# An Air Filter Intervention Study of Endothelial Function Among Healthy Adults in a Woodsmoke-Impacted Community

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Revised and resubmitted to
American Journal of Respiratory and Critical Care Medicine

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**Sources of support:** Funding for this work was provided by the British Columbia Lung Association, the British Columbia Ministry of Environment, and Health Canada.

**Running head:** Woodsmoke air pollution and endothelial dysfunction

**Descriptor number:** 6.01, Air Pollution Epidemiology

**Word count:** 3,745

**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.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

## ABSTRACT

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

**Objectives:** To assess the impact of an intervention on particle exposures and endothelial function among healthy adults in a woodsmoke-impacted community. In addition, we investigated the underlying role of oxidative stress and inflammation in relation to reductions in particle exposures.

**Methods:** Portable air filters were used in a randomized crossover intervention study of 45 healthy adult participants exposed to consecutive 7-day periods of filtered and non-filtered 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 over 60%. Filtration was associated with a 9.4% (95% CI: 0.9 – 18%) increase in reactive hyperemia index and a 32.6% (4.4 – 60.9%) decrease in C-reactive protein. Lower indoor concentrations of 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 those residing in 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 a reduction of indoor particle concentrations.

**Abstract Word Count:** 264

**Keywords:** Air pollution, particulate matter, HEPA filter, cardiovascular, intervention.

## INTRODUCTION

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 thought 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 $PM_{2.5}$ exposure in a woodsmoke-impacted airshed. Our main objectives were to better understand the mechanisms underlying air pollution-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 since 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; interleukin-6, 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). This study is registered at ClinicalTrials.gov.

## 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 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 prior to enrolment. More details on the methods are available in the Online Supplement.

Each participant's home was monitored for two consecutive seven-day periods, during which time a HEPA filter (Honeywell model 50300) 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 non-filtration 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 endoethelial 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 enzyme-linked immunosorbent assays (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 (PMN). 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 (RH) 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 prior to analysis (for band cells, which had 0 values, we added 0.5 prior to 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 gender, age, body mass index (BMI), and temperature. We explored effect modification by filtration/placebo order, age (> or ≤ 43 years, the median age), gender, overweight (BMI > or ≤ 25 $kg/m^2$), time spent indoors at home (> or ≤ 75%), and use of a woodstove.

***Data Reduction***

We enrolled a total of 56 participants from 31 homes. Prior to analysis, we excluded 8 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, prior to analysis we removed 1 pregnant participant, 1 participant with Raynaud's syndrome, and 1 participant who reported being highly exposed to ETS 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 gender 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 woodstove. 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 $^{0}$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's $r \geq 0.82$, Table E1) indicated a major contribution of woodsmoke to outdoor $PM_{2.5}$ concentrations. Lower levoglucosan/$PM_{2.5}$ ratios (mean ≤ 1%, Table 2) and 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 $\mu g/m^3$, -6.3 $\mu g/m^3$, and -44 $ng/m^3$ in woodburning homes; while in non-woodburning homes the median reductions were -6.2 $\mu g/m^3$, -2.1 $\mu g/m^3$, and -58 $ng/m^3$, 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 malondialdehyde 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 (Spearman's *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 prior to 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 gender. 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 years), gender, overweight status (BMI > 25 or ≤ 25), percent of time spent indoors at home (> 75% or ≤ 75%), and woodstove use (Figures 3 and 4). Though 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 (Figure 3). There was also a general pattern across endpoints of more pronounced effects among 23 subjects living in homes with woodburning 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 et al. (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 yrs) 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 et al. (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 diabetics 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, though the results 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 PMN, 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 thought 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 days) 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-day 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 240-280 $\mu g/m^3$ 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 et al. (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 – 2,000 $\mu g/m^3$ 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 et al. (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 woodburning homes, males, and participants with BMI > 25 kg/m$^2$. For the systemic inflammation markers, there was also some indication of more pronounced effects among younger participants. The findings in woodburning 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 woodburning homes, which is supported by the observation that during HEPA filtration woodburning 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 yrs) adults (47). Gender 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 et al. (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 > 30 kg/m$^2$. Similarly, Dubowsky et al. (3) reported stronger associations between 5-day $PM_{2.5}$ concentrations and both CRP and IL-6 among older adults with BMI > 30 kg/m$^2$, while 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, while 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 GC-MS (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, while Kuvin et al. (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.

**ACKNOWLEDGMENTS**

We thank the study participants and our field technicians, Thurza Aspinall and Amelia Mattson. The BC Ministry of Environment and the Bulkley Valley District Hospital generously provided laboratory access. We are also grateful to Dr. Winnie Chu and the UBC School of Environmental Health Laboratory staff for their assistance with sample preparation and analysis.

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**Table 1. Study population characteristics for 45 participants with complete data.**

| Variable | Mean ± SD or Number (%) |
|---|---|
| Age (years) | 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%) |

**Table 2. Summary statistics (mean ± SD) for exposure variables by HEPA status at 25 homes with complete data.**

| Variable | HEPA Off | | HEPA On | | Paired t-test p-value |
|---|---|---|---|---|---|
| | Mean ± SD | Median | Mean ± SD | Median | |
| 7-day Avg. Outdoor Temperature ($^0$C) | -2.5 ± 4.6 | -2.3 | -3.6 ± 6.1 | -1.7 | 0.32 |
| 7-day Avg. Indoor Temperature ($^0$C)* | 19.7 ± 1.4 | 19.4 | 19.8 ± 1.7 | 19.4 | 0.75 |
| 7-day Avg. Indoor Relative Humidity (%)* | 35.1 ± 3.3 | 36.0 | 35.3 ± 3.4 | 33.7 | 0.90 |
| $PM_{2.5}$ Outdoors (ug/m$^3$) | 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 (ug/m$^3$) | 11.2 ± 6.1 | 10.5 | 4.6 ± 2.6 | 3.9 | <0.01 |
| $PM_{2.5}$ Outdoor-Generated (ug/m$^3$) | 3.5 ± 2.3 | 3.6 | 1.5 ± 0.9 | 1.4 | <0.01 |
| $PM_{2.5}$ Indoor-Generated (ug/m$^3$) | 7.6 ± 6.6 | 6.3 | 3.0 ± 2.8 | 2.1 | <0.01 |
| Levoglucosan Outdoors (ng/m$^3$)† | 613 ± 548 | 415 | 530 ± 358 | 471 | 0.18 |
| Levoglucosan Indoors (ng/m$^3$) | 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 |

*From 13 homes with indoor HOBO data loggers.

† Excluding one highly influential outdoor levoglucosan observation.

**Table 3. Summary statistics for time-activity patterns and health measurements by HEPA status among 45 participants.**

| Variable | HEPA Off | | HEPA On | | Paired t-test p-value |
|---|---|---|---|---|---|
| | Mean ± SD | Median | Mean ± SD | Median | |
| Room Temperature During RHI Measurement ($^0$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 (mmHg)* | 112.4 ± 10.8 | 113 | 112.2 ± 11.5 | 112 | 0.88 |
| Diastolic Blood Pressure (mmHg)* | 68.6 ± 7.6 | 68 | 68.4 ± 8.2 | 67 | 0.80 |
| Reactive Hyperemia Index | 2.06 ± 0.63 | 1.93 | 2.28 ± 0.72 | 2.32 | 0.03 |
| C-reactive Protein (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 (uM) | 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 |

*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.

**Figure 1. Distributions of indoor $PM_{2.5}$ and levoglucosan concentrations by use of a wood-burning stove during periods without HEPA filtration (upper plot) and with HEPA filtration (lower plot). P-values are for 2-sample t-tests comparing woodburning and non-woodburning homes.**

Note: outliers not shown. Lines in the boxes are the median concentrations.

**Figure 2. Model estimates of exposure reduction effects on health indicators.**

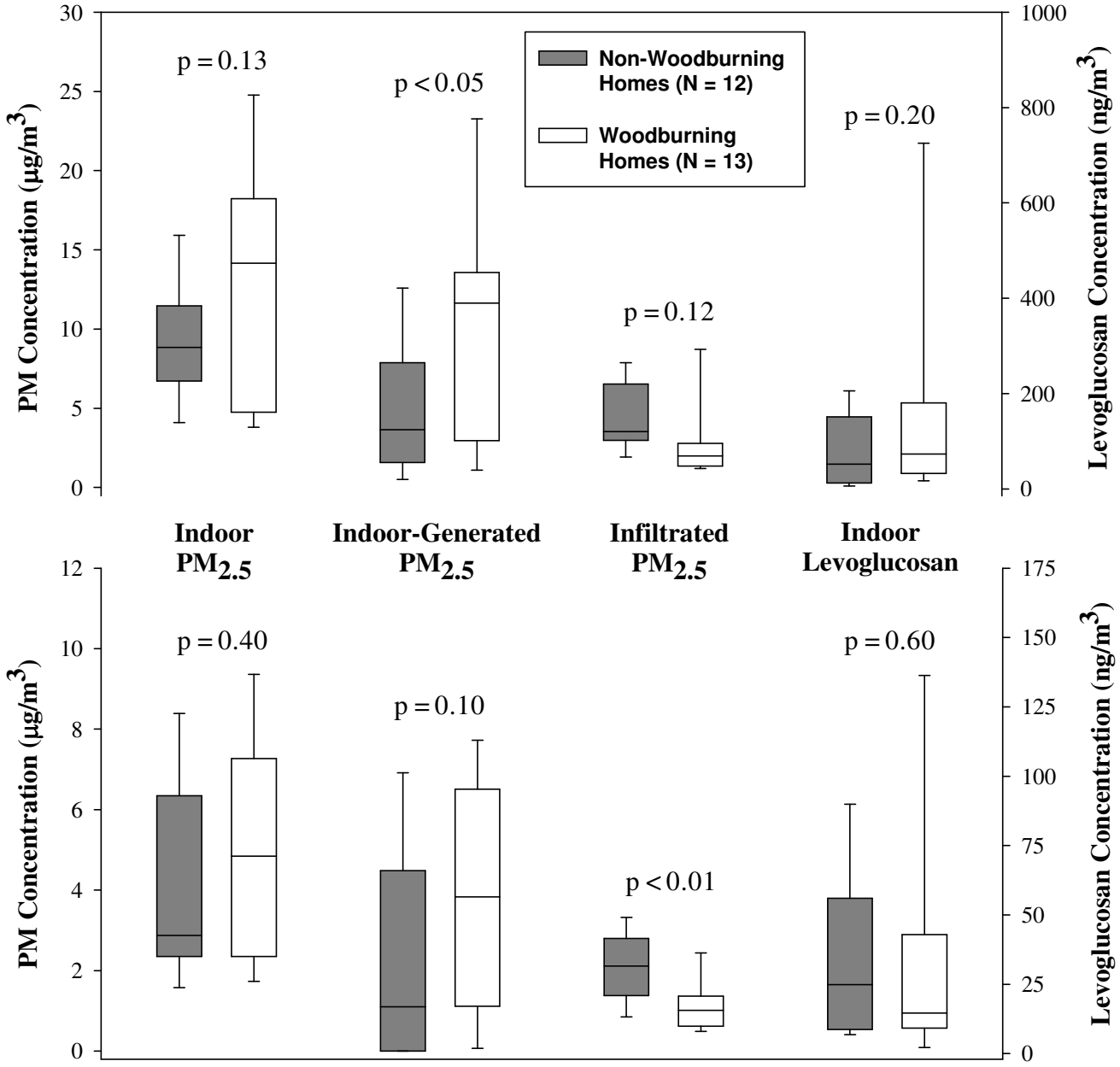
*Exposure contrasts for continuous exposure metrics are the median within-participant change between non-HEPA and HEPA periods to allow for a comparison of effect sizes between exposure metrics with different distributions. The exposure contrasts are: Indoor $PM_{2.5}$ = -6.6 $\mu g/m^3$; Indoor-Generated $PM_{2.5}$ = -4.4 $\mu g/m^3$; Outdoor-Generated $PM_{2.5}$ = -1.3 $\mu g/m^3$; Indoor Levoglucosan = -57.6 $ng/m^3$. Abbreviations: RHI = reactive hyperemia index; CRP = C-reactive protein; IL-6 = interleukin-6; MDA = malondialdehyde; 8-iso = 8-iso-prostaglandin F2α.

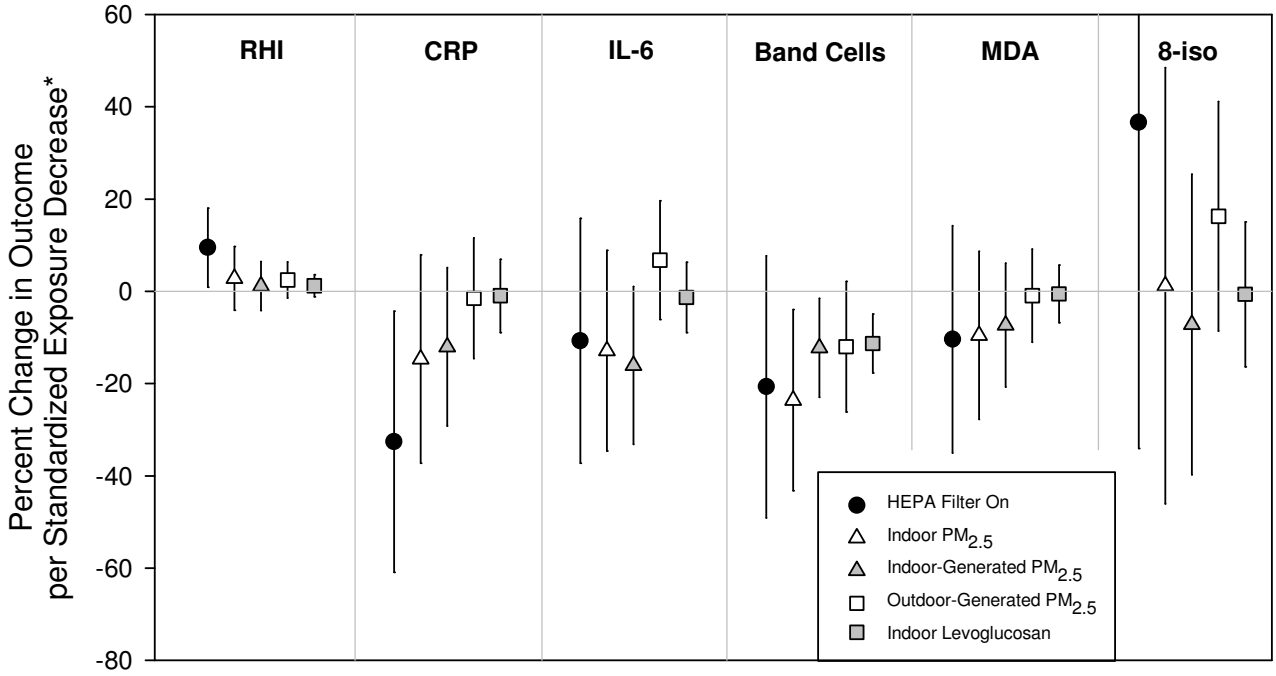
**Figure 3. Model estimates of HEPA filter effects on RHI (upper panel) and blood and urine markers (lower panel) stratified by age, gender, body mass index (BMI), and time spent indoors at home.**

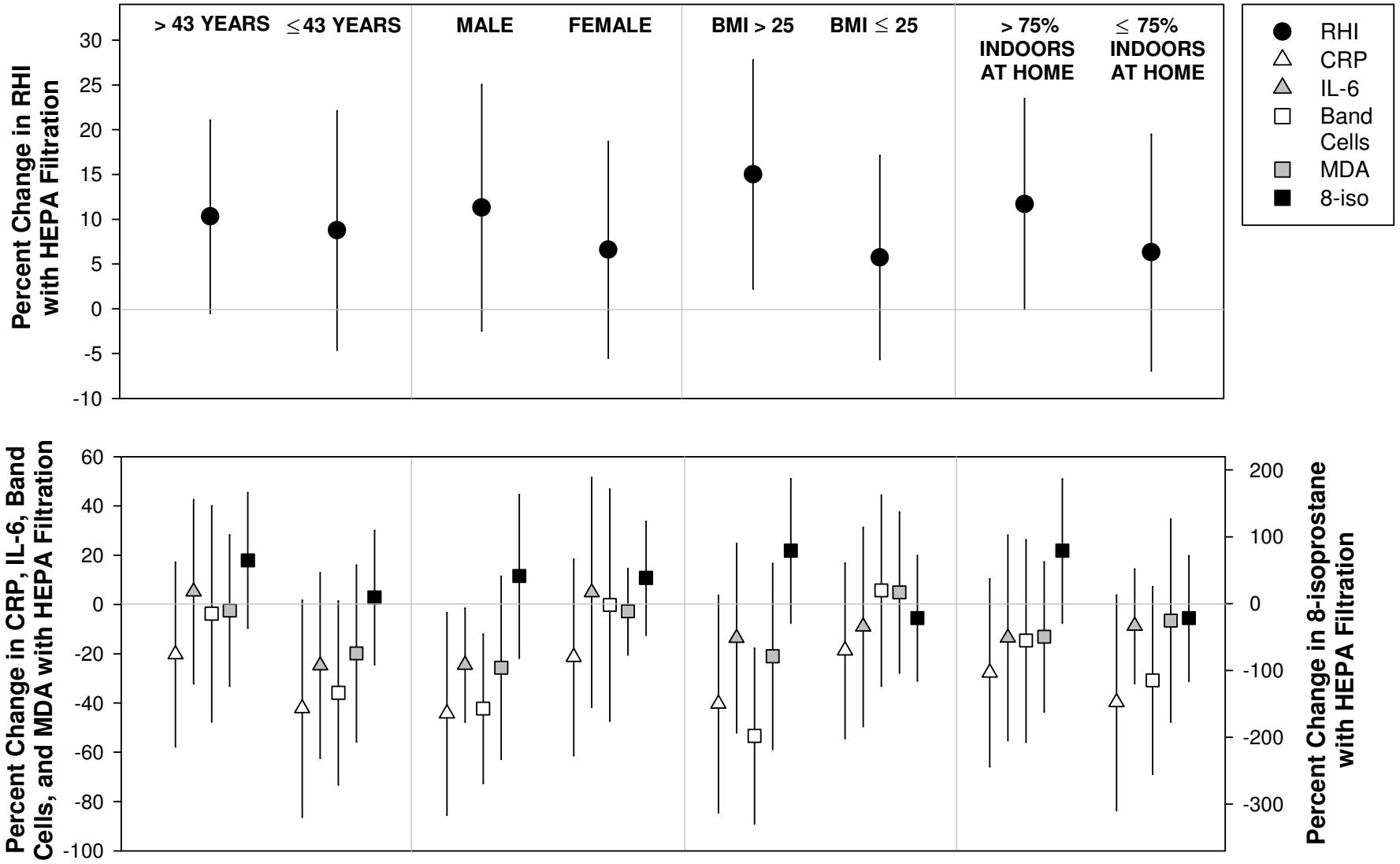
Abbreviations: RHI = reactive hyperemia index; CRP = C-reactive protein; IL-6 = interleukin-6; MDA = malondialdehyde; 8-iso = 8-iso-prostaglandin F2α.

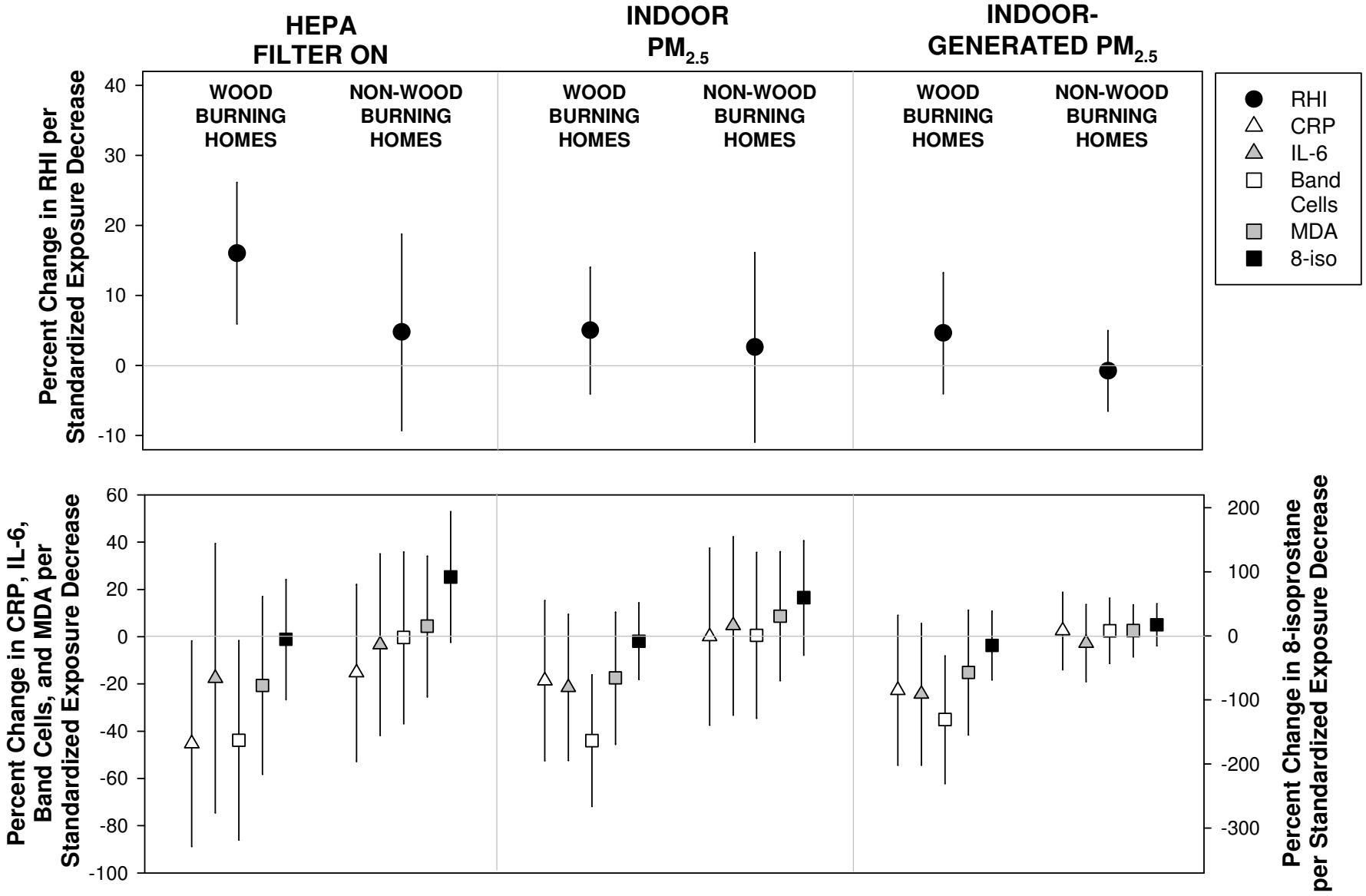
**Figure 4. Model estimates of exposure reduction effects on RHI (upper panel) and blood and urine markers (lower panel) stratified by use of a woodburning stove.**

*Exposure contrasts for continuous exposure metrics are the median within-participant change between non-HEPA and HEPA periods to allow for a comparison of effect sizes between exposure metrics with different distributions. The exposure contrasts are: Indoor $PM_{2.5}$ in woodburning homes = -7.5 $\mu g/m^3$; Indoor $PM_{2.5}$ in non-woodburning homes = -6.2 $\mu g/m^3$; Indoor-generated $PM_{2.5}$ in woodburning homes = -6.3 $\mu g/m^3$; Indoor-generated $PM_{2.5}$ in non-woodburning homes = -2.1 $ng/m^3$. Abbreviations: RHI = reactive hyperemia index; CRP = C-reactive protein; IL-6 = interleukin-6; MDA = malondialdehyde; 8-iso = 8-iso-prostaglandin F2α.


Non-Woodburning Homes (N = 12)
Woodburning Homes (N = 13)
PM Concentration (µg/m3)
Levoglucosan Concentration (ng/m3)
p = 0.13
p < 0.05
p = 0.12
p = 0.20
p = 0.40
p = 0.10
p < 0.01
p = 0.60
Indoor PM2.5
Indoor-Generated PM2.5
Infiltrated PM2.5
Indoor Levoglucosan




> 43 YEARS
≤43 YEARS
MALE
FEMALE
BMI > 25
BMI ≤ 25
> 75% INDOORS AT HOME
≤ 75% INDOORS AT HOME
RHI
CRP
IL-6
Band Cells
MDA
8-iso
Percent Change in RHI with HEPA Filtration
Percent Change in CRP, IL-6, Band Cells, and MDA with HEPA Filtration
Percent Change in 8-isoprostane with HEPA Filtration


HEPA FILTER ON
INDOOR PM2.5
INDOOR-GENERATED PM2.5
WOOD BURNING HOMES
NON-WOOD BURNING HOMES
WOOD BURNING HOMES
NON-WOOD BURNING HOMES
WOOD BURNING HOMES
NON-WOOD BURNING HOMES
Percent Change in RHI per Standardized Exposure Decrease
40
30
20
10
0
-10
RHI
CRP
IL-6
Band Cells
MDA
8-iso
Percent Change in CRP, IL-6, Band Cells, and MDA per Standardized Exposure Decrease
60
40
20
0
-20
-40
-60
-80
-100
Percent Change in 8-isoprostane per Standardized Exposure Decrease
200
100
0
-100
-200
-300

# An Air Filter Intervention Study of Endothelial Function Among Healthy Adults in a Woodsmoke-Impacted Community

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Stephan van Eeden, Sverre Vedal, Imelda Wong, and Michael Brauer



**Online Supplement**

## METHODS

Each participant's home was monitored for two consecutive seven-day periods, during which time a HEPA filter (Honeywell model 50300) 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 non-filtration was random. Because of noise produced by these HEPA filters participants were asked to operate the units on the highest comfortable setting.

### *Participant Recruitment*

We recruited participants 19 years or older by distributing letters through schools and large employers in the study area, and we excluded individuals who resided in tobacco smoking households from participating. We prioritized the inclusion of participants without morbidities (heart disease, diabetes, hypertension, metabolic syndrome, etc.) that might modify the air pollution effect on outcomes. We also gave highest priority to participants living in high woodsmoke areas and those with multiple eligible participants in the same home. 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 prior to enrolment.

### *Health Measurements*

At the end of each 7-day period a study technician measured microvascular endothelial function and collected blood and urine samples for assessment of systemic inflammation and oxidative

stress. Technician visits were scheduled in the morning before the participant had eaten breakfast or consumed caffeine, and all health measurements were made at approximately the same time of day to minimize the influence of diurnal variations on measurements. Biological sample collections and endothelial measurements for all participants were conducted by the same field technician.

Microvascular endoethelial function was measured via peripheral artery tonometry using the portable EndoPAT 2000 instrument (Itamar Medical Ltd, Cesari, Israel). This technology uses pneumatic fingertip probes to measure the change in pulse wave amplitude (PWA) before and after occlusion with a blood pressure cuff (E1, E2). Reactive hyperemia index (RHI) is determined by a computer algorithm and is based on the ratio of post-occlusion PWA to pre-occlusion PWA, normalized to changes in the control (non-occluded) arm. Room temperature at the time of RHI measurement was recorded to account for potential effects of temperature changes on within-participant differences in RHI. RHI measurements were made in a quiet room with low light (usually the participant's bedroom), and both measurements for a given participant were made in the same location under similar conditions.

Participants were asked to collect a sample from the first urination on the day of the technicians' home visits. Blood samples were collected following measurement of RHI. Blood and urine samples were processed in the local laboratory within 6 hours of sample collection and stored at -80 $^{0}$C. After completion of the sample collection phase of the study in April 2009, the blood and urine samples were transported on dry ice to the University of British Columbia in Vancouver. Serum samples were analyzed for C-reactive protein (CRP) and interleukin-6 (IL-6) by enzyme-

linked immunosorbent assays (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 PMN. Urine samples were analyzed for two markers of oxidative stress, malondialdehyde (MDA) and 8-iso-prostaglandin $F_{2\alpha}$ ("8-isoprostane"), via gas chromatography mass spectrometry (GC-MS) and ELISA, respectively. Technicians performing all laboratory analyses were blinded to the HEPA filter conditions under which samples were collected.

***Partitioning Indoor $PM_{2.5}$ into Indoor- and Outdoor-Generated Components***

We partitioned the 7-day average indoor $PM_{2.5}$ concentrations into their indoor- and outdoor-generated components by first estimating the $PM_{2.5}$ infiltration efficiency ($F_{inf}$, the fraction of the outdoor concentration that penetrates indoors and remains suspended under steady-state conditions) for each 7-day period in each home. The particle light scattering coefficient ($b_{sp}$) was measured continuously as a $PM_{2.5}$ surrogate indoors and outdoors using integrating nephelometers (Radiance Research model 903). The infiltration efficiency of outdoor $PM_{2.5}$ indoors was assessed using the continuous indoor and outdoor $b_{sp}$ data and a recursive form of the mass balance model (RM) (E3). Home specific 7-day estimates of $F_{inf}$ were used, in combination with measured concentrations of outdoor ($C_{out}$) and indoor ($C_{in}$) $PM_{2.5}$, to estimate the indoor-generated ($C_{in}^{ig}$) and infiltrated indoor concentrations ($C_{in}^{inf}$):

$$C_{in}^{inf} = F_{inf} \text{ x } C_{out} \quad (1)$$

and

$$C_{in}^{ig} = C_{in} - C_{in}^{inf} \quad (2)$$

***Statistical Methods***

Outcome variables were skewed, so we log-transformed prior to analysis (for band cells, which had 0 values, we added 0.5 prior to log-transforming). We used a mixed model approach, accounting for measurements clustered within individuals and individuals clustered within homes, to assess the impact of HEPA filtration and continuous exposure variables on the log-transformed outcomes. The mixed model for measurement *i* on participant *j* living in home *k* was:

$$\log Y_{ijk} = \alpha_j + \gamma_k + \beta_0 + \beta_1 HEPA_{ijk} + \beta_2 Gender_{jk} + \beta_3 Age_{jk} + \beta_4 BMI_{jk} + \beta_5 Temp_{ijk} + e_{ijk} \quad (3)$$

where $\alpha_j$ and $\gamma_k$ are random participant- and home-specific intercepts, respectively, $\beta_1$ represents the fixed effect of HEPA filtration on the log-transformed outcome variable, $\log Y_{ijk}$, and gender, age, body mass index (BMI), and temperature are fixed effects included to adjust for potential confounding. Because HEPA filtration only modifies PM exposure for participants inside their homes, and since this study was conducted among working-age adults, we also explored models that adjusted for the percent of time spent indoors at home during each 7-day period by each participant. In addition to modeling HEPA filtration as a binary variable, we also modeled the effect of continuous pollution concentrations in place of HEPA filter status. Effect estimates from all models are reported as a percent change in the outcome for the change in HEPA filter status or a standardized contrast (the median within-participant change in exposure between HEPA and non-HEPA conditions) in pollution concentration. The main "exposures" of interest were HEPA filtration and indoor $PM_{2.5}$. In addition, to better understand the role of pollution sources we examined the effects of indoor-generated $PM_{2.5}$, infiltrated (outdoor-generated) $PM_{2.5}$, and measured indoor levoglucosan. We explored modification of the HEPA filter effect by filtration/placebo order, gender, overweight (BMI > or < 25 kg/m$^2$), use of a woodstove, and

time spent indoors at home (> or < 75%).

***Sensitivity Analysis of Indoor $PM_{2.5}$ Averaging Times***

The averaging period for the $PM_{2.5}$ measurements (7 days) may not have matched the relevant exposure-response period for some of these outcomes (E4). To evaluate the impact of the indoor $PM_{2.5}$ averaging time, we used indoor nephelometer data to estimate average $PM_{2.5}$ concentrations over increasing durations from 12 hr to 7 days prior to the RHI measurement and collection of blood and urine samples. Because the nephelometer measures the particle light scattering coefficient ($b_{sp}$) as a surrogate for $PM_{2.5}$ concentration, we converted $b_{sp}$ to $PM_{2.5}$ using the relationship between $b_{sp}$ and 7-day average $PM_{2.5}$ concentrations inside the study homes. Consistent with previous studies (E5, E6), there was a strong correlation indoors between $b_{sp}$ and $PM_{2.5}$ ($r$ = 0.89). Among the 39 participants with complete nephelometer data at all averaging times, there was no clear pattern of $PM_{2.5}$ averaging time influencing the effect estimates for any of the outcomes (Figure E1).

## References

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E3. Allen, R., T. Larson, L. Sheppard, L. Wallace, and L. J. S. Liu. 2003. Use of real-time light scattering data to estimate the contribution of infiltrated and indoor-generated particles to indoor air. *Environmental Science & Technology* 37(16):3484-3492.
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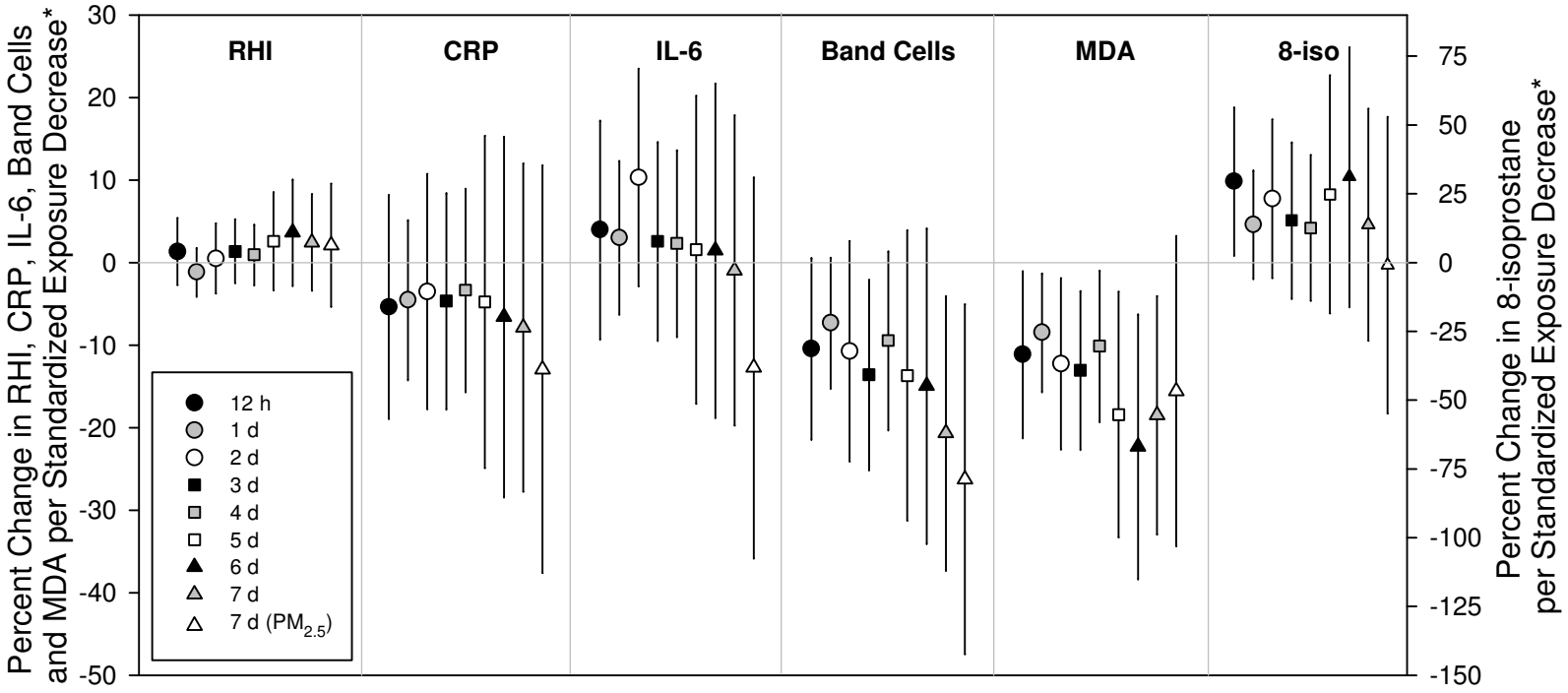
**Figure E1. Model estimates of indoor $PM_{2.5}$ effects for $PM_{2.5}$ estimated from light scattering and averaged over different periods prior to the health measurements for 39 participants in 22 homes. The modeled effect of the 7-day average indoor $PM_{2.5}$ concentration measured gravimetrically is shown for comparison.**

**Table E1. Spearman's correlation coefficients between exposure indicators at 25 homes during periods with HEPA filters off (below the diagonal) and on (above the diagonal).**

| | Outdoor $PM_{2.5}$ | Indoor $PM_{2.5}$ | $F_{inf}$ | Outdoor-Generated $PM_{2.5}$ | Indoor-Generated $PM_{2.5}$ | Outdoor Levoglucosan | Indoor Levoglucosan |
|---|---|---|---|---|---|---|---|
| Outdoor $PM_{2.5}$ | ----- | -0.11 | -0.51*** | -0.02 | -0.10 | 0.93*** | 0.31 |
| Indoor $PM_{2.5}$ | 0.07 | ----- | -0.14 | -0.20 | 0.91*** | -0.07 | 0.14 |
| $F_{inf}$ | -0.05 | -0.14 | ----- | 0.83*** | -0.41** | -0.60*** | 0.10 |
| Outdoor-Generated $PM_{2.5}$ | 0.61*** | -0.10 | 0.74*** | ----- | -0.49** | -0.18 | 0.28 |
| Indoor-Generated $PM_{2.5}$ | -0.18 | 0.89*** | -0.38* | -0.43** | ----- | -0.01 | 0.04 |
| Outdoor Levoglucosan | 0.82*** | 0.20 | -0.07 | 0.45** | 0.00 | ----- | 0.31 |
| Indoor Levoglucosan | 0.47** | 0.53*** | -0.19 | 0.19 | 0.38* | 0.55*** | ----- |

* p<0.10
** p<0.05
*** p<0.01




*Exposure contrasts are the median within-participant change between non-HEPA and HEPA periods to allow for a comparison of effect sizes between exposure averaging times with different distributions. The exposure contrasts are: 12 hour = -2.8 µg/m$^3$; 1 day = -2.6 µg/m$^3$; 2 day = -3.3 µg/m$^3$; 3 day = -3.6 µg/m$^3$; 4 day = -3.0 µg/m$^3$; 5 day = -4.4 µg/m$^3$; 6 day = -4.5 µg/m$^3$; 7 day = -4.2 µg/m$^3$; 7 day $PM_{2.5}$ = -6.4 µg/m$^3$. Abbreviations: RHI = reactive hyperemia index; CRP = C-reactive protein; IL-6 = interleukin-6; MDA = malondialdehyde; 8-iso = 8-iso-prostaglandin F2α.

**Note: Reviewer comments are listed individually (C1, C2, etc.) and are followed by our responses (R1, R2, etc.) in bold. The location of the change in the revised manuscript, where applicable, is provided in parentheses at the end of each response. Please note: the reference numbers quoted in our responses are sequential in this document and do not match those in the manuscript.**

*Editor*

C1: The reviewers raised a concern regarding the novelty of your study with respect to previously published work, specifically that described in reference 8. As the Journal publishes work that represents a major advance in the field, it will be important to address this concern in your resubmission.

**R1: This work represents an important contribution for several reasons. First, there is limited epidemiological evidence of air pollution effects on vascular/endothelial function. The recent American Heart Association's Scientific Statement on PM and Cardiovascular Disease characterized the strength of the evidence as "moderate" (1). Second, our focus on a population residing in a woodsmoke-impacted community is novel. Interestingly, when Brauner et al. (2) evaluated elements in the $PM_{2.5}$ samples they found that only potassium, which is present in relatively high concentrations in biomass smoke (3), was independently associated with RHI. In spite of this, all of the previous observational (2, 4-7) and experimental (8, 9) research on human endothelial/vascular function has focused on the typical urban pollution mixture and/or vehicle-generated pollution. Thus, our study of a woodsmoke-dominated airshed makes a valuable contribution, consistent with the recommendations in a recent review paper to "undertake studies among populations exposed primarily to woodsmoke particles" and to "conduct studies focused on cardiovascular...effects to compare with risks from fossil fuel-derived ambient particles..." (3). Third, much of the previous research on air pollution and endothelial function (including Brauner et al.) has focused on groups thought to be susceptible, including older adults (2) and diabetics (4, 5, 7). Our focus on healthy, young adults is meaningful because, as pointed out by Reviewer 2 (in comment C25) based on a controlled exposure study by Brauner et al. (10), "younger individuals could be less susceptible."**

**Thus, we believe that this paper makes an important, novel contribution for several reasons and although we used a similar study design to Brauner et al. (2), we focused on a very different population exposed to a very different air pollution mixture.**

C2: Finally, while this study represents an interventional trial, it does not appear to have been registered in a public database. Please explain the rationale for this omission in your revised manuscript.

**R2: This study is registered at ClinicalTrials.gov. (Page 3, Line 16)**

C3: In your revision, please ensure that your references include the most current articles and information. In compliance with the Journal's policy on Prior Publication, you need to cite any abstracts (related to the research contained in the manuscript) in the last sentence of the Introduction section and also include those citations in the list of References. The recommended format is: "Some of the results of these studies have been previously reported in the form of an abstract(s)(References)." In addition, the abstract(s) must be listed in the References section of the manuscript.

**R3: The last sentence of the Introduction now reads: "Some of the results of this study have been previously reported in the form of an abstract (11)". (Page 3, Line 15)**

*Reviewer 1*

C4: The last sentence of the conclusions (abstract and main text) should be reworded, i.e.: 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.

**R4: Done. (Page 1, Line 23 & Page 14, Line 23)**

C5: PM concentrations were obviously derived from 7-day cumulative mass measurements, using Harvard impactors. Therefore, the outcome-specific most relevant time-period of exposure (in most cases shorter than 7 days as the authors explain in the discussion) might suffer from relevant exposure misclassification, most likely biasing the effect of the 7-day cumulative exposure metric towards the null. Please discuss the (partial mis)matching of the used exposure metric with the outcome-specific relevant exposure periods.

**R5: In response to this comment (and comments C23 and C24) we have added the following discussion: "...the averaging period for the $PM_{2.5}$ measurements (7 days) may not have matched the relevant exposure-response period for some of these outcomes (12), although continuous indoor measurements did not reveal a clear influence of averaging times on the $PM_{2.5}$ associations (Table E1). Repeated measurements of outcomes during the 7-day monitoring periods, which would have allowed us to evaluate the time course of the biological responses, were not possible in this study." (Page 10 Line 23 through Page 11 Line 3)**

C6: What are the HEPA filtration efficiencies for different particle sizes? High efficiency at smaller sizes could also lead to the stronger results observed for HEPA filtration in contrast to PM2.5 mass concentration effects, if especially the ultrafine particles are responsible for vascular or inflammatory effects, as some studies suggest.

**R6: We agree with this comment, and in response we have added the following text: "...the observed HEPA effects may be due to specific components of the PM mixture, such as ultrafine (< 100 nm diameter) particles. HEPA filters are thought to effectively remove particles in the ultrafine range (10-100 nm) (13), and ultrafine**

**particles may play an important role in the inflammatory and endothelial effects of PM (1, 14, 15)." (Page 10, Lines 19 - 22)**

C7: It is not clear whether 8-Isoprostane was normalized to mg of urinary creatinine. Please clarify. ELISA measurements of 8-Isoprostanes are less reliable than the gold standard gas chromatography–mass spectrometry – this might be another possible reason for lack of findings in this outcome (including the large CIs).

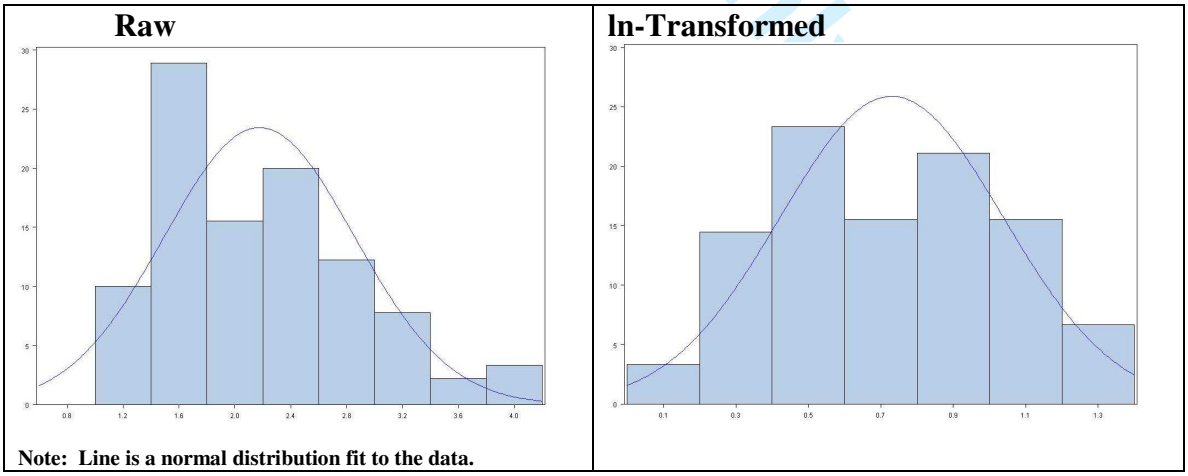
**R7: 8-isoprostane was not normalized to urinary creatinine: "Urine samples were analyzed for MDA and 8-isoprostane (not normalized to creatinine) via gas chromatography mass spectrometry and ELISA, respectively." ()**

**In addition, we have added these as potential limitations of the 8-isoprostane analysis: "...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 GC-MS (16, 17)." (Page 13, Lines 11 - 13)**

C8: I have a problem with the log transformation of the main outcome reactive hyperemia index. (1) the log transformation does not really seem to be necessary from looking at the descriptive table. In one subgroup of measurements the distribution is slightly skewed to the right, in the other to the left. How did the residuals look like? Is the assumption of normality of residuals met?

**R8: We chose to log-transform RHI for three reasons:**

**1. As indicated in the following figures the log-transformed data were more normally distributed than the raw data. (Note: the data distribution cannot be accurately determined from "from looking at the descriptive table" because the table divided the data into HEPA and non-HEPA periods, while the mixed models incorporate all data.) The assumption of normal residuals is met.**



**2. To be consistent with the two previous air pollution studies that used RHI as an outcome (2, 10). Both of these studies used log-transformed RHI as the outcome and reported effects as % changes.**

**3. Because the other outcomes in this analysis were also log-normally distributed, all variables were transformed to provide consistency in the way that results for different outcomes were reported and to ease interpretation.**

**4. To be consistent with a previous study that evaluated RHI in relation to cardiovascular events. Rubinshtein et al. (18) found that ln(RHI) independently predicted adverse cardiovascular events, suggesting that this measure does have relevance to the underlying biology and clinical outcomes.**

C9: (2) log transformation in linear mixed models assumes an exponential relationship between the exposure and the original not-transformed outcome variable. It is questionable, whether this is biologically reasonable.

**R9: Rubinshtein et al. (18) found that ln(RHI) independently predicted adverse cardiovascular events, suggesting that this measure does have relevance to the underlying biology and clinical outcomes.**

C10: (3) the log transformation results in a percent change per exposure unit. The original outcome however is already a ratio, so the estimate here is a percent change of a relative measure. Next to overinflation of the actual effect size, this estimate is not easily comprehensible and as already pointed out above, probably neither statistically necessary nor biologically plausible. So at the very least I recommend to analyse the main outcome untransformed in a sensitivity analysis.

**R10: In addition to the paired t-tests already presented in Table 3, in which there was a significant difference in mean RHI (on the original scale) between HEPA and non-HEPA periods, we have added a more formal analysis of the untransformed RHI data using fully adjusted mixed models. As expected, this analysis leads to the same conclusion as the analysis of ln-transformed data. The changes are as follows:**

**"As a sensitivity analysis we also modeled RHI without log-transforming." (Page 5, Lines 12 - 13)**

**"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)." (Page 7, Lines 16 - 18)**

C11: Similarly, band cells are given as a percentage of PMNs and transformed as ln(% of bands+0.5), so the back-transformed estimate is actually given as a percent change of the percentage of bands+0.5. How sensitive are the results to using a different summand (i.e.%bands+1, %bands+0.1)?

**R11: Since band cells were the outcome most clearly associated with the woodsmoke tracer levoglucosan, we ran additional levoglucosan models using summands of 0.1 and 1. The results are described as follows: "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 prior to 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." (Page 7 Line 22 through Page 8 Line 3)**

C12: Blood pressure as one important and possibly sensitive marker of vascular reactivity and sympathetic activation was obviously also measured. Was BP influenced by HEPA filtration, as some other intervention studies on reduction of home indoor biomass smoke indicate?

**R12: As indicated by the crude paired t-tests in Table 3 (which do not account for clustering or adjust for confounders), both systolic and diastolic BP were nearly identical during HEPA and non-HEPA periods. Similar results were observed in adjusted mixed models (not reported).**

C13: What are the proposed reasons for not observing an effect with particles of outdoor origin? Please discuss this important and surprising finding.

**R13: We have added the following possible explanation for this finding: "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)." (Page 10, Lines 11 - 14)**

C14: Abstract, line 18: … endothelial function and level of systemic inflammation…

**R14: Done. (Page 1, Line 17)**

C15: Introduction, page 2, line 11: please add Diez Roux et al. AJE 2008 and Hoffmann et al. Circulation 2007.

**R15: Done. (Page 2, Line 10)**

C16: Methods: When was the study conducted (year, months)? Please add in the main text that the measurements were conducted at the participants' homes.

**R16: In the originally submitted manuscript the first sentence of the Methods section stated: "This study was conducted from November, 2008 to April, 2009 in Smithers, British Columbia, Canada..." (Page 3, Lines 19 - 20)**

**We have modified the first sentence under "Health Measurements" to read: "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." (Page 4, Lines 12 - 13)**

C17: Statistical Methods, line 10 and 11: not all-inclusive categories (also in the figure), please correct.

**R17: We have re-written this sentence as follows: "We explored effect modification by filtration/placebo order, age (> or ≤ 43 years, the median age), gender, overweight (BMI > or ≤ 25 kg/m$^2$), time spent indoors at home (> or ≤ 75%), and use of a woodstove." (Page 5, Lines 15 - 17 and Figure 3)**

C18: Data reduction, line 15: Finf is used here for the first time, please explain

**R18: We have added the following text in the Exposure Assessment section: "…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 continuous indoor and outdoor measurements made with nephelometers (Radiance Research, Seattle, WA) (19)." (Page 5, Lines 1 – 5)**

C19: Model results, line 7: …HEPA filtration and air pollution ….

**R19: Done. (Page 17, Line 18)**

C20: Discussion, page 8 lines 21-22: The cited studies provide only very weak evidence for effects on endothelial function in diabetic patients.

**R20: We agree that the evidence is limited, but we still feel it is important to acknowledge the indication from 3 previous studies that diabetics may be more susceptible to the endothelial effects of air pollution. In response to the comment we have modified the language as follows: "In addition, although limited evidence suggests that diabetics may be more susceptible to endothelial dysfunction related to air pollution (4, 5, 7), our results provide additional evidence of endothelial effects among healthy individuals (6, 8, 9)." (Page 9, Lines 19 - 21)**

C21: Table 3 could be moved to the online supplement.

**R21: Done. (This is now Table E1, Online Supplement)**

*Reviewer 2*

C22: The title of the paper might be somewhat misleading considering that there is no evidence that it was the reduction of wood smoke particles, which was responsible for an increased microvascular function index. Thus, there was no association with levoglucosan as noted by the authors. Moreover, the RHI used cannot distinguish effects on endothelium dependent or independent vasodilatation and endothelial dysfunction should not be used in the title.

**R22: We have changed the title to "An Air Filter Intervention Study of Endothelial Function Among Healthy Adults in a Woodsmoke-Impacted Community."**

**We agree that our results do not clearly identify woodsmoke as the relevant exposure, and we have therefore changed "…Woodsmoke-Exposed" to "…in a Woodsmoke-Impacted Community." This change acknowledges the reviewer's concern while still identifying the dominant air pollution source in this airshed and one important aspect of our study that differs from previous research that has focused almost entirely on urban areas (with large impacts from mobile sources, industrial emissions, etc.). We would also like to point out that it is not entirely accurate to state that "there was no association with levoglucosan," because levoglucosan was associated with band cells.**

**While we agree that RHI does not definitively identify endothelium-dependent dilation, there is evidence suggesting that the vascular changes indicated by RHI are due, at least partially, to endothelial effects. We have enhanced discussion of this topic as follows: "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 (20). Moreover, Bonetti and colleagues (21) have reported a relationship between RHI and coronary artery endothelial function, while Kuvin et al. (22) 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 (23)." (Page 13, Lines 17 - 24)**

C23: Fig.2 suggest that the RHI effect was only significantly related to air filtration or not, whereas there was no significant association, i.e. dose-response relationship, with the indoor PM2.5 level which should represent the best estimate of actual exposure (at least for the time that air filtration is relevant). This surprising finding should be thoroughly discussed, as it otherwise cast doubt on causality. It appears that the PM2.5 level is a 7 day average and possibly only a certain time window/lag structure is relevant. Perhaps the authors could use the continuous measurements made for infiltration rate although that was done by nephelometer principle highly susceptible to humidity to assess possibly lagged effects.

**R23: In response to this comment (and comments C5, C6, and C24) we have added a new paragraph in the Discussion:**

**"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 thought to effectively remove particles in the ultrafine range (10-100 nm) (13), and ultrafine particles may play an important role in the inflammatory and endothelial effects of PM (1, 14, 15). Finally, the averaging period for the $PM_{2.5}$ measurements (7 days) may not have matched the relevant exposure-response period for some of these outcomes (12), 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-day monitoring periods, which would have allowed us to evaluate the time course of the biological responses, were not feasible in this study." (Page 10 Line 16 through Page 11 Line 3)**

**In addition, we have added Figure E1 and the following text to the online supplement: "The averaging period for the $PM_{2.5}$ measurements (7 days) may not have matched the relevant exposure-response period for some of these outcomes (12). To evaluate the impact of the indoor $PM_{2.5}$ averaging time, we used indoor nephelometer data to estimate average $PM_{2.5}$ concentrations over increasing durations from 12 hr to 7 days prior to the RHI measurement and collection of blood and urine samples. Because the nephelometer measures the particle light scattering coefficient ($b_{sp}$) as a surrogate for $PM_{2.5}$ concentration, we converted $b_{sp}$ to $PM_{2.5}$ using the relationship between $b_{sp}$ and 7-day average $PM_{2.5}$ concentrations inside the study homes. Consistent with previous studies (24, 25), there was a strong correlation indoors between $b_{sp}$ and $PM_{2.5}$ ($r$ = 0.89). Among the 39 participants with complete nephelometer data at all averaging times, there was no clear pattern of $PM_{2.5}$ averaging time influencing the effect estimates for any of the outcomes (Figure E1). (Online supplement)**

**Finally, in response to the comment about humidity, we would like to point out that at the low RH inside these homes (~35%), the relationship between light scattering and $PM_{2.5}$ is linear and highly correlated.**

C24: Given the 7 days intervention it would have improved the study if the RHI index had been determined during this period in order to follow the time course.

**R24: We have added the following text: "Repeated measurements of outcomes during the 7-day monitoring periods, which would have allowed us to evaluate the time course of the biological responses, were not feasible in this study." (Page 11, Lines 1 - 3)**

**Please also see response R23.**

C25: The authors report study of effect modification by intervention order, gender, BMI, wood stove use and percent time spent indoors. However, they have omitted the very interesting potential effect modification by age which could be done by stratification or as a continuous variable and an interaction term in the present study. The earlier intervention study by Brauner et al. (ref. 8) focused on elderly subjects and that group suggested in their other paper on no effect of traffic emission on microvascular function that younger individuals could be less susceptible.

**R25: We have added a stratification by the median age (43 years) to Figure 3. To accommodate this change we have removed HEPA filter order from Figure 3 and now report the results of stratifying by HEPA order (no consistent pattern) in the text only. (Figure 3)**

**In addition, since there is a general pattern of more pronounced effects among younger participants, we have added some additional discussion of age as an effect modifier: "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 (2), 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 yrs) adults (10). Gender 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, 9, 26, 27), and one study suggesting that the inflammatory effects of chronic PM exposure are more pronounced in men (28)." (Page 12, Lines 13 - 21)**

C26: The authors should spent a few more words discussing the possible mechanisms of particle effects on microvascular function, e.g. how inflammation could impair endothelial-dependent dilatation through reductions in the bioavailability of the vasodilator nitric oxide (p. 9 l 3-4). Part of this me could also have been addressed by administration of an endothelium independent nitric oxide donor such as nitroglycerin.

**R26: We have enhanced the discussion of mechanisms as follows: "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 (29, 30). ROS can be produced directly by the redox potential of the particles or through the activation of inflammatory cells (31). Inflammation may also play a role in reducing NO synthesis. For example, both CRP (32) and IL-6 (33) have been shown to decrease**

**expression of NO synthase in human aortic endothelial cells." (Page 9 Line 23 through Page 10 Line 5)**

**In response to the comment about nitroglycerin, we have added the following: "Administration of sublingual nitroglycerin, which would have allowed us to assess endothelium-independent effects on the RHI response (21), was not feasible in this residence-based study." (Page 13 Line 24 through Page 14 Line 2)**

C27: On p. 11 l. 17-20, the authors refer to their methodology for assessment of microvascular function as not implemented in the clinic. Whereas that is true, they could also refer to a recent publication that this measure actually predicts important cardiovascular outcomes in prospective setting. (Rubinshtein et al. Eur Heart J. 2010 May;31(9):1142-8).

**R27: We have added a reference to this paper in the discussion section: "First, our measure of microvascular endothelial function, RHI, has not been widely used for research or clinical purposes, but this measure is predictive of adverse cardiovascular events (18)." (Page 13, Lines 15 - 17)**

C28: On p. 11 l. 7-14 the authors discuss the use of biomarkers of oxidative stress and air pollution stating inconsistencies due to the measurements or dietary influences. However, a very recent review discuss these association systematically and find overall consistency between exposure and biomarker responses (Moller & Loft, Environ Health Perspect. 2010 Aug;118(8):1126-36).

**R28: While we generally agree with this comment, it is worth noting that the Moller & Loft review identified 4 papers that examined air pollution and lipid peroxidation products in urine, and 3 of the 4 reported confidence intervals that included the null.**

**Nevertheless, we may have overstated the limitations in the oxidative stress literature, and in response to this comment (and comment C7) we have added a reference to the Moller & Loft and revised this paragraph as follows: "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 (2, 16, 34-36). The experimental work of Barregard and colleagues (34) provides the only published evidence of an association between biomass smoke and systemic oxidative stress, while some experimental and observational studies of the urban pollution mixture have reported associations with oxidative stress markers among young adults and children (35, 37-40). The lack of observed effects in our study may have been due to other factors such as diet (41). 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 GC-MS (16, 17)." (Page 13, Lines 5 - 13)**

C29: Has this study been registered as a randomized clinical trial in an international database?

**R29: This study is registered at ClinicalTrials.gov. (Page 3, Line 16)**

*Reviewer 3*

C30: The authors state that "the study was conducted from November 2008 to April 2009." This time frame overlaps with both the winter and spring seasons in many parts of the northern hemisphere. The authors would perhaps want to indicate that the whole study period is winter in Smithers, BC since IL-6 may be subject to seasonal variation, and interaction between the effect of intervention on IL-6 and season may then be important.

**R30: We have addressed this comment by adding data on outdoor temperatures to indicate that the entire study was conducted during relatively cold periods: "Outdoor temperatures during 2-week home monitoring sessions ranged between -10.7 and 4.9 $^{0}$C, and outdoor temperatures were similar during HEPA and non-HEPA periods (Table 2)." (Page 6, Lines 11 - 12 & Table 2)**

C31: Since PM2.5 exposure was monitored using area sampling, and concentrations could vary substantially between micro-environments (resulting in exposure misclassification), it is important that the authors describe the location of the samplers – for example relative to sources of particulate matter and the HEPA filters, and the time spent at the location of the sampler by the subjects relative to other rooms, if these data are available.

**R31: We have added a sentence describing the location of the indoor sampling equipment: "Indoor pollution sampling equipment was placed in the home's main activity room." (Page 4, Lines 8 - 9)**

**Although information on participants' locations inside the home would have been useful, we did not obtain this level of detail due primarily to concerns about participant burden.**

C32: The authors should give a range or the confidence limits for the percentage reduction efficiencies of the HEPA filters. This will give the reader a better picture of the consistency associated with this important measure.

**R32: We have added the following sentences: "HEPA filters reduced indoor PM2.5 concentrations in 24 of 25 homes, and concentration changes during HEPA filtration in individual homes ranged between -15.7 and 2.2 µg/m3." (Page 6, Lines 15 - 17)**

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# An Air Filter Intervention Study of Endothelial Function Among Healthy Adults in a Woodsmoke-Impacted Community

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Revised and resubmitted to
American Journal of Respiratory and Critical Care Medicine

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**Sources of support:** Funding for this work was provided by the British Columbia Lung Association, the British Columbia Ministry of Environment, and Health Canada.

**Running head:** Woodsmoke air pollution and endothelial dysfunction

**Descriptor number:** 6.01, Air Pollution Epidemiology

**Word count:** 3,745

**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.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org

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## ABSTRACT

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

**Objectives:** To assess the impact of an intervention on particle exposures and endothelial function among healthy adults in a woodsmoke-impacted community. In addition, we investigated the underlying role of oxidative stress and inflammation in relation to reductions in particle exposures.

**Methods:** Portable air filters were used in a randomized crossover intervention study of 45 healthy adult participants exposed to consecutive 7-day periods of filtered and non-filtered 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 over 60%. Filtration was associated with a 9.4% (95% CI: 0.9 – 18%) increase in reactive hyperemia index and a 32.6% (4.4 – 60.9%) decrease in C-reactive protein. Lower indoor concentrations of 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 those residing in 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 a reduction of indoor particle concentrations.


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**Keywords:** Air pollution, particulate matter, HEPA filter, cardiovascular, intervention.

## INTRODUCTION

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 thought 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

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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 $PM_{2.5}$ exposure in a woodsmoke-impacted airshed. Our main objectives were to better understand the mechanisms underlying air pollution-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 since 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; interleukin-6, 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). This study is registered at ClinicalTrials.gov.

## 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 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

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written informed consent was obtained from all participants prior to enrolment. More details on the methods are available in the Online Supplement.

Each participant's home was monitored for two consecutive seven-day periods, during which time a HEPA filter (Honeywell model 50300) 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 non-filtration 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 endoethelial 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 enzyme-linked immunosorbent assays (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 (PMN). Urine samples were analyzed for MDA and 8-isoprostane (not normalized to creatinine) via gas chromatography mass spectrometry and ELISA, respectively.

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***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 (RH) 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 prior to analysis (for band cells, which had 0 values, we added 0.5 prior to 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 gender, age, body mass index (BMI), and temperature. We explored effect modification by filtration/placebo order, age (> or ≤ 43 years, the median age), gender, overweight (BMI > or ≤ 25 kg/m$^2$), time spent indoors at home (> or ≤ 75%), and use of a woodstove.

***Data Reduction***

We enrolled a total of 56 participants from 31 homes. Prior to analysis, we excluded 8 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, prior to analysis we removed 1 pregnant participant, 1 participant with Raynaud's syndrome, and 1 participant who reported being highly exposed to ETS the night

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## 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 gender 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 woodstove. 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 $^{0}$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's $r \geq 0.82$, Table E1) indicated a major contribution of woodsmoke to outdoor $PM_{2.5}$ concentrations. Lower levoglucosan/$PM_{2.5}$ ratios (mean ≤ 1%, Table 2) and correlations ($r \leq$ 0.53, Table E1) indoors indicated a smaller $PM_{2.5}$ contribution from woodsmoke to indoor

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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/m$^3$, -6.3 µg/m$^3$, and -44 ng/m$^3$ in woodburning homes; while in non-woodburning homes the median reductions were -6.2 µg/m$^3$, -2.1 µg/m$^3$, and -58 ng/m$^3$, 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 malondialdehyde 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 (Spearman's *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

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standardized reduction in levoglucosan. Band cell effect estimates were moderately sensitive to the summand used prior to 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 gender. 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 years), gender, overweight status (BMI ≥ 25 or ≤ 25), percent of time spent indoors at home (> 75% or ≤ 75%), and woodstove use (Figures 3 and 4). Though 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 (Figure 3). There was also a general pattern across endpoints of more pronounced effects among 23 subjects living in homes with woodburning stoves (Figure 4). The order of HEPA filtration did not modify the HEPA effect consistently across endpoints.

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## 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 et al. (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 yrs) 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 et al. (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 diabetics may be more susceptible to endothelial dysfunction related to air pollution , 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.

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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, though the results 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 PMN, 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 thought 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 days) may not have matched the relevant

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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-day 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 240-280 $\mu g/m^3$ 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 et al. (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 – 2,000 $\mu g/m^3$ 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 et al. (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

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sensitive to biomass smoke exposure.

There was limited evidence of more pronounced effects among participants residing in woodburning homes, males, and participants with BMI > 25 kg/m$^2$. For the systemic inflammation markers, there was also some indication of more pronounced effects among younger participants. The findings in woodburning 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 woodburning homes, which is supported by the observation that during HEPA filtration woodburning 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 yrs) adults (47). Gender 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, 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 et al. reported greater effects of short-term $PM_{2.5}$ on flow-mediated dilatation among persons with type 2





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diabetes and among those with BMI > 30 kg/m$^2$. Similarly, Dubowsky et al. (3) reported stronger associations between 5-day $PM_{2.5}$ concentrations and both CRP and IL-6 among older adults with BMI > 30 kg/m$^2$, while Chen and Schwartz 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. The experimental work of Barregard and colleagues (27) provides the only published evidence of an association between biomass smoke and systemic oxidative stress, while 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 GC-MS (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, while Kuvin et al. (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,

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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

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**ACKNOWLEDGMENTS**

We thank the study participants and our field technicians, Thurza Aspinall and Amelia Mattson. The BC Ministry of Environment and the Bulkley Valley District Hospital generously provided laboratory access. We are also grateful to Dr. Winnie Chu and the UBC School of Environmental Health Laboratory staff for their assistance with sample preparation and analysis.

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**Table 1. Study population characteristics for 45 participants with complete data.**

| Variable | Mean ± SD or Number (%) |
|---|---|
| Age (years) | 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%) |

**Table 2. Summary statistics (mean ± SD) for exposure variables by HEPA status at 25 homes with complete data.**

| Variable | HEPA Off | | HEPA On | | Paired t-test p-value |
|---|---|---|---|---|---|
| | Mean ± SD | Median | Mean ± SD | Median | |
| 7-day Avg. Outdoor Temperature ($^0$C) | -2.5 ± 4.6 | -2.3 | -3.6 ± 6.1 | -1.7 | 0.32 |
| 7-day Avg. Indoor Temperature ($^0$C)* | 19.7 ± 1.4 | 19.4 | 19.8 ± 1.7 | 19.4 | 0.75 |
| 7-day Avg. Indoor Relative Humidity (%)* | 35.1 ± 3.3 | 36.0 | 35.3 ± 3.4 | 33.7 | 0.90 |
| $PM_{2.5}$ Outdoors (ug/m$^3$) | 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 (ug/m$^3$) | 11.2 ± 6.1 | 10.5 | 4.6 ± 2.6 | 3.9 | <0.01 |
| $PM_{2.5}$ Outdoor-Generated (ug/m$^3$) | 3.5 ± 2.3 | 3.6 | 1.5 ± 0.9 | 1.4 | <0.01 |
| $PM_{2.5}$ Indoor-Generated (ug/m$^3$) | 7.6 ± 6.6 | 6.3 | 3.0 ± 2.8 | 2.1 | <0.01 |
| Levoglucosan Outdoors (ng/m$^3$)† | 613 ± 548 | 415 | 530 ± 358 | 471 | 0.18 |
| Levoglucosan Indoors (ng/m$^3$) | 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 |

*From 13 homes with indoor HOBO data loggers.

† Excluding one highly influential outdoor levoglucosan observation.

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**Table 3. Spearman's correlation coefficients between exposure indicators at 25 homes during periods with HEPA filters off (below the diagonal) and on (above the diagonal).** ¶

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**Table 3. Summary statistics for time-activity patterns and health measurements by HEPA status among 45 participants.**

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| Variable | HEPA Off | | HEPA On | | Paired t-test p-value |
|---|---|---|---|---|---|
| | Mean ± SD | Median | Mean ± SD | Median | |
| Room Temperature During RHI Measurement ($^0$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 (mmHg)* | 112.4 ± 10.8 | 113 | 112.2 ± 11.5 | 112 | 0.88 |
| Diastolic Blood Pressure (mmHg)* | 68.6 ± 7.6 | 68 | 68.4 ± 8.2 | 67 | 0.80 |
| Reactive Hyperemia Index | 2.06 ± 0.63 | 1.93 | 2.28 ± 0.72 | 2.32 | 0.03 |
| C-reactive Protein (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 (uM) | 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 |

*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.

**Figure 1. Distributions of indoor $PM_{2.5}$ and levoglucosan concentrations by use of a wood-burning stove during periods without HEPA filtration (upper plot) and with HEPA filtration (lower plot). P-values are for 2-sample t-tests comparing woodburning and non-woodburning homes.**

Note: outliers not shown. Lines in the boxes are the median concentrations.

**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-HEPA and HEPA periods to allow for a comparison of effect sizes between exposure metrics with different distributions. The exposure contrasts are: Indoor $PM_{2.5}$ = -6.6 µg/m$^3$; Indoor-Generated $PM_{2.5}$ = -4.4 µg/m$^3$; Outdoor-Generated $PM_{2.5}$ = -1.3 µg/m$^3$; Indoor Levoglucosan = -57.6 ng/m$^3$. Abbreviations: RHI = reactive hyperemia index; CRP = C-reactive protein; IL-6 = interleukin-6; MDA = malondialdehyde; 8-iso = 8-iso-prostaglandin F2α.

**Figure 3. Model estimates of HEPA filter effects on RHI (upper panel) and blood and urine markers (lower panel) stratified by age, gender, body mass index (BMI), and time spent indoors at home.**

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Abbreviations: RHI = reactive hyperemia index; CRP = C-reactive protein; IL-6 = interleukin-6; MDA = malondialdehyde; 8-iso = 8-iso-prostaglandin F2α.

**Figure 4. Model estimates of exposure reduction effects on RHI (upper panel) and blood and urine markers (lower panel) stratified by use of a woodburning stove.**

*Exposure contrasts for continuous exposure metrics are the median within-participant change between non-HEPA and HEPA periods to allow for a comparison of effect sizes between exposure metrics with different distributions. The exposure contrasts are: Indoor $PM_{2.5}$ in woodburning homes = -7.5 µg/m$^3$; Indoor $PM_{2.5}$ in non-woodburning homes = -6.2 µg/m$^3$; Indoor-generated $PM_{2.5}$ in woodburning homes = -6.3 µg/m$^3$; Indoor-generated $PM_{2.5}$ in non-woodburning homes = -2.1 ng/m$^3$. Abbreviations: RHI = reactive hyperemia index; CRP = C-reactive protein; IL-6 = interleukin-6; MDA = malondialdehyde; 8-iso = 8-iso-prostaglandin F2α.

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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 (8, 22, 40, 4110)

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**Table 3. Spearman's correlation coefficients between exposure indicators at 25 homes during periods with HEPA filters off (below the diagonal) and on (above the diagonal).**

| | Outdoor $PM_{2.5}$ | Indoor $PM_{2.5}$ | $F_{inf}$ | Outdoor-Generated $PM_{2.5}$ | Indoor-Generated $PM_{2.5}$ | Outdoor Levoglucosan | Indoor Levoglucosan |
|---|---|---|---|---|---|---|---|
| Outdoor $PM_{2.5}$ | ----- | -0.11 | -0.51*** | -0.02 | -0.10 | 0.93*** | 0.31 |
| Indoor $PM_{2.5}$ | 0.07 | ----- | -0.14 | -0.20 | 0.91*** | -0.07 | 0.14 |
| $F_{inf}$ | -0.05 | -0.14 | ----- | 0.83*** | -0.41** | -0.60*** | 0.10 |
| Outdoor-Generated $PM_{2.5}$ | 0.61*** | -0.10 | 0.74*** | ----- | -0.49** | -0.18 | 0.28 |
| Indoor-Generated $PM_{2.5}$ | -0.18 | 0.89*** | -0.38* | -0.43** | ----- | -0.01 | 0.04 |
| Outdoor Levoglucosan | 0.82*** | 0.20 | -0.07 | 0.45** | 0.00 | ----- | 0.31 |
| Indoor Levoglucosan | 0.47** | 0.53*** | -0.19 | 0.19 | 0.38* | 0.55*** | ----- |

* p<0.10
** p<0.05
*** p<0.01

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**Table 4.**