Supplementary Online Content

1. Research protocol and statistical analyses

2. Change in secondary outcomes (HRV parameters) by filtration type over the course of each 3-day intervention
1. Research Protocol

On Monday during study week 1, the study team placed blinded randomized portable air filter systems (HE-filtration, LE-filtration or no filter) in each participant's residence. The participants also wore small, battery-powered air monitors starting at 8:00 AM, and continued to carry the monitors until Thursday morning at 8:00 AM. Each subject had daily CV outcome testing performed in a fasting condition (>8 hrs) at the same time between 8-10 AM on 3 consecutive days starting 24 hours after filter system placement (Tues-Thurs). Questionnaires were used to collect information on factors influencing between-subject variance and factors constant over the study period. Subjects underwent each 3-day long intervention and outcome measurement block on 3 separate occasions with a washout period of at least one week between blocks (no presumed carry-over effect between blocks).

Intervention
Daily samples were collected in each subject's home throughout each 3-day filtration period, during which time either no filtration, low-cost, LE (Holmes HAPF30 filter) or more expensive, HE (Holmes HAPF300D filter) recirculating filtration was used. For the "w/o filter" condition, the air cleaner was operated normally without any filter element (i.e., sham filtration) so that filtration status was unknown to the subjects. All air cleaners exhibited similar noise (average of 46 decibels at 6 feet with no significant difference between scenarios) and identical outward appearance regardless of filtration status. The sequence of the filtration scenarios for each subject was randomized.

CV Outcome Measurements
Our prior studies have shown many of the selected CV health outcomes to be associated with PM$_{2.5}$ concentrations. The chosen CV assessment methodologies utilize equipment that can be readily transported and employed at the senior residential facility itself, and pose little or no risk to subjects. In addition, each outcome is a dynamic physiological parameter reflecting an important homeostatic CV mechanism well-known to respond within hours-to-days to an intervention or an insult (e.g., PM exposure). Finally, each outcome has been shown to be independently associated with or predict hard CV outcomes – thus they each represent important indicators of critical biological changes plausibly conveying an increase in risk for overt CV events. The outcomes provided complementary and differing biological information on CV health status, and employing them together thereby provided a comprehensive assessment of the CV impact of PM exposure and its reduction.

A single room was designated for all patient testing. Each examination was performed in this same room with constant internal lighting conditions and temperature held at approx. 72 °F. A portable subject examination bed was used for all patients to lie supine resting as required prior to and during examination. All testing was performed in a unified manner in this room at the same time each morning, with all outcomes being measured in a fasting state (>8 hrs). Subjects took any morning medications after performing these measurements. Completing all measurements took between 30-45 minutes, and they were performed each morning in the following order. Subjects rested seated for 5 minutes. Brachial BP was determined using the dominant arm resting at heart level per guidelines (Pickering et al., 2005). Afterwards, subjects rested seated for an additional 5 minutes and then had their resting arteriole tone measured by retinal photography. Next, subjects lay resting supine on a bed for 10 minutes and thereafter had the sphygmoCor outcomes performed. Repeat testing has been definitively demonstrated not to alter subsequent testing results (Harris 2006). We have performed repeat testing numerous times (on the same day 1-2 hours apart as well as on five subsequent mornings) and have observed no
effect of the order of testing on the outcomes for brachial FMD, endoPAT, or other testing results (Brook et al., 2011).

**Brachial blood pressure:** BP is a well-established predictor of the risk for CV events, and was measured according to guidelines (Pickering et al., 2005) using the validated BPTru device (http://www.bptru.com/html-pages/index.html). The BPTru is an automated oscillometric BP monitor that measures brachial BP without medical personnel present. This device was placed in a location where subjects had their BP measured while resting seated alone in a quiet room. The average of the last 5 of 6 automated BP measurements was recorded. This BP outcome is free of the white-coat response and is a superior indicator of 24-hour ambulatory BP and target organ status (Myers et al., 2011; Myers and Godwin 2012).

**Aortic compliance and central aortic hemodynamics:** The SphygmoCor device was used to measure these outcomes (http://www.atcormedical.com); the protocols are described in detail elsewhere (Pickering et al., 2005; Myers et al., 2011). Central aortic BP differs from arm levels depending on a variety of clinical parameters and is established to be a superior predictor of heart, brain, and kidney target organ status and CV events. The device also measures aortic augmentation index (Alx) (an index of arterial pressure wave reflection) and coronary perfusion index. These parameters are provided by pulse wave analyses of the radial artery using applanation tonometry and a mathematical generalized transfer function approved by the FDA. The device also provides the gold-standard measurement of arterial stiffness by measurement of pulse wave velocity (PWV) via carotid and femoral applanation tonometry. These results have independent CV prognostic abilities incremental to standard arm BP and are better measures of the hemodynamics faced by the heart, brain, and kidneys and are thus superior predictors of target organ damage/status (Laurent et al., 2006; Agabiti-Rosei et al., 2007; Nelson et al., 2010). Both Alx (central aortic hemodynamics) (Vlachopoulos et al., 2010) and PWV have been independently linked to adverse CV outcomes. We have extensive expertise with these techniques and have employed the SphygmoCor device in numerous past and ongoing studies.

**Exposure Assessment**  
**Outdoor air pollution sampling and measurement:** Ambient PM$_{2.5}$ samples were collected daily on Teflon filters using a dichotomous sequential air sampler (Partisol-Plus Model 2025, Rupprecht and Patashnick, Inc., Albany, NY) for subsequent gravimetric analysis of PM$_{2.5}$. This sampler maintained sampling flow rates of 16.7 L/min using integrated volumetric flow controllers, and also measured, averaged and stored ambient temperature, atmospheric pressure and relative humidity. The outdoor PM sampling equipment was positioned on the roof of a three-story building ~80 m NW of the residence due to power requirements and safety issues.

**Indoor air pollution sampling:** Indoor sampling of PM$_{2.5}$ was done concurrently with the daily outdoor sampling. Indoor air pollution sampling equipment was positioned at the furthest point in the room diagonally away from the air filtration unit. Indoor PM$_{2.5}$ samples were collected onto Teflon filter media using Teflon-coated aluminum cyclone sample inlets at a nominal flow rate of 16.7 L/min as determined via calibrated rotameters (Matheson Inc., Montgomeryville, PA). The indoor sampling used pump systems designed and fabricated at the University of Michigan Air Quality Laboratory for previous studies (Keeler et al., 2002). These pump systems used acoustically-insulated wood cases designed for operation in home environments, thus minimizing pump noise during sampling periods.

**Personal air sampling and measurement:** Each subject wore a battery-powered active air monitor – a personal particulate monitor (Thermo Scientific pDR-1500) – designed to continuously measure PM$_{2.5}$ concentrations, providing data logging and a continuous readout. The monitor
only weighed a total of 2 lb, minimizing physical burden on subjects. The personal particulate monitors were carried with the subjects throughout each day both indoors and outdoors. While the subjects slept, the monitors were placed on a nearby nightstand or equivalent. The pDR-1500 also collected sampled particles onto 37-mm Teflon filters for subsequent gravimetric analysis. Continuous data collected by these personal monitors (compared to 24-hr integrated mass values) facilitated assessment of short-term pollutant episodes and determination of contributions from outdoor local sources and indoor sources which can impact the subject on very short time frames.

**Sample analysis:** Sample handling, processing, and analysis took place in Class 100 and Class 1000 ultraclean rooms at the University of Michigan Air Quality Laboratory and Michigan State University Exposure Science Laboratory. Gravimetric determinations were made using a microbalance (MT-5, Mettler Toledo, Columbus OH) in a temperature/humidity-controlled environment as described in the Federal Reference Method (EPA 1997).

**Statistical Analyses**

We examined the distributions of outcomes and exposures under each intervention (filtration scenario), as well as individual and mean profiles over time. Graphical approaches such as boxplots and scatterplots with linear or non-linear (e.g., loess) methods were used, allowing identification of outliers, linearity, and correlation of measurements within person and across time. If outcome data did not appear normally distributed, transformations were employed. Because each subject’s outcomes and exposures were measured at more than one point in time, our analyses were based on linear mixed effects models (Diggle et al., 1994; Verbeke and Molenberghs 2000; Fitzmaurice et al., 2004). Our *a priori* time frame of interest after each intervention is 24-hours, although we also investigated models separately for 48- and 72-hours to explore different lag periods. Selection of important covariates and confounders was based on prior knowledge and bivariate associations assessed during exploratory data analyses. We expected no carryover since interventions were spaced at least one week apart. Tests to compare filtration scenarios were based on Wald and chi-squared tests based on maximum likelihood or restricted maximum likelihood methods. Alternative covariance structures (other than compound symmetry) were explored and model fit was examined using the Akaike Information Criteria (Burnham and Anderson 2004).

We tested whether LE and HE filters resulted in statistically better CV outcomes and lower PM\(_{2.5}\) levels than no filtration using models of the following form:

\[
CV_i = (b_0 + b_i) + (\beta_{11} \times LE_i + \beta_{12} \times HE_i) + \beta_2 \times CONFOUNDER_{it} + \epsilon_{it}
\]

where \(CV_i\) is the continuous CV health outcome (basal microvascular tone, brachial BP, central aortic BP, PWV, inflammatory biomarkers) in individual \(i\) at time \(t\); \(LE_i\) and \(HE_i\) are indicator variables indicating the filtration scenario for individual \(i\) at time \(t\) (with unfiltered air as the referent category); CONFOUNDER\(_{it}\) is a vector of potential confounders or covariates (e.g., age, gender, or time-varying covariates such as daily medication use); \(b_0\) is the overall intercept; \(b_1\) is the separate random intercept for individual \(i\) which accounts for correlation within repeated measures from an individual and is assumed to be an independently distributed random variable with mean zero and variance \(\sigma^2_{b_1}\); \(\beta_{11}\) and \(\beta_{12}\) are the overall effects of LE vs unfiltered air and HE vs unfiltered air, respectively; \(\beta_2\) is the overall effect of confounders or covariates; \(\epsilon_{it}\) is the random error (e.g., measurement error) of the \(t^{th}\) observation on the \(i^{th}\) individual and is assumed to be an independently distributed random variable with mean zero and variance \(\sigma^2\).

**Sample Size and Power Considerations:** The sample size for this study was based on a combination of logistical considerations and power required to detect important differences in key CV parameters (RHI and systolic BP) and PM\(_{2.5}\) levels in the context of a randomized
crossover design with repeated measures. Power was calculated for 40 individuals with 3 repeat samples under each intervention (no, LE, and HE filtration) using a 6-sequence, 3-period, 3-treatment crossover design. Brook et al (2011) provide estimates of the effect expected with significant (10 µg/m³) increase in personal-level PM$_{2.5}$ exposure: 0.17 difference in RHI and 1.4 mmHg elevation in systolic BP. Previous studies by these investigators support assumed intersubject variability of 4.0 µg/m³, 0.4, and 6.2 mmHg for PM$_{2.5}$, RHI, and systolic BP, respectively. Intrasubject variability was assumed to be the same for all samples at 9.2 µg/m³, 0.4, and 3.3 mmHg. Given that measurements were made more closely in time within interventions than across interventions, one might anticipate higher correlation for those samples; however, we lacked data to estimate that correlation structure so our power calculations may be slightly anti-conservative. For this reason we sized our study to achieve 90% power using a two-sided Type I error of 5% to allow for potentially alternate correlation structure and multiplicity. Results from past research (Brook et al., 2009) showing statistically significant findings for endothelial function further support our proposed sample size. Ultimately, we estimated 90% power to detect a 3.5 µg/m³ difference in PM$_{2.5}$, a 1.4 mmHg difference in systolic BP, and a 0.17 difference in RHI with filtration as compared to unfiltered air (Table 1) based on F tests in the crossover setting (Jones and Kenward 1989).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Mean Treatment Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP</td>
<td>1.4 mmHg</td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>3.5 µg/m³</td>
</tr>
</tbody>
</table>
2. Change in secondary outcomes (HRV parameters) by filtration type over the course of each 3-day intervention

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Intervention</th>
<th>Upper Limit</th>
<th>Lower Limit</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDNN</td>
<td>LE</td>
<td>0.091</td>
<td>-0.123</td>
<td>-0.022</td>
</tr>
<tr>
<td>SDNN</td>
<td>HE</td>
<td>0.073</td>
<td>-0.139</td>
<td>-0.039</td>
</tr>
<tr>
<td>SDNN</td>
<td>Any</td>
<td>0.061</td>
<td>-0.124</td>
<td>-0.036</td>
</tr>
<tr>
<td>LF</td>
<td>LE</td>
<td>-0.012</td>
<td>-0.526</td>
<td>-0.316</td>
</tr>
<tr>
<td>LF</td>
<td>HE</td>
<td>0.226</td>
<td>-0.416</td>
<td>-0.154</td>
</tr>
<tr>
<td>LF</td>
<td>Any</td>
<td>0.044</td>
<td>-0.475</td>
<td>-0.260</td>
</tr>
<tr>
<td>HF</td>
<td>LE</td>
<td>0.431</td>
<td>-0.276</td>
<td>0.018</td>
</tr>
<tr>
<td>HF</td>
<td>HE</td>
<td>0.388</td>
<td>-0.303</td>
<td>-0.016</td>
</tr>
<tr>
<td>HF</td>
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<td>0.378</td>
<td>-0.271</td>
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<tr>
<td>LF/HF</td>
<td>LE</td>
<td>0.020</td>
<td>-0.056</td>
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<tr>
<td>LF/HF</td>
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<td>0.032</td>
<td>-0.045</td>
<td>-0.008</td>
</tr>
<tr>
<td>LF/HF</td>
<td>Any</td>
<td>0.023</td>
<td>-0.047</td>
<td>-0.013</td>
</tr>
</tbody>
</table>

Values are % change compared to sham scenario. SDNN, standard deviation of normal-to-normal R intervals; LF, low frequency; HF, high frequency; LF/HF, low frequency-to-high frequency ratio. Upper and lower limits indicate 95% confidence interval bounds.
References:


