An Air Filter Intervention Study of Endothelial Function among Healthy Adults in a Woodsmoke-impacted Community

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***Rationale*: Particulate air pollution is associated with cardiovascular morbidity. One hypothesized mechanism involves oxidative stress, systemic inflammation, and endothelial dysfunction.**

***Objectives*: To assess an intervention's impact on particle exposures and endothelial function among healthy adults in a woodsmoke-impacted community. We also investigated the underlying role of oxidative stress and inflammation in relation to exposure reductions.**

***Methods*: Portable air filters were used in a randomized crossover intervention study of 45 healthy adults exposed to consecutive 7-day periods of filtered and nonfiltered air.**

***Measurements and Main Results*: Reactive hyperemia index was measured as an indicator of endothelial function via peripheral artery tonometry, and markers of inflammation (C-reactive protein, interleukin-6, and band cells) and lipid peroxidation (malondialdehyde and 8-iso-prostaglandin $F_{2\alpha}$) were quantified. Air filters reduced indoor fine particle concentrations by 60%. Filtration was associated with a 9.4% (95% confidence interval, 0.9–18%) increase in reactive hyperemia index and a 32.6% (4.4–60.9%) decrease in C-reactive protein. Decreases in particulate matter and the woodsmoke tracer levoglucosan were associated with reduced band cell counts. There was limited evidence of more pronounced effects on endothelial function and level of systemic inflammation among males, overweight participants, younger participants, and residents of wood-burning homes. No associations were noted for oxidative stress markers.**

***Conclusions*: Air filtration was associated with improved endothelial function and decreased concentrations of inflammatory biomarkers but not markers of oxidative stress. Our results support the hypothesis that systemic inflammation and impaired endothelial function, both predictors of cardiovascular morbidity, can be favorably influenced by reducing indoor particle concentrations.**

Clinical trial registered at www.clinicaltrials.gov (NCT01256957).

Keywords: air pollution; particulate matter; high-efficiency particulate air filter; cardiovascular; intervention

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Exposure to particulate air pollution is associated with cardiovascular morbidity. One hypothesized mechanistic pathway involves oxidative stress, systemic inflammation, and endothelial dysfunction.

What This Study Adds to the Field

Portable air filters reduced indoor particulate air pollution, improved microvascular endothelial function, and reduced markers of systemic inflammation among healthy adults in a community heavily impacted by residential wood combustion. The cardiovascular effects of particulate matter may be mediated through systemic inflammation and impaired endothelial function, and these effects may be favorably influenced by a reduction of particle concentrations

Many studies have linked exposure to air pollution, including particulate matter (PM), to cardiovascular morbidity and mortality (1). One hypothesized pathway through which air pollution might affect cardiovascular health involves pulmonary inflammation, the release of inflammatory and prothrombotic molecules into the circulation, impaired vascular function, and, ultimately, atherogenesis and plaque instability (1, 2). This hypothesized pathway is supported by epidemiologic evidence of links between air pollution and markers of systemic inflammation (3–6), endothelial dysfunction (7–12), and atherosclerosis (13–17). Inflammation and endothelial dysfunction are related phenomena that are both involved in the atherosclerotic disease process and have been linked with an increased risk of cardiovascular disease and cardiovascular events (18–24).

Combustion-derived pollution is believed to play a particularly important role in the cardiovascular effects of air pollution (1), and there is now strong evidence linking traffic-related air pollution with cardiovascular morbidity and mortality (25). Although there is limited evidence to assess the impact of woodsmoke on cardiovascular health, studies of occupationally exposed populations or in controlled experimental settings suggest that short-term exposures to high concentrations of biomass emissions may also elicit a systemic inflammatory response (4, 26, 27).

Residential wood combustion (RWC) is an important source of ambient particulate matter in mid- and high-latitude climates (26). The importance of RWC as a source of air pollution is likely to increase due to the rising costs of other fuels and the promotion of wood as a "carbon neutral" and renewable fuel (28).

In this study we used portable high-efficiency particulate air (HEPA) filters in a randomized intervention crossover study design (9) to study the subclinical cardiovascular effects of PM with a diameter less than 2.5 μm ($PM_{2.5}$) exposure in a woodsmoke-impacted airshed. Our main objectives were to better understand the mechanisms underlying air pollution–

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related cardiovascular morbidity and evaluate the potential for a simple intervention to reduce pollution-related cardiovascular health risks. HEPA filters are a potentially useful intervention because they are relatively inexpensive to purchase and operate and can effectively remove respirable particles (e.g., 99.97% of 0.3-μm diameter particles) to improve air quality inside homes, where the majority of time is spent (29–34). Our primary outcome was reactive hyperemia index (RHI), an indicator of microvascular endothelial function, because it represents an early pathology in the atherosclerotic process and predicts cardiovascular morbidity and mortality (19, 20, 35). Markers of oxidative stress (malondialdehyde [MDA], 8-iso-prostaglandin $F_{2\alpha}$, [8-isoprostane]) and inflammation (C-reactive protein [CRP], IL-6, and band cell counts) were considered exploratory endpoints to better understand potential pathways involved in endothelial dysfunction. Some of the results of this study have been previously reported in the form of an abstract (36).

METHODS

This study was conducted from November 2008 to April 2009 in Smithers, British Columbia, Canada (population ~ 5,300), where we have previously shown the outdoor air to be heavily impacted by RWC emissions (37). We recruited participants aged 19 years or older; individuals who resided in self-reported tobacco-smoking households were excluded from participating. The study protocol was approved by the research ethics boards at Simon Fraser University and the University of British Columbia, and written informed consent was obtained from all participants before enrollment. More details on the methods are available in the online supplement.

Each participant's home was monitored for two consecutive 7-day periods, during which time a HEPA filter (model 50300; Honeywell, Morristown, NJ) was operated in the main activity room and a quieter HEPA filter (Honeywell 18150) was operated in the participant's bedroom. HEPA filters were operated normally during one 7-day period and without the internal filters in place (i.e., placebo filtration) during the other period, thus blinding participants to the filters' status. The order of filtration or nonfiltration was random. Indoor pollution sampling equipment was placed in the home's main activity room.

Health Measurements

At the end of each 7-day period, a study technician measured microvascular endothelial function and collected blood and urine samples at the participant's home. Microvascular endothelial function was measured via peripheral artery tonometry using the portable EndoPAT 2000 instrument (Itamar Medical Ltd, Cesari, Israel), which determines RHI based on a computer algorithm. Serum samples were analyzed for CRP and IL-6 by ELISA. A trained technician performed manual band cell counts on thin blood smears that were air dried, fixed with methanol, and stained with Wright stain. Band cell counts are expressed as the percent of polymorphonuclear leukocytes. Urine samples were analyzed for MDA and 8-isoprostane (not normalized to creatinine) via gas chromatography mass spectrometry and ELISA, respectively.

Exposure Assessment

During each 7-day period, $PM_{2.5}$ filter samples were collected indoors and outdoors using Harvard Impactors (Air Diagnostics and Engineering, Harrison, ME). Filters were analyzed for $PM_{2.5}$ mass concentration and the woodsmoke tracer levoglucosan (26), and we partitioned indoor $PM_{2.5}$ concentrations into indoor- and outdoor-generated components by first calculating the $PM_{2.5}$ infiltration efficiency (F_{inf}, the fraction of the outdoor concentration that penetrates indoors and remains suspended) for each home during HEPA filtration and placebo filtration using indoor and outdoor measurements made with nephelometers (Radiance Research, Seattle, WA) (38). Indoor temperature and relative humidity were logged continuously using HOBO data loggers (Onset Computer Corporation, Pocaseset, MA) in a subset (n = 13) of homes. Each participant recorded their locations and proximity to potential sources of PM exposure at 60-minute resolution.

Statistical Methods

Outcome variables were skewed, so we log-transformed before analysis (for band cells, which had 0 values, we added 0.5 before log-transforming). As a sensitivity analysis we also modeled RHI without log-transforming. We used mixed models to account for measurements clustered within individuals and individuals clustered within homes. All models were adjusted for sex, age, body mass index (BMI), and temperature. We explored effect modification by filtration/placebo order, age ($>$ or $\leq$ 43 yr, the median age), sex, overweight (BMI $>$ or $\leq$ 25 kg/m^2), time spent indoors at home ($>$ or $\leq$ 75%), and use of a wood stove.

Data Reduction

We enrolled a total of 56 participants from 31 homes. Before analysis, we excluded eight participants who did not have complete $PM_{2.5}$ and F_{inf} data to allow for direct comparisons of effects between different exposure indicators. In addition, before analysis we removed one pregnant participant, one participant with Raynaud syndrome, and one participant who reported being highly exposed to environmental tobacco smoke the night before a technician visit.

RESULTS

Summary Statistics

The final study population for analysis consisted of 45 participants, from 25 homes, with complete paired HEPA and non-HEPA period data (Table 1). The mean age for the included participants was 43.0 ± 9.9 years (range, 20–63), there was a nearly even sex balance (53% female), and most (89%) of the participants reported working or volunteering outside the home. Twenty-three participants in 13 homes reported using a wood stove. Compared with the 45 participants with complete data, the 11 excluded participants were more likely to be female (8 out of 11, or 73%).

Outdoor temperatures during 2-week home monitoring sessions ranged between −10.7 and 4.9°C, and outdoor temperatures were similar during HEPA and non-HEPA periods (Table 2). Averages of F_{inf} and all indoor concentrations were significantly lower during HEPA filtration, with nearly 60% reductions in average concentrations of indoor $PM_{2.5}$ components and a 75% reduction in average indoor levoglucosan (Table 2). HEPA filters reduced indoor $PM_{2.5}$ concentrations in 24 of 25 homes, and concentration changes during HEPA filtration in individual homes ranged between −15.7 and 2.2 μg/m^3. $PM_{2.5}$ and levoglucosan concentrations outdoors were similar under HEPA and non-HEPA conditions (Table 2). During both HEPA and non-HEPA periods, indoor-generated $PM_{2.5}$ accounted for an average of 67% of the total indoor concentration. Consistent with our previous findings in this region (37), relatively high outdoor levoglucosan/$PM_{2.5}$ ratios (mean $>$ 5%; Table 2) and high $PM_{2.5}$-levoglucosan correlations (Spearman $r \geq 0.82$; *see* Table E1 in the online supplement) indicated a major contribution of woodsmoke to outdoor $PM_{2.5}$ concentrations. Lower levoglucosan/$PM_{2.5}$ ratios (mean $\leq$ 1%; Table 2) and

TABLE 1. STUDY POPULATION CHARACTERISTICS FOR 45 PARTICIPANTS WITH COMPLETE DATA

Variable	Mean ± SD or No. (%)
Age, yr	43.0 ± 9.9
BMI, kg/m^2	25.7 ± 3.5
Female	24 (53)
Asthma	2 (4)
Hypertension	1 (2)
Diabetes	0 (0)
Employed outside home	40 (89)
Wood stove used in home	23 (51)

Definition of abbreviation: BMI = body mass index.

TABLE 2. SUMMARY STATISTICS FOR EXPOSURE VARIABLES BY HIGH-EFFICIENCY PARTICULATE AIR FILTRATION STATUS AT 25 HOMES WITH COMPLETE DATA

	HEPA Off		HEPA On		
Variable	Mean ± SD	Median	Mean ± SD	Median	Paired *t* Test *P* Value
7-d Average outdoor temperature, °C	−2.5 ± 4.6	−2.3	−3.6 ± 6.1	−1.7	0.32
7-d Average indoor temperature,* °C	19.7 ± 1.4	19.4	19.8 ± 1.7	19.4	0.75
7-d Average indoor relative humidity,* %	35.1 ± 3.3	36.0	35.3 ± 3.4	33.7	0.90
$PM_{2.5}$ outdoors, μg/m³	10.8 ± 5.0	9.0	9.8 ± 4.2	8.9	0.26
$PM_{2.5}$ infiltration efficiency (unitless)	0.34 ± 0.17	0.30	0.20 ± 0.17	0.13	<0.01
$PM_{2.5}$ indoors, μg/m³	11.2 ± 6.1	10.5	4.6 ± 2.6	3.9	<0.01
$PM_{2.5}$ outdoor-generated, μg/m³	3.5 ± 2.3	3.6	1.5 ± 0.9	1.4	<0.01
$PM_{2.5}$ indoor-generated, μg/m³	7.6 ± 6.6	6.3	3.0 ± 2.8	2.1	<0.01
Levoglucosan outdoors,† ng/m³	613 ± 548	415	530 ± 358	471	0.18
Levoglucosan indoors, ng/m³	127 ± 191	73	33 ± 39	19	0.01
Levoglucosan/$PM_{2.5}$ outdoors,† %	5.1 ± 2.8	5.3	5.3 ± 1.8	5.1	0.79
Levoglucosan/$PM_{2.5}$ indoors, %	1.0 ± 1.1	0.7	0.9 ± 1.3	0.7	0.61

Definition of abbreviations: HEPA = high-efficiency particulate air filter; $PM_{2.5}$ = particulate matter with a diameter less than 2.5 μm.

* From 13 homes with indoor HOBO data loggers.

† Excluding one highly influential outdoor levoglucosan observation.

correlations ($r \leq 0.53$; Table E1) indoors indicated a smaller $PM_{2.5}$ contribution from woodsmoke to indoor concentrations. Indoor-generated $PM_{2.5}$ concentrations were generally higher in the 13 homes where participants reported burning wood (Figure 1). Median within-participant changes in indoor $PM_{2.5}$, indoor-generated $PM_{2.5}$, and levoglucosan were −7.5 μg/m³, −6.3 μg/m³, and −44 ng/m³ in wood-burning homes, whereas in non–wood-burning homes the median changes were −6.2 μg/m³, −2.1 μg/m³, and −58 ng/m³, respectively.

Participants' activity patterns were similar between HEPA and non-HEPA periods, as were durations spent cooking or exposed to environmental tobacco smoke (Table 3). The HEPA-related differences in biological measurements were generally in the hypothesized directions, with increases in median RHI and decreases in median CRP, band cell counts, IL-6, and MDA during periods of HEPA filtration. There was an increase in median concentrations of 8-isoprostane during HEPA filtration (Table 3). Only CRP and RHI were correlated

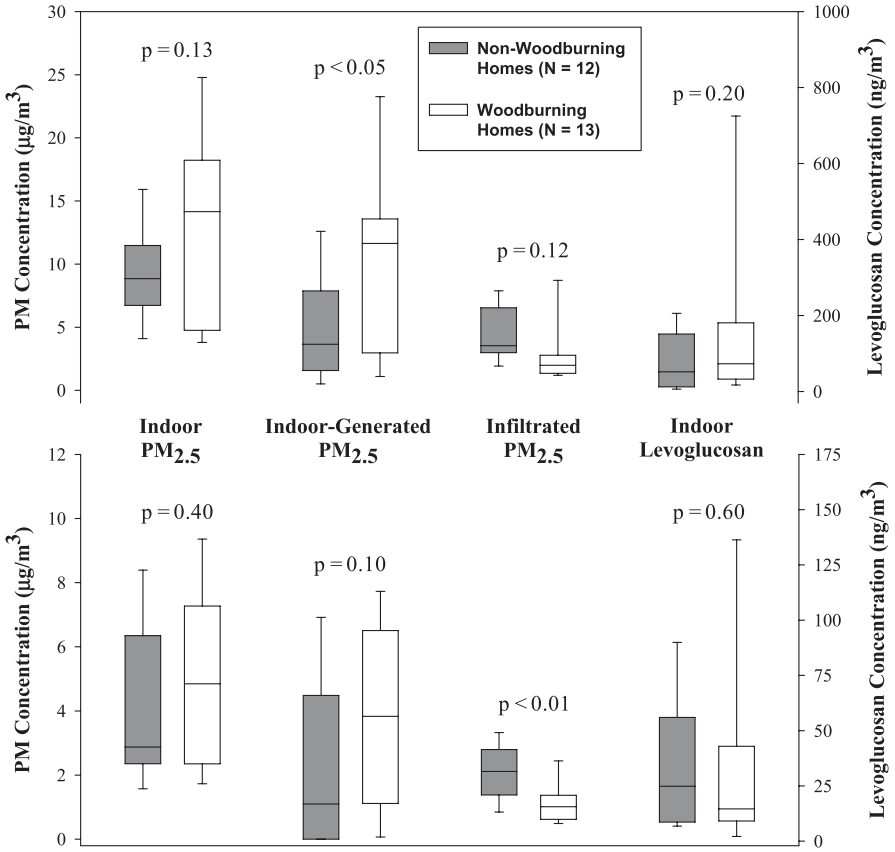

Figure 1. Distributions of indoor particulate matter (PM) with a diameter less than 2.5 μm ($PM_{2.5}$) and levoglucosan concentrations by use of a wood-burning stove during periods without high-efficiency particulate air (HEPA) filtration (*upper plot*) and with HEPA filtration (*lower plot*). *P* values are for two-sample *t* tests comparing wood-burning and non–wood-burning homes. Note: outliers not shown. Lines in the boxes are the median concentrations.

TABLE 3. SUMMARY STATISTICS FOR TIME-ACTIVITY PATTERNS AND HEALTH MEASUREMENTS BY HIGH-EFFICIENCY PARTICULATE AIR FILTRATION STATUS AMONG 45 PARTICIPANTS

	HEPA Off		HEPA On		
Variable	Mean ± SD	Median	Mean ± SD	Median	Paired *t* Test *P* Value
Room temperature during RHI measurement, °C	19.3 ± 1.4	19	19.1 ± 1.0	19	0.44
% of Time indoors at home	77.0 ± 13.2	78.4	76.0 ± 12.8	75.0	0.45
% of Time at work	14.8 ± 11.7	16.0	16.3 ± 11.9	17.4	0.29
% of Time in transit	5.0 ± 5.5	3.1	5.4 ± 5.6	3.1	0.49
% of Hours with ETS exposure reported	0.1 ± 0.4	0.0	0.1 ± 0.6	0.0	0.76
% of Hours cooking	7.2 ± 5.1	6.8	7.8 ± 4.9	8.8	0.35
Systolic blood pressure,* mm Hg	112.4 ± 10.8	113	112.2 ± 11.5	112	0.88
Diastolic blood pressure,* mm Hg	68.6 ± 7.6	68	68.4 ± 8.2	67	0.80
RHI	2.06 ± 0.63	1.93	2.28 ± 0.72	2.32	0.03
CRP, mg/L	1.00 ± 0.78	0.83	0.78 ± 0.74	0.48	0.06
IL-6, pg/mL	6.11 ± 19.34	1.66	4.12 ± 8.73	1.18	0.26
Band cells,† % of PMN	4.62 ± 3.49	4.00	3.57 ± 2.84	3.00	0.08
Malondialdehyde, μM	2.64 ± 1.78	2.14	2.61 ± 3.34	1.83	0.94
8-isoprostane,‡ pg/mL	8.78 ± 12.29	3.57	10.90 ± 14.32	4.58	0.48

Definition of abbreviations: CRP = C-reactive protein; ETS = environmental tobacco smoke; HEPA = high-efficiency particulate air filter; PMN = polymorphonuclear leukocytes; RHI = reactive hyperemia index.

* Blood pressure was measured at the time of the EndoPAT RHI measurement.

† Band cell counts were missing for one subject, so statistics are based on 44 participants.

‡ 8-isoprostane data were missing for two subjects, so statistics are based on 43 participants.

(Spearman *r*: −0.31, $P = 0.04$) during "baseline" (non-HEPA periods); endpoints were not correlated during HEPA filtration periods.

Model Results

In our mixed model analysis HEPA filtration was associated with a 9.4% (95% CI, 0.9–18%) increase in RHI and a 32.6% (4.4–60.9%) decrease in CRP (Figure 2). Similar to the crude results in Table 3, when RHI was modeled on the original scale HEPA filtration was associated with an RHI change of 0.22 (0.02–0.41). With the exception of 8-isoprostane, HEPA filtration and air pollution concentration effects on other endpoints were generally in the expected directions but with confidence intervals that included the null. For CRP, IL-6, and MDA, there was some suggestion of an association with total indoor $PM_{2.5}$ and indoor-generated $PM_{2.5}$, but no evidence of a relationship with outdoor-generated (infiltrated) $PM_{2.5}$ or indoor levoglucosan. Band cells were the only outcome for which there was any evidence of an indoor levoglucosan effect, with an 11.3% (5.0–17.7%) decrease in band cells per standardized reduction in levoglucosan. Band cell effect estimates were moderately sensitive to the summand used before log-transforming, with summands of 0.1 and 1 leading to modeled decreases of 13.2% (3.8–22.5%) and 10.1% (5.0–15.3%), respectively, per standardized levoglucosan reduction. As expected due to the crossover study design, model results were not sensitive to adjustment for age, BMI, or sex. Results were also insensitive to adjustment for indoor temperature at the time of sample collection or the percent of time spent indoors at home. Based on continuous indoor nephelometer light-scattering data, there was no clear influence of $PM_{2.5}$ averaging time on the effect estimates (Figure E1).

Effect Modification

We explored modification of the HEPA effect by HEPA order (filter installed first or placebo filtration first), age (> or ⩽ 43 yr), sex, overweight status (BMI > 25 or ⩽ 25), percent of time

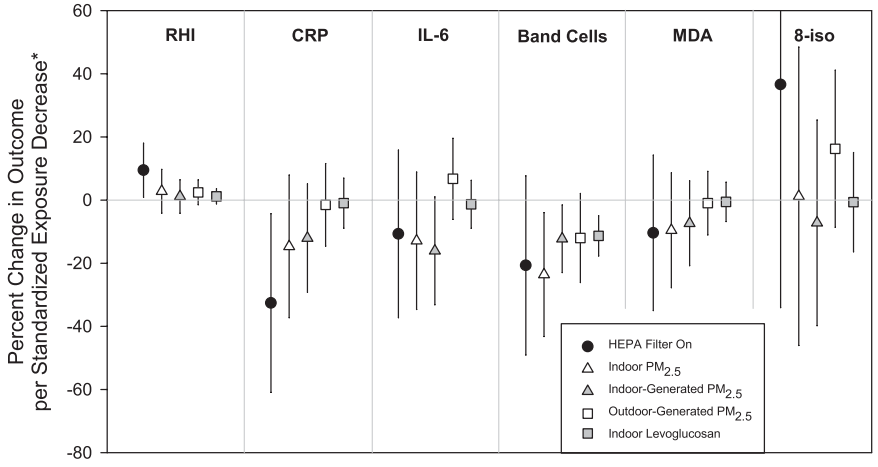

Figure 2. Model estimates of exposure reduction effects on health indicators. *Exposure contrasts for continuous exposure metrics are the median within-participant change between non–high-efficiency particulate air (HEPA) filtration and HEPA filtration periods to allow for a comparison of effect sizes between exposure metrics with different distributions. The exposure contrasts are: indoor particulate matter with a diameter less than 2.5 μm ($PM_{2.5}$) = −6.6 μg/m³; indoor-generated $PM_{2.5}$ = −4.4 μg/m³; outdoor-generated $PM_{2.5}$ = −1.3 μg/m³; indoor levoglucosan = −57.6 ng/m³. CRP = C-reactive protein; 8-iso = 8-iso-prostaglandin $F_{2\alpha}$; MDA = malondialdehyde; RHI = reactive hyperemia index.

spent indoors at home (> 75% or ⩽ 75%), and wood stove use (Figures 3 and 4). Although interactions were not statistically significant, with the exception of 8-isoprostane effects were generally more pronounced among males (n = 21) and overweight participants (n = 25) (Figure 3). Inflammatory effects, but not RHI effects, were generally more pronounced among participants 43 years of age or younger (Figure 3). There was also a general pattern across endpoints of more pronounced effects among 23 subjects living in homes with wood-burning stoves (Figure 4). The order of HEPA filtration did not modify the HEPA effect consistently across endpoints.

DISCUSSION

We used HEPA filters in a randomized crossover design to evaluate the relationship between relatively low $PM_{2.5}$ concentrations and microvascular endothelial function, our primary endpoint, and oxidative stress and systemic inflammation, our secondary endpoints, among healthy adults in an airshed heavily influenced by residential wood combustion. Consistent with previous results from this region (29, 37), the infiltration of outdoor $PM_{2.5}$ was relatively low, and the majority of indoor $PM_{2.5}$ was produced by indoor sources. HEPA filters reduced average indoor $PM_{2.5}$ and levoglucosan concentrations by approximately 60 and 75%, respectively. These reductions were anticipated based on numerous previous studies of HEPA filter effectiveness (30), including recent work in this region by Barn and colleagues (29), who concluded that HEPA filters effectively reduce PM exposures during periods of residential wood combustion.

Our RHI findings are similar to work by Brauner and colleagues (9), who also used a HEPA filter intervention design to investigate the subclinical cardiovascular health effects of traffic-related air pollution exposure among healthy older couples in Copenhagen. Their RHI results were quantitatively similar to ours, despite studying older participants (median age: 67 yr) exposed to an urban air pollution mixture. In their study, HEPA filtration reduced geometric mean indoor $PM_{2.5}$ concentrations by 7.9 $\mu g/m^3$ (from 12.6 to 4.7 $\mu g/m^3$) and was associated with an 8% increase in RHI, very similar to our observed 6.6 $\mu g/m^3$ reduction in median indoor $PM_{2.5}$ concentration and 9.4% increase in RHI. Brauner and colleagues (9) also evaluated several elements in the $PM_{2.5}$ samples and found that only potassium, which is present in relatively high concentrations in biomass smoke (26), was independently associated with RHI. They reported no associations with CRP, IL-6, or 8-isoprostane. Our study provides the first evidence of a link between air pollution and endothelial dysfunction in a woodsmoke-impacted airshed. In addition, although limited evidence suggests that people with diabetes may be more susceptible to endothelial dysfunction related to air pollution (7, 10, 11), our results provide additional evidence of endothelial effects among healthy individuals (8, 12, 39).

The mechanism(s) through which PM may affect endothelial function is not fully understood. Endothelial dysfunction is characterized by a reduction in the bioavailability of nitric oxide (NO) and other vasodilators, which occurs through scavenging by reactive oxygen species (ROS) and/or reduced synthesis (21, 35). ROS can be produced directly by the redox potential of the particles or through the activation of inflammatory cells (40). Inflammation may also play a role in the reduction of NO synthesis. For example, both CRP (23) and IL-6 (24) have been shown to decrease expression of NO synthase in human aortic endothelial cells. In our study, there was some indication of associations between air pollution and inflammatory markers CRP, IL-6, and band cells, although the results

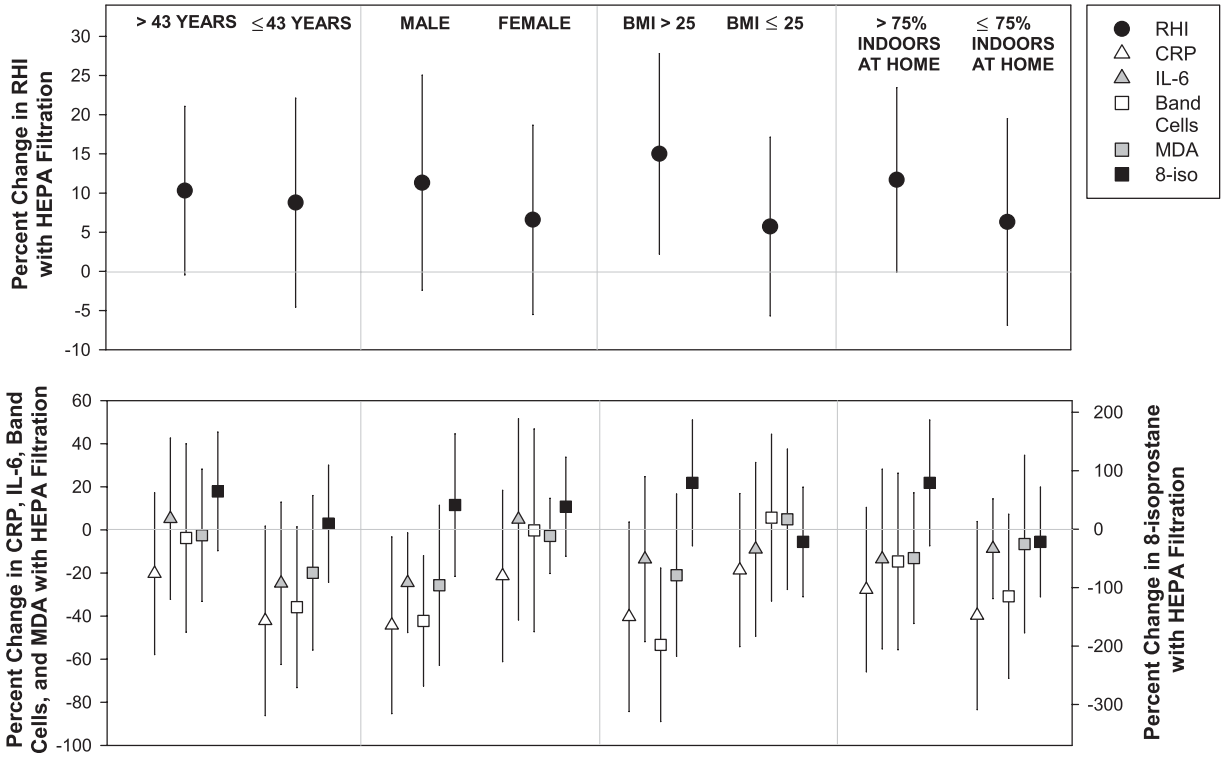

Figure 3. Model estimates of high-efficiency particulate air (HEPA) filtration effects on reactive hyperemia index (RHI) (*upper panel*) and blood and urine markers (*lower panel*) stratified by age, sex, body mass index (BMI), and time spent indoors at home. CRP = C-reactive protein; 8-iso = 8-iso-prostaglandin $F_{2\alpha}$; MDA = malondialdehyde.

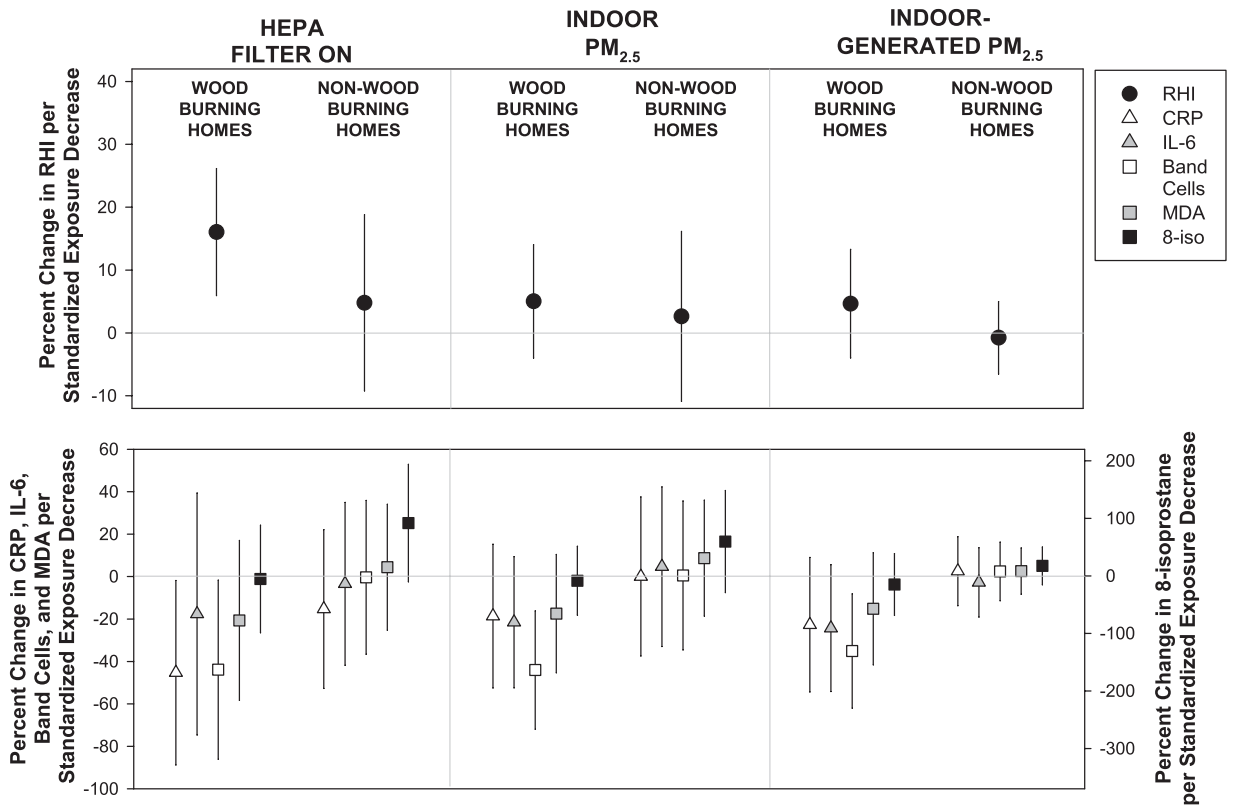

Figure 4. Model estimates of exposure reduction effects on reactive hyperemia index (RHI) (*upper panel*) and blood and urine markers (*lower panel*) stratified by use of a wood-burning stove. *Exposure contrasts for continuous exposure metrics are the median within-participant change between non–high-efficiency particulate air (HEPA) filtration and HEPA filtration periods to allow for a comparison of effect sizes between exposure metrics with different distributions. The exposure contrasts are: indoor particulate matter with a diameter less than 2.5 μm ($PM_{2.5}$) in wood-burning homes = −7.5 μg/m³; indoor $PM_{2.5}$ in non–wood-burning homes = −6.2 μg/m³; indoor-generated $PM_{2.5}$ in wood-burning homes = −6.3 μg/m³; indoor-generated $PM_{2.5}$ in non–wood-burning homes = −2.1 μg/m³. CRP = C-reactive protein; 8-iso = 8-iso-prostaglandin $F_{2\alpha}$; MDA = malondialdehyde.

were not entirely consistent across all exposure metrics. IL-6 is one of several cytokines that initiates the acute-phase inflammatory response, which involves the release of CRP and other proteins (41, 42). Band cells are immature polymorphonuclear leukocytes, and elevated numbers of band cells indicate stimulation of the bone marrow (4, 43). For both CRP and IL-6, there was some evidence of associations with total indoor $PM_{2.5}$ and indoor-generated $PM_{2.5}$, but less so for outdoor-generated $PM_{2.5}$ or levoglucosan. The lack of effects for outdoor-generated $PM_{2.5}$ and levoglucosan is possibly due to the low indoor concentrations of these constituents (due to low infiltration in these tightly sealed homes), which limited the exposure gradients introduced by HEPA filtration (Table 2).

There are at least three possible explanations for the observation that HEPA filtration, but not $PM_{2.5}$, was associated with changes in RHI and CRP. First, the lack of measurement error in the binary intervention variable may have allowed us to observe associations that were masked by error in the continuous pollution concentration variables. Second, the observed HEPA effects may be due to specific components of the PM mixture, such as ultrafine (< 100 nm-diameter) particles. HEPA filters are believed to effectively remove particles in the ultrafine range (10–100 nm) (44), and ultrafine particles may play an important role in the inflammatory and endothelial effects of PM (1, 45, 46). Finally, the averaging period for the $PM_{2.5}$ measurements (7 d) may not have matched the relevant exposure-response period for some of these outcomes (41), although continuous indoor measurements did not reveal a clear influence of averaging times on the $PM_{2.5}$ associations (Figure E1). Repeated measurements of outcomes during the 7-d monitoring periods, which would have allowed us to evaluate the time course of the biological responses, were not feasible in this study.

Although the literature is not totally consistent (47, 48), our results add to a growing body of evidence linking short-term PM exposure with a systemic inflammatory response (1). Traffic-related air pollution has been studied more extensively in relation to inflammation (3, 6), but there is also some evidence linking high concentrations of biomass smoke with a systemic inflammatory response. In an experimental crossover study, Barregard and colleagues (27) administered clean air and woodsmoke at $PM_{2.5}$ mass concentrations of 240 to 280 μg/m³ to healthy adult volunteers. They reported significant associations between woodsmoke and serum amyloid A, an acute-phase inflammatory protein, 8-isoprostane, and plasma factor VIII. Swiston and colleagues (4) studied 52 seasonal forest-fire fighters and reported significant increases in circulating white blood cells, band cells, IL-6, and monocyte chemotactic protein-1 levels after fire fighting. PM levels, estimated from measurements of carbon monoxide, were estimated in the 1,000 to 2,000 μg/m³ range.

In our study, band cells were the only endpoint for which there was persuasive evidence of an association with levoglucosan, a marker of woodsmoke PM. Similar to our results and those of Swiston and colleagues (4), Tan and colleagues (43) reported an association between air pollution from biomass

combustion and increased circulating band cells. They studied 30 men in Singapore exposed to biomass smoke during the 1997 Southeast Asian smoke haze. PM_{10} concentrations, which averaged 125 $\mu g/m^3$ during the event, were significantly associated with band cells at 0- at 1-day lags. The associations with band cells in these three studies suggest that this biomarker may be particularly sensitive to biomass smoke exposure.

There was limited evidence of more pronounced effects among participants residing in wood-burning homes, males, and participants with BMI greater than 25 kg/m^2. For the systemic inflammation markers, there was also some indication of more pronounced effects among younger participants. The findings in wood-burning homes were unexpected given the lack of associations with the woodsmoke tracer levoglucosan for all endpoints but band cells. This discrepancy may be explained by the presence of some other (non-woodsmoke) indoor $PM_{2.5}$ source in wood-burning homes, which is supported by the observation that during HEPA filtration wood-burning homes experienced much larger reductions in indoor-generated $PM_{2.5}$, but similar reductions in indoor levoglucosan, compared with homes where wood was not burned. Alternatively, the participants residing in these homes may have been more sensitive to the cardiovascular impacts of PM exposure.

Despite some inconsistency, previous research has suggested that older individuals may be more susceptible to the cardiovascular effects of air pollution (1). For example, in contrast to the results of their HEPA intervention study (9), Brauner and colleagues found that RHI and biomarkers of inflammation and oxidative stress were not associated with traffic-generated PM in a controlled exposure study among 29 healthy young (median age, 25 yr) adults (47). Sex has also not been definitively identified as an effect modifier. Nevertheless, our results are consistent with several previous studies that have reported short-term air pollution effects on endothelial function and inflammation among young male participants (6, 8, 12, 39, 43), and one study suggesting that the inflammatory effects of chronic PM exposure are more pronounced in men (5). The existing evidence for BMI/obesity as an effect modifier is somewhat stronger and is consistent with our results. Schneider and colleagues (10) reported greater effects of short-term $PM_{2.5}$ on flow-mediated dilatation among persons with type 2 diabetes and among those with BMI greater than 30 kg/m^2. Similarly, Dubowsky and colleagues (3) reported stronger associations between 5-day $PM_{2.5}$ concentrations and both CRP and IL-6 among older adults with BMI greater than 30 kg/m^2, whereas Chen and Schwartz (49) found that that metabolic syndrome modified the association between annual PM_{10} concentrations and white blood cell counts.

We did not find persuasive evidence that any exposure metrics were associated with MDA or 8-isoprostane, two products of lipid peroxidation that have been assessed in previous air pollution studies (9, 27, 50–52). The experimental work of Barregard and colleagues (27) provides the only published evidence of an association between biomass smoke and systemic oxidative stress, whereas some experimental and observational studies of the urban pollution mixture have reported associations with oxidative stress markers among young adults and children (50, 53–56). The lack of observed effects in our study may have been due to other factors, such as diet (57). In addition, the 8-isoprostane results may have been influenced by the lack of normalization to urinary creatinine and the use of ELISA, which is a less specific and less quantitative assay than gas chromatography mass spectrometry (51, 58).

Some additional limitations of this study should be noted. First, our measure of microvascular endothelial function, RHI, has not been widely used for research or clinical purposes. Nevertheless, this measure is predictive of adverse cardiovascular events (59). Although RHI does not directly distinguish between endothelium-dependent and endothelium-independent effects, inhibition of endothelial nitric oxide synthase attenuates the RHI response, suggesting that this measure is indicative of endothelial function (60). Moreover, Bonetti and colleagues (61) have reported a relationship between RHI and coronary artery endothelial function, whereas Kuvin and colleagues (62) demonstrated a correlation between RHI and endothelium-dependent brachial artery flow-mediated dilatation. In addition, RHI is associated with traditional cardiovascular risk factors, including diabetes mellitus, BMI, cholesterol, and smoking (63). Administration of sublingual nitroglycerin, which would have allowed us to assess endothelium-independent effects on the RHI response (61), was not feasible in this residence-based study.

An additional limitation was that we were not able to quantify air pollution exposure outside the home, where on average our study participants spent 25% of their time. Although time spent outside the home reduced the effectiveness of the in-home air cleaner intervention, pollution exposures outside the home are unlikely to explain the observed associations because of the crossover study design and the similarity in time-location patterns between HEPA and non-HEPA periods.

Carryover of effects between "treatments" is a concern in crossover study designs (64). However, in this study the 7-day exposure periods were long relative to the expected response time of the biological measurements (41). Therefore, our exposure periods were probably sufficient to wash out any effects from the previous exposure scenario. Moreover, carryover effects would likely have caused an underestimation of the effects (i.e., a bias toward no effect) and are therefore unlikely to be responsible for the observed associations.

In conclusion, portable HEPA filters reduced average indoor $PM_{2.5}$ concentrations by 60% and were associated with improved endothelial function and decreased concentrations of inflammatory biomarkers, but not markers of oxidative stress, among healthy adults residing in a woodsmoke-dominated airshed. There was limited evidence that effects were more pronounced among participants residing in homes that burned wood, males, younger participants, and overweight participants. Our results support the hypothesis that systemic inflammation and impaired endothelial function, both predictors of cardiovascular morbidity, can be favorably influenced by a reduction of indoor particle concentrations.

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