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Short Communication

Controlled exposure to diesel exhaust and traffic noise – Effects on oxidative stress and activation in mononuclear blood cells

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ABSTRACT

Particulate air pollution increases risk of cancer and cardiopulmonary disease, partly through oxidative stress. Traffic-related noise increases risk of cardiovascular disease and may cause oxidative stress. In this controlled random sequence study, 18 healthy subjects were exposed for 3 h to diesel exhaust (DE) at 276 μg/m^3 from a passenger car or filtered air, with co-exposure to traffic noise at 48 or 75 dB(A). Gene expression markers of inflammation, (*interleukin-8* and *tumor necrosis factor*), oxidative stress (*heme oxygenase* (*decycling-1*)) and DNA repair (*8-oxoguanine DNA glycosylase* (*OGG1*)) were unaltered in peripheral blood mononuclear cells (PBMCs). No significant differences in DNA damage levels, measured by the comet assay, were observed after DE exposure, whereas exposure to high noise levels was associated with significantly increased levels of hOGG1-sensitive sites in PBMCs. Urinary levels of 8-oxo-7,8-dihydro-2′-deoxyguanosine were unaltered. In auxiliary *ex vivo* experiments whole blood was incubated with particles from the exposure chamber for 3 h without effects on DNA damage in PBMCs or intracellular reactive oxygen species production and expression of CD11b and CD62L adhesion molecules in leukocyte subtypes.

Conclusion: 3-h exposure to DE caused no genotoxicity, oxidative stress or inflammation in PBMCs, whereas exposure to noise might cause oxidatively damaged DNA.

1. Introduction

Epidemiologic evidence shows that exposure to ambient air pollution, especially traffic-derived, is associated with cardiopulmonary disease and mortality [1,2]. Moreover, there is sufficient evidence to conclude that exposure to air pollution, particulate matter (PM) in air and diesel exhaust (DE) causes lung cancer [3,4]. Oxidative stress-induced genotoxicity is thought to be an important link to carcinogenesis and has consistently been associated with PM exposure in experimental models [5]. Similarly, elevated levels of biomarkers of oxidative damage to DNA in leukocytes and urine have been associated with exposure to ambient air pollution in relevant populations [6]. DNA damage has been assessed as guanine oxidation products in leukocytes either after DNA extraction, which has an inherent risk of artifacts due to spurious oxidation, or by the comet assay with formamidopyrimidine DNA glycosylase (FPG) for nicking, which is considered free of such potential artifacts [7]. Urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) has also been widely used as a valid biomarker of oxidative stress [8].

Combustion-driven vehicles are highly important sources of both local air pollution and noise. The latter is poorly investigated with respect to oxidative stress endpoints, whereas it is an important risk factor for vascular disease [9]. A study of human subjects exposed to airport noise overnight in their home showed endothelial dysfunction, which was attenuated by administration of the

Abbreviations: CD, cluster of differentiation; DE, diesel exhaust; FPG, formamidopyrimidine DNA glycosylase; hOGG1, human 8-oxoguanine DNA glycosylase; HMOX1, heme oxygenase (decycling-1); IL8, interleukin 8; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PBMCs, peripheral blood mononuclear cells; PM, particulate matter; ROS, reactive oxygen species; SB, strand breaks; TNF, tumor necrosis factor.

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antioxidant vitamin C [10]. Converging lines of evidence indicate that exposure to traffic noise is associated with the risk of breast cancer [11], of which risk factors for the latter could also include oxidative stress and urinary 8-oxodG excretion [12]. Accordingly, traffic-related air pollution and noise not only share sources, but also adverse health effects and these exposures might converge in some of the mechanistic pathways and act synergistically. Yet, little is known in terms of their short-term effects on such potential mechanistic pathways. Short-term effects of controlled exposure to DE have mainly been related to truck engines, whereas the number of diesel powered passenger cars has been increasing rapidly. Nevertheless, effects on oxidative stress and DNA damage have not yet been included in the outcomes as well as potential interactions with concomitant exposure to traffic noise have not been addressed.

The overall aim of this study was to assess the effects of controlled exposure to DE from a light-duty vehicle and traffic noise, each alone and in combination, on biomarkers of oxidative stress, inflammatory response, and DNA damage in both peripheral blood mononuclear cells (PBMCs) and urinary excretion of 8-oxodG. Previous observations from the same study have shown that the DE-exposure resulted in decreased lung function and increased leukocyte counts in peripheral blood [13]. In addition, PM collected in the exposure chamber was assessed for the ability to induce oxidative stress, DNA damage and inflammatory response in human whole blood *ex vivo* in order to simulate effects after potential translocation from the lungs to the circulation.

2. Materials and methods

2.1. Human exposure study

2.1.1. Study population and design

Eighteen subjects (9 male and 9 female, non-smoking and 40–66 years) were recruited for the study as further described in the supplement and previously [13,14]. The study was performed after approval by the Regional Ethical Review Board in Sweden. All the subjects gave their written informed consent. The subjects were exposed 4 times in random sequence inside a specially designed 22 m^3 exposure chamber for 3 h to a reference exposure (filtered air with ~2 $\mu g/m^3$ of PM_1 (<1 μm) and 46 dB(A) traffic noise), DE (276 $\mu g/m^3$ of PM_1 and 46 dB(A) traffic noise), traffic noise (filtered air with ~2 $\mu g/m^3$ and 75 dB(A) traffic noise), and DE + traffic noise (276 $\mu g/m^3$ of PM_1 and 75 dB(A) traffic noise) at rest, sitting relaxed. The traffic noise was recorded at a busy street crossing which was simulated by the high level (75 dB(A)) representing a real-life form of noise.

2.1.2. Collection and analysis of biomarker samples

Peripheral blood samples were collected immediately before and after the exposure. PBMCs were isolated in Vacutainer Cell Preparation Tubes (Vacutainer® CPT Becton Dickinson A/S, Brøndby, Denmark) and stored at −80 °C in preserving media. Urine was collected at three occasions: immediately before and after the exposure, as well as 20 h post-exposure, and analyzed for 8-oxodG.

The levels of DNA strand breaks (SB) and FPG- and human 8-oxoguanine DNA glycosylase (hOGG1)-sensitive sites in PBMCs were measured by the comet assay as further described in the supplement and previously [15]. We usually find substantially higher levels of FPG-sensitive sites than of hOGG1-sensitive sites in human PBMC samples, although the levels of FPG- and hOGG1-sensitive sites are similar and high after treatment with the photosensitizer Ro19-8022 or potassium bromate inducing specifically 8-oxodG [16–18].

The gene expression of inflammation markers (*IL8* and *TNF*), oxidative stress (*HMOX1*), and DNA repair (*OGG1*) were measured in PBMCs by using RT-PCR, as described in a previous study [19]. We selected these 4 genes as most relevant for the mechanisms of action related to inflammation, oxidative stress and possible change in repair of potential DNA damage as seen in a study of controlled exposure to wood smoke [20]. The eight samples collected per subject precluded assessment of a wider transcriptomics profile.

2.2. Ex vivo *study*

For the *ex vivo* tests a sample of the ultrafine (UF < 0.1 μm) fraction of the DEP was collected on a polytetrafluoroethylene filter using the same experimental set-up as in the human exposure study as further explained in the supplement. The particles were extracted with methanol and resuspended in ELGA® water.

Venous blood collected from an anonymous donor was added suspensions of DEP to final exposure concentrations of 0, 2.5, 12.5 and 25 μg/mL with incubation for 3 h in the dark to mimic the duration of the chamber study. After the exposure, whole blood was assigned to assessment of reactive oxygen species (ROS) production by 2′,7′-dichlorofluorescein diacetate and surface adhesion molecules expression in terms of CD62L and CD11b in leukocytes separated in three gates for lymphocytes, monocytes and granulocytes based on forward and side scatter to assess size and granularity by flow cytometry, whereas DNA damage was assessed in PBMCs by the comet assay as described for the human *in vivo* exposure study. The experiment was repeated on 3 different days.

2.3. Statistics

We used linear mixed effects models (*xtmixed*) to evaluate the DE and traffic noise exposure as single factors and the interaction between these two factors for effects on the biomarker measured after the exposure using Stata/IC software (version 13.0). The change with 95% confidence interval related to an exposure was calculated from the regression coefficient in the mixed effects model. The *ex vivo* study data were analyzed using an analysis of variance (ANOVA).

3. Results

3.1. Human exposure study

The concentration of PM_1 in the air was by mass 276 ± 27 $\mu g/m^3$ (mean ± 1 SD). The number size distribution peaked at 89 ± 9 nm (geometric SD: 1.98 ± 0.12 nm), and the mass size distribution at 195 ± 8 nm, determined as described by Wierzbicka and co-authors [14] from mobility number size distribution measured continuously combined with size resolved particle effective density measurements (from DMA-APM). Analysis by transmission electron microscopy revealed that the particles were aggregates, consisting of monomers with a diameter of ~30 nm [14], with the typical aggregate structure of diesel soot. The monomers had the typical microstructure of DEP. The soot aggregates had very little volatile coating (<10% by mass, volatile at 300 °C), and the level of coating was independent of the soot-aggregate size (classified according to the mobility diameter changed stepwise in the range 50–400 nm), indicating that only minor condensation of organics condensed unto the soot structures after the soot-aggregates were formed. The concentration of polycyclic aromatic hydrocarbons (PAHs) in the gas phase was 7.5 ± 0.2 $\mu g/m^3$, with less than 1% of the PAHs in the particle phase (60 ng/m^3) in the air [14]. The PM characteristics and estimated deposited dose to the lung are summarized in Supplemental Table S1.

The results on DNA damage in PBMCs are shown in Table 1. There was no significant interaction between DE and traffic noise

Table 1
Levels of strand breaks (SB) and formamidopyrimidine DNA glycosylase (FPG)- and 8-oxoguanine DNA glycolase (hOGG1)-sensitive sites as well as gene expression levels of *heme oxygenase* (*decycling*)*-1* (*HMOX1*), *8-oxoguanine DNA glycosylase* (*OGG1*), *interleukin 8* (*IL8*) *and tumor necrosis factor* (*TNF*) in PBMCs from humans before and after exposure to diesel exhaust (DE) and/or traffic noise.

	Reference		DE		Traffic noise		DE and traffic noise	
	Before	After	Before	After	Before	After	Before	After
SB/10^6 base pair	0.30 ± 0.07	0.24 ± 0.06	0.32 ± 0.04	0.30 ± 0.04	0.44 ± 0.3	0.30 ± 0.04	0.30 ± 0.07	0.26 ± 0.05
FPG/10^6 base pair	0.65 ± 0.05	0.60 ± 0.08	0.55 ± 0.05	0.55 ± 0.05	0.58 ± 0.05	0.69 ± 0.07	0.60 ± 0.04	0.58 ± 0.05
hOGG1/10^6 base pair	0.08 ± 0.03	0.06 ± 0.02	0.17 ± 0.06	0.04 ± 0.01	0.14 ± 0.04	0.17 ± 0.07*	0.10 ± 0.03	0.13 ± 0.05*
HMOX1/10^6 18S	8.0 ± 3.8	13 ± 4.9	4.8 ± 1.5	8.5 ± 2.8	11 ± 4.6	5.9 ± 2.3	5.8 ± 1.6	9.8 ± 4.7
OGG1/10^6 18S	3.9 ± 1.9	5.8 ± 2.2	1.8 ± 0.35	2.5 ± 0.75	5.9 ± 3.2	2.0 ± 0.4	1.9 ± 0.4	7.2 ± 3.8
IL8/10^6 18S	0.3 ± 0.2	0.07 ± 0.01	0.2 ± 0.1	0.05 ± 0.02	0.21 ± 0.09	0.06 ± 0.03	0.07 ± 0.02	0.15 ± 0.07
TNF/10^6 18S	1.0 ± 0.4	1.6 ± 0.6	0.47 ± 0.07	0.9 ± 0.2	1.6 ± 0.68	0.56 ± 0.15	0.56 ± 0.1	0.9 ± 0.25

The data are mean ± SEM ($n = 14$) reported as lesions/10^6 base pair.
* $P < 0.05$ for single factor effect of noise in mixed effects model with adjustment for before value.

exposure, or significant single factor effect of DE exposure, on levels of DNA damage in the mixed effects analysis with or without adjustment for the baseline value measured on the same day before entering the exposure chamber. As there was no sign of interaction, the single factor effect for each of DE exposure and noise exposure could be calculated from the regression coefficients of the mixed effects model. The DE exposure was associated with very small and non-significant changes estimated as the regression coefficients for the single factor effect in the mixed effects model: SB 0.01 lesions per 10^6 basepairs (95% CI: −0.05 to 0.08), FPG-sensitive sites: −0.08 lesions per 10^6 basepairs (95% CI: −0.18 to 0.03) and hOGG1–sensitive sites −0.05 lesions per 10^6 basepairs (95% CI: −0.13 to 0.04). The exposure to high level of traffic noise was associated with an increase of 0.11 lesions per 10^6 basepairs (95% CI: 0.03–0.21) in level of hOGG1-sensitive sites as regression coefficients for the single factor effect in the mixed effects model with adjustment for the value before entering the chamber, whereas there were no significant effects on levels of SB (regression coefficient: −0.01 lesions per 10^6 basepairs (95% CI: −0.07 to 0.06)) and a non-significant increase in FPG-sensitive sites (regression coefficient: 0.07 lesions per 10^6 basepairs (95% CI: −0.04 to 0.18)).

There was no significant change in gene expression of *HMOX1*, *OGG1*, *IL8* and *TNF* in PBMCs (Table 1), or exposure-related change in the level of 8-oxodG in the urine (Supplemental Table S2).

3.2. Ex vivo *study*

After collection and suspending the particles in ELGA water, the mean aggregate hydrodynamic size of the number size distribution was 141 ± 14 nm. The mode peaked at 99 nm, which is close to the 89 nm measured in the chamber room, although only the ultrafine fraction was collected. Note that the 100 nm cut-off of the particle sampler is with respect to the aerodynamic diameter. This corresponds to a cut-off of ~160 nm with respect to mobility diameter (see supplement). The hydrodynamic size measured in suspension and mobility diameter measured in air are diffusion-dependent and should be comparable, also for non-spherical particles.

Whole blood was exposed to DEP, generated by the same vehicle and system as used in the chamber study. The measurement of DNA damage in PBMCs, isolated from DEP-exposed whole blood, showed unaltered levels of DNA damage (Fig. 1). Similarly, there was no significant effect on intracellular ROS production in monocytes, granulocytes or lymphocytes, whereas the positive control (carbon black) was associated with increased intracellular ROS production in monocytes ($P < 0.039$) and granulocytes ($P < 0.003$) (Fig. 2). There were unaltered expression levels of CD62L and CD11b adhesion molecules on the membranes of monocytes, granulocytes or lymphocytes after exposure to DEP in whole blood (Supplemental Table S3).

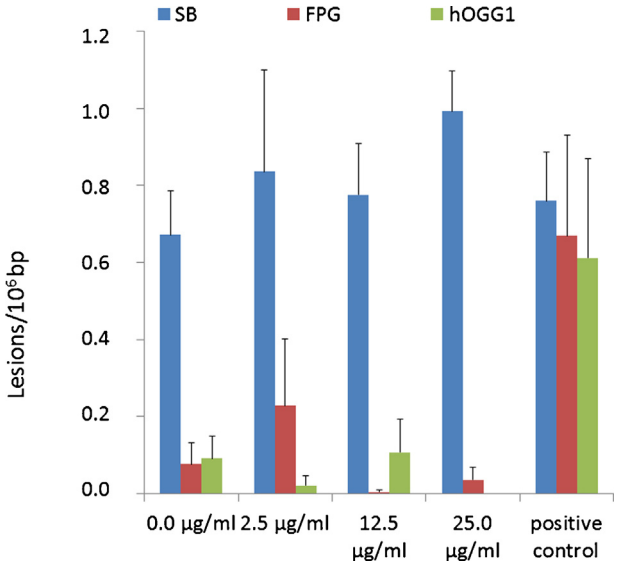

Fig. 1. DNA damage in human PBMCs measured as strand breaks (SB), formamidopyrimidine DNA glycosylase (FPG-) and 8-oxoguanine DNA glycolase (hOGG1)-sensitive sites after 3 h *ex vivo* exposure to diesel exhaust particles. There was no significant difference in levels of SB ($P = 0.54$), FPG- ($P = 0.26$) or hOGG1- ($P = 0.34$) sensitive sites. The positive control (Ro19-8022 photosensitizer plus white light) generated high levels of oxidatively damaged DNA. The results are mean ± SEM ($n = 3$).

4. Discussion

It has previously been shown that exposure to air pollution is associated with elevated levels of 8-oxodG, oxidized purines and SB in leukocytes/PBMCs as well as urinary excretion of 8-oxodG [6]. Yet, there was a surprising lack of effect on DNA damage in PBMCs or urine after DE exposure in the present study with 95% confidence interval of the effect estimate excluding an increase above 0.03 lesions per 10^6 basepairs for FPG- or hOGG1-sensitive sites. Similarly, a 2-h exposure period to DE (200 µg/m^3) resulted in no significant changes in systemic oxidative stress in terms of ascorbic acid in plasma or urinary excretion of 8-oxodG and F_2-isoprostanes in subjects with metabolic syndrome [21]. Most other previous studies have used cross-sectional or panel study designs susceptible to confounding and none included controlled exposure to entirely non-aged DE from solely one source [5].

Concomitant exposure to traffic-related noise and air pollution are considered important for health effects, especially cardiovascular disease, although the mutual confounding between the two factors appeared limited in a recent review [22], in which it is suggested that noise acts through stress responses from neural activation or cognitive interpretation as well as disturbed sleep

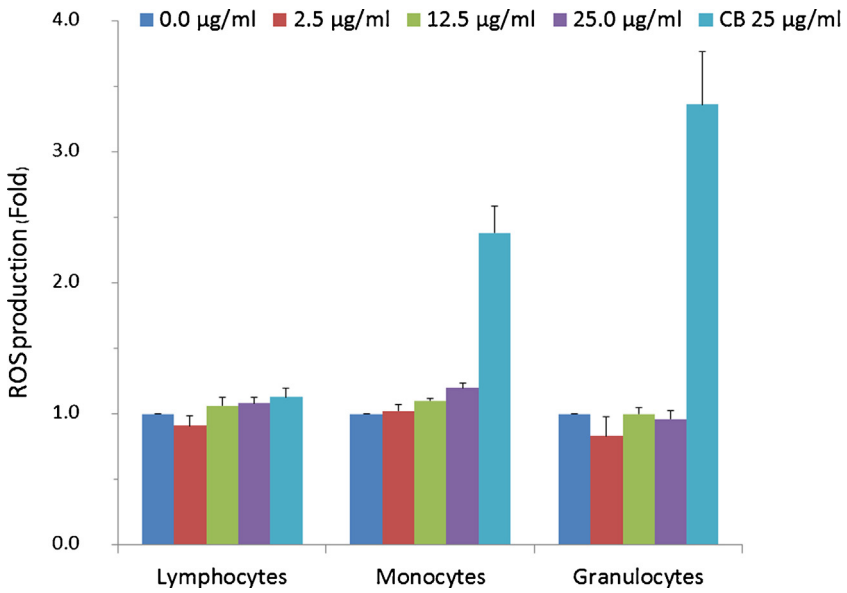

Fig. 2. Intracellular reactive oxygen species (ROS) production in lymphocytes, monocytes and granulocytes after *ex vivo* exposure to diesel exhaust particles in whole blood. The results are fold-difference as compared to unexposed cells. Carbon black (CB, 25 μg/ml) was included as a positive control. The results are mean ± SEM ($n = 3$).

patterns. Severe psychological stress, such as related to schizophrenia and depression, has been associated with systemic oxidative stress including DNA damage [23,24]. Accordingly, exposure to noise might contribute to systemic oxidative stress and not act directly as in the cochlea at intensity levels of no relevance for our study [25]. Overnight exposure to aircraft noise with a maximum of 60 dB showed no effect on blood cortisol, but impaired endothelial function alleviated by vitamin C administration, suggesting systemic oxidative stress as mechanism of action [10]. Similarly, exposure to chronic noise caused oxidative stress in cardiac tissue of rats [26]. An increased risk of breast cancer associated with chronic exposure to traffic noise was suggested related to sleep disturbances [11], but oxidative stress and urinary 8-oxodG excretion are also risk factors for breast cancer [12]. Thus, the significantly increased levels of hOGG1-sensitive sites, and similarly but not significant for FPG-sensitive sites in PBMCs, after the traffic noise exposure in the combined analyses, could be an issue worthwhile further investigation. However, there was large variation in the levels of hOGG1-sensitive sites that had to be adjusted for and the observation could be due to chance.

In observational studies associating air pollution and oxidative stress-related DNA damage the relevant exposure can be considerably longer than 3 h, which might be too short to induce measurable effects. In a similar chamber study, we have found no effect on the same biomarkers collected 0, 6 and 20 h after exposure to 354 μg/m^3 of wood smoke from a well-burning stove [19]. In another study with a 4-h exposure to similar levels of wood smoke, no effects were seen after another 3 h, whereas there was upregulation of *OGG1* and decreased level of SB on the following morning [27]. Still, a week-lasting exposure to wood smoke in a reconstructed Viking age house did not alter the levels of DNA damage in PBMCs, despite indoor $PM_{2.5}$ concentrations of 700–3600 μg/m^3 [28]. However, the characteristics of wood smoke PM depend on burning conditions which at optimum lead to much less lung deposition than seen for non-hygroscopic diesel and traffic-related ultrafine particles [29]. We have previously observed a strong association between levels of ambient ultrafine particles and SB and FPG-sensitive sites in PBMCs from subjects from Cotonou, Republic of Benin [30]. Elevated levels of SB and FPG-sensitive sites in PBMCs were also observed after 6 h of controlled exposure to outdoor air from a busy street in Copenhagen, Denmark, in a chamber with average PM_{10} levels of 22 μg/m^3 and particle counts of ~10,000/cm^3, *i.e.* far lower than in the present study. The effect was particularly associated with a 57 nm particle size mode, considered to represent the soot fraction in DE [31]. The emissions were also dominated by heavy duty vehicles under stop and go traffic and the coarse size PM fraction had a high content of transition metals possibly from break and road surface wear. We have earlier found that oxidative damage to DNA in PBMCs was particularly related to personal exposure to vanadium and chromium in $PM_{2.5}$ in ambient air [32]. Furthermore, the ambient air PM had significant contributions from other engine types, energy production facilities and long range transport, with potential DNA damaging properties. We used a passenger car to generate DE where the PM had physical properties and organic fraction similar to those reported for a heavy duty engine running in a transient cycle, but although engine and running conditions determine physical characteristics and chemical composition of PM, different running conditions of heavy duty diesel engines have induced similar adverse vascular responses [33].

Inflammation markers, including IL-8 and TNF in plasma or serum, have been linked to risk of cancer [34] and extensively studied in relation to exposure to ambient air pollution showing weak associations in with long-term population studies and often no associations in controlled exposure studies [35]. In the present exposure study increased counts of total leukocytes and monocytes were found at 20 h post-exposure [13]. We studied gene expression of *IL8* and *TNF* in PBMCs at the same time point as the genotoxicity measurements. This might be too early even for gene expression and no effect of DE or noise was observed. Controlled exposure to aircraft noise overnight at maximum intensity of 60 dB, showed similarly no effect on neutrophils, IL6 or C-reactive protein levels [10].

We also investigated *ex vivo* oxidative stress effects of DEP, collected from the same diesel engine as the human exposure study, as a hazard identification approach. The lack of observed effects *ex vivo* does preclude effects after *in vivo* exposure. Population-based studies related to air pollution more often show oxidative stress-induced DNA damage than inflammation, whereas the opposite is observed *in vitro* studies of PM [35]. Still, the collected DEP did not generate DNA damage after 3-h exposure to 2.5–25 μg/ml. Activation of leukocytes can lead to increased CD11b expression and

decreased CD62L expression important for vascular adhesion, but we found no change in these or in intracellular ROS in granulocytes, monocytes or lymphocytes. This was despite that carbon black induced intracellular ROS production in this experimental system and in other cell types also increased levels of oxidatively damaged DNA elevated mutant frequency with a spectrum related to ROS [36–39]. We have previously documented that combustion-derived PM from different diesel fuels and engines, including one small car engine complying with the EU 2 standard, and standard reference material of PM from diesel combustion down to concentrations of 2.5 μg/ml increased the level of SB and FPG-sensitive sites in lung (A549) epithelial cells [19,36,39–42]. The number size distribution in the liquid suspension used for the *ex vivo* exposure study was not far from that found in chamber air. Size and composition are important for intracellular ROS formation [43]. Our attempt to mimic part of *in vivo* exposure by using fresh whole blood with antioxidants and multiple proteins contributing to particle corona formation might have influenced the particle-cell interaction and reduced oxidative stress induced effects of DEP [44].

In conclusion, 3-h controlled exposure to high concentrations of DE from a passenger car with or without concomitant traffic noise had no effect on DNA damage or expression of oxidative stress, DNA repair or inflammation genes in PBMCs from healthy human subjects. Although, the lack of genotoxicity was supported by *ex vivo* observations of unaltered DNA damage levels in PBMCs directly exposed to DEP, longer exposure might show effects. Increased hOGG1-sensitive sites as sign of possible genotoxic oxidative stress after noise exposure may warrant further study.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Appendix A. Supplemental information

Supplemental information associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.mrfmmm.2015.03.009.

References

[1] R.D. Brook, S. Rajagopalan, C.A. Pope III, J.R. Brook, A. Bhatnagar, A.V. Diez-Roux, F. Holguin, Y. Hong, R.V. Luepker, M.A. Mittleman, A. Peters, D. Siscovick, S.C. Smith Jr., L. Whitsel, J.D. Kaufman, Particulate matter air pollution and cardiovascular disease: an update to the scientific statement from the American Heart Association, Circulation 121 (2010) 2331–2378.

[2] C.A. Pope III, R.T. Burnett, M.J. Thun, E.E. Calle, D. Krewski, K. Ito, G.D. Thurston, Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution, J. Am. Med. Assoc. 287 (2002) 1132–1141.

[3] IARC Monographs on the Evaluation of Carcinogenic Risk to Humans: Diesel and Gasoline Engine Exhausts and Some Nitroarenes, vol. 105, IARC, 2013, pp. 1–714.

[4] D. Loomis, Y. Grosse, B. Lauby-Secretan, G.F. El, V. Bouvard, L. Benbrahim-Tallaa, N. Guha, R.A. Baan, H. Mattock, K. Straif, The carcinogenicity of outdoor air pollution, Lancet Oncol. 14 (2014) 1262–1263.

[5] P. Moller, J.K. Folkmann, L. Forchhammer, E.V. Brauner, P.H. Danielsen, L. Risom, S. Loft, Air pollution, oxidative damage to DNA, and carcinogenesis, Cancer Lett. 266 (2008) 84–97.

[6] P. Moller, S. Loft, Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution, Environ. Health Perspect. 118 (2010) 1126–1136.

[7] P. Moller, M.S. Cooke, A. Collins, R. Olinski, R. Rozalski, S. Loft, Harmonising measurements of 8-oxo-7,8-dihydro-2′-deoxyguanosine in cellular DNA and urine, Free Radic. Res. 46 (2012) 541–553.

[8] S. Loft, D.P. Hogh, L. Mikkelsen, L. Risom, L. Forchhammer, P. Moller, Biomarkers of oxidative damage to DNA and repair, Biochem. Soc. Trans. 36 (2008) 1071–1076.

[9] W. Babisch, Transportation noise and cardiovascular risk: updated review and synthesis of epidemiological studies indicate that the evidence has increased, Noise Health 8 (2006) 1–29.

[10] F.P. Schmidt, M. Basner, G. Kroger, S. Weck, B. Schnorbus, A. Muttray, M. Sariyar, H. Binder, T. Gori, A. Warnholtz, T. Munzel, Effect of nighttime aircraft noise exposure on endothelial function and stress hormone release in healthy adults, Eur. Heart J. 34 (2013) 3508–3514.

[11] M. Sorensen, M. Ketzel, K. Overvad, A. Tjonneland, O. Raaschou-Nielsen, Exposure to road traffic and railway noise and postmenopausal breast cancer: a cohort study, Int. J. Cancer 134 (2014) 2691–2698.

[12] S. Loft, A. Olsen, P. Moller, H.E. Poulsen, A. Tjonneland, Association between 8-oxo-7,8-dihydro-2′-deoxyguanosine excretion and risk of postmenopausal breast cancer: nested case–control study, Cancer Epidemiol. Biomarkers Prev. 22 (2013) 1289–1296.

[13] Y. Xu, L. Barregard, J. Nielsen, A. Gudmundsson, A. Wierzbicka, A. Axmon, B.A. Jonsson, M. Karedal, M. Albin, Effects of diesel exposure on lung function and inflammation biomarkers from airway and peripheral blood of healthy volunteers in a chamber study, Part. Fibre Toxicol. 10 (2013) 60.

[14] A. Wierzbicka, P.T. Nilsson, J. Rissler, G. Sallsten, Y.Y. Xu, J.H. Pagels, M. Albin, K. Osterberg, B. Strandberg, A. Eriksson, M. Bohgard, K. Bergemalm-Rynell, A. Gudmundsson, Detailed diesel exhaust characteristics including particle surface area and lung deposited dose for better understanding of health effects in human chamber exposure studies, Atmos. Environ. 86 (2014) 212–219.

[15] L. Forchhammer, C. Johansson, S. Loft, L. Moller, R.W. Godschalk, S.A. Langie, G.D. Jones, R.W. Kwok, A.R. Collins, A. Azqueta, D.H. Phillips, O. Sozeri, M. Stepnik, J. Palus, U. Vogel, H. Wallin, M.N. Routledge, C. Handforth, A. Allione, G. Matullo, J.P. Teixeira, S. Costa, P. Riso, M. Porrini, P. Moller, Variation in the measurement of DNA damage by comet assay measured by the ECVAG inter-laboratory validation trial, Mutagenesis 25 (2010) 113–123.

[16] A. Jensen, M. Lohr, L. Eriksen, M. Gronbaek, E. Dorry, S. Loft, P. Moller, Influence of the OGG1 Ser326Cys polymorphism on oxidatively damaged DNA and repair activity, Free Radic. Biol. Med. 52 (2012) 118–125.

[17] M. Lohr, A. Jensen, L. Eriksen, M. Gronbaek, S. Loft, P. Moller, Age and metabolic risk factors associated with oxidatively damaged DNA in human peripheral blood mononuclear cells, Oncotarget 6 (2015) 2641–2653.

[18] P. Moller, D.M. Jensen, D.V. Christophersen, A. Kermanizadeh, N.R. Jacobsen, J.G. Hemmingsen, P.H. Danielsen, D.G. Karottki, M. Roursgaard, Y. Cao, K. Jantzen, H. Klingberg, L.G. Hersoug, S. Loft, Measurement of oxidative damage to DNA in nanomaterial exposed cells and animals, Environ. Mol. Mutagen. 56 (2015) 97–110.

[19] L. Forchhammer, P. Moller, I.S. Riddervold, J. Bonlokke, A. Massling, T. Sigsgaard, S. Loft, Controlled human wood smoke exposure: oxidative stress, inflammation and microvascular function, Part. Fibre Toxicol. 9 (2012) 7.

[20] P.H. Danielsen, E.V. Brauner, L. Barregard, G. Sallsten, M. Wallin, R. Olinski, R. Rozalski, P. Moller, S. Loft, Oxidatively damaged DNA and its repair after experimental exposure to wood smoke in healthy humans, Mutat. Res. 642 (2008) 37–42.

[21] J. Allen, C.A. Trenga, A. Peretz, J.H. Sullivan, C.C. Carlsten, J.D. Kaufman, Effect of diesel exhaust inhalation on antioxidant and oxidative stress responses in adults with metabolic syndrome, Inhal. Toxicol. 21 (2009) 1061–1067.

[22] L.F. Tetreault, S. Perron, A. Smargiassi, Cardiovascular health, traffic-related air pollution and noise: are associations mutually confounded? A systematic review, Int. J. Public Health 58 (2013) 649–666.

[23] M. Maes, P. Ruckoanich, Y.S. Chang, N. Mahanonda, M. Berk, Multiple aberrations in shared inflammatory and oxidative & nitrosative stress (IO&NS) pathways explain the co-association of depression and cardiovascular disorder (CVD), and the increased risk for CVD and due mortality in depressed patients, Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (2011) 769–783.

[24] A. Jorgensen, K. Broedbaek, A. Fink-Jensen, U. Knorr, S.M. Greisen, T. Henriksen, A. Weimann, P. Jepsen, J. Lykkesfeldt, H.E. Poulsen, J.M. Balslev, Increased systemic oxidatively generated DNA and RNA damage in schizophrenia, Psychiatry Res. 209 (2013) 417–423.

[25] L.E. Van Campen, W.J. Murphy, J.R. Franks, P.I. Mathias, M.A. Toraason, Oxidative DNA damage is associated with intense noise exposure in the rat, Hear. Res. 164 (2002) 29–38.

[26] N. Gannouni, A. Mhamdi, O. Tebourbi, M.M. El, M. Sakly, K.B. Rhouma, Qualitative and quantitative assessment of noise at moderate intensities on extra-auditory system in adult rats, Noise Health 15 (2013) 406–411.

[27] P.H. Danielsen, L. Risom, H. Wallin, H. Autrup, U. Vogel, S. Loft, P. Moller, DNA damage in rats after a single oral exposure to diesel exhaust particles, Mutat. Res. 637 (2008) 49–55.
[28] A. Jensen, D.G. Karottki, J.M. Christensen, J.H. Bonlokke, T. Sigsgaard, M. Glasius, S. Loft, P. Moller, Biomarkers of oxidative stress and inflammation after wood smoke exposure in a reconstructed Viking Age house, Environ. Mol. Mutagen. 55 (2014) 652–661.
[29] J. Londahl, A. Massling, J. Pagels, E. Swietlicki, E. Vaclavik, S. Loft, Size-resolved respiratory-tract deposition of fine and ultrafine hydrophobic and hygroscopic aerosol particles during rest and exercise, Inhal. Toxicol. 19 (2007) 109–116.
[30] P.H. Avogbe, L. Ayi-Fanou, H. Autrup, S. Loft, B. Fayomi, A. Sanni, P. Vinzents, P. Moller, Ultrafine particulate matter and high-level benzene urban air pollution in relation to oxidative DNA damage, Carcinogenesis 26 (2005) 613–620.
[31] E.V. Brauner, L. Forchhammer, P. Moller, J. Simonsen, M. Glasius, P. Wahlin, O. Raaschou-Nielsen, S. Loft, Exposure to ultrafine particles from ambient air and oxidative stress-induced DNA damage, Environ. Health Perspect. 115 (2007) 1177–1182.
[32] M. Sorensen, R.P. Schins, O. Hertel, S. Loft, Transition metals in personal samples of PM2.5 and oxidative stress in human volunteers, Cancer Epidemiol. Biomarkers Prev. 14 (2005) 1340–1343.
[33] S. Barath, N.L. Mills, M. Lundback, H. Tornqvist, A.J. Lucking, J.P. Langrish, S. Soderberg, C. Boman, R. Westerholm, J. Londahl, K. Donaldson, I.S. Mudway, T. Sandstrom, D.E. Newby, A. Blomberg, Impaired vascular function after exposure to diesel exhaust generated at urban transient running conditions, Part. Fibre Toxicol. 7 (2010) 19.
[34] D.R. Brenner, D. Scherer, K. Muir, J. Schildkraut, P. Boffetta, M.R. Spitz, L. LeMarchand, A.T. Chan, E.L. Goode, C.M. Ulrich, R.J. Hung, A review of the application of inflammatory biomarkers in epidemiologic cancer research, Cancer Epidemiol. Biomarkers Prev. 23 (2014) 1729–1751.
[35] P. Møller, P. Danielsen, D.G. Karotki, K. Jantzen, M. Roursgaard, H. Klingberg, D. Jensen, D.V. Christophersen, J.G. Hemmingsen, Y. Cao, S. Loft, Oxidative stress and inflammation generated DNA damage by exposure to air pollution particles, Mutat. Res. 762 (2014) 133–166.
[36] H. Frikke-Schmidt, M. Roursgaard, J. Lykkesfeldt, S. Loft, J.K. Nojgaard, P. Moller, Effect of vitamin C and iron chelation on diesel exhaust particle and carbon black induced oxidative damage and cell adhesion molecule expression in human endothelial cells, Toxicol. Lett. 203 (2011) 181–189.
[37] N.R. Jacobsen, A.T. Saber, P. White, P. Moller, G. Pojana, U. Vogel, S. Loft, J. Gingerich, L. Soper, G.R. Douglas, H. Wallin, Increased mutant frequency by carbon black, but not quartz, in the lacZ and cII transgenes of muta mouse lung epithelial cells, Environ. Mol. Mutagen. 48 (2007) 451–461.
[38] N.R. Jacobsen, P.A. White, J. Gingerich, P. Moller, A.T. Saber, G.R. Douglas, U. Vogel, H. Wallin, Mutation spectrum in FE1-MUTA(TM) Mouse lung epithelial cells exposed to nanoparticulate carbon black, Environ. Mol. Mutagen. 52 (2011) 331–337.
[39] L.K. Vesterdal, P.H. Danielsen, J.K. Folkmann, L.F. Jespersen, K. Aguilar-Pelaez, M. Roursgaard, S. Loft, P. Moller, Accumulation of lipids and oxidatively damaged DNA in hepatocytes exposed to particles, Toxicol. Appl. Pharmacol. 274 (2014) 350–360.
[40] P.H. Danielsen, S. Loft, P. Moller, DNA damage and cytotoxicity in type II lung epithelial (A549) cell cultures after exposure to diesel exhaust and urban street particles, Part. Fibre Toxicol. 5 (2008) 6.
[41] J.G. Hemmingsen, P. Moller, J.K. Nojgaard, M. Roursgaard, S. Loft, Oxidative stress, genotoxicity, and vascular cell adhesion molecule expression in cells exposed to particulate matter from combustion of conventional diesel and methyl ester biodiesel blends, Environ. Sci. Technol. 45 (2011) 8545–8551.
[42] K. Jantzen, M. Roursgaard, C. Desler, S. Loft, L.J. Rasmussen, P. Moller, Oxidative damage to DNA by diesel exhaust particle exposure in co-cultures of human lung epithelial cells and macrophages, Mutagenesis 27 (2012) 693–701.
[43] J.A. Araujo, A.E. Nel, Particulate matter and atherosclerosis: role of particle size, composition and oxidative stress, Part. Fibre Toxicol. 6 (2009) 24.
[44] Y. Yan, K.T. Gause, M.M. Kamphuis, C.S. Ang, N.M. O'Brien-Simpson, J.C. Lenzo, E.C. Reynolds, E.C. Nice, F. Caruso, Differential roles of the protein corona in the cellular uptake of nanoporous polymer particles by monocyte and macrophage cell lines, ACS Nano 7 (2013) 10960–10970.