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RESEARCH ARTICLE

Short-term chamber exposure to low doses of two kinds of wood smoke does not induce systemic inflammation, coagulation or oxidative stress in healthy humans

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Abstract

Introduction: Air pollution increases the risk of cardiovascular diseases. A proposed mechanism is that local airway inflammation leads to systemic inflammation, affecting coagulation and the long-term risk of atherosclerosis. One major source of air pollution is wood burning. Here we investigate whether exposure to two kinds of wood smoke, previously shown to cause airway effects, affects biomarkers of systemic inflammation, coagulation and lipid peroxidation.

Methods: Thirteen healthy adults were exposed to filtered air followed by two sessions of wood smoke for three hours, one week apart. One session used smoke from the start-up phase of the wood-burning cycle, and the other smoke from the burn-out phase. Mean particle mass concentrations were 295 µg/m³ and 146 µg/m³, and number concentrations were 140 000/cm³ and 100 000/cm³, respectively. Biomarkers were analyzed in samples of blood and urine taken before and several times after exposure. Results after wood smoke exposure were adjusted for exposure to filtered air.

Results: Markers of systemic inflammation and soluble adhesion molecules did not increase after wood smoke exposure. Effects on markers of coagulation were ambiguous, with minor decreases in fibrinogen and platelet counts and mixed results concerning the coagulation factors VII and VIII. Urinary F₂-isoprostane, a consistent marker of *in vivo* lipid peroxidation, unexpectedly decreased after wood smoke exposure.

Conclusions: The effects on biomarkers of inflammation, coagulation and lipid peroxidation do not indicate an increased risk of cardiovascular diseases in healthy adults by short-term exposure to wood smoke at these moderate doses, previously shown to cause airway effects.

Keywords

Biomarkers, F₂-isoprostane, health effects, human exposure studies, particles, systemic inflammation, wood smoke

History

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Introduction

Exposure to particulate air pollution increases short- and long-term cardiopulmonary morbidity and mortality (Brook et al., 2010; Pope & Dockery, 2006; Rückerl et al., 2011). Globally, exposure to particulate matter (PM) is an important and modifiable factor affecting public health, with an estimated 800 000 premature deaths per year caused by particles <2.5 µm (PM_{2.5}) (Brook et al., 2010) and 1.6 million premature deaths per year caused by biomass and coal smoke (Smith & Maezuhl-Feuz, 2004; WHO, 2006).

Half the world's population use solid fuels indoor for cooking and heating (Rehfuess et al., 2006). Exposure levels can be very high, especially indoors in the developing world, but burning wood for residential heating is common in the developed world as well and can be a major source of PM

(Naeher et al., 2007). In the European Union (EU-15), domestic wood stoves contributed 25% of annual PM_{2.5} emissions in the year 2000, an increase to 38% is expected by 2020 (Amann et al., 2005). Wintertime contributions can be much higher, with studies showing around 70% in northern Sweden (Hedberg et al., 2006), up to 90% in Seattle (Larson & Koenig, 1994) and more than 90% in Christchurch, New Zealand (McGowan et al., 2002).

Inhalation of PM causes pulmonary health effects, but the majority of adverse health effects involve the cardiovascular system. Researchers have suggested that PM may instigate cardiovascular health effects via three main pathways and these suggestions are supported by a number of studies. One suggestion is that PM induces airway oxidative stress and inflammation causing pulmonary cells to release systemic inflammatory and pro-thrombotic cytokines, leading to destabilization of plaques, increased risk of blood clotting and atherogenesis. Another suggestion is that a similar response may directly be caused by PM or particle constituents translocated into the systemic circulation. A third is that particle interactions with receptors in the lung may affect the

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autonomic nervous system, increasing the risk of arrhythmias (Brook, 2008; Brook et al., 2004, 2010; Mills et al., 2009).

However, health effects and mechanisms may vary between different kinds of PM. Air pollution is a complex heterogeneous mixture of particles of varying size, composition and origin. The toxicity is determined not only by weight and number but also by size, surface area and chemical composition, and the various components may interact synergistically or antagonistically (Brook et al., 2010; Donaldson & Tran, 2002; Schwarze et al., 2006). Specific studies of types of PM or emission source are therefore needed to determine their health effects.

Wood smoke is not as extensively studied as other major pollutants, but many studies have shown associations between it and respiratory health effects. The evidence available regarding cardiovascular outcomes is too limited to judge whether wood smoke particles are less toxic than other combustion-derived particles, and further experimental studies of acute effects of major types of biomass smoke have been called for (Naeher et al., 2007).

We therefore designed a study to compare the effects in healthy human subjects exposed to two different kinds of wood smoke PM, one from the start-up phase and one from the burn-out phase of the wood-burning cycle. In a previous paper, we reported effects on biomarkers of airway effects (increases of Clara Cell protein 16 in serum and urine and fraction of exhaled nitric oxide) (Stockfelt et al., 2012). These effects were similar to those in our first experimental wood smoke exposure study (Barregard et al., 2008), which used the same experimental chamber but higher doses of wood smoke PM. That study also showed effects on biomarkers of systemic inflammation, coagulation and lipid peroxidation (factor VIII, serum amyloid A (SAA) and 8-iso-prostaglandin $F_{2\alpha}$) (Barregard et al., 2006), which could indicate increased risks of cardiovascular disease (CVD) by short-term exposure to wood smoke.

In this study, we examine whether exposure-related effects on biomarkers of systemic inflammation, coagulation and lipid peroxidation in healthy subjects could be detected at these lower doses of wood smoke exposure, and whether there is a difference between wood smoke from the start-up phase and the burn-out phase of the combustion cycle.

Methods

Subjects

We recruited 16 healthy never-smokers (8 men and 8 women) aged 20–57 years of age (mean age 31) from among the staff of our department and students. All had normal spirometry values and none had symptomatic allergy. The subjects were not allowed to take any medication during the two days preceding each exposure session and had to be free from any known infections for at least one week prior to the session. Three subjects were excluded before data analysis because upper airway infections emerged just before or between the sessions, and thus all results presented below are based on 13 individuals (6 men and 7 women aged 20–57, mean age 34 years). The study was approved by the Regional Ethics Review Board of Gothenburg, and the subjects gave their written informed consent.

Experimental design

The design of the current study was similar to our previous wood smoke exposure study (Barregard et al., 2006, 2008; Sallsten et al., 2006), but differed in using (1) wood smoke from two different phases of the combustion cycle and (2) lower inhaled doses. Because the exposure time had been decreased from four to three hours and no periods of exercise were included (compared to 50 min), the volume of inhaled air was only about half that of the previous study.

The subjects were first exposed to filtered indoor air for three hours; one week later they were exposed to wood smoke from the start-up phase of the wood-burning cycle and another week later to wood smoke from the burn-out phase of the wood-burning cycle. The sessions were identical apart from the wood smoke exposure, and each started with blood and urine sampling before the subjects entered the exposure chamber. After exposure, samples were taken several times (Table 1). The subjects had free intake of soft drinks and water and a small snack in the middle of the exposure, but did not eat during the hour before the first blood sample was taken. For more details, see Stockfelt et al. (2012).

Exposure chamber and generation of wood smoke

A more detailed description of the exposure chamber and the principles of wood smoke generation has been reported (Sallsten et al., 2006). Briefly, the chamber was 29 m³ with walls covered in teflon-impregnated glass-fiber fabric. Wood smoke was generated in a small cast-iron wood stove placed just outside the chamber and was mixed with filtered air to achieve the target concentration. A mixture of hardwood and softwood was used (50% birch, 50% spruce). A 2.5–3 kg batch of wood logs was ignited, and another batch of 1.5 kg was added approximately every 40 min.

In the session using wood smoke from the start-up phase, smoke was supplied to the chamber for 12–14 min, immediately after the new wood logs were added. In the session using smoke from the burn-out phase, smoke was supplied for 15 min, starting 25 min after wood was added. The PM_{2.5} mass concentration was controlled online to maintain the target concentration.

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

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

*Timed from bedtime to morning.

†Timed from start to end of exposure.

Sampling and characterization of wood smoke

PM_{2.5} mass concentrations, number concentrations and size distributions of particles, black carbon, trace elements, NO, NO₂, CO, CO₂, particulate polycyclic aromatic hydrocarbons (PAHs), some volatile organic compounds (VOCs), temperature and relative humidity were measured in the center of the chamber. The complete results and further descriptions of sampling technique and apparatus have been described previously (Sallsten et al., 2006, Stockfelt et al., 2012). The most important data are summarized in Table 2.

Blood and urine sampling

Blood was collected by venipuncture in SST tubes for serum, citrate tubes for plasma and EDTA tubes for leukocyte counts. Leukocyte counts were performed on the same day, while the serum and plasma aliquots were stored frozen in polyethylene cryotubes until analysis. The urine samples were collected in polypropylene bottles at different time points, and the volumes were registered and aliquots stored frozen until analysis.

Biochemical analyses

Blood counts were performed with flow cytometry (Celldyn 400, Abbott, reagents from Abbott, Abbot Park, IL). High-sensitivity serum C-reactive protein (CRP) was measured by immunoturbidimetry using modular equipment and reagents from Roche Diagnostics (Mannheim, Germany). The STA-R equipment and reagents from Diagnostica Stago (Asnières,

France) were used to determine fibrinogen in plasma based on the coagulation time at high thrombin concentration, factor VII (FVII) using a one-stage thromboplastin method, and factor VIIIc (FVIII) using a one-stage clotting measurement. D-dimers were measured using a sandwich enzyme-linked immunosorbent assay (ELISA) with reagents from Diagnostica Stago, Triolab AB (Molndal, Sweden). Von Willebrand factor (vWf) antigen was determined with a sandwich ELISA using antibodies from DakoCytomation (Solna, Sweden). SAA, soluble intercellular adhesion molecule-1 (sCAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble P-selectin (sP-selectin), interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were analyzed using commercial ELISA kits, for SAA from Anogen (Ontario, Canada) and the others R&D Quantikine (Abingdon, UK). 8-Iso-prostaglandin F_{2 α} (8-iso-PGF_{2 α}) was analyzed in urine using a radioimmunoassay as developed by Basu (1998). Urinary 8-iso-PGF_{2 α} values are presented after adjustment for creatinine levels in urine.

Statistics

For all biomarkers, intra-individual differences at each time point were calculated by subtracting the changes after exposure to filtered air from the changes after exposure to each of the two wood smoke sessions, making each individual his or her own control. Statistical significance was tested with two-sided Student's *t* test for normally distributed biomarkers (FVII, FVIII, vWf, f8/vWf, leukocytes, sP-selectin, platelets)

Table 2. Time weighted averages of the exposure measurements in all sessions. More details are presented in Stockfelt et al. (2012).

	Filtered air session			Start-up session			Burn-out session		
	N	Mean	SD	N	Mean	SD	N	Mean	SD
PM _{2.5} ($\mu\text{g}/\text{m}^3$) (TEOM)	C	8.4	2.0	C	221*	121	C	148	48
PM ₁ mass ($\mu\text{g}/\text{m}^3$)	7	<15		16**	295	43	17	146	15
PM ₁ (#/cm ³)	C	2900	770	C	140 000	83 000	C	100 000	51 000
Ultrafine particles (%)	C	55	5.8	C	68	21	C	40	15
Geometric diameter (nm)					38	1.7		83	1.5
Trace elements (ng/m ³)									
K	6	<1500		6	9700	4500	6	8800	1200
Zn	6	70	14	6	2400	720	6	3100	340
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
Benzo(g,h,i)perylene	3	<0.01		6	41	9.6	6	11	1.4
VOCs ($\mu\text{g}/\text{m}^3$)									
Benzene	3	2.0	0.20	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
Naphthalene	2	1.6	0.08	3	10	0.79	3	4.1	0.60
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	0.10	C	0.30	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	2.0	C	15	5.1
Other									
Air temperature (°C)	C	22	1.2	C	22	1.3	C	22	1.4
Relative humidity (%)	C	49	2.2	C	50	1.5	C	29	2.7

C, continuous measurements.

*Registration missing for half an hour.

**One sample omitted due to leakage.

†Mean of PM₁ and PM_{2.5}.

and Wilcoxon's signed rank test for those not normally distributed (CRP, fibrinogen, SAA, 8-iso-PGF_{2α}, sICAM-1, sVCAM-1, D-dimer, IL-6, TNF-α). Point estimates of all significant differences are also presented, expressed as the median of the intra-individual differences divided by each individual's baseline value before wood smoke exposure, as a percentage. 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 replaced with the detection limit divided by the square root of 2 (Hornung & Reed, 1990). Statistical calculations were made using SAS 9.2.

Results

Exposure characterization

The mean PM mass concentration was below the detection limit in the filtered air session, was 295 µg/m³ in the start-up session, and was 146 µg/m³ in the burn-out session (Table 2). Average PM number concentrations were 2900/cm³ in the filtered air session and 140 000/cm³ and 100 000/cm³ in the start-up and burn-out sessions, respectively. Nearly all particles were <1 µm, with 55%, 68% and 40% respectively, being ultrafine (<100 nm, PM_{0.1}). The geometric mean diameter was 38 nm ($\sigma_g = 1.7$) in the start-up session and 83 nm ($\sigma_g = 1.5$) in the burn-out session.

The concentrations of particulate PAHs were higher in the start-up session than in the burn-out session (Table 2), and in both wood smoke sessions much higher than in the filtered air session. The concentrations of benzene, 1,3-butadiene, formaldehyde, acetaldehyde and naphthalene were also higher in the wood smoke sessions. The mean NO₂ 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 (Table 2).

Biomarkers of systemic effects

The main biomarkers of systemic inflammation did not increase after exposure to wood smoke compared to exposure to filtered air; the levels of IL-6, TNF-α and SAA did not change significantly after exposure at any sampling time (Table 3). The white blood cell (WBC) count did not change significantly either; but did show a clear diurnal variation with higher levels in afternoons than in the mornings. CRP was slightly lower four hours after exposure in the start-up session compared to exposure to filtered air (median difference −5%, *p* = 0.04).

The sCAMs did not increase after wood smoke exposure. sP-selectin did not change significantly at any sampling time. Neither did soluble ICAM-1 apart from an isolated minor net increase (+7%, *p* = 0.008) 47 h after exposure in the burn-out session due to a decrease after filtered air. sVCAM-1 was marginally decreased four hours after exposure in both wood smoke sessions compared to exposure to filtered air (−2% and −5%, *p* = 0.01 and *p* = 0.02, respectively).

The effects of wood smoke on biomarkers of hemostasis were ambiguous. Fibrinogen decreased in the morning after exposure in both the start-up session (−4%, *p* = 0.04) and the burn-out session (−5%, *p* = 0.02) compared to exposure to

filtered air. In the burn-out session, it was also lower four hours after exposure (−8%, *p* = 0.04, Figure 1). Platelet counts were also slightly lower four hours after exposure in the burn-out session (−6%, *p* = 0.003) and in the morning after exposure in both the start-up and burn-out sessions (−6% and −6%, *p* = 0.03 and 0.01, respectively). D-dimer showed a significant net decrease two mornings after exposure in the start-up session (−20%, *p* = 0.04). In the burn-out session, FVII showed a slight but significant increase compared to filtered air four hours after exposure, as well as two mornings after exposure (point estimates +6% and +12%, *p* = 0.006 and 0.04, respectively, Figure 2A). In the same session, FVIII showed a non-significant tendency to increase four hours after wood smoke exposure (+14%, *p* = 0.09, Figure 2B), and was significantly increased the next morning (+37%, *p* = 0.02), as was the FVIII/vWf ratio (+47%, *p* = 0.02). In the start-up session, FVIII also showed a tendency to increase the morning after exposure (+13%, *p* = 0.08), but had significantly decreased four hours after exposure (−29%, *p* = 0.03, Figure 2B).

Concerning lipid peroxidation, the urinary isoprostane 8-iso-PGF_{2α} adjusted for creatinine was markedly decreased after exposure to wood smoke; for the start-up session this occurred only the second morning after exposure (−53%, *p* = 0.02) and the burn-out session on the first and second mornings after exposure (−82% and −71%, *p* = 0.0002 and *p* = 0.006, respectively, Figure 3). This might in part be explained by an increase after exposure in the filtered air session. There was a diurnal variation with higher levels in the afternoons than in the mornings.

At most sample times, fibrinogen was highly correlated with both sP-selectin and leukocyte counts, CRP with SAA, FVIII with vWf and D-dimer, and FVII with platelet counts.

Discussion

This is the first study of systemic effects in humans of exposure to two kinds of wood smoke PM in the same experimental setup. Our a priori hypothesis was that this exposure would increase biomarkers of systemic inflammation, coagulation, and lipid peroxidation, thus indicating an increased risk of CVD. However, we found no consistent increases in any systemic biomarkers after exposure to smoke either from the start-up phase or the burn-out phase of the wood-burning cycle as compared to exposure to filtered air. As reported previously (Stockfelt et al., 2012), wood smoke at these exposure doses affected biomarkers of airway effects. In a previous study in the same exposure chamber with higher doses of wood smoke, we observed increases in biomarkers of both airway and systemic effects (Barregard et al., 2006, 2008). Taken together, these results indicate that local airway inflammation precedes systemic inflammation and has a lower threshold.

Markers of systemic inflammation and sCAMs

IL-6 and TNF-α are inflammatory cytokines that induce the production of acute phase reactants in the liver; among these reactants are CRP and SAA. They all increase in serum during inflammation, as does WBC count. WBCs are recruited from

Table 3. Biomarkers of systemic inflammation, coagulation and lipid peroxidation. Medians (ranges) in serum, plasma and urine in the filtered air session and the two sessions of wood smoke exposure at all sample times. For timing of samples see Table 1, $N=13$.

Parameter Sample #	After filtered air				After start-up smoke				After burn-out smoke			
	Before 1	2	3	4	Before 1	2	3	4	Before 1	2	3	4
IL-6 (plasma, pg/ml)	0.77 (0.28–1.14)	0.75 (0.33–1.81)	0.61 (0.32–2.31)	0.73 (0.20–1.03)	0.67 (0.38–1.44)	0.77 (0.22–1.34)	0.66 (0.35–0.93)	0.74 (0.24–1.84)	0.66 (0.42–1.72)	0.83 (0.42–1.33)	0.66 (0.15–1.29)	0.58 (0.11–1.58)
TNF- α (plasma, pg/ml)	0.99 (0.79–1.36)	0.75 (0.33–1.81)	0.97 (0.76–1.48)	0.90 (0.84–1.51)	1.01 (0.85–1.24)	0.94 (0.75–1.32)	0.96 (0.88–1.56)	0.99 (0.80–1.48)	1.00 (0.78–1.87)	0.88 (0.72–1.66)	0.95 (0.80–1.50)	1.04 (0.81–1.30)
SAA (serum, mg/L)	1.6 (0.27–2.1)	1.8 (0.77–7.3)	2.0 (0.78–8.8)	1.7 (0.72–8.5)	1.8 (0.85–1.3)	1.5 (0.75–2.0)	1.8 (0.8–1.2)	1.9 (0.77–1.5)	2.5 (1.1–4.4)	1.7 (0.52–8.1)	2.2 (0.63–8.7)	2.4 (0.66–1.6)
Leukocytes (blood, $\times 10^9/L$)	5.6 (4.2–7.3)	7.1 (5.6–8.3)	5.6 (4.5–8)	6.2 (4.2–8.6)	5.6 (3.1–9.7)	6.6 (5.1–8.2)	5.6 (4.1–7.4)	5.6 (3.6–8.8)	5.9 (3.8–7.7)	6.6 (4.8–8.1)	5.6 (4.2–7.2)	5.7 (3.8–7.4)
CRP (serum, mg/L)	0.87 (0.1–2.3)	0.76 (0.1–2.1)	0.62 (0.1–1.7)	0.62 (0.1–1.7)	0.64 (0.1–2.9)	0.60† (0.16–2.4)	0.52 (0.16–2.1)	0.63 (0.17–1.7)	0.84 (0.16–2.2)	0.74 (0.1–2.8)	0.72 (0.15–2.4)	0.69 (0.18–2.5)
sP-selectin (serum, ng/mL)	71 (44–126)	70 (30–105)	69 (31–99)	77 (32–99)	81 (35–109)	68 (35–106)	73 (31–103)	74 (36–102)	77 (50–105)	60 (43–98)	64 (42–87)	72 (53–86)
sICAM-1 (serum, ng/mL)	113 (47–148)	106 (46–129)	108 (54–135)	110 (42–136)	115 (40–147)	103 (42–141)	106 (41–144)	105 (43–146)	113 (43–142)	103 (45–140)	110 (44–134)	110* (50–143)
sVCAM-1 (serum, ng/mL)	604 (398–887)	601 (380–886)	566 (410–899)	578 (411–950)	620 (416–936)	618† (339–916)	600 (450–882)	619 (442–876)	651 (420–885)	637† (394–832)	611 (453–891)	634 (481–1000)
PFVII (plasma, kIU/L)	1.02 (0.74–1.5)	0.89 (0.58–1.3)	0.97 (0.59–1.4)	0.96 (0.67–1.4)	1.04 (0.71–1.4)	0.94 (0.64–1.4)	0.97 (0.68–1.4)	0.97 (0.64–1.3)	0.88 (0.65–1.3)	0.85* (0.64–1.2)	0.87 (0.71–1.4)	0.95* (0.72–1.5)
PFVIII (plasma, kIU/L)	1.1 (0.44–1.4)	1.2 (0.32–2.1)	1 (0.4–1.6)	1.1 (0.38–1.7)	1.1 (0.41–1.6)	1† (0.45–1.8)	1.2 (0.48–1.7)	1.2 (0.29–1.5)	0.9 (0.4–1.5)	1.4 (0.51–2.7)	1.2* (0.42–1.7)	1.1 (0.45–1.9)
vWF (plasma, kIU/L)	1.2 (0.36–1.6)	1.2 (0.3–2.1)	1 (0.38–1.5)	1.1 (0.36–1.7)	1 (0.28–1.4)	0.9 (0.28–1.2)	0.9 (0.37–1.2)	0.9 (0.29–1.5)	1.1 (0.43–1.6)	1.2 (0.42–1.6)	1 (0.32–1.4)	1 (0.33–1.5)
Fibrinogen (plasma, g/L)	2.6 (1.9–3.5)	2.5 (1.8–3.4)	2.7 (1.7–3.7)	2.5 (1.7–3.5)	2.6 (2.1–3.4)	2.4 (1.9–3.5)	2.5† (2–3.5)	2.5 (1.9–3.6)	2.6 (2.1–3.6)	2.5† (1.7–3.4)	2.7† (1.8–3.5)	2.7 (1.9–3.6)
Platelets (blood, $\times 10^9/L$)	251 (173–313)	246 (142–331)	239 (160–320)	234 (178–333)	259 (179–346)	269 (180–232)	250† (180–320)	255 (183–315)	282 (179–386)	264† (172–345)	256† (166–333)	267 (180–396)
D-dimer (plasma, mg/L)	0.15 (0.09–0.28)	0.15 (0.1–0.29)	0.15 (0.09–0.29)	0.16 (0.08–0.34)	0.20 (0.09–0.36)	0.17 (0.1–0.31)	0.18 (0.09–0.29)	0.15† (0.08–0.32)	0.16 (0.09–0.48)	0.16 (0.1–0.93)	0.17 (0.1–0.39)	0.16 (0.09–0.36)
8-iso-PGF _{2α} /creatinine (urine, nmol/mmol crea)	0.47 (0.33–0.94)	0.82 (0.42–1.7)	0.57 (0.39–1.3)	0.59 (0.39–1.2)	0.67 (0.31–1.7)	0.82 (0.33–1.5)	0.46 (0.34–1.5)	0.50† (0.32–1.1)	0.50 (0.36–0.81)	0.59 (0.37–1.4)	0.17† (0.05–0.52)	0.41† (0.15–0.66)

*Significant increase (two-sided p value <0.05) compared to filtered air exposure.†Significant decrease (two-sided p value <0.05) compared to filtered air exposure.

the blood to the site of inflammation with the aid of CAMs, the soluble forms of which are usually elevated in serum during inflammation. Inflammation plays a key role in the development of CVD. Of all the biomarkers of inflammation we measured, CRP is perhaps the best established predictor of CVD, but associations between increased serum levels and CVD/mortality have been found also for SAA, WBC, IL-6, TNF- α , sICAM-1, sVCAM-1 and sP-selectin (Harris et al., 1999; Huo & Ley, 2001; Libby, 2002; Pai et al., 2004; Pearson et al., 2003; Ridker et al., 2000; The Emerging Risk Factors Collaboration: Kaptoge, 2010, 2012).

For most biomarkers of systemic inflammation and soluble adhesion molecules, we found no significant changes after either of the wood smoke exposure session compared to exposure to filtered air (IL-6, TNF- α , SAA, WBC, sP-selectin), or only small sporadic changes, most likely explained by chance (CRP, sICAM-1, sVCAM-1).

However, our first wood smoke exposure study, which used higher exposure doses found an increase in SAA and a decrease in IL-6 (Barregard et al., 2006). This can be interpreted as a dose-dependent short-term effect in healthy humans. Surprisingly, few other wood smoke exposure studies have published any results about systemic effects on biomarkers of inflammation and soluble adhesion molecules. One study (PM_{2.5} about 180 $\mu\text{g}/\text{m}^3$) found an increased percentage of neutrophils but no increases in total WBC, IL-6 or TNF- α (Ghio et al., 2011); another study examining markers of inflammation and oxidative stress in peripheral blood monocytes found no increases in either CAMs or inflammatory cytokines (Forchhammer et al., 2012). In summary, moderate short-term exposure to wood smoke seems to have little effect on systemic inflammatory biomarkers. However, a recent real-world intervention study using indoor air filtration for one week

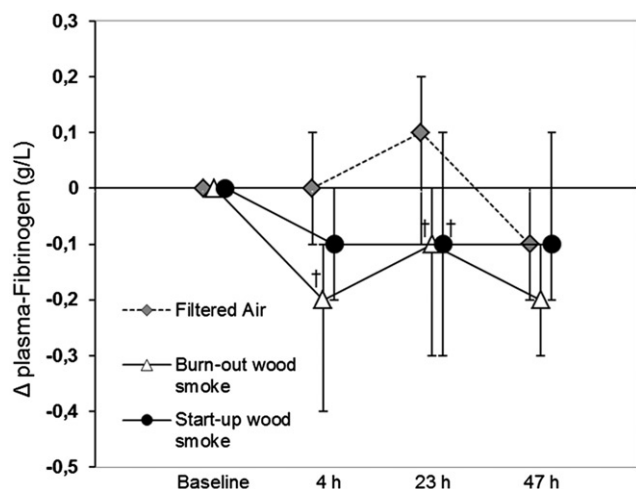


Figure 1. Median changes (Δ) from baseline and 90% CI for fibrinogen in plasma at all sample times in the filtered air session and both wood smoke sessions. †Significant net decrease after wood smoke exposure compared to after filtered air.

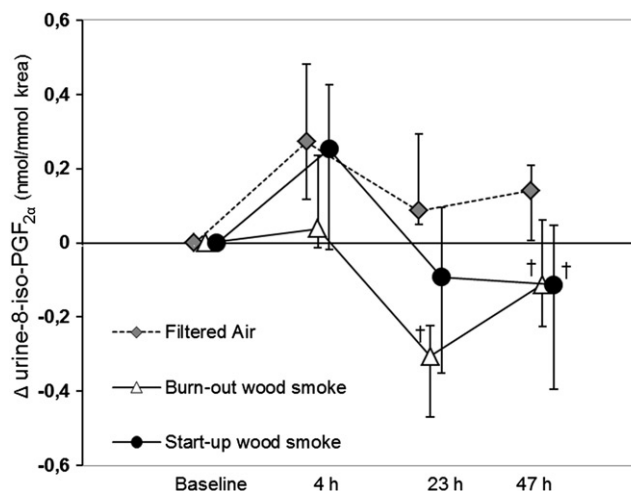


Figure 3. Median changes (Δ) from baseline and 90% CI for 8-iso-PGF_{2α} in urine corrected for creatinine concentrations at all sample times in the filtered air session and both wood smoke sessions. †Significant net decrease after wood smoke exposure compared to after filtered air.

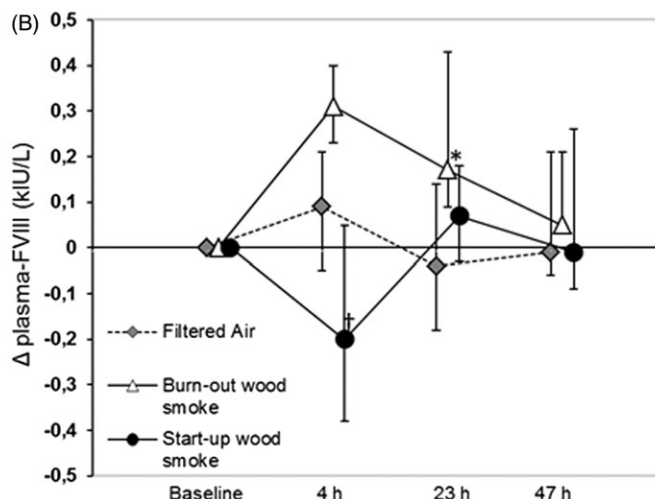
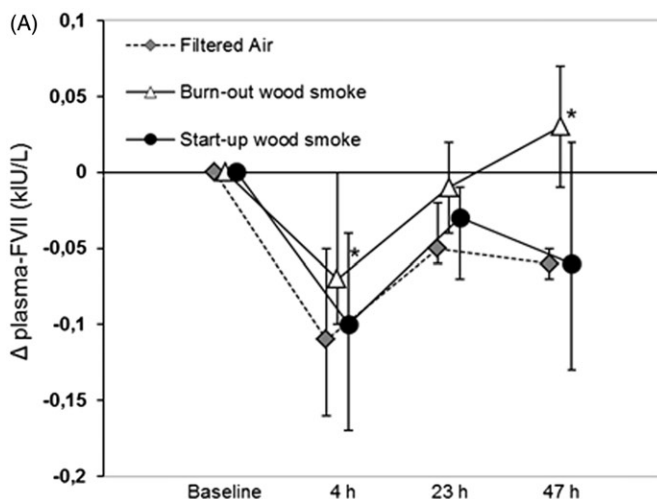


Figure 2. Median changes (Δ) from baseline and 90% CI for the coagulation factors VII (A) and VIII (B) at all sample times in the filtered air session and both wood smoke session. *Significantly net increase after wood smoke exposure; †Significant net decrease after wood smoke exposure compared to after filtered air.

in homes in a wood-burning community found decreases in CRP and IL-6 (Allen et al., 2011).

Biomarkers of coagulation and fibrinolysis

Inflammation and thrombosis are closely linked. Fibrinogen is an acute phase reactant that is increased in inflammatory conditions and an important determinant of blood viscosity and platelet aggregation. Plasma levels have been associated with CVDs (Fibrinogen Studies Collaboration: Danesh et al., 2005; Pearson et al., 2003; Stec et al., 2000; The Emerging Risk Factors Collaboration: Kaptoge, 2012). Platelets are important in both inflammation and thrombosis and are linked to the progression of atherosclerosis (Gawaz et al., 2005). Circulating levels of D-dimer reflects the degradation of cross-linked fibrin, and higher levels have been associated with coronary heart disease (Danesh et al., 2001).

Fibrinogen and platelet counts had both slightly but significantly decreased after exposure to wood smoke compared to exposure to filtered air at three sampling times. The few other wood smoke exposure studies investigating these biomarkers did not observe any effects, even though their exposure doses were higher (Barregard et al., 2006; Ghio et al., 2011). Studies of other air pollution PM sources have reported mixed results (Frampton et al., 2004; Ghio et al., 2000, 2003; Gong et al., 2003; Steinvil et al., 2008).

The coagulations factor VII and VIII and the vWf are part of the coagulation cascade. Factor VIII and vWf have been associated with inflammation and CVD (Folsom et al., 1997; Haverkate, 2002; Lacroix-Desmazes et al., 2008). The vWf was not affected in either of these studies nor in the only other study investigating it (Ghio et al., 2011). The coagulation factor VII was significantly increased at two sample times and factor VIII at one sample time after exposure to burn-out smoke compared with exposure to filtered air. However, the increase for factor VII was slight and was observed after four hours and after 47 hours but not in between these sampling times; and in the start-up session factor VIII instead decreased four hours after exposure. The only other published experimental wood smoke exposure study analyzing these coagulation factors observed an increase in factor VIII but not in FVII the morning after exposure, but the inhaled dose in that study was about twice as high (Barregard et al., 2006).

In summary, the effects on coagulation and fibrinolysis are unclear. While certain biomarkers increased at some time points after exposure to wood smoke, the increases were not consistent and were accompanied by a higher number of decreases. After putting these together, and taking other studies of wood smoke exposure into account, we do not interpret these results as a sign of increased risk of thrombosis in healthy adults after short-term exposure to moderate doses of wood smoke. Such effects may, however, occur at higher or longer exposures. Another possible interpretation of the results is that the decrease in fibrinogen and platelets represents a true effect by wood smoke exposure. Though slight, these effects were consistent for (the same) three sampling times for both biomarkers. A panel study that found a negative correlation between PM₁₀ exposure and both fibrinogen and platelets speculated that this might represent a consumption of these factors as a response to nanoparticles

(Seaton et al., 1999). However, the majority of studies find either a positive or null association between fibrinogen and particle exposure (Rückerl et al., 2011).

Lipid peroxidation

8-Iso-PGF_{2α}, a major F₂-isoprostane in urine, is considered to be a reliable marker of lipid peroxidation and oxidative stress *in vivo*. Cigarette smoking and CVDs are associated with elevated levels of F₂-isoprostanes (Basu, 2008). Increases in markers of oxidative stress have been reported in a large number of *in vitro* and animal air pollution studies (Risom et al., 2005). Our hypothesis was therefore that 8-iso-PGF_{2α} in urine (adjusted for creatinine or measured as excretion rate per hour) would increase after exposure to wood smoke. Surprisingly, there was a clear decrease in 8-iso-PGF_{2α} in urine after exposure to wood smoke, especially in the burn-out session. We cannot find any biologically plausible explanation for this finding. Increased 8-iso-PGF_{2α} in urine has been reported in one human experimental wood smoke exposure study (Barregard et al., 2006). Another wood smoke study found no changes in 8-iso-PGF_{2α} in exhaled breath condensate after exposure (Riddervold et al., 2012), but a study of concentrated ambient particles found an increase (Mills et al., 2008). There are also studies reporting no significant changes after diesel exposure (Allen et al., 2009) or indoor air filtration (Allen et al., 2011; Brauner et al., 2008).

Comparisons between experimental wood smoke exposure studies

It has previously been stated that experimental data of PM toxicity would benefit from increased comparability of studies (Schwarze et al., 2006), which seems especially true for studies of wood smoke. Emissions from burning wood vary considerably depending on the wood type, combustion appliance, combustion conditions and phase of the combustion cycle (Kocbach Bolling et al., 2009; McDonald et al., 2000; Naeher et al., 2007). Evidence on which characteristics are important for health effects is limited, but animal and *in vitro* studies indicate that the effects might be different for particles from different combustion conditions (Danielsen et al., 2010, 2011; Kocbach Bolling et al., 2009), and that the organic fraction might be most important for the biological effects (Kocbach Bolling et al., 2012). Considering this, it is not surprising that the results are not completely consistent for the published experimental human wood smoke chamber exposure studies (Barregard et al., 2006, 2008; Forchhammer et al., 2012; Ghio et al., 2011; Pope et al., 2011; Riddervold et al., 2011, 2012; Sehlstedt et al., 2010; Stockfelt et al., 2012). There is great heterogeneity in experimental setups, wood smoke generation, subjects, exposure doses and outcomes measured, making a comparative analysis tentative at best. Compared to the experimental studies of wood smoke exposure, studies of real-world exposure more consistently report clear effects on biomarkers (Allen et al., 2011; Hejl et al., 2013; Swiston et al., 2008; Tan et al., 2000; Van Eeden et al., 2001). This might be explained by higher doses of wood smoke in most of these studies – mainly because of longer times of exposure. In the studies of firefighters

(Hejl et al., 2013; Swiston et al., 2008) the levels of PM were also very high.

It would be valuable if future studies on wood smoke were more comparable. Biomarkers that are risk factors for CVDs may be especially important to study, considering the vast burden of disease due to particulate air pollution, and the evidence that the effects of wood smoke exposure is less for cardiovascular outcomes than for respiratory effects (Naeher et al., 2007). Also, testing several kinds of wood smoke in the same setup may provide evidence on differences in toxicity and using different doses of the same type of wood smoke may increase the understanding of the dose–response relationships. Moreover, it is important that also negative results are published to provide a complete picture and to avoid publication bias.

Strengths and limitations

One strength of this study is that we mainly used biomarkers associated with clinically relevant adverse outcomes, such as cardiovascular morbidity and mortality. Another strength is that we used the same experimental setup for two different wood smoke exposures, as well as for a previous wood smoke exposure study, enabling comparisons between the studies. The experimental chamber is large enough to house all the subjects in the same chamber, decreasing exposure variability compared to exposing subjects on many different days. A limitation is that we did not use a randomized cross-over design due to time limitations, making it difficult to exclude carry-over effects or effects of learning with certainty. This seems biologically unlikely though as we did not see any clear effects on the biomarkers by exposure to either filtered air or wood smoke, and we do not see any evidence of this in the pre-exposure levels of biomarkers. Moreover, since the smell of wood smoke is obvious and difficult to mask, we could not do a blind study regarding exposure to filtered air or wood smoke; however, the subjects were unaware of wood-burning cycles. One possible reason for non-positive findings that must be considered is lack of power. Post hoc power calculations show that with 80% power (two-tailed) we can detect changes of about 8–15% of pre-exposure values, which we think is acceptable power. The fact that the biomarkers often changed in the direction opposed to the hypothesis also argues against a true but not detected effect.

We used a relatively straight-forward analysis of intraindividual differences in the outcome variables, adjusted for effects at filtered air. Other statistical methods to analyze the data might be considered, but do not seem to alter the results substantially. A repeated-measures analysis of variance using PROC MIXED in SAS comparing effects of wood smoke to filtered air showed similar non-positive results for most biomarkers, a few but conflicting significant effects on the coagulation factors VII and VIII, and a decrease in 8-iso-PGF_{2α}.

Conclusions

The results indicate that there is no increase in systemic inflammation or coagulation in healthy adults at these doses of wood smoke exposure, which has previously been shown to cause airway effects. Instead a few biomarkers associated with

coagulation and lipid peroxidation showed a tendency to decrease after wood smoke exposure.

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Declaration of interest

The authors declare no conflicts of interest.

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