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Oxidatively damaged DNA and its repair after experimental exposure to wood smoke in healthy humans

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ABSTRACT

Particulate matter from wood smoke may cause health effects through generation of oxidative stress with resulting damage to DNA. We investigated oxidatively damaged DNA and related repair capacity in peripheral blood mononuclear cells (PBMC) and measured the urinary excretion of repair products after controlled short-term exposure of human volunteers to wood smoke. Thirteen healthy adults were exposed first to clean air and then to wood smoke in a chamber during 4 h sessions, 1 week apart. Blood samples were taken 3 h after exposure and on the following morning, and urine was collected after exposure, from bedtime until the next morning. We measured the levels of DNA strand breaks (SB), oxidized purines as formamidopyrimidine-DNA-glycosylase (FPG) sites and activity of oxoguanine glycosylase 1 (hOGG1) in PBMC by the comet assay, whereas mRNA levels of hOGG1, *nucleoside diphosphate linked moiety X-type motif 1* (*hNUDT1*) and *heme oxygenase 1* (*hHO1*) were determined by real-time RT-PCR. The excretion of 8-oxo-7,8-dihydro-oxoguanine (8-oxoGua) and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in urine was measured by high performance liquid chromatography purification followed by gas chromatography with mass spectrometry. The morning following exposure to wood smoke the PBMC levels of SB were significantly decreased and the mRNA levels of *hOGG1* significantly increased. FPG sites, hOGG1 activity, expression of *hNUDT1* and *hHO1*, urinary excretion of 8-oxodG and 8-oxoGua did not change significantly. Our findings support that exposure to wood smoke causes systemic effects, although we could not demonstrate genotoxic effects, possibly explained by enhanced repair and timing of sampling.

1. Introduction

Adverse health effects of ambient particulate matter (PM) in urban air have been evaluated in more than 500 epidemiological studies [1,2]. Dose-response relationships of mortality, reduction of life expectancy, hospital admissions, asthma attacks, etc. based on mass concentrations of PM have been characterised. Similarly, a large number of studies have related various estimates of exposure to traffic emissions to health effects. Although wood combustion contributes significantly to ambient PM levels in many parts of the world little is known with respect to the contributions to the health effects associated with exposure to wood smoke [3,4]. The epidemiological database in this respect is limited to acute airway effects and mortality in areas with significant contribution from wood smoke to ambient PM levels identified by Boman et al. [5], who concluded that wood smoke appeared no less harmful than PM from other sources [5]. One study suggests risk of chronic obstructive pulmonary disease associated with long-term exposure to wood smoke [6]. Despite limited evidence of carcinogenicity in humans caused by wood smoke exposure a working group from the International Agency for Research on Cancer concluded that indoor emissions from household combustion of biomass fuel (mainly wood) are "probably carcinogenic to humans (Group 2A)" [7]. Accordingly, there is urgent need for knowledge on health effects related to wood combustion.

The mechanisms of action behind the adverse effects caused by PM are thought to involve induction of inflammation and oxidative stress [8–10]. In this context, particles with an aerodynamic diameter less than 0.1 μm defined as ultrafine particles (UFP) appear to be more potent than fine or coarse particles per unit mass and they are more readily deposited in the alveolar region as well as translocated to the circulation for systemic

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effects [11–15]. However, with respect to wood smoke, experimental studies are mainly limited to traditional assessment of effects on lungs and immune function, whereas the mode of action, systemic effects, oxidative stress and genotoxicity have received little attention [4,16]. Oxidative stress causes damage to DNA, so far mainly studied as formation of 8-oxo-7,8-dihydroguanine (8-oxoGua) and strand breaks (SB) when investigating PM exposure effects [17]. Wood smoke particles have been shown to induce ROS formation and DNA SB in vitro [18,19]. Oxidatively damaged DNA is mutagenic, relevant for carcinogenesis and may be studied as a biomarker of systemic or target organ oxidative stress. In DNA, oxoguanine glycosylase 1 (hOGG1) removes 8-oxoGua which is excreted into the urine. The nucleoside diphosphate linked moiety X-type motif 1 (hNUDT1) enzyme removes 8-oxo-7,8-dihydro-2′-deoxyguanosine 5′-triphosphate (8-oxo-dGTP) from the nucleotide pool, which prevents the incorporation of 8-oxoGua during the repair processes or replication and possibly results in 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) excretion in the urine [20]. Experimental studies indicate that although exposure to PM induces DNA oxidation in target organs, the expression of *hOGG1* as well as oxidative stress response and defence genes such as *heme oxygenase 1* (*hHO1*) are upregulated, potentially resulting in measured unchanged levels of damage in DNA [17,21,22].

Application of biomarkers in exposed human populations can allow substantiation of biological effects and causal relationships. We have shown significant relationships between individual exposure to PM with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) or traffic generated UFP and systemic biomarkers of oxidatively damaged DNA, proteins and lipids among healthy subjects [23–28]. Recently, a study of experimental human exposure to wood smoke showed adverse effects in terms of increased levels of biomarkers of lung and systemic inflammation and coagulation and indication of lipid peroxidation [29,30]. However, there is little information concerning possible damage to DNA from wood smoke, except that increased levels of chromosomal aberrations and micronuclei have been found in Indian women cooking with biofuels [31].

The aim of this study was to investigate the relationship between generation of DNA damage and the DNA repair system after controlled short-term exposure of healthy adults to wood smoke. The level of systemic oxidative stress with damage to DNA in terms of SB and oxidized purines and expression of the related *hOGG1*, *hNUDT1* and *hHO1* genes was assessed in human peripheral blood mononuclear cells (PBMC). The excretion of the corresponding repair products, 8-oxoGua and 8-oxodG, was measured in urine.

2. Materials and methods

2.1. Subjects, blood and urine sampling

Details of the study design and results on other biomarkers have been published previously [29,30,32]. In short, 13 healthy never-smoking subjects (6 men and 7 women) aged 20–56 (mean 34) years were recruited from the staff of Department of Occupational and Environmental Medicine, Goteborg University, Sweden, after informed consent. The study was approved by the Ethics Committee of Goteborg University.

Subjects were exposed to filtered indoor air for 4 h on one occasion and to wood smoke for 4 h 1 week later in an exposure chamber. The wood smoke session was performed after the clean air session to avoid carryover effects. The experiment was carried out in two rounds with seven and six subjects, respectively. There were two 25 min periods of light exercise (about 70 W).

Blood samples were obtained 3 h after leaving the exposure chamber and the following morning (20 h after leaving the exposure chamber). All urine from bedtime until the following morning was collected and timed. Samples were stored frozen until analysis. The urinary sampling was incomplete in 3 subjects; hence the results regarding urinary excretion are only based on 10 subjects. PBMC were collected and isolated in Vacutainer® Cell Preparation Tubes (CPT, Becton-Dickinson, NJ) according to the manufacturer's instructions. PBMC for DNA damage and repair capacity were stored frozen at −80 °C in a mixture containing 50% foetal bovine serum (FBS, GibcoRBL, Grand Island, NY), 40% culture medium (RPMI 1640, GibcoRBL, Grand Island, NY), and 10% dimethyl sulfoxide (DMSO, Applichem, Darmstadt, Germany). PBMC for mRNA expression levels were stored at −80 °C in TRIzol® Reagent (Invitrogen, Denmark). For all assays, the samples from each subject were run in one batch blinded to exposure and time of sampling.

2.2. Exposure characterization

This has previously been described in detail [32]. In brief, the exposure chamber, built at the Swedish National Testing and Research Institute, was 7.4 m × 6 m × 2.9 m. Wood smoke was generated in a wood stove placed just outside the chamber. A standardized mixture (50/50) of hardwood/softwood (birch/spruce), dried for 1 year (moisture content 15–18%), was used in the wood stove. A partial flow of the smoke was mixed with filtered indoor air to the target concentration. PM mass and number concentrations differed somewhat between the two rounds. The median PM mass concentrations were 279 μg/m^3 and 243 μg/m^3, respectively. The PM number concentrations were 180,000 cm^{-3} and 95,000 cm^{-3}, respectively, with 65% and 28% UFPs (<100 nm). Particle size distributions showed a geometric mean diameter of 42 nm in the first session and 112 nm in the second session. In the clean-air sessions mass concentrations were low, less than 30 μg/m^3. The majority (70–80%) were UFPs and the total number concentrations were 4400 and 7500 cm^{-3}, respectively. The median benzene and 1,3-butadiene concentrations were 30 μg/m^3 and 3.9 μg/m^3, respectively, in the first wood smoke session and 20 μg/m^3 and 6.3 μg/m^3 in the second. The levels in the clean air sessions were at least 10 times lower. The PAH concentrations were only measured in the second wood smoke session and the levels were relatively high, with a benzo(*a*)pyrene concentration about 20 ng/m^3 [32].

2.3. DNA damage and repair activity measured by the comet assay

The levels of SB and modified purines as formamidopyrimidine-DNA-glycosylase (FPG) sites were measured in PBMC by the single cell gel electrophoresis (comet) assay as previously described [23,33]. This assay has been validated in an interlaboratory trial by the European Standards Committee on Oxidative DNA damage [34]. Coded samples from each participant were analysed in the same batch along with a quality control PBMC sample. We visually scored 100 comets per slide using a five-class scoring system (arbitrary score range: 0–400). The arbitrary scores for SB and the FPG sites as the difference between scores of slides incubated with and without FPG were converted into lesions per 10^6 base pairs (bp) by means of an investigator-specific calibration curve based on induction of SB by X-rays, which has a known yield. We used a conversion factor of 0.0263 Gy equivalents per score corresponding to 0.0051 lesions (SB and FPG sites) per 10^6 bp and the assumption that a diploid cell contains 4×10^{12} Da DNA (6×10^9 bp) [35].

The OGG1 repair activity of PBMC was determined as the DNA incision activity measured in substrate cells treated with Ro19-8022/white light, which generates 8-oxodG in DNA [36,37]. The Ro19-8022 photosensitizer was a gift from Hoffman-La Roche, Basel, Switzerland. The substrate cells were PBMC from multiple donors from our laboratory. The assay was optimized and performed as previously described [25,38].

2.4. Measurement of 8-oxoGua and 8-oxodG in urine

The excretion of 8-oxoGua and 8-oxodG in urine was measured by high performance liquid chromatography purification followed by gas chromatography with mass spectrometry as described elsewhere [39]. The excretion rate per hr was calculated from the urinary volume and collection time.

2.5. Expression levels of hHO1, hOGG1, and hNUDT1 mRNA by real-time RT-PCR

On the day of analysis the stored PBMC samples were rapidly thawed and the RNA was extracted according to the manufacturer's instructions. After RT-mediated cDNA synthesis quantitative real-time PCR reactions were carried out in ABI PRISM® 7900HT (Applied Biosystems, Naerum, Denmark), using primers and cDNA specific probes purchased from Applied Biosystems, Naerum, Denmark. We used 18S rRNA as reference gene which is commercially available as a probe and primer solution (Eukaryotic 18S rRNA Endogenous Control, 4352930E, Applied Biosystems, Naerum, Denmark). Probes and primers for the genes were as follows:

hHO1—forward primer: 5′-CAT GAG GAA CTT TCA GAA GGG C-3′, reverse primer: GAT GTG GTA CAG GGA GGC CAT-3, TaqMan probe: 5′-6-FAM-TGA CCC GAG ACG GCT TCA AGC AGC TG-TAMRA-3′ (NM_002133).
hOGG1—forward primer: 5′-AAA TTC CAA GGT GTG CGA CTG-3′, reverse primer: 5′-GCG ATG TTG TTG TTG GAG GA-3′, TaqMan probe: 5′-6-FAM-CAA GAC CCC ATC GAA TGC CTT TTC TCT TT-TAMRA-3′ (U96710).
hNUDT1—forward primer:5′-CAT CGA GGA TGG GGC TAG-3′, reverse primer: CAG AAG ACA TGC ACG TCC ATG A-3′, TaqMan probe: 5′-6-FAM-TCG CCC ACG AAC TCA AAC ACG ATC T-TAMRA-3′ (D16581).

The PCR reactions were performed as described previously [25]. The level of gene expression is reported as the ratio between the mRNA level of the

Table 1
Means ± S.D. of DNA damage, repair activity (hOGG1), mRNA levels (per 18S mRNA) in peripheral blood mononuclear cells (PBMC) and urine excretion of 8-oxoGua and 8-oxodG according to exposure and time after exposure

	Time after exposure to filtered air		Time after exposure to wood smoke	
	3 h	20 h	3 h	20 h
PBMC				
hHO1 × 10^{-6}	14.2 ± 6.45	19.0 ± 8.61[a,b]	11.2 ± 4.36	22.0 ± 13.5[b]
hNUDT1 × 10^{-6}	0.41 ± 0.09	0.63 ± 0.34[a,b]	0.37 ± 0.13	0.55 ± 0.32[b]
hOGG1 × 10^{-6}	1.07 ± 0.28[c]	1.05 ± 0.35[a]	1.29 ± 0.59	2.02 ± 1.18[d]
hOGG1 activity[e]	5.44 ± 4.78	6.57 ± 3.53	6.46 ± 4.22	7.09 ± 4.41
FPG sites (per 10^6 bp)	0.25 ± 0.14	0.26 ± 0.098	0.24 ± 0.15	0.21 ± 0.11
SB (per 10^6 bp)	0.071 ± 0.053	0.085 ± 0.043	0.042 ± 0.036[f]	0.035 ± 0.019[d]
Urine				
8-OxodG (nmol/h)[g]	–	0.73 ± 0.19[h]	–	0.84 ± 0.35[h]
8-OxoGua (nmol/h)[g]	–	4.24 ± 2.31[h]	–	8.43 ± 6.48[h]

[a] $n = 11$.
[b] $P < 0.05$ versus samples collected after 3 h.
[c] $n = 12$.
[d] $P < 0.05$ versus 20 h after clean air exposure.
[e] Data are expressed as % DNA incisions compared to an internal standard of FPG enzyme.
[f] $P < 0.05$ versus 3 h after clean air exposure.
[g] Urine was collected from bedtime until next morning.
[h] $n = 10$.

target gene and the 18S rRNA reference gene using the comparative $2^{-\Delta C_t}$ method.

2.6. Statistics

We investigated the effect of wood smoke exposure and time after exposure on the outcome variables SB, FPG sites, mRNA expression of DNA repair genes and hOGG1 repair activity using mixed effects models by the PROC MIXED procedure of SAS v9.1 (SAS Inst. Inc., Cary, NC). Missing values of mRNA expression levels after exposure to filtered air precluded the use of repeated measures ANOVA. Subject nested in gender was included as random factor variable to account for inter-individual variation and age was included as continuous explanatory variable. Exposure in terms of presence or absence of wood smoke, time after exposure (3 or 20 h) and round was included as categorical explanatory variables. The distributions of the DNA damage and mRNA expression biomarkers were skewed; therefore all statistical analyses were performed on their natural logarithms. The urinary excretion of 8-oxoGua and 8-oxodG was only measured once in each exposure scenario and therefore analysed by paired *t*-tests. Spearman rank correlation coefficients (R_s) were calculated between all measured variables. Ratios of parallel levels measured after wood smoke and clean air exposure were calculated with 95% confidence intervals (CI). In all analyses, $P < 0.05$ was considered to be statistically significant.

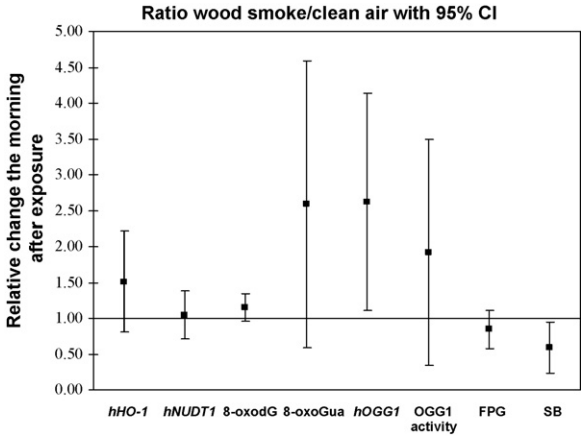

Fig. 1. The relative change of all measured biomarkers, 20 h after exposure. The data are shown as the mean of the individual ratios between wood smoke and filtered air, with 95% confidence intervals.

3. Results

A summary of the levels of DNA damage, hOGG1 activity and mRNA levels according to exposure and time after exposure is presented in Table 1. The relationship between exposure and endpoints is presented as the relative % change in Table 2 and the ratio between exposed and unexposed levels of each biomarker is shown graphically in Fig. 1.

The level of SB was significantly decreased at both 3 and 20 h after exposure to wood smoke ($P < 0.05$). mRNA expression lev-

Table 2
The relationship between biomarkers and exposure expressed as the relative % change[a]

	Total exposure effects		Effects after 3 h		Effects after 20 h	
	% change (CI)	*P*-value	% change (CI)	*P*-value	% change (CI)	*P*-value
PBMC						
hHO1 mRNA	−8.29 (−27.5, 16.0)	0.46	−19.4 (−40.7, 9.50)	0.15	6.8 (−30.1, 63.2)	0.74
hNUDT1 mRNA	−14.4 (−32.1, 7.63)	0.18	−14.7 (−33.7, 9.89)	0.20	−15.9 (−46.7, 32.6)	0.42
hOGG1 mRNA	40.4 (8.3, 81.9)	0.012	14.2 (−14.4, 52.2)	0.33	73.9 (6.25, 184)	0.031
hOGG1 activity	18.4 (−34.4, 113)	0.57	59.1 (−34.6, 287)	0.28	−12.0 (−64.9, 121)	0.77
FPG sites	−15.0 (−31.1, 4.9)	0.13	−5.21 (−33.4, 35.0)	0.75	−23.7 (−41.9, 0.15)	0.051
SB	−50.9 (−65.0, −31.0)	0.0001	−45.7 (−68.9, −5.18)	0.034	−56.89 (−74.0, −28.6)	0.0037
Urine						
8-OxodG					16.4 (−6.89, 45.5)	0.16
8-OxoGua					79.3 (−12.9, 269)	0.10

[a] Mixed model regression with subject nested in gender as a random factor was used. All models were adjusted for age and time after exposure. Exposure to wood smoke: categorical (yes/no) was included as a predictor and the natural logarithm of the effect marker included as a continuous outcome variable. The predictive value of the estimates (% change) is expressed relative to wood smoke exposure.

els of *hOGG1* were significantly increased 20 h after wood smoke exposure as compared with 20 h after filtered indoor air exposure ($P < 0.05$). There was no significant effect of exposure on modified purines measured as FPG sites, hOGG1 activity, urinary excretion of 8-oxodG and 8-oxoGua or mRNA expression levels of *hNUDT1* and *hHO1*. However, as seen in Fig. 1, the data were compatible with increased levels of hOGG1 activity and excretion of 8-oxoGua and 8-oxodG. The expression levels of *hNUDT1* and *hHO1* were significantly lower in the afternoon as compared to the following morning (Table 1). We found no relevant significant correlations between the biomarkers on an individual level (data not shown).

4. Discussion

This is the first study of the effects of controlled wood smoke exposure on DNA damage and repair in humans. We found a significant up-regulation in the expression of the repair gene *hOGG1*, which is central in the base excision repair of 8-oxoGua in DNA. This was accompanied by a significant decrease in DNA SB after exposure to wood smoke. We observed no other significant effects of wood smoke exposure on other biomarkers included in this study, although the data showed a trend toward increased oxidatively damaged DNA and its repair, especially with respect to excretion of 8-oxoGua.

8-OxoGua may be formed by oxidation of guanine or incorporated during replication or repair as oxidized nucleotides (8-oxodGTP). If not repaired 8-oxoGua may lead to GC to TA transversion mutations in the DNA and accumulation of these irreversible mutations may be a part of initial events in carcinogenesis. In animals, exposure to diesel exhaust particles by inhalation or oral gavage have resulted in increased levels of *hOGG1* mRNA and increased or unchanged 8-oxodG levels in lungs, liver and colon [21,22,40]. The 4 h exposure to wood smoke was of sufficient length and strength to cause a similar up-regulation of *hOGG1* in PBMC. This is important per se, because a high expression of *hOGG1* was associated with increased risk of lung cancer among never-smokers in a prospective study [41]. We had expected increased levels of DNA damage after the exposure but this could have been obscured by changes in repair capacity in the present study, although the increase in the measured repair capacity of hOGG1 failed to reach statistical significance.

The FPG enzyme recognizes mainly modified purines such as 8-oxodG and ring-opened formamidopyrimidine bases [37]. The DNA SB measured by the comet assay may arise because of direct breakage of the DNA strand by genotoxic compounds or it may be transient breaks created by DNA repair enzymes, including hOGG1 [42]. In addition, the alkaline version of the comet assay also detects lesions that are converted to SB because of the high pH (i.e. alkali labile sites). This version of the comet assay has been used extensively as biomarker of exposure in biomonitoring studies of genotoxic effects in environmental and occupational settings [33,37]. In the present study we found no significant effect of exposure on the level of FPG sites, which might be due to rapid repair with enhanced capacity after the exposure was terminated, as reflected in the increased *hOGG1* mRNA level on the following morning. In support of this, a non-significant increase in excretion of 8-oxoGua, the product of hOGG1 mediated repair of the lesion in DNA, was found in the urine collected overnight after exposure to wood smoke. We even found a decreased level of SB in the morning following exposure, which might also be due to enhancement of the repair of SB, although no biomarker is presently available to address this hypothesis. Religation of SB is considered to be faster than the repair of 8-oxoGua which has a half-life of a couple of hours [43,44]. We have previously observed consistently elevated levels of modified purines measured as 8-oxodG or FPG sites in PBMC in relation to exposure to PM [23–26]. In two of these studies we also found exposure-related increased levels of SB [24,25]. Measurement of *hOGG1* mRNA and activity was included in one study, which found no effect of exposure to street air PM [25]. However, these studies have been mainly focused on traffic-related PM exposure, including controlled exposure to PM from a busy street for 24 h [25]. Moreover, the exposure has been at rather low levels, e.g. 10–20 $\mu g/m^3$ $PM_{2.5}$, and mainly ongoing until blood sampling [23,25,26]. Accordingly, the present study had qualitatively as well as quantitatively different exposure and timing of sampling compared to our previous studies. Other systemic effects of this high dose of 240–280 $\mu g/m^3$ of wood smoke for four hr were demonstrated by increased levels of biomarkers of inflammation, coagulation and an indication of lipid peroxidation as published elsewhere [29]. It is not known whether the systemic effects including oxidative stress relate to uptake of intact particles or chemical substances released from them and taken up from the lung. It is possible that the high dose was sufficient to induce DNA repair, whereas DNA damage might have reached a ceiling level and/or ceased after exposure was terminated. Unfortunately, samples were not collected for assessment of DNA damage and repair before, during and immediately after exposure for further elucidation of this hypothesis. Even intense continuous exposure to particles and gaseous oxidants in terms of tobacco smoking has not shown consistent effects on DNA strand breakage assessed by the comet assay [45], whereas smokers may have increased levels of both 8-oxodG and OGG1 activity in their leukocytes as well as consistently increased urinary excretion of 8-oxodG [20,46,47]. Finally, we have lower statistical power in the present study with only 13 subjects and a set of measurements per scenario whereas our previous exposure studies have included at least 30 subjects or repeated the exposure scenarios within the same subjects [23–26].

The background level of 8-oxoGua in cellular DNA represents a dynamic balance between the rate of DNA damage and the rate of repair where the urinary excretion of 8-oxoGua and 8-oxodG is considered to reflect the ongoing oxidative stress and rate of repair [20]. The excretion of 8-oxodG has been extensively studied and it is increased by many occupational exposures, including air pollution, whereas ambient PM levels had no effect [26,48,49]. Although the exact contribution from different tissues to 8-oxodG excretion is unknown, the excretion appears to predict the risk of lung cancer among never-smokers [50]. 8-OxodG is a putative product of hNUDT1, which prevents the incorporation of 8-oxodGTP in DNA by degradation to 8-oxodGMP. In the present study we did not find significant effects of wood smoke exposure on the excretion of 8-oxodG or *hNUDT1* expression. Similarly, there was no significant effect on the expression of *hHO1*, which has not been previously studied in human PBMC with respect to PM, whereas hyperbaric oxygen, exercise and some cardiovascular diseases appear to have inducing properties [51–53].

5. Conclusion

In conclusion, controlled exposure to wood smoke was followed by significant up-regulation of *hOGG1* and decreased levels of DNA SB, whereas oxidized purines were not significantly changed in PBMC. Our findings support that exposure to wood smoke PM at relatively high doses causes systemic effects although, we could not demonstrate direct genotoxic effects, which we have previously observed at lower exposure levels of traffic-related PM. This could be due to enhanced repair and timing of sampling. Further studies of the effect of wood smoke on DNA damage and repair in humans are required.

Conflict of interest

All authors declare no competing financial interests.

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