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# Indoor Particles Affect Vascular Function in the Aged

## An Air Filtration–based Intervention Study

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**Rationale:** Exposure to particulate matter is associated with risk of cardiovascular events, possibly through endothelial dysfunction, and indoor air may be most important.

**Objectives:** We investigated effects of controlled exposure to indoor air particles on microvascular function (MVF) as the primary endpoint and biomarkers of inflammation and oxidative stress as secondary endpoints in a healthy elderly population.

**Methods:** A total of 21 nonsmoking couples participated in a randomized, double-blind, crossover study with two consecutive 48-hour exposures to either particle-filtered or nonfiltered air (2,533–4,058 and 7,718–12,988 particles/cm<sup>3</sup>, respectively) in their homes.

**Measurements and Main Results:** MVF was assessed noninvasively by measuring digital peripheral artery tone after arm ischemia. Secondary endpoints included hemoglobin, red blood cells, platelet count, coagulation factors, P-selectin, plasma amyloid A, C-reactive protein, fibrinogen, IL-6, tumor necrosis factor- $\alpha$ , protein oxidation measured as 2-aminoadipic semialdehyde in plasma, urinary 8-iso-prostaglandin F<sub>2 $\alpha$</sub> , and blood pressure. Indoor air filtration significantly improved MVF by 8.1% (95% confidence interval, 0.4–16.3%), and the particulate matter (diameter < 2.5  $\mu$ m) mass of the indoor particles was more important than the total number concentration (10–700 nm) for these effects. MVF was significantly associated with personal exposure to iron, potassium, copper, zinc, arsenic, and lead in the fine fraction. After Bonferroni correction, none of the secondary biomarkers changed significantly.

**Conclusions:** Reduction of particle exposure by filtration of recirculated indoor air for only 48 hours improved MVF in healthy elderly citizens, suggesting that this may be a feasible way of reducing the risk of cardiovascular disease.

**Keywords:** atherosclerosis; biomarkers; cardiovascular disease; indoor air pollution; inflammation

### AT A GLANCE COMMENTARY

#### Scientific Knowledge on the Subject

Increased cardiovascular risk is associated with exposure to air pollution.

#### What This Study Adds to the Field

Particles in indoor air affect endothelial function in elderly subjects. A significant improvement was shown after reduction of particles in the indoor air achieved by air filtration in their homes.

Short-term and chronic exposure to ambient levels of particulate matter (PM) is associated with increased morbidity and mortality related to respiratory and cardiovascular disease (1, 2).

Biological mechanisms of action of PM are believed to involve altered pulmonary inflammation, cardiac autonomic function, endothelial dysfunction, systemic inflammation, oxidative stress, and altered balance between blood clotting and fibrinolysis, with small particles being more potent per unit mass than larger particles due to their higher surface area and reactivity (3–5). Traffic-related PM may be particularly relevant, as indicated by risk of cardiovascular events shortly after exposure in traffic (6). These particles may also penetrate indoors, and previous studies have described increased mortality associated with a residential address close to major roads with dense traffic as well as acute mortality associated with source allocation (7, 8).

Abnormal endothelial function (EF) is a strong predictor of adverse cardiovascular outcomes (9, 10) and widely recognized in patients with atherosclerosis and its risk factors (11, 12). Inhalation of high concentrations of diesel exhaust particles has recently been shown to impair two important and complementary aspects of vascular functions in healthy humans: the regulation of vascular tone and fibrinolysis (13).

A number of personal monitoring studies have found that biomarkers related to cardiovascular diseases show stronger association with personal exposure than with ambient levels (14, 15). This indicates that the exposure to particles generated indoors could cause an additional increase in adverse effects. However, studies of the health effects of indoor air on healthy humans are lacking. In addition, most of the mechanistic evidence comes from experimental human or animal studies with high levels of exposure, or from observational panel studies with associated difficulties in exposure assessment and control of confounders. Indoor penetration of ambient air particles is variable, and there are many indoor sources, such as cooking,

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candles, human activity, heating appliances, and environmental tobacco smoke (15, 16). This underlines the importance of understanding details of indoor PM levels, and there is a specific need to study this in the elderly, as they appear to have elevated susceptibility (17) and have the largest attributable risk related to indoor PM, as they spend more time indoors (18).

The primary aim of this study was to use controlled exposure to real-life indoor air particles to delineate the relationship between intervention and microvascular function (MVF), as a measure of EF in a healthy population of 42 elderly volunteers. We used two consecutive 48-hour periods in each private home, and an intervention was achieved by using high-efficiency particle air (HEPA) filters during one of these periods. Changes in peripheral artery tone due to enhanced flow after arm ischemia was used to assess MVF as the *a priori*-defined primary endpoint. Secondary endpoints in terms of blood pressure, hematological parameters, markers of inflammation, and hemostasis, as well as lipid and protein oxidation products, were included to elucidate potential mechanisms of action.

## METHODS

Further details of all methods are available in the extended METHODS in the online supplement.

### Study Population and Design

A total of 21 couples, aged 60–75 (median, 67) years and mean body mass index of 25 (SD, 3.24), were recruited, and one female was later excluded. All participants were healthy nonsmokers, and each was his/her own control, excluding confounding by factors that are stable within an individual over time but vary between participants.

The project design was a double-blind crossover intervention with randomized order of 48-hour exposure to recirculated particle-filtered and nonfiltered indoor air in the volunteer's homes located in Copenhagen in proximity (<350 m) to major roads (>10,000 vehicles/24 h). Two filter units (Airshower; Airsonett AB, Ängelholm, Sweden), running continuously with airflow of 540 m<sup>3</sup>/hour, sound level less than 35 dB, and filter exhaust height of 2.15 m, were placed in the bedroom and living room of each apartment during the study period (either with or without a HEPA filter, according to scenario; see Figure E2 in the online supplement). When the HEPA filter was removed, filtration efficiency of the unit was less than 10%, with unchanged noise, airflow, and appearance. The air pollution monitoring equipment was always positioned at the furthest point diagonally away from the filter.

The size distribution and number concentration (NC) of particles (10–700 nm) in each residence were continuously monitored. Particle samples were collected using Dichotomous Stacked Filter Units (University of Hertfordshire, Hatfield, UK) (19), as fine (diameter < 2.5 μm [PM<sub>2.5</sub>]) and coarse (10–2.5 μm diameter [PM<sub>10–2.5</sub>]) fractions. Particle mass was determined gravimetrically, and elemental composition was determined using a proton-induced X-ray emission method (20).

Blood and spot-urine was sampled on the morning after each 48-hour scenario, and, to avoid problems due to diurnal variation, each scenario started at the same time of morning. For the duration of the study, we asked participants to stay indoors (equivalent to 92–94% of time) and keep windows closed.

The study was approved by the local ethics committee and in accordance with the Declaration of Helsinki. All participants gave written, informed consent before inclusion.

### Primary Endpoint

**Vascular function.** MVF was measured immediately before blood sampling and noninvasively using reactive hyperemia–peripheral arterial tonometry (RH-PAT), as previously described in detail (21–23). This technique uses finger-mountable pneumatic sensors (Endo-PAT 2000; Itamar Medical Ltd., Cesaria, Israel) specifically designed to continuously record the digital arterial pulse wave amplitude. A blood pressure cuff was placed above the elbow of the right arm for hyperemia testing, while the left arm served as a control. The plethysmographic finger probes were placed on the index fingers of both hands.

The test consisted of three stages: baseline, brachial arterial occlusion, and a postocclusion recording of the induced reactive hyperemia response. Data was digitally stored as pulse wave tracings from both hands and an MVF score describing the extent of hyperemia was computed using an automated algorithm in an operator independent manner.

### Secondary Endpoints

**Hematological measurements, markers of inflammation, hemostasis, and oxidative stress.** We measured hemoglobin, red blood cells, fibrinogen, platelets, coagulation factors (II + VII + X), C-reactive protein (CRP), IL-6, tumor necrosis factor (TNF)-α, plasma amyloid A, 2-aminoapicid semialdehyde, and urinary 8-iso-prostaglandin F<sub>2α</sub> (8-iso-PGF<sub>2α</sub>).

### Statistical Analysis

Due to three missing data points for the MVF score and one missing blood sample, we used mixed-effect model repeated measures analysis to investigate the effect of intervention on the primary and secondary outcome variables. Partner cluster and participant nested in partner cluster were included as random factor variables to account for interindividual variation and the effects of partners living at the same address. Exposure in terms of filtration status was included as a fixed categorical explanatory variable. Gender was included as a categorical variable, and age, body mass index, and indoor temperature as continuous variables. We investigated dose–response relationships related to the total NC, area and volume of particles, as well as mass concentration of PM<sub>2.5</sub> and PM<sub>10–2.5</sub> up to blood sampling. These were analyzed in single-exposure models and in a multiple-component, backward stepwise selection procedure. Similarly, the mass concentration of each element analyzed in the PM<sub>2.5</sub> and PM<sub>10–2.5</sub> fractions were analyzed with and without adjustment for mass concentration of the fraction. The significance threshold was *P* less than 0.05 in all analyses.

## RESULTS

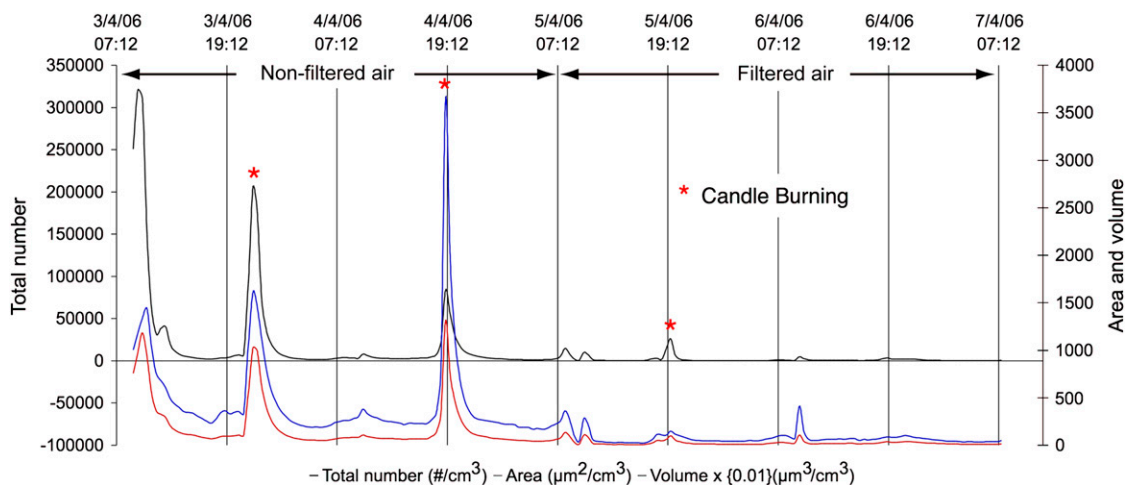
### Exposure Characterization

Table 1 summarizes indoor levels of PM characteristics and NO<sub>2</sub> during the two different exposure scenarios. The HEPA filter placed in homes effectively removed ultrafine, fine, and coarse particles, whereas levels of NO<sub>2</sub> were unaltered. Filtration mean efficacy within each apartment was uniform: 59.8% (bedroom) and 61.2% (living area), and this was not significantly correlated with NC<sub>urban background</sub>, maximum traffic density within a 100-m radius, room volume, or carpeting coverage (see Table E1). Figure 1 depicts NC<sub>10–700 nm</sub>, Area<sub>10–700 nm</sub>, and Volume<sub>10–700 nm</sub> of particles with and without filtration of the air in one of the included homes. Occasional peaks could be related to candle burning and cooking. The average change in

**TABLE 1. GEOMETRIC MEAN AND 95% CONFIDENCE INTERVAL OF INDOOR CONCENTRATIONS OF PARTICULATE MATTER NUMBER CONCENTRATION<sub>10–700 nm</sub>, AREA<sub>10–700 nm</sub>, VOLUME<sub>10–700 nm</sub>, PARTICULATE MATTER MASS, INDOOR NO<sub>2</sub>, RELATIVE HUMIDITY, AND TEMPERATURE**

Variable	Nonfiltered Air	Particle-filtered Air
	Geometric Mean (95% CI)	Geometric Mean (95% CI)
NC <sub>10–700 nm</sub> , no./cm <sup>3</sup>	10,016 (7,718–12,998)	3,206 (2,533–4,058)
Area <sub>10–700 nm</sub> , μm <sup>2</sup> /cm <sup>3</sup>	173 (144–209)	47 (38–58)
Volume <sub>10–700 nm</sub> , μm <sup>3</sup> /cm <sup>3</sup>	5.7 (4.7–6.8)	1.6 (1.3–2.0)
PM <sub>10–2.5</sub> , μg/m <sup>3</sup>	9.4 (8.1–1.0)	4.6 (3.5–6.0)
PM <sub>2.5</sub> , μg/m <sup>3</sup>	12.6 (11.2–14.1)	4.7 (3.9–5.7)
NO <sub>2</sub> , ppb	20 (18–21)	20 (18–22)
Relative humidity, %	34.0 (30.9–37.4)	34.0 (31.1–37.1)
Temperature, °C	21.6 (21.2–22.0)	21.5 (21.1–21.9)

*Definition of abbreviations:* CI = confidence interval; NC = number concentration; PM = particulate matter.



**Figure 1.** Number concentration (NC<sub>10-700 nm</sub>; black line), Area<sub>10-700 nm</sub> (red line), and Volume<sub>10-700 nm</sub> (blue line) of particles during 48 hours without and 48 hours with filtration of the indoor air in one of the included homes. Peaks in NC<sub>10-700 nm</sub> (asterisks) occurred during candle burning and cooking, as indicated.

NC<sub>10-700 nm</sub> over the entire study period within the included apartments is presented in the online supplement (Figure E1); peaks were not related to rush hour traffic, but rather to indoor activities. The concentrations of the elements in the indoor fine fraction of particles (PM<sub>2.5</sub>) per indoor air unit of nonfiltered and particle-filtered indoor air are presented in the online supplement (Table E2). There was a relatively high concentration of sulfur, consistent with substantial penetration from outdoor air originating from long-range transport, and this fraction was also relatively rich in metals. All concentrations were substantially reduced by HEPA filtration. With the exception of chromium, antimony, arsenic, and chlorine, the concentrations of all the elements within the PM<sub>2.5</sub> fraction of indoor air were significantly correlated with Area<sub>10-700 nm</sub> and Volume<sub>10-700 nm</sub> of particles (Table E3).

**Biomarkers and Function Tests**

MVF score, hematological parameters, oxidative products, and markers of inflammation and hemostasis are presented in Table 2 according to exposure scenario.

**Primary endpoint.** Three pulse wave tracings of RH-PAT were not recorded due to instrument failure. The MVF score was significantly improved by 8.1% (95% confidence interval [CI], 0.4–16.3%; *P* = 0.03) during air filtration, as assessed in the mixed-effects model with inclusion of filtration as a categorical variable (Table 2). The MVF score was significantly and inversely associated with mass concentration of PM<sub>2.5</sub> and NC<sub>10-700 nm</sub> during both scenarios in the single-exposure models, whereas PM<sub>10-2.5</sub>, Area<sub>10-70 nm</sub>, and Volume<sub>10-700 nm</sub> were not significant predictors (Table 3). After applying a backward stepwise selection approach and the stepwise exclusion of exposure variables: PM<sub>10-2.5</sub> mass, Volume<sub>10-700 nm</sub>, NC<sub>10-700 nm</sub>, and Area<sub>10-70 nm</sub>, only PM<sub>2.5</sub> mass was a significant predictor of MVF score in the reduced model. Of the elements in PM<sub>2.5</sub> fraction, we found that iron, copper, potassium, zinc, lead, and arsenic were all significantly and inversely associated with MVF score. When these single-element associations were adjusted for the mass concentration of the PM<sub>2.5</sub> fraction, we found that only potassium was significantly and independently associated with MVF score (Table E2). There was no effect of exposure on the

**TABLE 2. GEOMETRIC MEAN AND 95% CONFIDENCE INTERVAL OF MICROVASCULAR FUNCTION AND BIOMARKERS ACCORDING TO FILTRATION SCENARIO AND RELATIONSHIP BETWEEN BIOMARKERS AND INTERVENTION FILTRATION IN THE HOMES OF 41 ELDERLY SUBJECTS**

Effect Marker	Nonfiltered Air	Particle-filtered Air	<i>P</i> Value	% Change (95% CI) <sup>a</sup>
Microvascular function score*	1.78 (1.68 to 1.89)	1.95 (1.80 to 2.11)	0.040	8.1 (0.4 to 16.3)
Hemoglobin, mmol/L	9.0 (8.8 to 9.2)	9.1 (8.8 to 9.3)	0.029	0.9 (0.1 to 1.8)
Red blood cell count × 10 <sup>12</sup> /L	4.8 (4.6 to 4.9)	4.8 (4.8 to 4.9)	0.115	0.7 (−0.2 to 1.5)
Plasma fibrinogen, μmol/L	9.8 (9.5 to 10.1)	9.8 (9.7 to 10.0)	0.639	0.7 (−2.3 to 3.7)
Platelet count × 10 <sup>9</sup> /L	227 (209 to 246)	230 (212 to 249)	0.372	1.3 (−1.5 to 4.1)
Coagulation factors (II + VII + X)	1.00 (0.96 to 1.04)	1.02 (0.97 to 1.07)	0.061	1.9 (−0.1 to 3.9)
Plasma C-reactive protein, mg/L	1.5 (1.3 to 1.9)	1.6 (1.4 to 1.9)	0.755	2.0 (−10.4 to 16.1)
Plasma IL-6, ng/L	1.2 (1.0 to 1.6)	1.2 (0.9 to 1.5)	0.130	−6.6 (−14.5 to 2.1)
TNF-α, ng/L	1.1 (1.0 to 1.4)	1.2 (1.0 to 1.4)	0.848	0.5 (−4.4 to 5.6)
Plasma amyloid A, mg/L	3.8 (3.0 to 4.9)	3.7 (2.9 to 4.8)	0.486	−3.1 (−11.7 to 6.2)
Plasma-selectin, μg/L	72.7 (65.8 to 80.2)	76.8 (67.3 to 87.7)	0.412	5.6 (−7.6 to 20.7)
8-iso-PGF <sub>2α</sub> , nmol/mmol <sup>†</sup>	0.5 (0.4 to 0.5)	0.4 (0.4 to 0.5)	0.173	−6.3 (−14.8 to 3.0)
PLAAS, pmol/mg protein	31.7 (26.1 to 38.6)	32.3 (26.7 to 39.1)	0.986	−0.2 (−19.4 to 24.5)
Systolic blood pressure, mm Hg	81 (78 to 84)	81 (78 to 84)	0.443	−0.2 (−3.8 to 3.5)
Diastolic blood pressure, mm Hg	136 (131 to 141)	133 (129 to 139)	0.893	−1.4 (−4.7 to 2.2)

*Definition of abbreviations:* 8-iso-PGF<sub>2α</sub> = 8-iso-prostaglandin F<sub>2α</sub>; CI = confidence interval; PLAAS = 2-aminoapicid semi-aldehyde in plasma proteins; PM = particulate matter; TNF = tumor necrosis factor.

Mixed model regression with partner cluster and subject nested in partner cluster used as random factors. All model estimates adjusted for age, gender, body mass index, and indoor temperature. Exposure to particle-filtered indoor air: categorical (yes/no) included as a predictor and the natural logarithm of the effect marker in question included as a continuous outcome variable. The predictive value of the estimates (%-change) expressed relative to exposure to particle-filtered indoor air.

\* The microvascular function score described in detail in the extended methods section of the online supplement.

<sup>†</sup> 8-Iso-prostaglandin F<sub>2α</sub>: creatinine corrected concentration.

**TABLE 3. THE RELATIONSHIP BETWEEN THE MICROVASCULAR FUNCTION SCORE AND CONTINUOUS PARAMETERS OF EXPOSURE MEASURED WITH AND WITHOUT FILTRATION OF THE INDOOR HOME AIR**

Exposure Variable	Single-Exposure Component Model	
	% Change (95% CI)	P Value
NC <sub>10-700 nm</sub>	-3.2 (-6.2 to -0.0)	0.048
Area <sub>10-700 nm</sub>	-3.1 (-6.2 to 0.1)	0.060
Volume <sub>10-700 nm</sub>	-3.2 (-6.3 to 0.1)	0.060
PM <sub>2.5</sub>	-5.5 (-9.2 to -1.6)	0.007*
PM <sub>10-2.5</sub>	-2.5 (-6.2 to 1.4)	0.200

Definition of abbreviations: CI = confidence interval; NC = number concentration; PM = particulate matter.

Mixed model regression with partner cluster and subject nested in partner cluster used as random factor variables. All model estimates are adjusted for age, gender, and body mass index. The natural logarithm of the exposure variable in question and the microvascular function score were included as a continuous predictor and outcome variables, respectively. The relative predictive value (%-change) of estimates is expressed per doubling in exposure variable numbers.

\* In a stepwise backward selection process with all variables of exposure included, only PM<sub>2.5</sub> was a significant predictor of the microvascular function score. Exclusion order: PM<sub>10-2.5</sub> ( $P = 0.345$ ); volume ( $P = 0.475$ ); number ( $P = 0.564$ ); and area ( $P = 0.737$ ).

baseline peripheral arterial tone amplitude in either the ischemic or control arm.

**Secondary endpoints.** Filtration of the indoor air was significantly associated with an increase in hemoglobin concentration in the blood (Table 2). None of the other biomarkers was significantly associated with exposure as categorical or continuous variables. However, an association between NC<sub>10-700 nm</sub> and 8-iso-PGF<sub>2 $\alpha$</sub>  was borderline significant ( $P = 0.055$ ), and a doubling in NC<sub>10-700nm</sub> corresponded to a 4.31 (95% CI, -0.09 to 8.91) % increase. After Bonferroni correction, none of the secondary endpoints changed significantly in accordance with exposure as either a continuous or categorical variable. None of these secondary endpoints correlated with MVF score ( $P \geq 0.099$ ).

NO<sub>2</sub> was not a significant predictor when included in the above models of primary and secondary endpoints ( $P \geq 0.20$ ). The intake of medication with antiinflammatory properties had no significant effects on the predictive value of exposure on these endpoints and was not significantly associated with any of the markers studied here ( $P \geq 0.79$ ). Finally, the order of randomization had no affect on the parameters studied.

## DISCUSSION

EF constitutes an independent predictor of cardiovascular events (24, 25), and the clinical implications of endothelial dysfunction and the association between endothelial cell dysfunction and cardiac events are well established (26, 27). There have been a number of studies showing associations between improved EF after interventions of increased exercise (28), smoking cessation (29), and weight reduction (30). In this study, we investigated the effects of intervention using HEPA filtration of indoor air particles for 48 hours. Our main finding was a significant improvement in MVF demonstrated by an increased flow-mediated vasoresponse after reduction of indoor air particles, most likely indicating a general improvement in EF.

A recent experimental study including 30 young healthy volunteers was the first to demonstrate that inhalation of diesel emission in high doses (300  $\mu\text{g}/\text{m}^3$ ) could impair vasomotor responses to both endothelium-dependent (acetylcholine and

bradykinin) and endothelium-independent (sodium nitropruside) vasodilators (13). Moreover, this group showed that endothelium-dependent vasodilatation occurring in the presence of mild systemic inflammation was persistent 24 hours after the same exposure in 15 volunteers (31). We found an effect on MVF at a 10-times lower exposure level in an elderly population, which may be more susceptible. Indeed, EF measured in connection with previous exposure studies was only negatively associated with ambient levels of PM<sub>2.5</sub> and sulfate and/or black carbon among diabetics, who are particularly susceptible, and diabetes enhances vulnerability to particulate air pollution-associated impairment in vascular reactivity and EF (32). Moreover, EF measured as acetylcholine-induced vasodilatation in aorta segments 1 hour after systemic administration of diesel exhaust particles was only reduced in hyperlipidemic apolipoprotein E knockout mice, whereas there was a tendency toward enhanced vasodilatation in wild-type mice, and no effect on endothelium-independent vasodilatation in any type of mouse (33). Thus, EF appears to be negatively affected by exposure to particulate air pollution in susceptible individuals, and this could provide part of a mechanistic link to acute cardiovascular events as well as progression of atherosclerosis.

Digital MVF was defined as our primary endpoint because this functional measure reflects coronary EF and can be considered a more specific predictor of cardiovascular risk than the secondary biomarkers that were included to elucidate potential mechanisms. The method has been validated in clinical settings (23, 34, 35), but ours is the first group to use it in relation to the effects of air pollution. The portable, user-independent, automated, and noninvasive equipment allowed us to study the most relevant exposure in terms of indoor air within the homes of elderly volunteers. In another experimental study using this technique, we included 29 young, healthy volunteers and found that the MVF score geometric mean was higher (2.14; 95% CI, 2.08-2.19), reflecting the general consensus that aging increases susceptibility. In a study of patients referred for coronary angiography, those showing endothelial dysfunction had an average MVF score of 1.27, whereas patients without coronary endothelial dysfunction had an average MVF score of 1.78 (34), which is comparable to our healthy, elderly volunteers. Endothelium-independent vasodilatation assessed by the peripheral artery tone response to nitroglycerin was similar in the two groups in that study (34). These data support the application of RH-PAT as a convenient, noninvasive measure of EF.

An assessment of endothelium-independent vasodilation using PAT to measure the hyperemic response to sublingual nitroglycerine would have clarified the mechanism by which particulate filtration improved MVF in our study. This was not performed, as the administration of nitroglycerine in the home environment posed unacceptable risks of adverse effects to the elderly study participants. Previous studies have suggested a role for oxidative stress and reduced nitric oxide bioavailability in mediating adverse vascular effects of PM (13, 31). As the digital hyperemic response is largely dependent on nitric oxide (35), we believe that the improvement in MVF after particle filtration in our study represents a generalized improvement in EF.

Limitations to the use of MVF score for EF assessment include the limited data on associations with outcomes and other risk factors such as smoking, hypertension, and hypercholesterolemia.

We attempted to address the mechanisms of the association between MVF and particle exposure by means of biomarkers as secondary endpoints. There was a borderline significant ( $P = 0.055$ ) association between particle NC<sub>10-700 nm</sub> and excretion of free 8-iso-PGF<sub>2 $\alpha$</sub> , a major F<sub>2</sub>-isoprostane, although this was far from significant after Bonferroni correction. The F<sub>2</sub>-isoprostanes are

formed from arachidonic acid through nonenzymatic, free radical-catalyzed reaction, and are reliable markers of lipid peroxidation in a variety of conditions, including acute and chronic inflammatory conditions (36). Excretion of free 8-iso-PGF<sub>2α</sub> was elevated in cigarette smokers (37) and in subjects after high-dose exposure to woodsmoke (38), and has been shown to be associated with coronary artery disease (39). We have previously found correlations between lipid and protein oxidation products in plasma and personal exposure to black carbon in PM<sub>2.5</sub> in individuals living in Copenhagen (40). However, in the present study, we found no effect of particle exposure on protein oxidation assessed by plasma protein 2-aminoapicid semialdehyde, although this lack of effect may have been due to the use of heparinized plasma, which is not ideal for the measurement.

The biomarkers related to inflammation responses and coagulation (IL-6, TNF-α), the acute-phase reactants (fibrinogen, CRP, and serum amyloid A), as well as coagulation factors II, VII, and X showed no sign of effect. Previously, experimental exposure to concentrated ambient air particles at mean concentrations of 120 μg/m<sup>3</sup> caused increased fibrinogen levels among 15 volunteers (41). In another study including 13 healthy subjects exposed to woodsmoke particles at 280 μg/m<sup>3</sup>, serum amyloid A as well as factor VIII in plasma and the factor VIII/von Willebrand factor ratio were significantly increased, whereas IL-6, TNF-α, CRP, and fibrinogen showed no increase (38). High exposure to diesel emission at 300 μg/m<sup>3</sup> caused diminished fibrinolytic capacity in other studies, whereas the plasma concentration of IL-6, TNF-α, von Willebrand factor activity, prothrombin fragments, CRP, and fibrinogen were unaltered, despite reduced EF (13, 42). A recent study showed some association between ambient PM levels and global coagulation function, whereas fibrinogen was unaffected (43). In other panel studies, CRP levels have been found to be associated with ambient or personal PM exposure (44–46). Accordingly, acute-phase reactants, such as CRP, fibrinogen, and amyloid A in plasma, may respond at relatively high levels of particle exposure, whereas cytokine levels in plasma do not seem to be sensitive for the detection of inflammatory responses in this respect. Moreover, there are no obvious associations between biomarkers of inflammation or oxidative stress and MVF. In accordance with this observation, we found no sign of correlations in the present study. The recent finding of association between expression of adhesion molecules on leukocytes or in plasma and ambient levels of PM in observational panel studies suggest these as promising biomarkers for experimental exposure studies, (46–48), although we found no effect on P-selectin in the present study. We found that PM exposure was significantly associated with a decrease in hemoglobin, without Bonferroni correction, which is in agreement with earlier results (49); however, in another previous study, we observed a positive association with exposure to black smoke among young women (40). Our results may suggest that some component of PM causes sequestration of red blood cells in the circulation, but the effect on hemoglobin may also be due to chance, considering the large number of secondary endpoints.

Indoor air contains a mixture of PM from both indoor and outdoor sources with various chemical species and trace elements. We analyzed size distribution and elemental composition of particles within the PM<sub>2.5</sub> and PM<sub>10–2.5</sub> fractions in both scenarios and found that PM<sub>2.5</sub> mass was the only remaining independent predictor of the MVF score in the reduced multi-exposure model, indicating that the indoor fine particle mass, rather than numbers or surface area of particles, are important for the effect on EF. However, this may be due to the fact that indoor sources, such as cooking and candle burning, contributed

substantially to indoor NC, whereas vehicle emissions probably contributed less. Of the elements in the PM<sub>2.5</sub> fraction, we found significant associations between individual increases in iron, copper, potassium, zinc, lead, and arsenic concentrations and reduced MVF score, whereas other elements, including the transition metals, vanadium, titanium, chromium, and nickel, had no effects. Iron and copper are typical elements associated with brake dust from vehicles, and their presence indoors may be due to penetration (20). Transition metals, including iron and copper, catalyze the formation of reactive oxygen species via Haber-Weiss reactions (50), which may explain the effects observed for iron and copper. However, the associations between the metals and the MVF score were not independent of the PM<sub>2.5</sub> mass, and we found no clear associations between the MVF score and markers of oxidative stress, as discussed previously here. Only potassium showed an independent association with the MVF score. Potassium is a typical element associated with particles generated from the burning of biomass and smoking (51, 52), and indoor penetration of ambient particles from biomass burning, including long-range transport, and/or penetration of environmental tobacco smoke from neighboring apartments, may have contributed to the effect on MVF. A relatively high level of indoor sulfur in the PM<sub>2.5</sub> fraction, which correlated with the urban background levels (Table E3), suggests substantial penetration of long-range transported particles. These findings are in keeping with a recent source apportionment study in Copenhagen, which showed associations between daily cardiovascular-related admissions and daily urban background concentrations of secondary sulfate-rich particles and biomass particles in PM<sub>10</sub> (53). Unfortunately, we could not address contributions from elemental carbon or organic compounds directly in the present study.

NO<sub>2</sub> was not a significant predictor of any of the endpoints, and this result was expected, as the filtration of recirculated indoor air had no effects on NO<sub>2</sub> levels. Furthermore, the intake of minor medications had no effect on our results, which was also expected, as these medications were constant within these individuals throughout the study, and each individual was his/her own control.

The effects we show in this study were measured after a 48-hour intervention. It is possible that the effects occurred much earlier, and it may also be speculated that further improvement may occur after prolonged intervention by 6 months to 1 year, and that this could result in further reduction in cardiovascular risk in this healthy, elderly age group.

## Conclusions

The results of this study indicate that reduction of particles in recirculated indoor air by filtration significantly improves MVF in a healthy, nonsmoking, elderly population. The improvement could not be ascribed to significant reductions in inflammation or oxidative stress by means of biomarkers. Indoor air sources differ from outdoor air and indoor PM<sub>2.5</sub> mass, rather than total numbers or surface area of particles had the most important association with MVF. Indoor air filtration represents a feasible means of reducing cardiovascular risk and suggests long-term and large-scale studies with cardiovascular events as endpoints.

**Conflict of Interest Statement:** None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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