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Blood pressure changes in association with black carbon exposure in a panel of healthy adults are independent of retinal microcirculation

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ABSTRACT

Exposure to ambient particulate matter and elevated blood pressure are risk factors for cardiovascular morbidity and mortality. Microvascular changes might be an important pathway in explaining the association between air pollution and blood pressure. The objective of the study was to evaluate the role of the retinal microcirculation in the association between black carbon (BC) exposure and blood pressure.

We estimated subchronic BC exposure based on 1-week personal measurements (μ-Aethalometer, AethLabs) in 55 healthy nurses. Blood pressure and retinal microvasculature were measured on four different days (range: 2–4) during this week.

Subchronic BC exposure averaged (± SD) 1334 ± 631 ng/m^3 and ranged from 338 ng/m^3 to 3889 ng/m^3. An increased exposure of 631 ng/m^3 BC was associated with a 2.77 mm Hg (95% CI: 0.39 to 5.15, $p = 0.027$) increase in systolic blood pressure, a 2.35 mm Hg (95% CI: 0.52 to 4.19, $p = 0.016$) increase in diastolic blood pressure and with 5.65 μm (95% CI: 1.33 to 9.96, $p = 0.014$) increase in central retinal venular equivalent. Mediation analysis failed to reveal an effect of retinal microvasculature in the association between blood pressure and subchronic BC exposure.

In conclusion, we found a positive association between blood pressure and subchronic black carbon exposure in healthy adults. This finding adds evidence to the association between black carbon exposure and cardiovascular health effects, with elevated blood pressure as a plausible intermediate effector. Our results suggest that the changes in a person's blood pressure as a result of subchronic black carbon exposure operate independently of the retinal microcirculation.

1. Introduction

Short-term and long-term exposures to particulate matter air pollution contribute to cardiovascular morbidity and mortality (Brook et al., 2010; Laden et al., 2006). Altered autonomic function of the heart, changes in micro- and macrovascular reactivity, induction of systemic inflammation, endothelial dysfunction and altered peripheral resistance of the blood vessels can mediate these cardiovascular effects (Mills et al., 2009). The microcirculation determines the overall peripheral resistance and microvascular alterations may lead to blood pressure elevation and an increased risk for developing hypertension (Boudier et al., 1992; Levy et al., 2001). Adar et al. (2010) and Louwies et al. (2013) have studied the impact of air pollution on the retinal microcirculation. These authors found that retinal arteriolar narrowing is associated with long-term and short-term exposure to air pollution (Adar et al., 2010; Louwies et al., 2013). Additionally, retinal arteriolar narrowing has been associated with increased blood pressure and hypertension (Wong and Mitchell, 2007). Thus, microcirculatory changes in the retina are potentially relevant in the association between air pollution exposure and blood pressure changes.

Epidemiological research and animal studies have produced positive, negative and null associations between blood pressure and ambient air pollution (Brook, 2007). These outcomes can be explained by study-specific differences such as population characteristics, dose and duration of the exposure that are different between studies. Furthermore, the chemical composition of particulate matter is heterogeneous and varies between studies. For instance, $PM_{2.5}$ (particulate matter with a diameter smaller than 2.5 μm) exposure in high-traffic areas had a stronger effect on blood pressure compared with $PM_{2.5}$ in low-traffic

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areas (Auchincloss et al., 2008; Brook et al., 2009). Furthermore, spatial and temporal variability in pollution sources may obscure associations between PM and blood pressure. Most epidemiological studies rely on central monitor data or complex models to estimate PM concentrations at the participant's residence. Exposure is however strongly related to an individual's time–activity patterns and time spent indoor and outdoor (Dons et al., 2011).

Exposure misclassification may occur with central monitor data and may lead to incorrect estimation of cardiovascular health effects associated with air pollution exposure (Brook et al., 2011; Padro-Martinez et al., 2012).

Black carbon (BC), a by-product of fuel combustion and a constituent of particulate matter, has been associated with systemic inflammation and oxidative stress, decreased flow-mediated dilation of the brachial artery and reduced parasympathetic tone (Alexeeff et al., 2011; O'Neill et al., 2005; Park et al., 2008; Schneider et al., 2010). Mordukhovich et al. (2009) and Wilker et al. (2010) reported positive associations between short-term BC exposure, measured as the concentration averaged over the 7 days preceding each study visit, and systolic and diastolic blood pressure (Mordukhovich et al., 2009; Wilker et al., 2010). Schwartz et al. (2012) reported an association between blood pressure and modelled long-term BC concentrations. A 0.32 μg/m^3 increase in BC was significantly associated with a 2.64 mm Hg increase in systolic blood pressure and a 2.41 mm Hg increase in diastolic blood pressure (Schwartz et al., 2012). Zhao et al. (2014) measured personal BC exposure using portable measuring devices in a study that investigated the effects of BC on blood pressure in 65 persons suffering from the metabolic syndrome. A short-term BC increase of 1 μg/m^3, 10 h prior to the study visit, was associated with a 0.53 mm Hg increase in systolic blood and a 0.37 mm Hg increase in diastolic blood pressure (Zhao et al., 2014).

We explore the association between blood pressure, short-term and subchronic BC exposure in this study. Subchronic BC exposure was calculated based on personal monitoring during one week with portable measuring devices and data from a reference station. During this 1-week period we repeatedly measured blood pressure and retinal vessel diameters. The retinal microcirculation was measured to study the potential mediating effect of the microcirculation in the relationship between BC exposure and blood pressure.

2. Methods

2.1. Study design

A total of 130 nurses working in the north of Belgium were invited and 99 (76%) agreed to participate. Fifty five nurses (56%) could be monitored in this study. The predominantly female participants were aged between 22 and 59 years and reported to be free of cardiovascular diseases and diabetes. Every participant was monitored during one average working week between April and May 2013. Clinical examinations were scheduled for every participant on Tuesday, Thursday, Saturday and Monday between 7 am and 9 pm [mean difference between repeated measurements was 1 h (range, 0.1–1.9 h)]. 75% of the participants underwent all 4 examinations, 23% completed 3 examinations, whereas 2% completed 2 examinations. Participants were not asked to fast before the visits. Blood pressure measurements and retinal images were collected during each examination. A venous blood sample was collected on the last day of the study. Gamma-glutamyl transpeptidase (γ-GT) was measured as a marker for liver function and alcohol consumption. Haemoglobin A1C was measured as a glycemic index and metabolic marker for diabetes. Participants completed a questionnaire on their smoking status, medical history and current medication use. All participants provided written informed consent. The ethics boards of Hasselt University and University Hospital Antwerp approved the study.

2.2. Blood pressure measurement

Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were measured with an automated device (Stabilograph, Stolberg, Germany) according to the guidelines of the European Society of Hypertension (Parati et al., 2008). After the participants had rested in a sitting position for 5 min, SBP, DBP and HR were measured five times consecutively during each of the 4 study visits. The average of the last three measurements collected during the examinations was used in the analysis.

2.3. Retinal photography and grading

A Canon 45° 6.3 megapixel digital nonmydriatic retinal camera (Hospithera, Brussels, Belgium) was used. The fundus of the right eye and the left eye of each participant were photographed twice during each study visit. Participant characteristics were masked for the trained grader before review and analysis of the retinal images. IVAN retinal image analysis software was used to measure retinal vessel diameters according to previously reported protocols (Knudtson et al., 2003; Wong et al., 2004a). Retinal vessel calibres were summarized as the Central Retinal Arteriolar Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE) in each picture. The equivalents represent a summary of vessel diameters within an area equal to 0.5–1 disc diameters from the optic disc margin. Average CRAE and CRVE values were calculated for each study visit based on the four images.

2.4. Exposure assessment

2.4.1. Personal black carbon exposure

Personal exposure to black carbon (BC) was measured continuously for 7 consecutive days (from Tuesday to next week's Monday) with a portable MicroAeth Model AE51 (Aethlabs, San Francisco, California, US) on a 1-min time resolution. A short tube was attached to the inlet of the aethalometer, giving the participants the opportunity to put the device in a purse or backpack while still sampling ambient air. Air was drawn over a Teflon-coated borosilicate glass fibre filter at a flow rate of 100 ml/min, resulting in BC accumulation on the filter. The attenuation of light at 880 nm was measured and converted into a BC concentration (ng/m^3). The filter was replaced every two days to prevent filter saturation. The participants were instructed to carry the device with them at all times, but for indoor activities they were allowed to leave it in the room where the majority of the time was spent. Raw BC data were processed before they were used. Measurements with high attenuation (ATN > 75) or an error code were excluded (Dons et al., 2011). Next, data were smoothened with an algorithm that was developed by the Environmental Protection Agency (Hagler et al., 2011).

Short-term exposure windows (24 h and 48 h) were calculated by taking the average of all BC measurements 24 h or 48 h before the clinical visit.

2.4.2. Calculated personal subchronic black carbon exposure

Subchronic BC exposure was calculated based on the personal BC exposure measured during the study period. This was done using the following formula: Subchronic BC exposure = Personal BC measurement × (Refsite yearly average / Refsite week average). The personal BC measurement was calculated as the average BC exposure over the whole week for each participant. This timeframe can capture the time–activity pattern during an average working week (Dons et al., 2012). "Refsite yearly" represents the average BC concentration during the year 2013. "Refsite week average" represents the average BC concentration at the BC reference monitor during the same week as the personal BC measurements. The latter factor allows correcting for varying ambient concentrations during the study period. The monitoring station of Dessel that is operated by the Flemish Environment Agency was chosen as reference. The station is equidistant from both

study locations. The personal subchronic BC exposure represented the main exposure variable in our study (Dons et al., 2012).

2.5. Outdoor temperature

The 24 h mean outdoor temperature measured at the meteorological stations of Diepenbeek and Sint–Katelijne–Waver was obtained from the Belgian Royal Meteorological Institute (Ukkel, Belgium).

2.6. Traffic-related GIS-variables

The home address of the nurses was geocoded. The coordinates were manually adapted when they differed from the actual position of the residence. Residential distance to major roads was calculated with the Tele Atlas MultiNet dataset in ArcGIS 9.3. Attributes include name of street, route number, speed class, length and road classification (0: Motorways; 1: Roads belonging to 'Main road' major importance; 2: Other major roads; 3: Secondary roads; 4: Local connecting roads; 5: Local roads of high importance; 6: Local roads; 7: Local roads of minor importance; 8: Others). All roads of classes 0, 1 and 2 were classed as major roads.

2.7. Statistical analysis

Statistical analysis was carried out using SAS software (version 9.3, SAS Institute Inc., Cary, NC, USA). Continuous data were presented as mean ± standard deviation (SD) and categorical data as percentages (%) and frequencies. We used mixed models to investigate the association between blood pressure and BC exposure, the association between retinal vessel calibres (CRAE and CRVE) and BC exposure, and the association between blood pressure and CRAE and CRVE. We used random subject effects accounting for repeated measures and we applied an unstructured covariance structure. Models were adjusted for the following fixed effects: sex, age, body mass index (BMI), smoking behaviour, use of anti-hypertensive medication, γ-GT, haemoglobin A1c, distance to major road, location where the clinical visit took place, day of the week and average weekly temperature. In a sensitivity analysis, we excluded persons that were on anti-hypertensive medication and persons with a smoking history.

To assess the role of the microcirculation in the association between blood pressure and BC exposure, we first included CRAE and CRVE as additional covariates in our previously described models. Secondly, we performed a formal mediation analysis, which decomposes the total observed effect of BC exposure on blood pressure into a direct effect (DE) and an indirect effect (IE) that acts via the mediator of interest. In this analysis we used the average blood pressure of the study week as a response variable (i.e. one value per participant) and we adjusted for the same covariates as the mixed models, except day of the week. The direct effect, indirect effect and total effect were estimated by using the SAS macro developed by Valeri and Vanderweele (2013).

3. Results

The characteristics of the study population are summarized in Table 1. 93% of the 55 participants were women with a mean age ± SD of 41 ± 11 years. The mean BMI ± SD was 24.2 ± 4.5 kg/m^2. 39 study participants (71%) had never smoked and 14 persons (25)% were former smokers, whereas 2 persons (4%) were current smokers. Three participants (5%) used β-blockers as antihypertensive medication. All participants had a similar college degree and socioeconomic background. Average values ± SD of heart rate, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were 75 ± 25 bpm, 116 ± 12 mm Hg and 73 ± 8 mm Hg, respectively. Mean Central Retinal Arteriolar Equivalent (CRAE) and Central Retinal Venular Equivalent (CRVE) were 152.15 ± 12.65 μm and 211.28 ± 17.35 μm, respectively. Average values ± SD for γ-GT and haemoglobin A1C were 17.71 ± 12.21 U/l and 5.36 ± 0.25%, respectively. None of the participants had divergent values for γ-GT or haemoglobin A1C. Participants lived on average ± SD at a distance of 1714 ± 1629 m from a major road. This variable was introduced as a proxy for traffic noise exposure. The mean personal Black Carbon (BC) concentration measured during the week was 866 ± 425 ng/m^3. The derived subchronic BC exposure was 1334 ± 631 ng/m^3 and ranged from 338 ng/m^3 to 3889 ng/m^3.

Table 1
Population characteristics (n = 55).

Parameter	Mean ± SD or number (%)
	41 ± 11
Sex	
Female	51 (93%)
Ethnicity	
Caucasian	54 (99%)
African	1 (1%)
Health indicators	
Body mass index, kg/m^2	24.2 ± 4.5
Systolic blood pressure, mm Hg	116 ± 12
Diastolic blood pressure, mm Hg	73 ± 8
Heart rate, bpm	75 ± 25
Central retinal arteriolar equivalent, μm	152.15 ± 12.65
Central retinal venular equivalent, μm	211.28 ± 17.35
Gamma-glutamyl transpeptidase, U/Ll	17.71 ± 12.21
HemoglobineHaemoglobin A1c, %	5.36 ± 0.25
Haemoglobin A1c, %	
Smoking status	
Never/former	53 (96%)
Current	2 (4%)
Antihypertensive medication	3 (5%)
Distance to major road, m	1714 ± 1629
Personal black carbon exposure, ng/m^3	866 ± 425
Subchronic black carbon exposure, ng/m^3	1334 ± 631

Blood pressure components were significantly associated with CRAE and CRVE. All models were adjusted for sex, age, BMI, smoking behaviour, use of anti-hypertensive medication, γ-GT, haemoglobin A1c, distance to major road, location of the clinical visit, day of the week and average weekly temperature. SBP and DBP decreased with 0.33 mm Hg (95% CI: −0.49 to −0.18, p = 0.0001) and 0.25 mm Hg (95% CI: −0.38 to −0.13, p = 0.0002) for each 1 μm increase in CRAE. The corresponding estimates for CRVE were 0.16 mm Hg decrease (95% CI: −0.27 to −0.06, p = 0.0033) and 0.14 mm Hg decrease (95% CI: −0.22 to −0.06, p = 0.0014), respectively.

We did not find any association between short-term BC exposure (lag 24 h and lag 48 h) and SPB, DBP, CRAE or CRVE (results not shown).

In contrast to short-term exposure, subchronic BC exposure was associated with both SBP and DBP. Independent of the aforementioned covariates, a SD-increase in BC concentration was associated with a 2.77 mm Hg (95% CI: 0.39 to 5.15, p = 0.027) increase in SBP and a 2.35 mm Hg (95% CI: 0.52 to 4.19, p = 0.016) increase in DBP (Fig. 1A, Model 1). Subsequently, the association between BC exposure and the retinal microcirculation was assessed. An association between BC exposure and CRVE was identified. A SD-increase in BC exposure was associated with a 4.76 μm (95% CI: 0.27 to 9.24, p = 0.044) increase in CRVE (Fig. 1B, Model 1). Addition of SBP and DBP to the model, did not change the association: a SD-increase in BC exposure was associated with a 5.65 μm (95% CI: 1.33 to 9.96, p = 0.014) increase in CRVE (Fig. 1B, Model 2). The association between CRVE and BC remained significant when an additional correction for fellow vessel diameter was included (Fig. 1B, Model 3). No associations between BC exposure and CRAE were identified (Fig. 1B).

We explored the role of the microcirculation as a mediator of the association between BC and blood pressure. In a first analysis, we tested the mediating effect of CRAE and/or CRVE by adding these factors as covariates to our previously described model. After correction for CRAE, a SD-increase in BC exposure was associated with a 2.98 mm Hg (95% CI: 0.68 to 5.28, p = 0.015) increase in SBP and a 3.09 mm Hg (95% CI: 1.49 to 4.69, p = 0.0005) increase in DBP (Fig. 1A, Model 2). When both

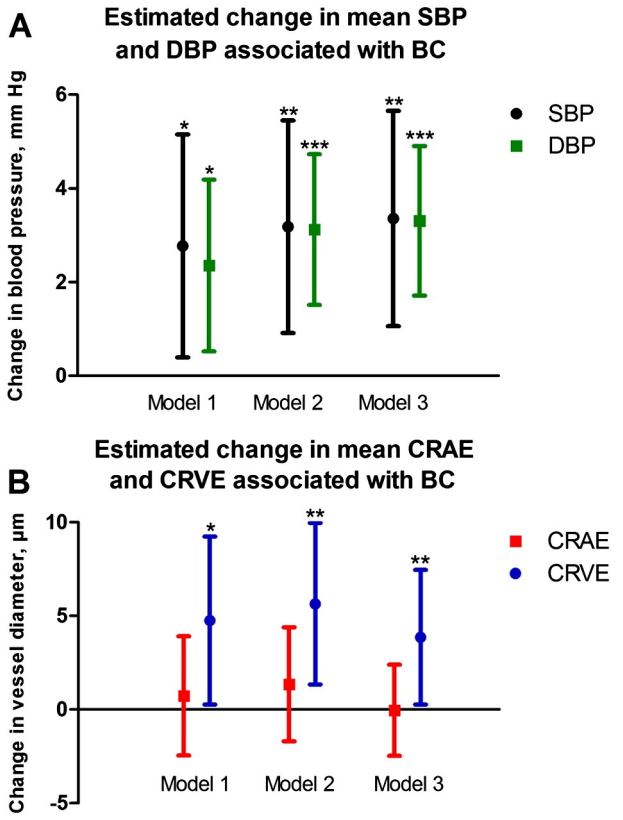

Fig. 1. A. Effect sizes (95% confidence interval) express the change in systolic/diastolic blood pressure (SBP/DBP) (mm Hg) for an SD (631 ng/m^3) increase in subchronic BC exposure. All models include 55 persons. Model 1 is corrected for sex, age, BMI, smoking behaviour, use of anti-hypertensive medication, γ-GT, haemoglobin A1c, location where the clinical visit took place, distance to major road, day of the week, average weekly temperature. Model 2, includes all aforementioned covariates and is further corrected for central retinal arteriolar equivalent. Model 3 also includes central retinal venular equivalent. Statistical significance is expressed as: *p < 0.05, **p < 0.01, ***p < 0.001. B. Effect sizes (95% confidence interval) express the change in Central Retinal Arteriolar/Venular Equivalent (CRAE/CRVE) (µm) for an SD (631 ng/m^3) increase in subchronic BC exposure. All models include 55 persons. Model 1 is corrected for sex, age, BMI, smoking behaviour, use of anti-hypertensive medication, γ-GT, haemoglobin A1c, location where the clinical visit took place, distance to major road, day of the week, average weekly temperature. Model 2 also includes SBP and DBP. Model 3 additionally includes fellow vessel diameter. Statistical significance is expressed as: *p < 0.05, **p < 0.01.

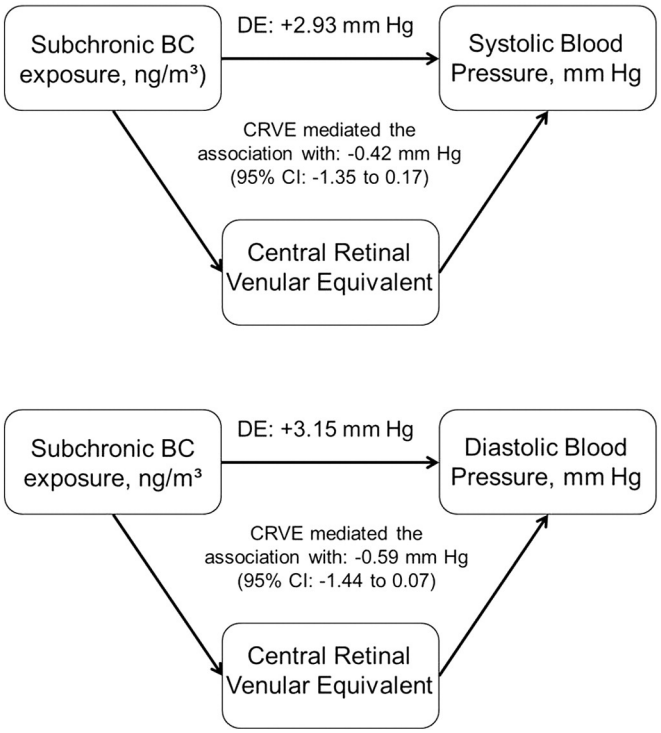

Fig. 2. Mediation of the effect subchronic BC exposure (ng/m^3) on blood pressure through Central Retinal Venular Equivalent. The figure shows Central Retinal Venular Equivalent as a potential mediator in the association between systolic/diastolic blood pressure and subchronic BC exposure. The estimates of the mediation through CRVE and the estimates of the direct effect (DE) of subchronic BC exposure on systolic/diastolic blood pressure are presented. The models were adjusted for sex, age, BMI, smoking behaviour, use of anti-hypertensive medication, γ-GT, haemoglobin A1c, distance to major road, location of the where the clinical visit took place and average weekly temperature.

CRAE and CRVE were considered in the fully adjusted model, the effect estimates changed slightly. A SD-increase in BC exposure was associated with a 3.10 mm Hg (95% CI: 0.77 to 5.43, p = 0.012) increase in SBP and a 3.25 mm Hg (95% CI: 1.66 to 4.85, p = 0.0002) increase in DBP (Fig. 1A, Model 3). Secondly, we performed a formal mediation analysis. CRVE was significantly associated with BC exposure and BP. Therefore, the mediation analysis was conducted with CRVE as a potential mediator. The results of the mediation analysis are shown in Fig. 2. The total effect of BC on SBP or DBP is decomposed into a direct and indirect effect, the latter mediated by CRVE. The effects of BC on blood pressure mediated by CRVE, were not significant and were respectively 0.42 mm Hg decrease in SBP (95% CI: −1.35 to 0.17) and 0.59 mm Hg decrease in DBP (95% CI: −1.44 to 0.07).

Finally, we conducted a sensitivity analysis to investigate the effect of anti-hypertensive medication use and smoking behaviour on the reported associations in our previously reported models (Model 1, Model 2 and Model 3). First, we excluded persons on antihypertensive treatment (n = 3). This did not change the reported associations in any of the presented models (Supplementary Table 1). Second, we excluded current smokers (n = 2) from the analysis. The associations were not affected in any of the models (Supplementary Table 2).

4. Discussion

Blood pressure in healthy nurses was positively associated with subchronic black carbon (BC) exposure. The microcirculation, assessed with retinal imaging, did not mediate the observations. The associations were identified at ambient BC exposure levels in healthy individuals. This is suggestive for the absence of a threshold value at which BC can induce health effects. Small increases in SBP or DBP in the normotensive range may eventually lead to a chronically elevated blood pressure or hypertension. The latter are associated with an increased long-term risk for cardio- and cerebrovascular events (Lewington et al., 2002; Vasan et al., 2001). A SD-increase of 631 ng/m^3 in BC concentrations was associated in our model with a 2.49 mm Hg increase in SBP (95% CI: 0.08 to 4.91, p = 0.049) and a 2.65 mm Hg increase in DBP (95% CI: 0.93 to 4.37, p = 0.0041). Our findings have public health relevance. Assuming that BC concentrations could be lowered to background level and this leads to a population-wide reduction of 2.49 mm Hg in SBP, such an effect is then likely to result in a 9% decrease in coronary heart disease and a 13% decrease in stroke (30), and a 5 to 10% decrease of cardiovascular disease (32, 33).

Our findings are comparable with other studies that investigated the association between long-term BC exposure and blood pressure. We recalculated the effect estimates presented in these studies in order to allow for a direct comparison with our effect estimates. For an identical increase in annual BC exposure, Wilker et al. (2010) reported increases in SBP and DBP of 2.14 mm Hg (95% CI: 0.15 to 4.14) and 1.28 mm Hg (95% CI: 0.22 to 2.33), respectively (Wilker et al., 2010). For an identical increase in annual BC exposure, Schwartz et al. (2012) found increases of 5.20 mm Hg (95% CI: 2.90 to 7.49) and 4.75 mm Hg (95% CI: 3.49 to 6.01) for SBP and DBP, respectively (Schwartz et al., 2012). Zhao et al. (2014) used personal measurements of BC exposure and reported, for

a comparable increase in BC, an acute increase in SBP and DBP of respectively 0.26 mm Hg (95% CI: 0.08 to 0.43) and 0.18 mm Hg (95% CI: 0.05 to 0.31) (Zhao et al., 2014). We did not find an association between blood pressure changes and short-term BC exposure. Differences in study design and exposure range might explain this.

Our study was concerned with the effects of subchronic BC exposure on blood pressure. Furthermore, Zhao et al. reported an average exposure of 5.08 $\mu g/m^3$ whereas our average short-term exposure was 0.87 $\mu g/m^3$.

The microcirculation determines the peripheral resistance and thus exerts a great influence on blood pressure. Adverse manifestations of cardiovascular diseases are also likely to occur in microvascular beds (Levy et al., 2001; Mulvany, 1991). Therefore, we investigated the association between the microcirculation and BC exposure in our current study. We observed a positive association between retinal venules and BC exposure. During our previous work that focused on short-term microcirculatory effects of ambient BC exposure we observed retinal arteriolar narrowing in association with BC exposure (Louwies et al., 2013). In contrast, the current study focused on subchronic BC exposure. Our current study panel was confronted with a narrow range of BC exposure levels, whereas exposure levels were a tenfold higher in our previous study.

The influence of the microvasculature on the association between BC exposure and blood pressure was assessed by including CRAE and CRVE parameters in the statistical models. This approach did not change the associations between BC exposure and blood pressure. We also conducted a mediation analysis to formally test the interference of the microvasculature on the association between blood pressure and BC exposure. Mediation analysis requires a significant association between the exposure and the mediator, a significant association between the mediator and the outcome, and a significant association between the exposure and the outcome (Baron and Kenny, 1986). In the presence of mediation, the effect of the exposure on the outcome is expected to be reduced after controlling for the mediator. Only CRVE was considered as a candidate for mediation because the significant association between CRAE and BC exposure, one of the requirements to conduct mediation analysis, was not met in our study. The mediation analysis did not reveal evidence that supported our hypothesis that the microvasculature, as measured in the retina, mediates the association between blood pressure and subchronic BC exposure. However, this statement should be interpreted with caution because of the small size of the study.

The exact pathophysiological mechanism underlying the rise in blood pressure caused by BC exposure remains to be further elucidated. BC inhalation may favour the sympathetic nerve activity via alterations in the cardiovascular autonomic nervous system (Gold et al., 2000; Magari et al., 2001; Pieters et al., 2012). Activation of α-adrenergic receptors leads to vasoconstriction and blood pressure increase (Bartoli et al., 2009). The stress imposed on the arterial vessel walls may lead to hypertrophic remodelling and an increase of medial thickness, which can further increase peripheral resistance (Heagerty et al., 2010; Mulvany, 2002). Regular incidents that trigger a blood pressure increase may result in narrowing of retinal arterioles. In this respect, research has indicated that the narrowing of the retinal blood vessels precedes hypertension (Ikram et al., 2006; Wong et al., 2004b). At the same time it should be note that blood pressure is very tightly controlled by several feedback mechanisms. The microcirculatory response is only one effector pathway in this complex mechanism in which the renin–angiotensin pathway and the baroreceptor reflex also play an important role (Reid, 1992).

The strength of our study is the combination of personal monitoring data and ambient BC concentrations from a reference station to estimate subchronic BC exposure. It has been shown before that this approach prevents exposure misclassification. The cost of personal monitoring devices typically limits the size of these personal monitoring studies over longer time periods. After correction for variation in ambient BC exposure, our subchronic exposure estimate is a reliable proxy and preferable over modelled exposure estimates. Furthermore, it has been shown that a 1-week monitoring of a representative working week can capture in a reliable way the time–activity patterns that are known to influence BC exposure (Dons et al., 2011). Blood pressure is a highly variable phenotype and we have anticipated this by measuring blood pressure at four distinct time points with 5 measurements during each study visit. The circadian rhythm influences blood pressure. We accounted for this by measuring blood pressure of each study participant at the same time at each study visit. When the time of day was added to our models, our reported associations did not change (data not shown). Because the time difference between study visits was on average only 1 h, blood pressure was not influenced by a circadian pattern. A third strength is that we have studied a panel of mostly female participants who reported to be free from clinically diagnosed cardiovascular diseases. A homogeneous study population reduces between-individual variability and increases the statistical power in a small panel. To our knowledge, we are introducing the first study with a retinal microvascular measurement to assess the role of the microvasculature in the association between blood pressure and subchronic BC exposure in healthy adults. We have observed a blood pressure increase associated with subchronic BC exposure. The microvasculature, assessed by retinal image analysis, did not mediate these effects in our study.

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Disclosures

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