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Personal exposure to traffic-related air pollutants and relationships with respiratory symptoms and oxidative stress: A pilot cross-sectional study among urban green space workers

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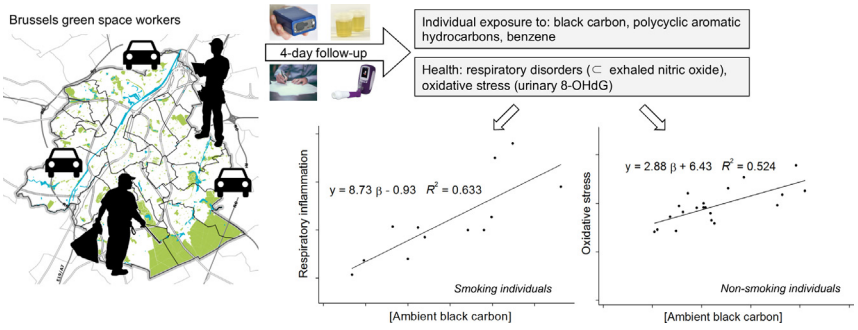
HIGHLIGHTS

- Individual exposure to and health effects of black carbon are underexplored.
- A pilot cross-sectional study using personal/bio monitoring was set-up for green workers.
- A positive linear association existed between BC and respiratory inflammation.
- A positive linear association existed between BC and oxidative stress.
- Associations were influenced by smoking status.

GRAPHICAL ABSTRACT



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ABSTRACT

Exposure to ambient air pollution has been associated with various adverse health effects including respiratory, cardiovascular and neurological diseases. Exposure data for some specific pollutants and settings are however still insufficient and mechanisms underlying negative health outcomes are not fully elucidated. This pilot study aimed to assess individual exposure to three traffic-related air pollutants, black carbon (BC), polycyclic aromatic hydrocarbons (PAHs) and benzene, and the relationship with respiratory and oxidative stress outcomes in a cross-sectional sample of 48 green space workers in Brussels, Belgium. Participants were followed during four consecutive working days in 2016–2017 during which their individual exposure to BC, PAHs, benzene and more generally air pollution was measured using aethalometers, urinary biomarkers (1-hydroxypyrene, 1-naphthol, 2-naphthol, *S*-phenylmercapturic acid) and questionnaires. Data on respiratory health and oxidative stress were collected using questionnaires and respiratory/urinary biomarkers (exhaled nitric oxide [NO], 8-hydroxydeoxyguanosine [8-OHdG]). Associations between exposure and health outcomes were investigated

Abbreviations: 8-OHdG, 8-hydroxy-2′-deoxyguanosine; BC, Black Carbon; CI, Confidence Interval; LOQ, Limit of Quantification; LOD, Limit of Detection; MS, Mass Spectrometry; NO, Nitric Oxide; PAHs, Polycyclic Aromatic Hydrocarbons; SD, Standard Deviation; SPMA, S-PhenylMercapturic Acid; UPLC, Ultra Performance Liquid Chromatography; WHO, World Health Organization.

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using comparison tests and linear regression models, after stratification by present-day smoking status. Spatial variation in BC exposure was high, with concentrations varying between 0.26 and 5.69 μg/m^3. The highest levels were recorded during transport and, to a lesser extent, in green spaces located in the vicinity of roads with high traffic intensity. Concentrations of PAHs and benzene biomarkers did not systematically exceed the limits of detection. Among smokers, respiratory inflammation increased linearly with exposure to BC measured over the four days of follow-up (β = 8.73, 95% CI: 4.04, 13.41). Among non-smokers, oxidative stress increased linearly with BC measured on the fourth day (β = 2.88, 95% CI: 1.52, 4.24). Despite some limitations, this work supports the hypothesis that BC induces respiratory inflammation and oxidative stress. It also highlights the value of this compound as well as exhaled NO and urinary 8-OHdG biomarkers to detect early/mild effects of air pollution.

1. Introduction

Ambient air pollution represents a major environmental issue affecting all earth's systems. For humans, in vitro, in vivo and epidemiological studies have shown that short term and long term exposure to air pollution, at levels frequently recorded in Europe, contribute to the expression of various non-communicable diseases (WHO, 2013). Effects can be acute or chronic and include minor respiratory irritations, asthma, chronic obstructive pulmonary disease, cardiovascular disorders, lung cancer or neurological effects (Brunekreef and Holgate, 2002; Kampa and Castanas, 2008). Although each pollutant presents its own specific toxic properties, induction of a localized/systemic inflammation and oxidative stress appear as important potential pathways (WHO, 2013). Overall, air pollution could be responsible for one out of ten deaths worldwide (World Bank, Institute for Health Metrics and Evaluation, 2016).

Despite substantive evidence, experts of the "Health risks of air pollution in Europe" (HRAPIE) project led by the World Health Organization (WHO) concluded in 2013 that several significant knowledge gaps persist (Henschel and Chan, 2013). "Road transport" was identified as a key emission source of concern due to its emission at low height, contributing to surface pollution in often densely populated areas (EEA, 2017). "Particles" were as for them recognized as important pollutants and "Respiratory health effects", "Cancer" as health risks of interest (Henschel and Chan, 2013). Urban populations and some professions highly exposed to traffic and particles appear in this context at particular risk. Part of these knowledge gaps arises from some frequent study limitations: the use of ecological study design, determination of exposure through modelling, focus on some common pollutants or exclusive use of self-reported symptoms for health assessment. While these approaches present strengths (easy implementation, work with large sample sizes, etc.), they also show limitations. Exposure concentrations derived from fixed-monitoring stations can for instance greatly differ from those observed in some microenvironments or at the individual level due to local urban characteristics, variation of pollution over time, personal's activity patterns, etc. (Dons et al., 2011; Park and Kwan, 2017; Steinle et al., 2013, 2015; Violante et al., 2006). Self-reported symptoms can suffer from information bias (Althubaiti, 2016). By taking into account both the spatiotemporal variability of air pollution and human mobility, personal mobile air pollution sensors and biomarkers appear as interesting alternatives to adequately estimate exposure, objectively measure health and so accurately assess the relationships between air quality and health. In the field of exposure assessment, interest is growing for black carbon (BC) because of its potential intrinsic toxicological properties (considered, among others, as possibly carcinogenic to humans) and its recognition as a valuable metric for evaluating the health risks associated with particles from road traffic (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2006; Janssen et al., 2012; WHO, 2013). Studies highlighted associations between short-term rise in personal BC exposure and increased arterial stiffness in healthy adults (Provost et al., 2016) or between personal BC exposure and respiratory inflammation in subjects with respiratory diseases (Jansen et al., 2005). Considering their toxic/carcinogenic properties and their relationships with traffic, the study of polycyclic aromatic hydrocarbons (PAHs) and benzene is also relevant (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2009; WHO, 2013).

Located in the centre of Belgium, the Brussels-Capital Region constitutes the most urbanized and densely populated territory of the country (DG Statistics - Federal Public Service Economy, 2017). This region counts few polluting industries or agricultural activities but has high volumes of traffic with around 370,000 cars daily running in Brussels (Brussels Environment, 2015) and two-thirds of BC concentrations are traffic-related. Although air quality has improved over the last years, the region still often records high levels of air pollution at the local scale, with frequent exceedances of the WHO guideline values (Fierens et al., 2017). In 2016, annual average concentrations of $PM_{2.5}$ reached 13.4 μg/m^3, a value exceeding the WHO guideline value of 10 μg/m^3 (Fierens et al., 2017; WHO, 2006). Few studies investigated the adverse health effects of this exposure to urban air pollution in the region. One ecological time series demonstrated a temporal relationship between daily changes in air pollutant concentrations (PM_{10}, NO_2) and daily asthma/chronic obstructive pulmonary disease medication sales (Casas et al., 2016) while a field study carried out among Brussels policemen and foresters from the countryside investigated the potential of several serum proteins to detect effects of air pollution on the pulmonary epithelium (Berthoin et al., 2004).

Based on these elements, a pilot cross-sectional study was set-up in the Brussels-Capital Region with the goal of investigating personal exposure to BC, PAHs, benzene and the relationship with respiratory health and oxidative stress outcomes. This work focused on a sample of urban green space workers who, due to the nature of their outdoor work, are continuously exposed to emissions from road traffic.

2. Material and methods

2.1. Study design and population of interest

A pilot cross-sectional study was conducted in the Brussels-Capital Region in Belgium. The population of interest consisted of workers employed by Brussels Environment (the local administration responsible for the environment and energy) in charge of the daily maintenance of several parks, gardens and reserves of the region. To limit the impact of confounding factors, the recruitment focused on individuals (1) aged between 20 and 55 years, (2) who had no pathology limiting their understanding of the study and (3) who did not suffer from a respiratory disease in the 15 days preceding their involvement. Participation relied on volunteering and participants gave prior written informed consent.

48 workers accepted to participate in the research project: 26 were park wardens, 11 gardeners, 5 work supervisors, 4 forestry/river maintenance agents and 2 repairmen. Their intervention zones were distributed over the region and participants were exposed during their working time to different air pollution levels according to the traffic intensity surrounding their workplace. Data collection was carried out between August 2016 and April 2017. Individuals were followed for four consecutive working days during which their individual exposure to

air pollution and their health status were assessed. The researcher in charge of data collection met each participant at the workplace at the beginning and end of follow-up to ensure a good guidance and provide/recover the measuring devices, questionnaires, the biological samples and perform the necessary tests.

This study conformed to the standards of the Declaration of Helsinki and was approved by the Ethics committee of the University Hospitals of Leuven (Ref.: S58976; B322201628202). Biological samples were analysed respecting the ISO 17025 standard.

2.2. Exposure assessment

Individual exposure to traffic-related air pollution was measured using different methods. First, individual exposure to BC was assessed for each participant, during the four days of follow-up, from breakfast till bedtime, using a pocket-sized aerosol monitor microAeth AE51® (Aethlab, San Franciso, USA) on a 1-min time resolution. Measurements relied on the continuous collection of air samples (air flow fixed at 100 mL/min) through a Teflon filter analysed by infrared radiation (880 nm) (Aethlabs, 2016). Each device was maintained, calibrated annually and intercomparisons were carried out on a regular basis. The filter was replaced every day to prevent saturation. The aethalometer was carried on a shoulder bag and collected air through a tube attached to the sensor inlet. Daily activities were recorded in a log book. This permitted to assess participant's general exposure to BC as well as exposure in three microenvironments: outdoor workplace, transport and home, for each day and the whole four-day follow-up. For all of these conditions and following the Van Poppel's approach (Van Poppel et al., 2013), two BC concentration types were considered: (1) the total BC concentration corresponding to the one measured by the device and including both the local and background components. Representative of the real exposure of participants, it was mainly used in the statistical analyses; (2) the local BC concentration, obtained after subtracting the background BC concentration (measured in fixed monitoring stations located in remote areas of the Brussels-Capital Region) from the total BC concentration. This value was more representative of the local traffic contribution and facilitated comparisons between measurements carried out on different days. In both cases, median concentrations (expressed in $\mu g/m^3$) were used due to the non-normal distribution of BC concentrations. These calculations were preceded by a data cleaning step during which all data showing an error code were excluded from the analyses. Negative values, corresponding to false decrease in measured absorption, were not eliminated as offset in the next observations (their exclusion would have led to an overestimation of BC concentrations) (Dons et al., 2012). Temporary technical issues encountered with the aethalometers (10 cases) and refusal of certain participants to carry out measurements with the device outside of their working hours (2 cases), led to the exclusion of some individuals for part of the further analyses. Reasons given by the latter included: discomfort caused by carrying the device and constraints associated with filling out the log book.

Secondly, individual exposure to PAHs was determined using three urinary biomarkers: 1-hydroxypyrene, 1- and 2-naphthol. 1-hydroxypyrene is a metabolite of pyrene. It has been used in various research contexts since 1985 and is now identified as a solid and sensitive indicator of exposure to PAHs mixture (Hansen et al., 2008; Jongeneelen, 2001). Its estimated urinary excretion half-life ranges from 6 to 35 h (Jongeneelen, 2001). 1- and 2-naphthol are the two most frequently analysed metabolites of naphthalene, a second group of PAHs (Preuss et al., 2003). 2-naphthol has notably been identified as equally or even more representative of exposure to PAHs in ambient air than 1-hydroxypyrene (Yoon et al., 2012). A urine sample (about 40 mL) was collected from each participant (one refusal, for personal reasons) at the end of the fourth day of follow-up in a Falcon tube. Samples were kept at 4 °C during transport and then stored at −80 °C in the lab prior to the analysis. To reduce bias, participants were asked not to eat grilled/fried/barbecued food during the 72 h preceding the urine sampling. Samples were analysed using a UPLC-MS-MS instrument (Acquity Xevo TQ-S triple quadrupole ®, Waters, Milford, USA). For 1-hydroxypyrene, 1- and 2-naphthol, concentrations were measured in negative electrospray ionization mode using an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm). The solvents were composed of 0.2% NH_4OH in water (solvent A) and 0.2% NH_4OH in acetonitrile (solvent B, used for the gradient elution) and the flow rate was 0.3 mL/min. Total runtime per sample was 10 min. As these analytes can exist in a free and glucuronidated form, two injections were carried out. To measure the free form, 500 μL of urine were diluted with 400 μL of solvent A and 100 μL of 13C-heavy labelled internal standard (100 ppb in methanol) before injection of 10 μL. To measure the glucuronidated form, a deconjugation step was carried out. To 500 μL of urine, 100 μL of 1 M NH_4-acetate and 10 μL of a β-Glucuronidase/Arylsulfatase (*Helix pomatia*, Roche Diagnostics) solutions were added. The enzymatic reaction was done overnight at 37 °C. After that, 100 μL of 13C-heavy labelled internal standard (100 ppb in methanol) and 290 μL of solvent A were added before centrifugation and injection of 10 μL. For each batch of samples, blanks and control samples (low and high concentrations) were added at the beginning, middle and end of the series to control the sensitivity and accuracy of the method. During data analysis, all chromatograms were processed using the TargetLynx™ software (Waters, Milford, USA) and manually reviewed. The limits of detection (LOD) and quantification (LOQ) for 1-hydroxypyrene were respectively 0.07 ng/mL and 0.2 ng/mL. For 1-naphthol, LOD and LOQ reached respectively 0.6 ng/mL and 2.0 ng/mL. For 2-naphtol, corresponding values equalled 0.15 ng/mL and 0.5 ng/mL. For further analyses, measurement values between the LOD and LOQ were treated “as is” (without modification) (Guo et al., 2010; Harel et al., 2014) while values below the LOD were replaced by the respective half-value LOD.

A fourth urinary biomarker, *S*-phenylmercapturic acid (SPMA), was used to measure participants' exposure to benzene. This metabolite was selected over trans, trans-muconic acid because of its better reliability (Boogaard and van Sittert, 1996; Hoet et al., 2008). Its average urinary elimination half-life may vary between 9 h and 13 h (Hoet et al., 2008). This compound was assayed under UPLC-MS-MS conditions similar to the ones applied for 1-hydroxypyrene, 1- and 2-naphthol, except that this method lasted only 8 min. The LOD and LOQ were respectively 0.2 ng/mL and 0.7 ng/mL.

Knowing urinary concentrations of biomarkers can be influenced by sample collection timing or urine output of each person, biomarker concentrations were normalized to the urinary concentrations of creatinine (expressed in μg metabolite/g creatinine) (Tang et al., 2015). Creatinine assay relied on UPLC-MS-MS with positive electrospray ionization mode using a HSS T3 C18 column. The solvents were composed of 0.1% NH_4OH in water (solvent A) and 0.1% NH_4OH in acetonitrile (solvent B, used for the gradient elution). 100 μL of urine were diluted 10,000 times in solvent A, in three steps. 100 μL of 13C-heavy internal standard (1000 ppb in methanol) were added in the last step. 10 μL of sample were injected and the MS method lasted 5 min. The LOD and LOQ were respectively 3 ng/mL and 10 ng/mL.

In addition, participants were asked to fill in a self-administered questionnaire to provide information on their work conditions (place, type of tasks carried out, potential use of protective equipment, job history, etc.), home characteristics (surrounding environment, potential passive smoking, problems of humidity, chemicals used for house cleaning, recent renovation works, etc.), transport habits (means, duration) and leisure (do-it-yourself, etc.).

2.3. Health assessment

The study focused on various health endpoints using different assessment methods. First, detailed information on respiratory health (current and past diseases, nonspecific symptoms, etc.) and general health (global wellbeing, serious pathologies other than respiratory,

healthcare consumption, body mass index, etc.) were collected with a self-administered questionnaire. This document was specifically developed for this study based on validated questionnaires used in the "European Community Respiratory Health Survey" (Burney et al., 1994) and the "Belgian Health Interview Survey" (Sciensano, n.d.). Answers were analysed "as is" (without any changes) and after construction of indicators, including suffering from general respiratory symptoms if reporting sneezing, respiratory tract irritation, blocked/runny nose, chest tightness or coughing, abnormal expectorations.

Secondly, participants' airway inflammation was assessed using a quantitative non-invasive method: the measurement of the exhaled Nitric Oxide (NO). NO is a biological mediator synthesized in the respiratory tract and recognized as a marker of up-regulation of airway inflammation (Dweik et al., 2011). It has been analysed since several years for respiratory diseases monitoring (ATS and ERS, 2005) and is now increasingly used to assess the effect of air pollution on airways (Annesi-Maesano and Dinh-Xuan, 2016; Jacobs et al., 2010; Koenig et al., 2003). The measurement was carried out at the end of the fourth day of follow-up using a portable device NObreath® (Bedfont Scientific Ltd., Maidstone, UK). Measurements relied on the online analysis of exhaled breath by an electrochemical sensor (Bedfont, 2017). Recommendations of the American Thoracic Society, the European Respiratory Society and the manufacturer were respected (ATS and ERS, 2005; Bedfont, 2017). Participants were, among others, asked to exhale with a flow rate of 50 mL/s and not to eat, drink or smoke in the 2 h preceding the measurements. Arithmetic mean concentrations of best acceptable measurements were calculated (expressed in ppb).

Thirdly, a measure of oxidative stress was carried out using the urinary biomarker 8-hydroxy-2′-deoxyguanosine (8-OHdG). Resulting from the oxidative attack of DNA, this molecule is recognized since several years as a relevant marker of oxidative stress and carcinogenesis process (Valavanidis et al., 2009). It was analysed in the same urine sample as the one used for PAHs and benzene exposure assessment. The compound was quantified with UPLC-MS-MS in positive electrospray ionization mode using an Acquity UPLC BEH C18 column (2.1 × 100 mm, 1.7 μm). Solvents used were composed of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B, used for the gradient elution). The sample was diluted twice by adding 400 μL of solvent A and 100 μL of 13C-heavy labelled internal standard (100 ppb in methanol). 10 μL of sample were injected and the method lasted 10 min. The LOD and LOQ were respectively 0.15 ng/mL and 0.5 ng/mL. As for biomarkers of exposure, concentrations of 8-OHdG were normalized to the creatinine level.

2.4. Additional individual information

In addition to food and leisure habits, data on demographic, socio-economic characteristics and lifestyle (participants were classified as smokers or non-smokers at the time of the study) were collected through questionnaires.

2.5. Statistical analyses

Variables identified as non-normal (Shapiro-Wilk test) were log10-transformed and treated as such if enabling use of parametric tests. If not, non-parametric tests were used. All analyses linking exposure to air pollution and health parameters were carried out after stratification by smoking status (current versus non-smokers). Based on these principles, comparison of continuous variables between two groups (smokers or non-smokers, participants reporting or not general respiratory symptoms) was performed by Student *t*-test (preceded by a Bartlett variance comparison test) or Wilcoxon rank-sum (Mann-Whitney) test. Differences in more than two groups (BC concentrations among the different microenvironments) were assessed using the Spearman's rank correlation coefficient. Relationships between measures of exposure to air pollution and biomarkers of effects were investigated using linear regression methods. Models were checked for normality, homoscedasticity of residuals, linearity and model specifications. The level of statistical significance was set at $P < 0.05$ for all analyses. The STATA ® 13.0 software was used (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.).

Table 1
Characteristics of the study population (urban green space workers in Brussels; $n = 48$).

Variables	Value
Sex (male, %)	93.8
Age (year, median)	40
Education level (%)	
Primary education or no diploma	12.8
Secondary education	74.5
Higher education	12.8
Body mass index (kg/m^2, median)	24.2
Smoking status (non-smoker, %)	68.8
Residence environment (%)	
Urban	63.0
Semi-urban	21.7
Rural	15.2

3. Results

3.1. Characteristics of the study population

Males accounted for 93% of the sample and the median age equalled 40 years at the time of the study (Table 1). The majority of the participants had a weight in the normal range (63.6% had a body mass index below the overweight threshold: 24.9 kg/m^2). Most participants (68.8%) were non-smokers with non-significant difference with smokers with regard to age (Student *t*-test, $P = 0.453$) and body mass index (Student *t*-test, $P = 0.553$). Lastly, almost two thirds of the sample (63.0%) lived in an urban environment.

3.2. Exposure to pollutants

Total general exposure to BC (without distinction of the microenvironment) ranged from 0.29 μg/m^3 to 3.47 μg/m^3 with a median concentration of 1.11 μg/m^3 (Fig. 1). The lowest concentrations were measured

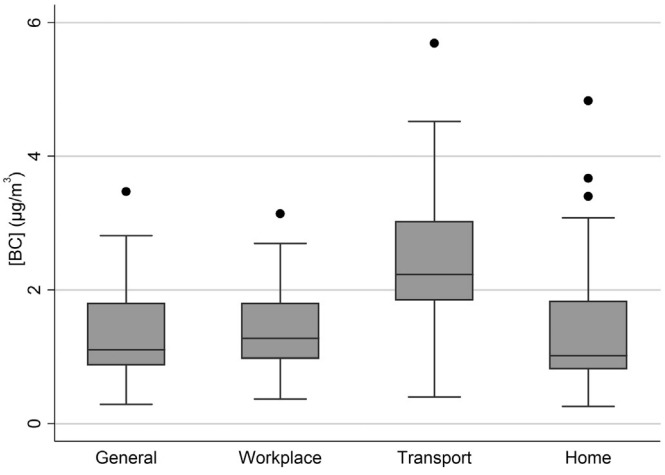

Fig. 1. Individual exposure to total black carbon measured in workplace, transport and home microenvironments over a four-day follow-up in a group of 48 urban green space workers in Brussels. Measurements carried out with mobile aethalometers. Boxplots represent percentiles 5, 25, 50, 75 and 95. Dots correspond to points beyond the upper quartile +1.5 interquartile range or lower quartile - 1.5 interquartile range. BC, Black carbon.

Table 2
Concentrations of urinary PAHs biomarkers measured in a group of 48 urban green space workers in Brussels.

PAHs biomarkers (μg/g creatinine; number of detectable observations)	Mean	SD	Min	P25	P50	P75	Max
1-hydroxypyrene							
All (14)	0.12	0.13	0.01	0.03	0.07	0.15	0.59
Non-smoking participants (5)	0.07	0.05	0.01	0.02	0.04	0.105	0.21
Smoking participants (9)	0.22	0.17	0.01	0.09	0.17	0.40	0.59
1-naphthol							
All (23)	4.31	7.49	0.12	0.33	0.77	3.62	30.12
Non-smoking participants (12)	0.78	0.77	0.15	0.29	0.46	1.04	3.62
Smoking participants (11)	11.84	9.69	0.12	1.25	9.92	21.9	30.12
2-naphthol							
All (47)	8.71	7.23	0.34	2.68	6.85	12.33	28.19
Non-smoking participants (32)	5.69	5.05	0.34	2.10	4.47	7.88	23.40
Smoking participants (15)	15.16	7.09	6.85	9.83	14.91	16.81	28.19

PAHs, Polycyclic aromatic hydrocarbons; SD, Standard deviation; P, Percentile.

in the home microenvironment while the highest were recorded in transport (all modes considered). Values in this latter environment were significantly more elevated than the ones measured in the others (Dunn test, $P < 0.001$ for both comparisons with workplace and home). There was no significant difference in exposure between smokers and non-smokers except in homes where higher concentrations were detected for smokers (Student *t*-test, $P = 0.049$ for home, $P = 0.471$ for workplace, $P = 0.806$ for transport). When specifically looking at exposure at the workplace, important variations were observed from one site to another: local BC concentrations ranged from 0.20 μg/m^3 in a park located on the outskirts of the capital to 2.59 μg/m^3 in a park of the urban centre.

When looking at PAH exposure, creatinine-normalized urinary concentrations of 1-hydroxypyrene and 1-naphtol were below the limit of detection for respectively 70% and half of the sample (Table 2). For this reason, they were not considered in further statistical analysis. Only 2-naphtol was quantifiable in all specimens, with concentrations ranging from 0.34 to 28.19 μg/g creatinine. These levels were significantly higher in smoking participants than in non-smoking ones (Wilcoxon rank-sum, $P < 0.001$). No significant correlation was observed between the total general exposure to BC measured the four-day or day 3 or day 4 and concentrations of 2-naphthol among non-smokers or smokers (Spearman's rank correlation, $P > 0.05$ for all combinations). Regarding benzene exposure, concentrations of SPMA never exceeded the LOD in the whole sample.

20.8% of the participants was exposed to second-hand smoke at home. More than 90% used mainly gas (83.0%) or electricity (10.6%) for domestic heating (remaining percentage used fuel oil or wood burning stove). For cooking, 66.7% of the respondents used gas, 44.4% used electricity (some using both) and 82.1% had a cooker hood.

3.3. Health condition

Few participants reported respiratory diseases diagnosed by a medical doctor and that manifested in the last twelve months. No asthma case was identified and only one person had chronic obstructive pulmonary disease. Conversely, 33.3% of non-smoking respondents indicated regularly suffering from upper respiratory symptoms such as sneezing, respiratory tract irritation, blocked/runny nose, 15.2% from chest tightness and 15.2% from coughing, abnormal expectorations. Percentages for smoking respondents reached respectively 20.0%, 20.0% and 6.7%. In total, 39.4% of the non-smokers and 26.7% of the smokers reported one or more of these general respiratory symptoms.

Concentrations of exhaled NO ranged between 1 and 119 ppb (Table 3). The mean was significantly higher among non-smokers (27 ppb) than smokers (12 ppb) (Student *t*-test, $P = 0.001$). According to the American Thoracic Society clinical practice guidelines for interpretation of this biomarker, four participants over 48 suffered from airway inflammation (NO concentration above 50 ppb) (Dweik et al., 2011).

Table 3
Concentrations of exhaled NO and urinary 8-OHdG measured in a group of 48 urban green space workers in Brussels.

Biomarker (number of quantifiable observations)	Mean	SD	Min	P25	P50	P75	Max
NO (ppb)							
All (48)	22	20	1	10	17	26	119
Non-smoking participants (33)	27	22	6	13	19	33	119
Smoking participants (15)	12	9	1	4	10	19	28
8-OHdG (μg/g creatinine)							
All (47)	10.76	2.83	7.05	9.12	10.00	11.55	20.92
Non-smoking participants (32)	10.22	2.35	7.05	9.03	9.64	11.16	17.36
Smoking participants (15)	11.93	3.46	7.14	10.00	11.00	13.75	20.92

NO, Nitric oxide; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; SD, Standard deviation; P, Percentile.

Creatinine-normalized urinary concentrations of 8-OHdG varied between 7.05 and 20.92 μg/g creatinine (Table 3). Non-smokers tended to show lower mean concentrations (10.22 μg/g creatinine) than smokers (11.93 μg/g creatinine) but this difference was not statistically significant (Student *t*-test, $P = 0.058$).

3.4. Associations between exposure to air pollution and health parameters

For both non-smokers and smokers, there was no significant difference in the total general four-day BC concentrations between individuals reporting general respiratory symptoms and those who did not (Student *t*-test, $P = 0.823$, $P = 0.636$ respectively). Likewise, no significant difference in 2-naphthol concentrations was observed between individuals suffering from general respiratory symptoms and "healthy" ones (Student *t*-test, for non-smokers: $P = 0.184$, for smokers: $P = 0.060$).

In contrast, a positive linear relationship appeared between the total general four-day BC concentrations and exhaled NO concentrations among smokers ($\beta = 8.73$, 95% CI [4.04, 13.41], $P = 0.002$, adjusted $R^2 = 0.633$). For non-smokers, data were more scattered and no significant relationship was observed between these two variables ($P = 0.851$) (Fig. 2). No significant correlation was detected when looking

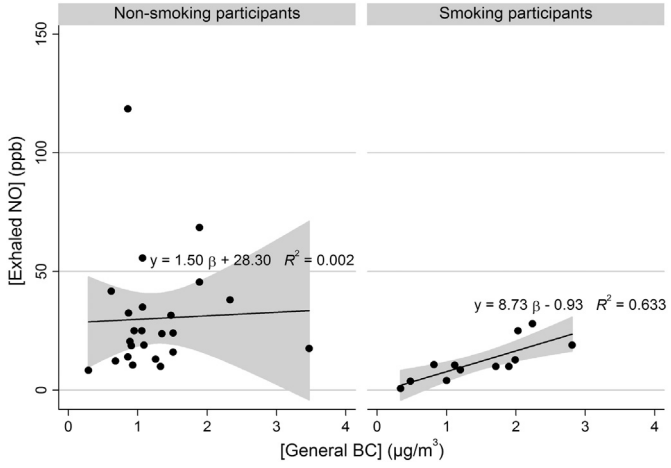

Fig. 2. Linear regression between individual exposure to total general black carbon measured over a four-day follow-up and exhaled nitric oxide concentrations in a group of 48 urban green space workers in Brussels. Measurements carried out with mobile aethalometers and a portable exhaled nitric oxide monitor. Regression stratified by smoking status and accompanied by 95% confidence interval (grey overlay). NO, Nitric oxide; BC, Black carbon.

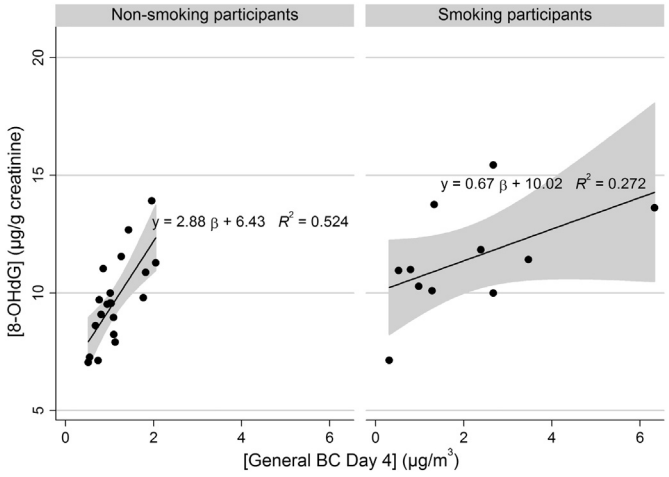

Fig. 3. Linear regression between individual exposure to total general black carbon measured the fourth day of follow-up and 8-OHdG concentrations in a group of 48 urban green space workers in Brussels. Measurements carried out with mobile aethalometers and a urinary biomarker. Regression stratified by smoking status and accompanied by 95% confidence interval (grey overlay). 8-OHdG, 8-hydroxy-2′-deoxyguanosine; BC, Black carbon.

at the total general concentration of BC measured the same day of exhaled NO measurement (fourth day) or with concentrations of 2-naphthol. Besides, exhaled NO concentrations did not show significant association with use of gas as main heating (Student *t*-test, for non-smokers: $P = 0.695$, for smokers: $P = 0.803$) or cooking (Student *t*-test, for non-smokers: $P = 0.840$, for smokers: $P = 0.813$) energy source.

Total general BC concentrations measured the fourth and last day of follow-up were linearly and significantly related with urinary 8-OHdG concentrations among non-smoking participants ($\beta = 2.88$, 95% CI [1.52, 4.24], $P < 0.001$, adjusted $R^2 = 0.524$) (Fig. 3). Relationship for smoking individuals was positive but non-significant ($P = 0.100$). No similar trend was observed when considering the total general four-day BC concentrations or the 2-naphthol measurements.

4. Discussion

The Brussels-Capital Region has high volumes of car traffic but few studies have investigated the possible health effects using data collected at the individual level. This cross-sectional pilot study aimed to assess individual exposure of Brussels green space workers to several traffic-related air pollutants and the relationship with respiratory and oxidative stress outcomes.

When first looking at BC exposure, important variations were observed across participants, working places and microenvironments. General total exposure to BC measured over the four days of follow-up varied between 0.29 and 3.47 µg/m^3. Such disparities can partially be explained by difference in exposure at working place where up to 13-fold variations in BC concentrations were observed. Several parameters may influence these levels: the distance between the parks and the traffic roads, the corresponding traffic intensity, density and structure of park plants, surrounding urban architecture (influencing ventilation and pollutants dispersion), the possible presence of air vents linked with nearby metros or underground car parks, among others. Variations in individual exposure are also likely to be driven by differential exposure during transport. It was indeed during transport (all modes considered) that the highest median concentration was reached: 2.24 µg/m^3. This is coherent with other studies having investigated BC exposure according to time-activity patterns (Dons et al., 2012; Paunescu et al., 2017). A Belgian study notably showed that even if only 6% of time is dedicated to transport, this activity may be responsible for 21% of personal exposure to BC and 30% of inhaled dose (Dons et al., 2012). These results were related to the large share of diesel engines in the Belgian car fleet: at the time of the study, this technology equipped 60% of private cars, a higher percentage than in the neighbouring countries (Dons et al., 2012).

Regarding PAHs exposure, only the 2-naphthol biomarker could be detected in all samples. Overall, its concentrations appeared higher than the ones detected among the general population for whom tobacco is often the main source of exposure to naphthalene: for non-smokers, mean/median concentrations may not exceed 3 µg/L (here 4.18 µg/L) (Preuss et al., 2003; Wheeler et al., 2014). Mean 1-hydroxypyrene concentrations measured in the present study were slightly lower than the ones assayed among Brussels policemen in the early 2000s (here 0.07 versus 0.13 µg/g creatinine among non-smokers and here 0.22 versus 0.31 µg/g creatinine among smokers) (Berthoin et al., 2004).

Regarding health parameters, almost no respondent reported serious and diagnosed respiratory problems but a substantial number indicated to regularly suffer from general respiratory symptoms (sneezing, respiratory tract irritation, etc.) and/or was diagnosed as suffering from respiratory inflammation based on the exhaled NO measurements. Concentrations of this biomarker ranged from 1 to 119 ppb with significant and expected differences between non-smoking and smoking participants (tobacco is recognized as an inhibitor of the enzyme NO synthase) (ATS and ERS, 2005; Kharitonov et al., 1995). For non-smokers, the median reached 19 ppm, a value below the geometric mean observed among Flemish cyclists after they rode 20 min in a busy street (29 ppm) (Jacobs et al., 2010). In terms of oxidative stress, mean urinary concentrations of 8-OHdG reached 10.76 µg/g creatinine with a difference close to significance between non-smoking (10.22 µg/g creatinine) and smoking (11.93 µg/g creatinine) individuals.

No significant association was observed between measures of exposure to BC or 2-naphthol concentrations and report of general respiratory symptoms. Such result may reflect a gap between exposure measurements, primarily representative of recent exposure to air pollution and symptoms investigated, likely to result from chronic exposure. In contrast, a positive linear relationship was shown between total general concentrations of BC measured over the four days of follow-up and exhaled NO levels among smokers. This supports the hypothesis that air pollution causes airway inflammation, playing a key role in the development of respiratory diseases (Brunekreef and Holgate, 2002; Kampa and Castanas, 2008; WHO, 2013). Such relationship has already been demonstrated but mainly in studies focusing on some specific microenvironments (Cornell et al., 2012; Fischer et al., 2002; Lin et al., 2011), age groups (often children) (Cornell et al., 2012; De Prins et al., 2014; Fischer et al., 2002; Lin et al., 2011; Steerenberg et al., 2001), disease status (Jansen et al., 2005) or regions experiencing much higher BC concentrations than here (Fischer et al., 2002; Lin et al., 2011). In the present study, the relationship was only observable for smoking participants. This might be explained by the ability of cigarette smoke to alter airway epithelium (US Department of Health, Education and Welfare, 1964) making smokers more sensitive to air pollution. Besides, record of some very high exhaled NO concentrations may have prevented to reveal similar association among non-smokers. These values might be due to some individual characteristics such as undiagnosed, untreated allergies (considering no association was observed between exhaled NO and use of gas for heating or cooking).

A positive linear relationship was apparent between total general concentrations of BC measured the fourth day of follow up and urinary concentrations of 8-OHdG. This observation adds to the evidence that oxidative stress may mediate air pollution toxicity (Brunekreef and Holgate, 2002; Kampa and Castanas, 2008; WHO, 2013). It may more specifically act by damaging biomolecules (proteins, lipids, DNA, etc.), disturbing biological pathways (especially redox-sensitive ones) leading to various metabolic dysregulations including promotion of inflammation, cell death and mutations (Lodovici and Bigagli, 2011). Few

research works investigated the specific associations between BC and 8-OHdG concentrations. The only study identified failed to demonstrate a significant relationship in a population of American veterans (Ren et al., 2011). Associations have nonetheless been observed for $PM_{2.5}$, number of particles, nitrogen dioxide, ozone, sulphate and organic carbon in other (occupational) studies (Benson et al., 2013; Huang et al., 2012). In the present work, the relationship between BC and 8-OHdG concentrations was only significant for non-smoking participants. For smoking ones, tobacco consumption might have had a greater impact on oxidative stress than BC (Loft et al., 1993; Valavanidis et al., 2009).

Linking specific adverse health effects with specific air pollutants is difficult due to the complex composition of the atmosphere in the urban environment. Toxic compounds are indeed various, with changing, often correlated concentrations and a capacity to interact with and act on several biological systems. In the case of BC, the WHO concluded that this element may not be the major responsible of fine PM toxicity but may rather operate by interaction, as a carrier of various chemicals (such as PAHs) to the inner body (including pulmonary, cardiovascular targets) (Janssen et al., 2012; National Research Council, 1983). In this study, BC and 2-naphthol concentrations were not correlated, even when restricting analyses to non-smoking participants. This may be related to the small sample size or translate a real lack of association due to the impact of specific particle composition and other PAH sources such as food, insect repellents, etc. (Choi et al., 2010). Influence of individual factors should also not be dismissed when associations between exposure to air pollution and health are investigated. Thus, exhaled NO levels are influenced by tobacco consumption (controlled for in this study), concurrent diseases (asthma, allergic rhinitis, etc.), medication use, recent physical activity (controlled for in this study), genetic or potentially age, morphology or food (ATS and ERS, 2005). 8-OHdG concentrations are affected by smoking status, physical activity, age, morphology and gender (Aldini et al., 2011).

The protocol adopted for this project shows several strengths. First, a detailed assessment of exposure was carried out. Three pollutants of concern were studied: 1) BC, recognized by the WHO as "*a better indicator of harmful particulate substances from combustion sources (especially traffic) than undifferentiated particulate matter (PM) mass*" (based on short-term studies) (Janssen et al., 2012), 2) PAHs and 3) benzene, ubiquitous pollutants showing, among others, carcinogenic properties (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, 2009; WHO, 2013). Individual exposure was assessed by combining personal monitor and biomarkers. For BC, both total and local concentrations were determined for different microenvironments, allowing the assessment of real exposure and easier comparison across measurements carried out on different days. For PAHs, several metabolites were analysed to reflect the wide diversity of compounds forming this group. Taking better into account the interaction between continuous spatio-temporal variability of pollution and individual mobility, this approach permitted to be more representative of real personal exposure and to limit exposure misclassification compared with fixed monitoring stations or models (Dons et al., 2011; Park and Kwan, 2017; Steinle et al., 2013, 2015; Violante et al., 2006). For health assessment, different endpoints with varying degrees of seriousness were investigated, combining questionnaire and non-invasive biomarker methods. Analyses of exhaled NO and urinary 8-OHdG appeared sensitive enough to detect slight metabolic changes that do not necessarily translate into symptoms. This is especially interesting in the case of relatively low exposure to air pollution and/or investigation of symptoms subject to subjective assessment such as respiratory problems.

This study is also subject to limitations, mostly due to its pilot nature. First, the project involved 48 participants, a "low" number compared with similar works in which close to 100 persons were followed (Autrup et al., 1999; Barbieri et al., 2008; Berthoin et al., 2004; Tomei et al., 2001). This limited the ability to adjust analyses for confounders other than tobacco consumption and to detect significant associations. The method used to assess exposure to benzene appeared for its part not sensitive enough: no detectable level of SPMA was observed in any of the samples (LOD = 0.2 ng/mL) despite a mean ambient concentration of 0.63 ± 0.28 μg/m^3 in Brussels in 2016–2017 (regional measurements). One alternative would have consisted in assessing directly urinary benzene. This requires however very limited handling/storage (due to the volatility of benzene and to limit the risk of contamination), hard to implement in such field study (Hoet et al., 2008). This study presents also some more general limitations, common to other similar works. Thus, selection bias (due to voluntary participation) and information bias (for data collected by self-administered questionnaire) should not be dismissed. Despite their advantages, biomarkers present also some weaknesses including (inter)individual variability, effect of confounders or cost (Mayeux, 2004). Lastly, extrapolation of the present results to the general population should be done cautiously as the ordinary citizens are likely to spend less time outdoor and have different activities than the studied group of workers.

5. Conclusions

In summary, this study highlighted positive linear relationships between exposure to BC and exhaled NO, urinary 8-OHdG concentrations in a sample of Brussels urban green space workers, supporting the crucial role of inflammation and oxidative stress in mechanistic pathways of air pollution toxicity. These results underline the usefulness of BC as metric to evaluate the harmfulness of fine particles released by combustion processes and of exhaled NO, urinary 8-OHdG biomarkers to detect early signs or mild symptoms in humans. These results pave the way for more comprehensive projects. The objective would be to involve a wider sample of the population, showing a higher contrast in air pollution exposure. It would also be advantageous to make more use of electronic systems and biomarkers to facilitate data collection and get more standardized answers. Paper questionnaires could be replaced by online secured survey tools and log books by smartphone apps or personal digital assistants that are capable of tracking the whereabouts of the participants by means of GPS. Such systems have already been successfully used in the UK, Spain and Belgium (de Nazelle et al., 2013; Dons et al., 2011; Vigo et al., 2018). Regarding biomarkers, a study recently developed a method to assay urinary BC and concluded on the reliability of this marker to assess medium-term up to chronic exposure to combustion-related air pollution (Saenen et al., 2017).

Ethics approval and consent to participate

This study has been approved by the Ethics committee of the University Hospitals of Leuven (Ref.: S58976; B322201628202).

Competing interests

The authors declare that they have no competing interest.

Authors' contributions

AG contributed to the study design, data collection, data analysis, results interpretation and drafted the article.

KDC was in charge of the urinary biomarker assays and contributed to results interpretation.

BH was involved in black carbon data processing and results interpretation.

CD and RA assisted data analysis and results interpretation.

PD, OB and AVN took part to the study design, the supervision of the research and results interpretation.

All authors critically revised the article and gave their final approval for publication.

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