
Lunch	13.00
Breath and blood	15.30
Samples #3 – next morning	
Urine[a]	At home
Breath and blood	07.00
Samples #4 – morning the second day	
Urine[a]	At home
Breath and blood	07.00

[a]Timed from bedtime to morning.
[b]Timed from start to end of exposure.

The exposure chamber and generation of wood smoke

The exposure chamber is at the SP Technical Research Institute of Sweden (SP), in Borås, Sweden. A more detailed description of the exposure chamber and principles of wood smoke generation have been reported previously (Sallsten et al., 2006). Briefly, the chamber measured 7.4 m × 6 m × 2.9 m, and the walls were covered by Teflon-impregnated glass-fiber fabric. Wood smoke was generated in a small cast-iron wood stove placed just outside the chamber. A partial flow of the generated wood smoke was mixed with indoor air (filtered using a high-efficiency particulate air filter) to achieve the target concentration. A mixture of hardwood and softwood was used (50% birch, 50% spruce, moisture content 14–16% and 17–19% respectively). A 2.5–3 kg batch combining small and large logs was ignited, and approximately every 40 minutes another three logs of 1.5 kg were added until the session was over. The chamber walls, floor, and ceiling were cleaned between exposure sessions.

In the session when wood smoke was generated from the start-up phase, smoke was supplied to the chamber for 12–14 minutes starting immediately after new wood logs were added. In the session using smoke from the burn-out phase, smoke was supplied for 15 minutes starting 25 minutes after wood was added. The aim in both sessions was to generate a $PM_{2.5}$ mass concentration in the chamber of about 200 μg/m^3. The $PM_{2.5}$ mass concentration was controlled online (see below) to maintain the target concentration.

Sampling and characterization of wood smoke

Sampling and characterization of wood smoke in the chamber was performed similarly to that in Sallsten et al. (2006). All measurements were taken in the center of the chamber throughout the whole session.

The $PM_{2.5}$ mass concentration was measured online using a tapered element oscillating microbalance (TEOM) (1400 TEOM; Thermo Inc., USA) instrument with inbuilt data correction algorithm (reported concentration = 1.03 × measured concentration + 3). Number concentrations and size distributions of particles (0.007–6.7 μm) were measured by an electric low pressure impactor (ELPI) from Dekati, Finland. In addition, stationary measurements of $PM_{2.5}$ and PM_1 mass concentrations were performed during each session using cyclones and sampling pumps. Some filters were analyzed for trace elements using an energy dispersive X-ray fluorescence (EDXRF) spectrometer (Molnar et al., 2005), and for black carbon (BC) content by an optical method (Magee Inc OT21 Transmissometer, calculation according to classical BC). Other filters were analyzed for particulate polycyclic aromatic hydrocarbons (PAHs) using high-resolution gas chromatography and low resolution mass spectrometry (HRGC/LRMS) (Agilent Technologies, Inc, Santa Clara, CA, USA) (Johannesson et al., 2009, Kliucininkas et al., 2011).

We measured NO and NO_2 online using a chemiluminescence instrument (MEMonitor Europe Chemiluminescence Analyser, ML, UK) and CO_2 as well as CO using infrared instruments (Metrosonics, Inc. NDIR analyzer, aq 5001, USA and Unor 6N; Maihak AG, Germany). Stationary measurements of benzene and 1,3-butadiene were performed using SKC-Ultra diffusive samplers (SKC Inc., USA) filled with Carbopack X (60–80 mesh adsorbent) and the samples were analyzed with an automatic thermal desorber (ATD) (Perkin Elmer Corporation, USA) and gas chromatograph flame ionization detection (Strandberg et al., 2005). Active sampling of formaldehyde and acetaldehyde was performed using pumps and Sep-Pak 2,4-dinitrophenylhydrazine (DNPH)-impregnated silica cartridges (Waters, USA). The aldehydes were analyzed using high performance liquid chromatography (HPLC) (Levin et al., 1988). Measurements of naphthalene, toluene, ethylbenzene and xylenes (o-, m-, and p-xylene) were made using active sampling with Perkin Elmer ATD tubes filled with Tenax TA. The samples were analyzed using ATD and gas chromatograph flame ionization detection (Egeghy et al., 2003, Strandberg et al., 2005). Finally, we registered the temperature and relative humidity in the chamber (Tinytag Ultra TGU-1500; Gemini Data Loggers, UK).

Symptoms and sampling of blood, breath and urine

Subjective symptoms were measured and scored (0–10) according to the Borg scale (Borg, 1982), using a self-administered questionnaire in the last 15 minutes of each session. The symptoms included in the questionnaire were headache, dizziness, nausea, tiredness, chest pressure, cough, shortness of breath, irritation of the eyes, irritation of the nose, unpleasant odor, irritation of the throat and bad taste in the mouth.

Blood was collected by venipuncture in SST-tubes for serum and aliquots were stored frozen in polyethylene cryotubes (Nunc) until analysis. Timed urine samples were collected in polypropylene bottles, males discarding the first 100 mL as described in Andersson et al. (2007), the volumes were registered and aliquots frozen until analysis.

Exhaled breath condensate (EBC) was collected and fraction of exhaled nitric oxide (FENO) measured with the same equipment and as described previously (Barregard et al., 2008). The FENO-measurements were performed in duplicates according to the 2005 ATS/ERS recommendation and at several exhalation flow rates – 50 and 270 mL/s presented as $FENO_{50}$ and $FENO_{270}$. A plus or minus 10% deviation of the instant flow and plus or minus 5% of the mean flow during the plateau phase was accepted.

Biochemical analyses

Clara cell protein 16 in serum and urine (S/U-CC16) and surfactant protein D (SP-D) were analysed using commercial ELISA kits from Biovendor. Surfactant protein A (SP-A) was determined using a home-made ELISA using two different antibodies against human SP-A, one polyclonal and one monoclonal (Ellingsen et al., 2010). EBC was analyzed for MDA, as previously described

(Barregard et al., 2008). U-CC16 is presented adjusted for creatinine levels in urine.

Statistics

For all biomarkers, intra-individual differences at each time point were calculated by subtracting changes after filtered air from changes after each of the two wood smoke sessions separately, making each individual his/her own control. Comparison of symptoms was performed in the same way. Since no biomarkers were normally distributed statistical significance was tested with Wilcoxon's signed rank test. All *p*-values presented are two-sided. Associations between biomarkers were assessed using Spearman's rank correlation coefficient (r_s), separately for each sample time. Biomarker levels below the detection limit were imputed with the detection limit divided by the square root of 2 (Hornung, 1990). Statistical calculations were made using SAS 9.2.

Results

Exposure characterization

The mean air temperature and relative humidity in the exposure chamber was 22°C and 49% in the filtered air session, 22°C and 50% in the start-up session, and 22°C and 29% in the burn-out session, respectively.

The mean PM mass concentration was 295 μg/m^3 in the start-up session and 146 μg/m^3 in the burn-out session and in the filtered air session below the detection limit (Table 2). In the filtered air session TEOM concentration was 8.4 μg/m^3. Results of the online TEOM measurements for the wood smoke sessions are shown in Figure 1 and Table 2. In Figure 1 each peak represents a new supply of wood smoke into the chamber. The ratio between the mean TEOM readings to the mean gravimetric mass concentration was close to one in the burn-out session.

Table 2. Time weighted averages of the online measurements (PM mass, particle number concentration, NO, NO_2, CO), means of replicate filter samples (PM mass, BC, elements and PAHs) and volatile organic carbons (VOCs) in all sessions.

	Filtered air session			Start-up session			Burn-out session		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
$PM_{2.5}$ (μg/m^3) (TEOM)	C	8.4	2	C	221[a]	121	C	148	48
PM[b] mass (μg/m^3)	7	<15		16[c]	295	43	17	146	15
PM_1 (#/cm^3)	C	2900	770	C	140 000	83 000	C	100 000	51 000
Ultra fine particles (%)	C	55	5.8	C	68	21	C	40	15
BC (μg/m^3)	–	–		6	90	17	6	115	10
Trace elements (ng/m^3)									
K	6	<1500		6	9700	4500	6	8800	1200
Zn	6	70	14	6	2400	720	6	3100	340
Rb	6	<30		6	73	30	6	105	18
Pb	6	47	43	6	170	24	6	400	55
Particulate PAHs (ng/m^3)									
Benzo(b)fluoranthene	3	0.03	0.05	6	20	6	6	4.9	1.3
Benzo(k)fluoranthene	3	<0.01		6	23	8.6	6	4.5	1.1
Benzo(a)pyrene	3	0.01	0.006	6	36	15	6	4.8	1.6
Perylene	3	<0.01		6	5.5	2.1	6	1.1	0.26
Indeno(1,2,3-cd)pyrene	3	0.02	0.02	6	49	14	6	14	2.3
Dibenzo(a,h)anthracene	3	<0.01		6	4.3	1.9	6	3	0.5
Benzo(g,h,i)perylene	3	<0.01		6	41	9.6	6	11	1.4
VOCs (μg/m^3)									
Benzene	3	2	0.2	3	33	0.78	3	21	1.1
1,3-Butadiene	3	0.16	0.02	3	8.5	0.17	3	4.2	0.11
Toluene	2	15	3	3	28	0.95	3	17	0.49
Ethylbenzene	2	4.8	0.56	3	4	0.25	3	4.7	0.28
Xylenes	2	19	0.58	3	13	1.3	3	20	1.4
Naphthalene	2	1.6	0.08	3	10	0.79	3	4.1	0.6
Formaldehyde	2	11	0	3	94	4.7	3	81	9.5
Acetaldehyde	2	13	0	3	71	4.2	3	37	4.4
Gaseous (ppm)									
NO	C	0.08	0.02	C	0.14	0.1	C	0.3	0.07
NO_2	C	0.01	0.004	C	0.03	0.01	C	0.05	0.02
CO	C	0.73	0.04	C	5.6	2	C	15	5.1

C, continuous measurements.
[a]Registration missing for half an hour.
[b]Mean of PM_1 and $PM_{2.5}$.
[c]One sample omitted due to leakage.

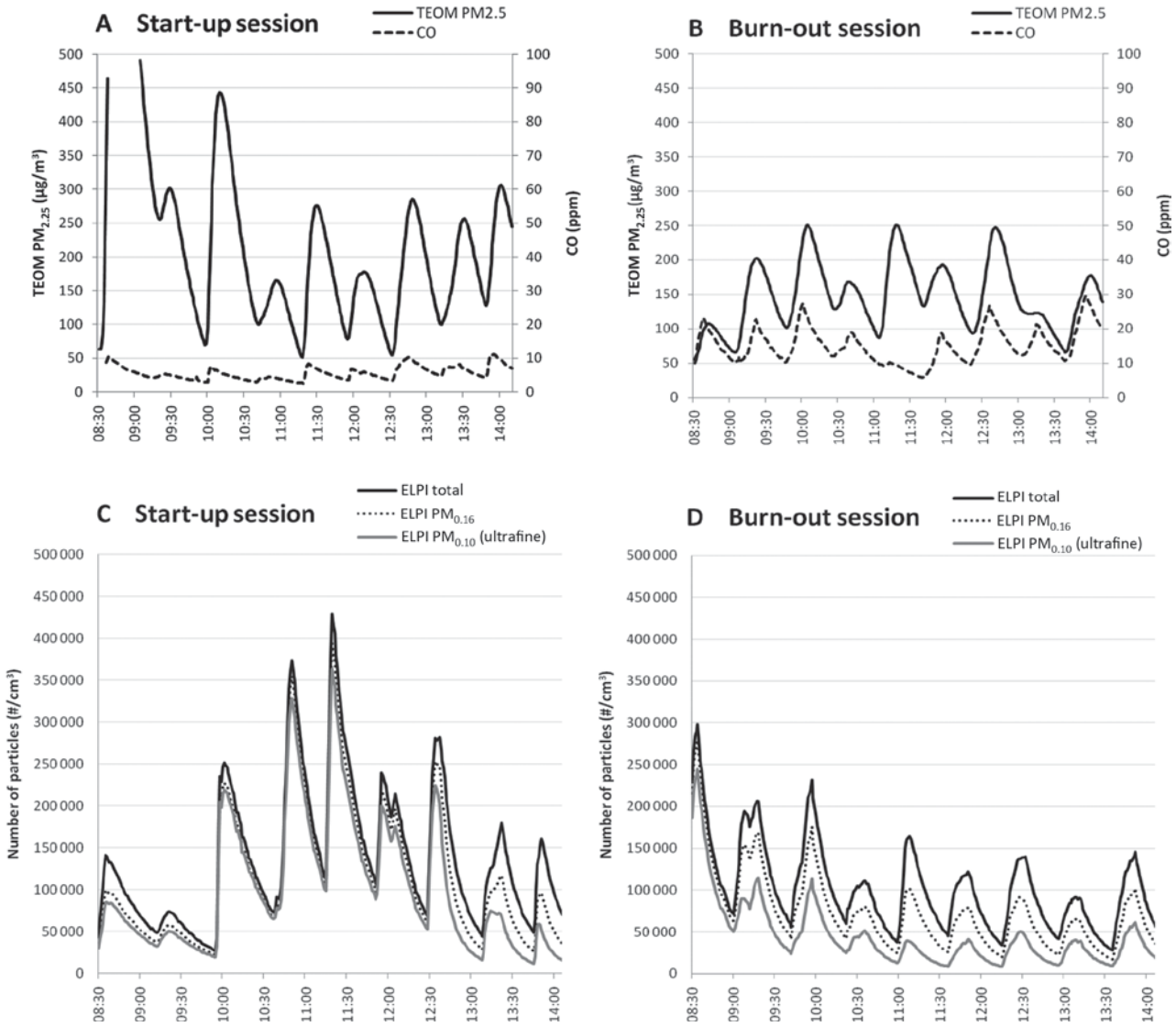

Figure 1. Particle mass ($PM_{0.25}$) and carbon monoxide (CO) concentration during 5.8 h in the exposure chamber for both wood smoke sessions (A and B), measured online using a taperedelement oscillating microbalance (TEOM). Particle number concentrations (C and D) during the same period, in total and in the smallest size intervals ($PM_{0.10}$, or $PM_{0.16}$), as measured using an electric low-pressure impactor (ELPI).

In the start-up session the PM mass concentration was clearly higher during the first half hour with levels exceeding 500 µg/m³ (data not shown due to instrument limitation; Figure 1).

The results of the ELPI measurements (number concentrations) showed that nearly all particles were <1 µm. Particle size distributions resulted in a geometric mean diameter of 38 nm ($\sigma_g = 1.7$) and 83 nm ($\sigma_g = 1.5$), in the start-up and the burn-out session, respectively. The average PM number concentrations were 140 000/cm³ and 100 000/cm³ in the start-up session and burn-out session, respectively, with 68% and 40% of the particles ultrafine (< 100 nm, $PM_{0.1}$) (Table 2 and Figure 1). If we add another stage ($PM_{0.16}$), the corresponding figures are 80% and 69%. In the filtered air session, about half (55%) of the particles were ultrafine, and the total number concentration was 2900/cm³.

BC levels on the analyzed filters ($N = 6$ in each session) were slightly higher in all samples in the burn-out session compared to the start-up session (mean 115 vs. 90 µg/m³). In both wood smoke sessions, the concentrations of K and Zn were much higher compared to the filtered air session where all filters were low or below the detection limit.

The concentrations of particulate PAHs were clearly higher in the wood smoke sessions compared to the filtered air session, and the start-up phase produced higher PAH concentrations than the burn-out phase (Table 2). The average concentration of benzo(a)pyrene (BaP) was 3600 times higher in the start-up session (36 ng/m³) and 480 times higher in the burn-out session (4.8 ng/m³) compared to the filtered air session (0.01 ng/m³). In the start-up session strong correlations were found between almost all PAHs and between the PAHs and PM

(r_s = 0.83–1.0). In the burn-out session fewer significant correlations were found.

Of the volatile organic compounds (VOCs), the concentrations of benzene and 1,3-butadiene were much higher in the wood smoke sessions compared to the filtered air session (Table 2). Higher concentrations of formaldehyde and acetaldehyde and somewhat higher concentrations of naphthalene were also seen. As for the PAHs, the levels of these were higher in the start-up session.

For NO_2, the mean concentration was 0.01 ppm in the filtered air session and 0.03 ppm and 0.05 ppm in the start-up and burn-out sessions, respectively. The highest peak concentrations of NO_2 (0.1 ppm), and CO (30 ppm), were registered in the burn-out session (data not shown). The concentrations of NO and CO were also higher in the burn-out session (Table 2), while the concentrations of CO_2 were similar in all sessions, about 1300 ppm.

Pneumoproteins

In all sessions CC16 had a clear circadian trend with lower S-CC16 and higher U-CC16 in the afternoon than in the mornings. In the start-up session S-CC16 was significantly increased 4 hours after exposure to wood smoke (point estimate 1.4 μg/L, 19%, $p = 0.04$. Table 3, Figure 2A), and U-CC16 the next morning (point estimate 0.23 μg/L, 59%, $p = 0.01$. Table 3, Figure 2B), compared to the filtered air session. No significant changes were found in the burn-out session.

The surfactant proteins SP-A and SP-D showed no significant changes in the start-up session, while there was a significant but small net decrease of SP-D 4 hours after exposure in the burn-out session (4%, $p = 0.03$). SP-D showed a circadian variation with lower afternoon than morning levels, and was correlated with increasing age.

Exhaled NO and MDA in exhaled breath

Adjusted for filtered air, $FENO_{50}$ showed a significant increase after exposure in the burn-out session (point estimates mornings post-exposure 12% and 19%; $p < 0.001$ and $p = 0.003$, respectively. Figure 3B), but did not change significantly in the start-up session.

$FENO_{270}$ increased significantly after exposure in both the start-up session (the first and second morning post-exposure; $p = 0.03$ and $p = 0.02$, respectively) and the burn-out session (4 hours after exposure, and in both mornings post-exposure; $p = 0.006$, $p = 0.01$, and $p = 0.006$, respectively), compared to the filtered air session. This relative increase after wood smoke was, however, in part due to a high $FENO_{270}$ baseline the morning of the filtered-air session leading to a significant decrease after exposure to filtered air (Table 3 and Figure 3A). Without adjustment for changes after exposure to filtered air there was no increase in the start-up session, and the tendency to increase in the burn-out session was no longer significant.

$FENO_{50}$ and $FENO_{270}$ were highly correlated with each other, and often correlated with MDA – which had a slight

Table 3. Biomarkers of airway effects. Median concentrations (ranges) in serum, plasma, urine and breath after filtered air and the two sessions wood smoke exposure at all sample times. For timing of samples see Table 1. $N = 13$.

Parameter	Before	After filtered air			Before	After start-up smoke			Before	After burn-out smoke		
Sample	1	2	3	4	1	2	3	4	1	2	3	4
S-CC16	8.6	6.7	8.1	7.2	8.3	6.7[a]	8.3	8.3	8.1	6.4	8.7	7.8
(Serum, μg/L)	(5.3–17.6)	(2.5–11.9)	(4.7–13.4)	(4.3–19.2)	(3.9–16.2)	(3.1–13.5)	(4.9–14.3)	(5.8–17.3)	(5.7–13.3)	(0.01–13.2)	(5.2–11.3)	(3.7–13.2)
U-CC16	0.07	1.7	0.4	0.5	0.12	1.4	0.5[b]	0.3	0.2	1.3	0.4	0.12
(Urine, μg/g krea)	(0.02–3.2)	(0.06–18.9)	(0.03–3)	(0.03–3.7)	(0.02–5.5)	(0.18–16.9)	(0.04–5.9)	(0.03–3.7)	(0.01–3.7)	(0.03–13.7)	(0.03–4)	(0.02–3)
$FENO_{270}$	6.3	5.1	5.1	5	4.7	5.2	5.0[c]	4.9[c]	5.1	7.1[c]	6.2[c]	5.0[c]
(Exhaled air, ppb)	(3.4–12.9)	(1.3–13.1)	(2.1–11.1)	(1–9.5)	(2.6–10.2)	(2.9–10.8)	(2.6–10.2)	(3–12.5)	(2.67–9.9)	(3.4–10.4)	(2.2–10.1)	(3.5–11.6)
$FENO_{50}$	17	16	19	17	14	17	17	17	15	19	18[c]	19[c]
(Exhaled air, ppb)	(7.8–52)	(6.9–44)	(7.6–44)	(8.8–46)	(6.8–43)	(8.4–47)	(5.9–47)	(8.3–53)	(6.9–45)	(7.9–43)	(8.8–44)	(7.7–50)
SP-A	6	6.7	6.1	6.5	6.1	6	6.1	5.9	5.9	5.5	5.6	5.8
(Serum, ng/mL)	(0.23–244.4)	(0.29–227.1)	(0.26–246.9)	(0.24–246.8)	(0.23–269)	(0.23–246.2)	(0.26–241.8)	(0.22–243)	(0.23–227.8)	(0.23–224.7)	(0.23–246.9)	(0.22–259.4)
SP-D	61	63	70	73	65	56	61	57	59	54[d]	63	70
(Serum, mg/mL)	(31–175)	(28–146)	(30–161)	(35–178)	(29–173)	(30–151)	(28–190)	(31–190)	(28–182)	(29–148)	(34–168)	(30–153)
MDA	0.02	0.02	0.01	0.03	0.01	0.04	0.02	0.01	0.02	0.01	0.02	0.01
(Breath condensate, μmol/L)	(0.003–0.08)	(0.003–0.06)	(0.003–0.07)	(0.003–0.1)	(0.003–0.09)	(0.003–0.53)	(0.003–0.07)	(0.003–0.07)	(0.003–0.07)	(0.003–0.07)	(0.003–0.08)	(0.003–0.07)

[a]Median of individual differences between changes after exposure to wood smoke and filtered air 1.4 μg/L, $p = 0.04$.
[b]Median of individual differences between changes after exposure to wood smoke and filtered air 0.23 μg/L, $p = 0.01$.
[c]Significant increase (two-sided p-value <0.05) compared to filtered air.
[d]Significant decrease (two-sided p-value <0.05) compared to filtered air.

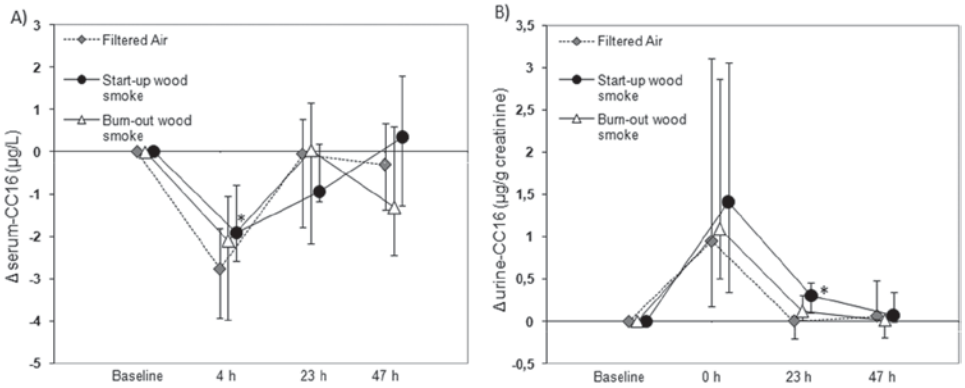

Figure 2. Median changes (Δ) from baseline and 90% confidence intervals for (A) S-CC16 and (B) UCC16 at all sample times in the filtered air session and the both wood smoke sessions. *significant net increase after wood smoke exposure.

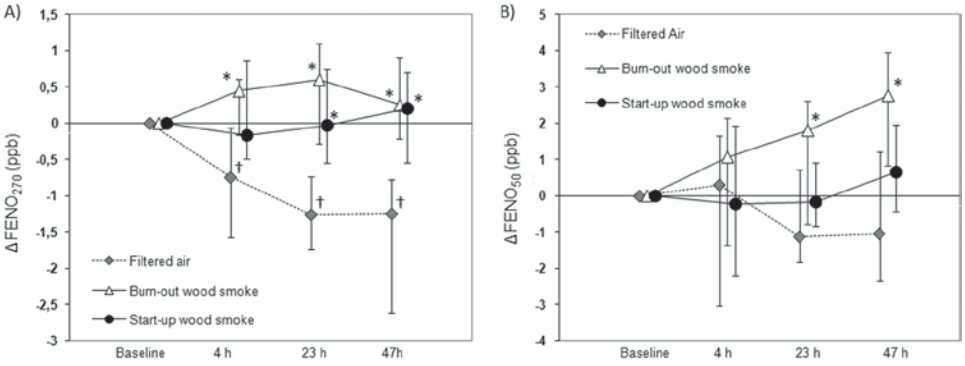

Figure 3. Median changes (Δ) from baseline and 90% confidence intervals for (A) FENO270 and (B) FENO50 at all sample times in the filtered air session and both wood smoke sessions. *significant net increase after wood smoke exposure. †significant decrease from baseline.

tendency ($p = 0.1$) to increase 4 hours after exposure in the start-up session but did not change significantly at any times.

Symptoms

Subjective symptoms were weak, most subjects scoring 0–2 on most symptoms and a few subjects scoring 0 on all symptoms in all sessions. The most commonly reported symptoms in all sessions were (in this order) tiredness, irritation of the eyes, irritation of the nose and throat and headache. There were generally somewhat higher symptom-scores during exposure to wood smoke than during filtered air, and slightly more so in the start-up session than in the burn-out session. For irritation of the eyes, the increase in symptom score was statistically significant ($p = 0.02$ in both wood smoke sessions). For irritation of the nose higher symptom scores were reported in the burn-out session ($p = 0.03$) and in the start-up session ($p = 0.06$) compared to filtered air.

Discussion

This is the first study to test the effects of wood smoke from two different phases of the combustion cycle in the same experimental setup. We observed effects on airway epithelial permeability (CC16) after exposure to smoke from the start-up phase of wood burning, where the combustion was less complete and the smoke contained more particles and higher PAH-concentrations. Airway inflammation (FENO), however, was affected more after exposure to smoke from the burn-out phase of the combustion cycle.

The effects on FENO and CC16 have not previously been observed at these relatively low inhaled doses of wood smoke. We have, however, in a previous study with the same chamber and similar setup but higher doses of wood smoke (Barregard et al., 2008) reported similar increases in FENO and CC16. Using the same experimental setup and outcomes for different exposure doses and combustion phases in two studies provides an opportunity to compare the effects of specific exposures.

CC16 and other pneumoproteins

CC16 was one of our primary outcome markers as it represents a direct effect on airways. The increase in CC16 after exposure to start-up smoke occurred after 4 hours in serum and then the next morning in urine, which is biologically plausible. The effect size in S-CC16 was moderate and similar (about 20% net increase after wood smoke compared to filtered air) to our previous study (Barregard et al., 2008). The circadian variation (lower S-CC16 and

higher U-CC16 in the afternoon than in the morning) is concurrent with other studies (Helleday et al., 2006, Andersson et al., 2007).

Other studies have also found increased CC16 in serum after short-term exposures to, for example, polypropylene smoke (Bernard et al., 1997) and ozone (Broeckaert et al., 2000a, Blomberg et al., 2003) but for the latter also no effects have been reported (Bernard et al., 2005). Cigarette smokers have instead lower S-CC16 due to loss of Clara cells by long-term exposure (Bernard et al., 1994, Shijubo et al., 1997, Hermans et al., 1998, Ellingsen et al., 2010). Some air pollution studies have seen an effect on U-CC16 (Timonen et al., 2004, Barregard et al., 2008, Jacquemin et al., 2009) while others have not (Bräuner et al., 2009).

Although the exact function of CC16 is not known, it is believed to protect the respiratory tract against inflammation and oxidative stress. CC16 is secreted by Clara cells into the epithelial lining fluid (ELF) of the lung, and a small fraction normally passes through the lung epithelial barrier into serum where it is rapidly eliminated through renal clearance. CC16 can thus be measured both in ELF, blood and urine. Increased levels of CC16 in serum may come from increased secretion in the respiratory tract, increased leakage through the lung-blood barrier or decreased renal clearance (Broeckaert et al., 2000b). In acute exposure situations increased leakage may be more important than the CC16-levels in ELF (Hermans et al., 1999).

The surfactant proteins A and D are also present in the ELF, and although they are larger than CC16 they penetrate the epithelium into the systemic circulation to some extent. Damage to the air-blood barrier increases leakage, and increased serum levels have been observed in many pulmonary diseases (Hermans and Bernard, 1999). The fact that CC16 but not SP-A and SP-D increased in serum in our study suggests that CC16 is a more sensitive biomarker of epithelial damage, and thus suitable for short-term studies of air pollution. The rapid normalization of S-CC16 indicates that the epithelial damage in our experiment was minor. CC16 is a small protein with a high concentration gradient, and subtle defects in the lung epithelial barrier may be enough to cause a detectable increase in S-CC16 (Broeckaert et al., 2000b). The circadian variation of CC16 needs to be taken into account, however, and for U-CC16 correct sampling methods are needed to avoid including secretion from the prostate (Andersson et al., 2007).

The decrease in SP-D 4 hours after exposure in the burn-out session was slight (4%), smaller than the variability between or within individuals, and chance is a likely explanation. The circadian variation with lower levels in the afternoon compared to morning is similar to previously reported (Hoegh et al., 2010). For SP-A no changes were observed after exposure, but the inter-assay variability of the method was relatively large, limiting the power to detect small differences.

Exhaled nitric oxide – a marker of airway inflammation

FENO is a proposed but not yet clinically established marker of airway inflammation. It is often used to study asthmatics but has also been associated with other diseases (ATS/ERS, 2005). A number of studies have reported increased FENO with high levels of air pollutants in both asthmatics and non-asthmatics (Steerenberg et al., 1999, Mar et al., 2005, Jansen et al., 2005, Delfino et al., 2006, Barraza-Villarreal et al., 2008, Berhane et al., 2011), while no such effects were found in other experimental studies (Olin et al., 2001b, Pietropaoli et al., 2004, Langrish et al., 2010). Our previous chamber study (Barregard et al., 2008) observed an increase in $FENO_{270}$ after wood smoke exposure, but two other recent chamber studies did not find any effect (Sehlstedt et al., 2010, Riddervold Skogstad, 2011). FENO can be measured at several exhalation flow rates, with variation between different studies regarding which are used. At low flow rates (such as 50 mL/s, $FENO_{50}$) exhaled NO-levels represent mostly the conducting airways, while at higher flow rates (such as 270 mL/s, $FENO_{270}$) a larger fraction represents NO derived from the alveolar compartment (Jorres, 2000).

There was a net increase of $FENO_{270}$ after exposure in both wood smoke sessions, but a large part of that effect was based on a substantial decrease after exposure to filtered air which we cannot explain (see Figure 3A). We believe that while there may be a true effect on $FENO_{270}$ by wood smoke in this study, chance is an alternative explanation, so interpretation should be made with caution.

$FENO_{50}$ increased in the burn-out session both one and two mornings after exposure, indicating inflammation in the conducting airways. $FENO_{50}$ did not decrease after filtered air in the same way as $FENO_{270}$ (see Figure 3b), and the results might therefore be more reliable. This increase is, however, not in accordance with our previous exposure study using higher inhaled doses (Barregard et al., 2008), where an increase in $FENO_{270}$ but not $FENO_{50}$ was seen.

MDA – a marker of oxidative stress

Levels of MDA, an indicator of lipid peroxidation, in EBC showed a non-significant tendency to increase 4 hours after exposure to start-up wood smoke. MDA did increase significantly after exposure in our previous wood smoke exposure study (Barregard et al., 2008) and has been associated with exposure to air pollutants (Isık et al., 2005, Romieu et al., 2008, Bae et al., 2010), but there are also other studies with negative (Allen et al., 2011) or mixed results (Sorensen et al., 2003). Possible interpretation relates to dose and variability, since studies with higher exposures (Barregard et al., 2008, Işık et al., 2005), or more subjects (Romieu et al., 2008, Bae et al., 2010) seemed to detect an effect on MDA. Another explanation is that collection of EBC and measurement of MDA *in vivo* is still a method fraught with difficulties. MDA in EBC is often below the detection limit (26% of samples in

our study, 25% in Romieu et al., 2008), which increases the risk of both false positive and negative findings.

Symptoms

Almost all symptoms reported were mild, and a few subjects did not report any symptoms at all. Both 'irritation of the eyes' and 'irritation of the nose' were reported significantly more during exposure to wood smoke compared to filtered air. This is comparable to the results of our previous study, where only 'irritation of the eyes' was significantly more reported after exposure and not 'irritation of the nose', but more participants reported an 'unpleasant smell' than in the present study (Sallsten et al., 2006). The results are also similar to those in a pellet burner study (Sehlstedt et al., 2010). Another exposure study using more sophisticated measurements of symptoms also reported significantly increased but weak symptoms (Riddervold et al., 2011). One interpretation of this is that symptoms are mild even during exposures that cause measurable effects on biomarkers. Another interpretation is that symptoms are significantly increased in all studies even at the lowest exposure (which no single biomarker is) and therefore seem to be the most sensitive effect of wood smoke exposure.

Differences between the exposure sessions

Our theory was that in the start-up phase the combustion would be less complete, and the smoke thus contain more toxic carbonaceous material and large particles, while the combustion in the burn-out phase should be more complete producing smoke with more small particles of alkali salts (Kocbach Bolling et al., 2009).

From the exposure characteristics (Table 2) we can see that this, to some extent, was the case. There were higher levels of particulate PAHs and of most VOCs in the start-up session than in the burn-out session, but more similar level of trace elements, and actually higher levels of ultrafine particles and a lower geometric mean diameter in the start-up session. It has been shown that the particle size distribution can be more variable during the burn-out phase and is dependent on the air supply (Hueglin et al., 1997, Hedberg et al., 2002). The burning conditions have also been shown to have greater influence on the organic emissions than the type of wood burned, with strongly enhanced organic emissions like levoglucosan in the start-up phase and high levels of oxygenated organic species in the burn-out phase (Weimer et al., 2008). As CC16, one of our primary biomarkers of airway effects, increased significantly only in the start-up session, one interpretation might be that the wood smoke particles in that session affected the air-blood barrier to a higher extent. Also, in our previous study (Barregard et al., 2008) the exposure in the first round was most similar to the start-up session, and in the first round there were more pronounced effects on CC16.

Particle mass and numbers were, however, also higher in the start-up session, and CC16 showed a non-significant tendency to increase also after exposure to smoke from the burn-out session. It is thus difficult to determine whether a difference in effects on biomarkers depends on different particle levels or different toxicity of the particles. Moreover, while CC16 was more affected in the start-up session, the possible increases in $FENO_{270}$ and in $FENO_{50}$ representing airway inflammation instead occurred primarily in the burn-out session, for which we do not find any explanation.

An *in vitro* study using wood smoke particles from the same combustion equipment as the present study, as well as other particles, did not find any difference in cytotoxicity and release of inflammatory cytokines between particles from the different phases of the combustion cycle (Kocbach-Bolling, Manuscript). This could indicate that the difference in effects on CC16 in the present study was due to a difference in dose rather than toxicity. That study also observed that the PM from medium temperature combustion using this wood stove were more cytotoxic than wood smoke particles from high-temperature combustion using another wood stove, and suggested that wood stove type and combustion conditions might be more important for toxicity than the phase of the combustion cycle. This is interesting, as the differences in results from wood smoke exposure studies are surprisingly large.

Comparisons with other wood smoke exposure studies

A recent study of wood smoke from a pellet burner found only limited effects in healthy adults, reporting an increase in glutathione (GSH), in bronchoalveolar lavage (BAL) and mild upper airway symptoms, though the levels of $PM_{2.5}$ were comparable to the start-up session in this study and the inhaled doses higher (Sehlstedt et al., 2010) (Table 4). Another study exposed healthy atopic adults to aged wood smoke from a cast iron stove, in one session with levels of $PM_{2.5}$ comparable to the present study and in another session with exposure almost twice as high. They observed increased symptoms (Riddervold et al., 2011) and some inflammatory interleukins in nasal lavage (IL-1β and IL-6) but no effects on other biomarkers (Riddervold Skogstad, 2011). This is in contrast with our previous study, which found effects on biomarkers of airway effects (Barregard et al., 2008). In that study inhaled doses were higher than in the current study but similar to the high exposure session in the Danish study (Riddervold et al., 2011).

One possible explanation for the difference in results is that most effects are probably caused by particles in the ultrafine range (< 100 nm). Small particles have, compared to large particles, a relatively larger surface area, which is important for particle toxicity (Donaldson and Tran, 2002), and many experimental studies have demonstrated the especially toxic properties and oxidative potential of ultrafine particles (Brook et al., 2010). Even when $PM_{2.5}$ mass concentrations are similar, PM number concentrations are higher in the present study and in our previous wood smoke study

(Barregard et al., 2008) than in the chamber studies using a pellet burner (Sehlstedt et al., 2010) or aged smoke (Riddervold et al., 2011) (Table 4). In both the present study and in our previous study (Barregard et al., 2008) the effects on biomarkers were stronger in the sessions with higher particle numbers. This might also explain the striking effects reported after using high efficiency particulate air (HEPA) filters to clean indoor air in a wood burning community (Allen et al., 2011), as HEPA-filters are believed to effectively remove particles in the ultrafine range.

There are also other differences between the exposure studies which might, by influencing particle numbers or particle toxicity, account for the differences in effects on health outcomes between studies. Some examples are type of wood used, combustion equipment and combustion temperature. A standardized exposure setup varying one parameter, for example type of wood, while keeping the others constant, is desirable to determine which is most important for the adverse health effects of exposure to biomass smoke.

Comparisons with real world wood smoke exposures

The mass concentrations in our and other chamber studies are much higher than the typical contribution of 1–10 μg/m^3 to personal or indoor $PM_{2.5}$ concentrations for those using wood for residential heating in Sweden (Molnar et al., 2005). However, the exposure times in the chambers are short, in our study 3 hours which corresponds to an exposure of 20–40 μg/m^3 for 24 hours. Indoor levels of PM and B(a)P may, however, be in the same range as in our start-up session if non-airtight wood stoves are used (Traynor et al., 1987). In developing countries exposures can be much higher and daily average concentrations of several hundreds of μg/m^3 are often found (Naeher et al., 2007).

Strengths and limitations

One strength of this study is that we used the same experimental setup to test the effects of two different kinds of wood smoke, allowing us to compare the effects of different exposures. We were also able to compare the results with those in a previous exposure study, in which we used the same experimental setup. Another strength is that our chamber is large

Table 4. Subject characteristics, study design, exposure measurements and airway outcomes for different wood smoke exposure studies.

		Barregard 2008		Present study			Riddervold 2011	
Study		Session 1	Session 2	Start-up	Burn-out	Sehlstedt 2010	Low exp	High exp
Subjects	*N*	13[c]		13		19	20	
	Characteristics	Healthy		Healthy		Healthy	Healthy atopics	
	Mean age (range)	34 (20–56)		34 (20–57)		24 (21–31)	25 (19–55)	
Setup	Burner	Cast iron wood stove		Cast iron wood stove		Pellet burner	Cast iron wood stove	
	Wood type	Birch/spruce 50/50		Birch/spruce 50/50		Pine/spruce pellets/sawdust	Beech	
	Combustion conditions etc	Optimal		Optimal		Low O_2, high temp	Optimal (aged smoke)	
Exposure	$PM_{2.5}$ (μg/m^3)	272	241	295	146	224	222	385
	PM_1 # conc (10^3/cm^3)	180	95	140	100	67	29	71
	Time (hours)	4		3		3	3	
	Exercise	2*25 minutes		No		15/15 min rest/exercise	No	
	Volume of inhaled air (liters)	2580[d]		1260[e]		3744[f]	1260[e]	
Outcomes	Symptoms	Yes (weak)		Yes (weak)		Yes (weak)	Yes (weak)	
	S-CC16	↑		↑	0	–	[g]	
	U-CC16	↑		↑	0	–	[g]	
	$FENO_{270}$	↑		(↑?)	(↑?)	0	0	0
	$FENO_{50}$	0		0	↑	0	0	0
	SP-A, SP-D	–		0	0	–	[g]	
	MDA in EBC	↑		0	0	–	–	–
	GSH in BAL	–		–	–	↑	–	–
	GSH in BW[a]	–		–	–	0	–	–
	Other markers in BAL and BW	–		–	–	0	–	–
	FVC, FEV1	–		–	–	0	0	0
	PEF	–		–	–	–	0	0
	Inflammation in NAL[b]	–		–	–	–	0 (?)	

[a]BW, Bronchial wash.
[b]Nasal lavage.
[c]7 in session 1, 6 in session 2.
[d]Estimate based on an average minute ventilation of 7 liters/minute at rest and 25 liters/minute during 70 Watt bicycle exercise.
[e]Estimate based on an average minute ventilation of 7 liters/minute at rest.
[f]Estimate based on figures in that paper and an average body surface of 1.73 m^2.
[g]Not yet reported.

enough to include the entire group of subjects in the same session. This decreases variability in exposure compared to testing a few subjects per day in many different days.

A limitation is the variation in exposure during the sessions. Because of this there is a difference in exposure levels between those who entered the chamber first and last. Exposure was also higher in the start-up session, making it difficult to compare toxicity of the smoke from the two sessions.

We cannot exclude carry-over effects in the burn-out session by exposure to wood smoke in the start-up session 1 week before, though it seems biologically unlikely and we do not see any evidence of this in the pre-exposure levels of the biomarkers. Likewise, an effect on symptom reporting by adaptation to consecutive exposures cannot be excluded.

We did not inform the subjects which exposure they were exposed to each session, but the smell of wood smoke is obvious and difficult to mask. The study was therefore not blinded regarding exposure versus control, but it might be considered blinded concerning the two exposure sessions.

As multiple comparisons were made, there is a risk of false positives. Bonferroni correction does not seem suitable in this type of study (Rothman, 1990). Instead we report standard values for statistical significance ($p < 0.05$, two-sided), but are cautious in interpretation, focusing on effects that are consistent at several sampling times, are biologically plausible, or find support in other studies.

Biomarkers may always vary for other reasons than the exposures tested, such as circadian rhythms, gender, age, diet, menstrual cycles or infectious diseases. We control for this by excluding subjects with signs of infections or taking medicines, by performing the experiments during a limited time period, and by keeping all the sessions as similar as possible except for the exposure, and by using each individual as its own control.

Conclusions

Our results indicate that at these relatively low doses of wood smoke there is an effect on airways in healthy adults. CC16 in blood and urine seems to be a good biomarker for studies of air pollution and chamber studies, but is not (yet) clearly connected to adverse health outcomes. Regarding the two combustion phases we found more effects on biomarkers after exposure to smoke from the start-up phase than after the burn-out phase of the wood-burning cycle, but because of differences in exposure levels and limited effects we cannot draw certain conclusions. High particle number seems to be associated with more effects in wood smoke exposure studies.

This is the first study testing wood smoke from two different phases of the wood burning cycle in the same setup. A standardized setup with well-defined outcomes testing different types of combustion equipment, combustion conditions, wood types and groups of subjects is desirable to determine the toxicity of different kinds of wood smoke.

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Declaration of interest

The authors report no declarations of interest.

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