Free Radical Research, September 2005; 39(9): 963–972

Analysis of 8-hydroxydeoxyguanosine among workers exposed to diesel particulate exhaust: Comparison with urinary metabolites and PAH air monitoring

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Accepted by Professor H. Poulsen

(Received 17 May 2005; in revised form 31 May 2005)

Abstract

Oxidative DNA damage and repair, as measured by 8-hydroxy-2′-deoxyguanosine (8-OHdG) in urine and DNA samples were studied in association with work-related diesel exhaust exposure among garage and waste collection workers. Seasonal variations of the urinary 8-OHdG levels in pre- and two post-workshift urine samples of 29 exposed workers and 36 control persons were evaluated. The mean ± SE levels of post-workshift 8-OHdG (μmol/mol crea) were 1.52 ± 0.44 in winter and 1.61 ± 0.33 in summer for the exposed workers, and 1.56 ± 0.61 in winter and 1.43 ± 0.49 in summer for the controls, respectively. No significant difference in the urinary 8-OHdG levels between exposed workers and control subjects in winter ($p = 0.923$) and summer ($p = 0.350$) was observed. A linear mixed model, adjusted for years of employment, age, ex/non-smoking and BMI, indicated no significant dose exposure-relationships between the urinary 8-OHdG and 15 PAH air concentrations nor between the 8-OHdG and 7 PAH monohydroxy-metabolites analyzed in the same workers. 8-OHdG was also analyzed in the mononuclear cell DNA of 19 exposed and 18 control subjects. The mean value of 8-OHdG/non-modified 2′-deoxyguanosine (8-OHdG/105 dG ± SE) were 4.89 ± 0.17 for the exposed and 4.11 ± 0.16 for the control persons, which showed no correlation with the urinary 8-OHdG levels ($r = 0.01$, $n = 28$, $P = 0.96$). The PAH exposure at workplaces was mainly composed of volatile compounds, particularly naphthalene, suggesting low exposure through the respiratory tract and a low effect of PAH in ROS induction.

Keywords: *8-Hydroxy-2′-deoxyguanosine, oxidative stress, diesel exhaust exposure, reactive oxygen species, polycyclic aromatic hydrocarbons (PAH), PAH monohydroxy-metabolites*

Introduction

The formation of reactive oxygen species (ROS), by environmental, occupational and lifestyle factors, generates oxidative stress, resulting in the damage of cellular macromolecules [1–3]. Urinary 8-hydroxydeoxyguanosine (8-OHdG) is a useful biomarker to evaluate oxidative stress and has been related to human diseases, such as cancer, diabetes and neurological disorders [3–5]. Exposure to diesel exhaust particles has been shown to induce oxidative damage and harmful health effects, including increased lung cancer risk [6–7]. Epidemiological studies and results from animal experiments support the human carcinogenicity of diesel exhaust emissions, although the mechanism and the responsible agents for the carcinogenic effects still remain to be clarified [8–10]. Diesel exhaust contains polycyclic aromatic hydrocarbons (PAH), which have been shown to induce toxic health

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ISSN 1071-5762 print/ISSN 1029-2470 online © 2005 Taylor & Francis Group Ltd
DOI: 10.1080/10715760500190115

effects and DNA damage. The particles in diesel exhaust also produce ROS, including hydroxyl radicals (.OH) that may react with DNA [11]. As a marker of oxidative DNA damage, the formation of 8-OHdG can be analyzed in samples obtained from the exposed humans, animals, and from the experimental systems [9,10,12–15]. DNA lesions, such as 8-OHdG, can be repaired in human cells by the base excision repair (BER) process, in which the damaged guanine bases are cleaved by 8-oxoguanine DNA glycosylase 1 (OGG1), and the subsequent synthesis restores the damaged site and removes the sugar residue by short or long patch repair, requiring polymerases and ligases [16–18]. Although the origin of urinary 8-OHdG is still unclear, associations between increased levels of 8-OHdG and environmental and occupational exposures, diseases, aging, smoking and apoptosis have been shown in several studies, in which 8-OHdG is one of the most frequently analyzed markers of DNA damage [4–5,12–15,19–23]. In addition to DNA damage, free radicals can modify other cellular macromolecules, such as lipids and proteins, and are also involved in the process of declining mitochondrial functions [24–26]. Many methods have been used to analyze markers of oxidative DNA base damage, to identify the most reliable method [3,27]. For the analysis of urinary 8-OHdG, the methods include immunoassay (ELISA) [28], an automated method using high performance liquid chromatography (HPLC) with electrochemical detection (EC) [29], mass spectrometry (MS) [30] or tandem mass spectrometry (MS/MS) detection [31], and ^{32}P-postlabeling [32].

We previously published PAH air monitoring and urinary PAH-monohydroxy metabolite data on exposure to diesel exhaust in bus garages, maintenance and household garbage collection [33–35]. We used the same urine samples in this investigation to study whether oxidative DNA damage is related to PAH deriving from occupational diesel exposure. As a marker of oxidative damage, 8-OHdG was analyzed in pre- and post-shift urine samples.

Materials and methods

Study population

About 29 workers participated in this study, including 9 garage workers and 20 waste collection workers (garbage collection and maintenance workers) in the winter, and 16 garage workers and 13 waste collection workers in the summer with the same 36 control persons both in the winter and summer. Garage workers were exposed to diesel exhaust inside the large fleet during the seven and a half-hour work-shift. The garage workers comprised mechanics, workers who cleaned, washed and re-fuelled the buses, special workers (electricians, welders, painters) and foremen. In addition to the diesel exhaust, some workers were exposed to solvents, paints, oils and lubricants. Typically, the buses were driven from outdoors into a large hall, where the engine repair, washing, cleaning and re-fuelling were carried out. Inside the halls, the engines were started and stopped several times, and the amount of diesel exhaust occasionally became high, although all of the garages had efficient ventilation systems. Especially in the winter, the amount of diesel exhaust was elevated and drifted into the repair halls when the engines were cold-started outdoors, close to the garages. The second group of participants was waste collection workers, consisting of garbage truck drivers collecting household waste in Helsinki suburbs and maintenance personnel at the waste collection center. The garbage collection work was partly outdoor work, when emptying the containers and tipping the load at the waste management base, and partly sitting inside the cabin, when driving the trucks from house to house and back to the waste handling center. The truck drivers were exposed to diesel exhaust during their 8h-work-shift when they were working next to the diesel trucks. The maintenance work of the two waste handling centers included the indoor monitoring of the garbage loads while they were weighed, emptied and compressed into the transport containers for further transportation to the large metropolitan region's landfill. The control group consisted of 36 white-collar office and maintenance workers, and research scientists who worked an average 8 h workday indoors. The control persons were matched as closely as possible with the demographic characteristics of the exposed group. All voluntary participants signed an informed consent and were interviewed regarding their personal data, employment, and smoking habits.

Measurements of urinary 8-OHdG

Three spot urine samples were collected in the winter and summer, during a two work week sampling period. Pre-shift samples were collected after two days away from work, before the work-shift started, and two post-shift urine samples were collected at the end of each work week. One urine sample was collected from each control person before the work week started. All urine samples were stored at -20°C until analyzed. The 8-OHdG analyses were performed using an automated HPLC-ECD method, according to Kasai [29]. The creatinine concentrations of the urine samples were analyzed by a standard automated colorimetric determination [36]. Before the analysis, a 50 μl aliquot of a homogenous urine sample was mixed with 50 μl of a solution containing an 8-hydroxyguanosine marker (120 μg/ml) in 4% acetonitrile, 130 mM NaOAc (pH 4.5) and 0.6 mM H_2SO_4 (final pH $<$ 7). Urine samples were stored in a refrigerator at 5°C for 2–3 h before being centrifuged at 13,000 rpm for 5 min. The 8-hydroxyguanosine

marker was mixed with each sample for the purpose of automatic peak detection, to exactly collect the following 8-OHdG fraction during the separation by HPLC. The analysis apparatus consisted of three pumps (Shiseido Nanospace SI-2), the sampling injector (Gilson 231 XL), the UV detector (Toso UV-8020, micro cell), and the Coulochem II EC detector (ESA Coulochem II) with a guard cell (5020, 400 mV) and an analytical cell (5011, $E1 = 280$ mV, $E2 = 350$ mV). The analyses were performed using three solvents: 0.3 mM sulfuric acid in 2% acetonitrile (A), 10 mM sodium phosphate buffer (pH 6.7) in 5% methanol (B), and 0.5 M ammonium sulfate: Acetonitrile (7:3 v/v) (C). Urine samples were first fractionated by an anion exchange chromatography using solvent (A). A 20 μl aliquot of the diluted urine sample was injected into the first HPLC column (MCI GEL CA08F, 7 μm, 1.5 × 150 mm, 37 μl/min, 65°C), and the 8-OHdG-fraction was precisely recognized after the automatic peak detection of the 8-hydroxyguanosine marker at 254 nm. The fraction containing 8-OHdG was then automatically injected into the second HPLC column (Shiseido, Capcell, Pak C 18, 5 μm, 4.6 × 250 mm, 1 ml/min, 40°C), and the 8-OHdG was separated using solvent (B). Thirteen minutes after each injection, the guard column was washed using solvent (C) for 30 min, at a flow rate of 37 μl/min.

Measurements of 8-OHdG in DNA

Peripheral blood samples were collected at the end of each work week. Separation of lymphocytes and mononuclear cells from plasma and red blood cells was carried out by centrifugation at 2500 rpm for 15 min using the LeucoSep tubes (Greiner Bio-One Inc., FL, USA) and Ficoll-Paque (Amersham Biosciences, USA). Mononuclear cells, containing approximately 85% lymphocytes and 15% monocytes, were washed three times with phosphate buffered saline (PBS), pH 7.2. DNA was isolated from the cells by using Qiagen columns, without the phenol/chloroform extraction, according to the Qiagen handbook (Qiagen Inc. CA, USA). Due to the large amount of DNA required for the HPLC/EC, the 8-OHdG levels from the summer samples of the 12 exposed workers and the 16 control persons were measured according to Park et al. [37] Briefly, the DNA (100 μg) was dissolved in 100 μl of distilled water, denatured at 95 °C for 3 min, and digested with 2 μl of 1 M sodium acetate (pH 4.8) and 2 U of nuclease P1 at 37°C for 1 h. Before the treatment with 1.1 U of alkaline phosphatase, 1 M Tris–HCl (16 μl; pH 8) was added, and the digestion was carried out at 37°C for 2 h, followed by centrifugation at 12000 rpm for 10 min. The hydrolyzed DNA sample (40 μl) was analyzed by HPLC (Shimadzu LC-10AD) using an ODS-18 column (Chemoco Pak, 4.6 × 1.0 × 250 mm, 1 ml/min, 40°C). The solvent was composed of 10% methanol, 20 mM sodium acetate and 10 mM citric acid (pH 4.5). The electrochemical detection (BAS LC-4C and BAS Heating Box Ft-1) was carried out with an amperometric cell using an applied potential of +600 mV (5 nA output potential and cell filter set to 0.10 Hz). The molar ratio of 8-OHdG to deoxyguanosine (dG) was determined based on the peak height of 8-OHdG and the peak area of dG, measured by the EC detector and the UV absorbance at A280, respectively. The results were calculated as the number of 8-OHdG molecules per 10^5 2′-deoxyguanosine (dG). Duplicate or triplicate analyses were performed, and the linearity of the molar range, and the day to day variations were verified by injections (40 μl) of 8-OHdG (5 ng/ml) and dG (0.1 mg/ml) standards.

Statistical methods

The Spearman non-parametric test, linear regression and a linear mixed regression model were used to study the correlation and the regression between the urinary 8-OHdG and air PAH concentrations, and the 8-OHdG and the urinary seven PAH monohydroxy-metabolites [38,39]. To compare the 8-OHdG concentrations obtained from the repeated sampling design of the seasonal pre- and post-workshift urine samples, a linear mixed regression model was used. The unstructured correlation function for the three repeated 8-OHdG measurements of the pre- and two post-workshift samples was used, due to the unequal time points of measurements (winter/summer). The linear regression model was applied to study the 8-OHdG data obtained from the control samples, and to study the association of the 8-OHdG levels with creatinine, DNA 8-OHdG, work years, age, non-smoking, ex-smoking, and BMI parameters. Statistical analysis of the data was performed using the SAS software, version 8.2.

Results

The data of the study population characteristics are shown in Table I. Samples from 29 exposed workers in winter and summer were collected for the urinary 8-OHdG measurements. The same 19 workers were studied in winter and summer; however, 7 more garage workers and 7 fewer waste collection workers participated in the summer, as compared to the study group in winter. The years of employment and age respectively ranged from 1 to 43 years (average ± SE from 12.1 ± 10.9 in winter to 18.7 ± 13 in summer) and from 23 to 61 (average ± SE from 41.6 ± 11.7 in winter to 44.9 ± 11.7 in summer). The study group consisted of 13 and 12 non-smokers (43%), 13 ex-smokers (45%), and 3 and 4 smokers (12%) in winter and summer, respectively. The cessation of smoking

Table I. Characteristics of exposed workers and control subjects.

	Exposed workers		Control subjects	
	Winter	Summer	Winter	Summer
Urinary 8-OHdG measurements				
Total participants (*n*)*	29	29	36	36
Employment (mean years ± SE)	12.1 ± 10.9	18.7 ± 13.0	16.3 ± 10.4	17.0 ± 10.7
Age (mean years ± SE)	41.6 ± 11.7	44.9 ± 11.7	46.0 ± 10.0	45.7 ± 10.6
Non-smokers (*n*)	13	12	19	19
Ex-smokers† (*n*)	13	13	16	16
Smokers‡ (*n*)	3	4	1	1
Body mass index (kg/m^2) ± SE	27.0 ± 5.7	26.9 ± 4.8	25.0 ± 3.3	25.3 ± 3.5
DNA 8-OHdG measurements¶				
Total participants (*n*)*		19		17
Employment (mean years ± SE)		20.7 ± 11.6		17.8 ± 11.3
Age (mean years ± SE)		44.7 ± 10.3		47.8 ± 10.2
Non-smokers (*n*)		6		9
Ex-smokers† (*n*)		11		8
Smokers‡ (*n*)		2		–
Body mass index (kg/m^2) ± SE		26.9 ± 5.1		26.4 ± 4.4

*Number of persons in each group. †Cessation of smoking was 9.9 ± 7.2 (urinary 8-OHdG measurements) and 11.1 ± 8.0 (DNA 8-OHdG measurements). ‡Cigarettes smoked per day were 9 ± 9.6 (urinary 8-OHdG measurements) and 13.0 ± 8.2 (DNA 8-OHdG measurements). ¶8-OHdG was analyzed in the mononuclear, mainly lymphocytes, cell DNA.

was, on average, 10 years ago after 9 cigarettes per day were smoked. The body mass indexes (BMI) were 27 and 25 for the exposed workers and control persons, respectively. The control group consisted of 36 subjects, and their employments and ages ranged from 1 to 43 years (average ± SE from16.3 ± 10.4 in winter to 17.0 ± 10.7 in summer) and from 25 to 61 (average ± SE from 46.0 ± 10.0 in winter to 45.7 ± 10.6 in summer). Nineteen were non-smokers, 16 were ex-smokers, and one smoker was both in the winter and summer groups. The BMI values were 25 and 25.3 in winter and summer, respectively. The data also apply to the characteristics of the 19 exposed workers and the 17 control persons participating in summer for the sampling of blood, from which the 8-OHdG analyses in the DNA were carried out.

Urinary 8-OHdG measurements

In total, 173 pre- and post-workshift urine samples of the exposed workers and 72 urine samples of the control persons were analyzed for 8-OHdG. Typical examples of the HPLC-ECD chromatograms of the 8-OHdG standard and the urinary 8-OHdG from an exposed worker are shown in Figure 1A and B, respectively. The 8-OHdG levels were normalized with the creatinine concentrations, while considering the individual variations in the urinary flow rates. A statistically significant correlation ($R = 0.829$, $n = 245$, $P < 0.0001$) was observed when the urinary 8-OHdG (ng/ml) was compared with the creatinine concentration (g/l) (Figure 2). No large variations

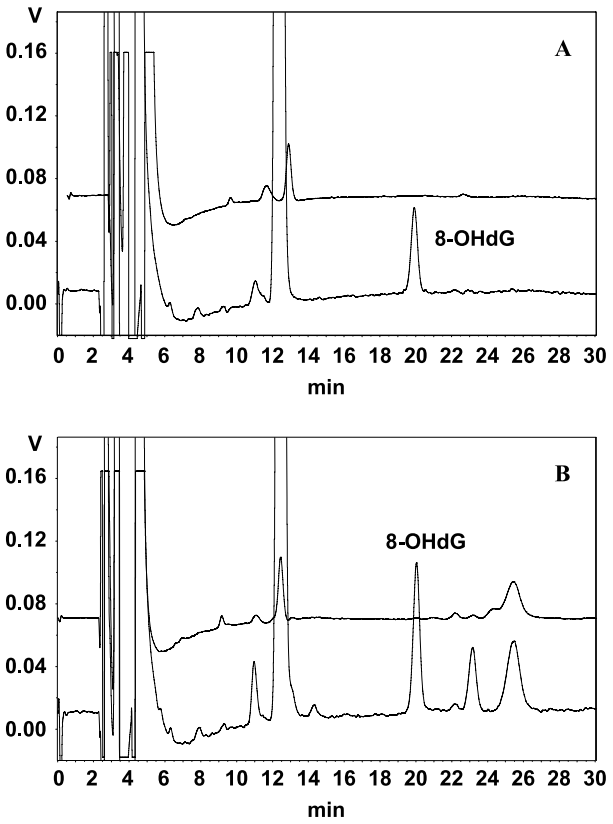

Figure 1. Examples of the coulometric HPLC-ECD chromatograms for the 8-OHdG standard (A) and an exposed worker (B) obtained from a urine sample. The upper and lower panels show chromatogram signals analyzed in channel EC-1 and EC-2, respectively.

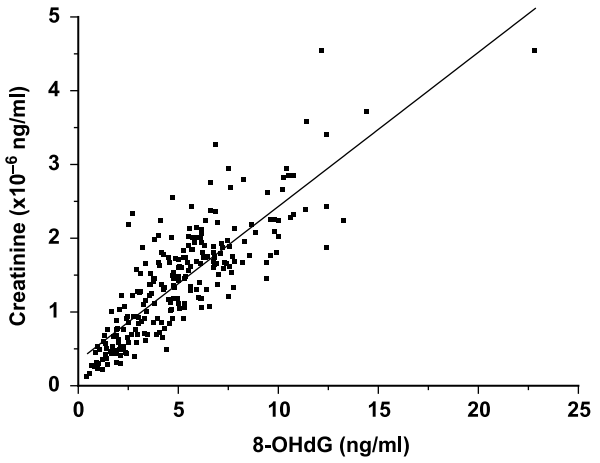

Figure 2. Correlation between creatinine and 8-OHdG levels measured in the urine samples of the exposed workers and controls, $n = 245$, $R = 0.829$, $P < 0.0001$.

in the creatinine-adjusted 8-OHdG levels, in the pre- and post-workshift samples of the exposed workers in winter and summer, were detected. Figure 3 shows that a slightly higher mean level of 8-OHdG was measured in the first post-workshift samples (Post1-WS) than in the second post-workshift samples (Post2-WS); however, no clear difference in the mean 8-OHdG levels in winter between the exposed and control samples was detected. In summer, somewhat lower mean concentrations of 8-OHdG in the pre-workshift samples were measured, while nearly the same 8-OHdG levels were detected in the post-workshift and control samples. Table II summarizes the 8-OHdG concentrations (mean ± SE) in the pre- and two post-workshift urine samples of the exposed and control persons, divided into groups according to the smoking status. In the pre-workshift samples of the exposed workers, the 8-OHdG levels were 1.48 ± 0.46 and 1.39 ± 0.48; 1.44 ± 0.28 and 1.43 ± 0.44; 1.49 ± 0.46 and 1.26 ± 0.40 for non-smokers, ex-smokers and smokers in winter and summer, respectively. The mean 8-OHdG values in the two post-workshift samples were 1.61 ± 0.48, 1.43 ± 0.48, and 1.53 ± 0.36 in winter and 1.47 ± 0.34, 1.59 ± 0.46, and 1.77 ± 0.19 in summer for non-smokers, ex-smokers and smokers, respectively. The lowest mean 8-OHdG level (1.35 ± 0.41) was found for the ex-smokers from the post-workshift samples in winter, whereas the highest mean level (1.77 ± 0.19) was obtained for smokers in summer. The total mean 8-OHdG value for all exposed workers was slightly lower (1.52 ± 0.44) in winter than (1.61 ± 0.33) in summer, whereas the mean 8-OHdG level of all control persons (1.56 ± 0.61) was higher in winter than in summer (1.43 ± 0.49). Both the non-smoking workers and control subjects had higher mean 8-OHdG levels in winter, in contrast to those of the ex-smoking and smoking workers and the ex-smoking controls in summer (Table II). According to the adjusted regression model, no significant difference in the total mean 8-OHdG level between the exposed workers and the control subjects in winter ($p = 0.923$) and summer ($p = 9.350$) was observed.

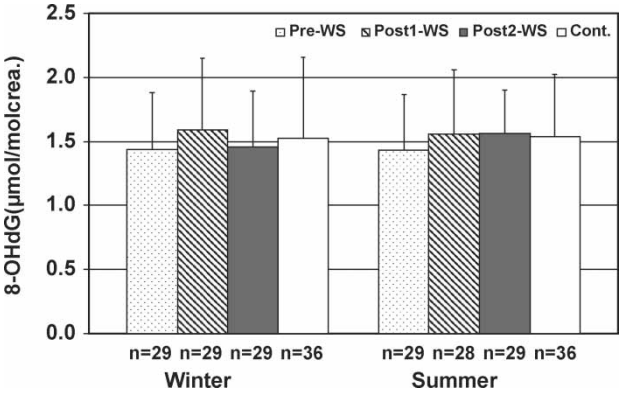

Figure 3. Mean 8-OHdG concentrations of pre- and post-shift urine samples measured in the exposed ($n = 173$) and control persons ($n = 72$) in winter and summer. Pre-WS, Post1-WS and Post2-WS correspond to the samples collected after the weekend before the work-shift started, and at the ends of the 1st and 2nd workweeks, respectively.

8-OHdG in DNA samples

The blood samples collected in the summer were analyzed for the oxidative DNA marker, which revealed 8-OHdG /10^5 dG levels in the mononuclear cells of the exposed workers between 2.98 and 6.67, while those of the control persons were between 1.56 and 7.91 (Figure 4). The mean values of 8-OHdG/10^5 dG for the exposed workers and control persons were 4.84 ± 0.17 and 4.11 ± 0.16, respectively. No significant differences were detected between the mean 8-OHdG /10^5 dG values of 5.42, 4.54, and 4.69 for the non-smoking, ex-smoking and smoking workers, and 3.96 and 4.25 for the non-smoking and ex-smoking control persons, respectively. No significant correlation between the urinary and DNA 8-OHdG was found ($r = 0.011$, $n = 28$, $p = 0.957$).

Association between 8-OHdG and ambient air monitoring and urinary metabolites of PAH

A linear mixed regression model, using the crude data and an analysis adjusted for years of employment, age, smoking and BMI in pre-and post-shift samples, was used to analyze the relationship between the urinary 8-OHdG and the air PAH concentrations and seven PAH monohydroxy-metabolites (Table III). The air PAH concentrations (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[a]anthracene, benzo[b] fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene, and

Table II. Summary of mean (± SE) urinary and DNA 8-OHdG concentrations analyzed by HPLC/EC in exposed workers and control persons.

	Exposed workers				
	Urinary 8-OHdG (μmol/mol creatinine)				
Season	Pre-shift	Post-shift-1	Post-shift-2	Mean of post-shift 1 and 2	Control subjects*
Winter					
Non-smokers ($n = 13/19$)†	1.48 ± 0.46	1.70 ± 0.57	1.53 ± 0.49	1.61 ± 0.48	1.57 ± 0.79
Ex-smokers ($n = 13/16$)	1.39 ± 0.48	1.52 ± 0.58	1.35 ± 0.41	1.43 ± 0.48	1.46 ± 0.42
Smokers ($n = 3/1$)	1.44 ± 0.28	1.44 ± 0.54	1.61 ± 0.32	1.53 ± 0.36	1.65
All ($n = 29/36$)	1.44 ± 0.11	1.55 ± 0.56	1.50 ± 0.41	1.52 ± 0.44	1.56 ± 0.61
Summer					
Non-smokers (12/19)‡	1.43 ± 0.44	1.36 ± 0.35	1.54 ± 0.34	1.47 ± 0.34	1.49 ± 0.51
Ex-smokers (13/16)	1.49 ± 0.46	1.63 ± 0.61	1.56 ± 0.38	1.59 ± 0.46	1.62 ± 0.48
Smokers (4/1)	1.26 ± 0.40	1.88 ± 0.23	1.65 ± 0.26	1.77 ± 0.19	1.18
All (29/36)	1.39 ± 0.43	1.62 ± 0.40	1.58 ± 0.33	1.61 ± 0.33	1.43 ± 0.49
	8-OHdG/10^5 dG in mononuclear cell DNA¶				
Summer					
Non-smokers ($n = 6/9$)‡			5.43 ± 0.17		3.96 ± 0.17
Ex-smokers ($n = 11/8$)			4.54 ± 0.14		4.25 ± 0.16
Smokers ($n = 2/-$)			4.69 ± 0.20		-
All ($n = 19/17$)			4.89 ± 0.17		4.11 ± 0.16

*Only one urine sample was collected from the control persons at the end of the work week. †In parentheses, the number of exposed and control participants in winter, respectively. ‡In parentheses, the number of exposed and control participants in summer, respectively. ¶Blood samples were collected at the end of the two-week sampling period.

indeno[1,2,3-cd]pyrene) and the urinary PAH monohydroxy-metabolites of 2-naphthol, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 1 + 9-hydroxyphenanthrene, 4-hydroxyphenanthrene, and 1-hydroxypyrene were previously analyzed in the same pre- and post-workshift samples in a separate study [33–35]. According to the crude linear mixed regression model, the urinary 8-OHdG levels, respectively in winter/summer, were not associated with the air PAH concentrations ($p = 0.826/0.679$) and the PAH monohydroxy-metabolites ($p = 0.219/0.817$). The adjusted model did neither show a significant relationship in the winter/summer samples between 8-OHdG and air PAH ($p = 0.618/0.688$) and between 8-OHdG and PAH monohydroxy-metabolites ($p = 0.106/0.777$). A statistically significant relationship was obtained in the crude analysis between the urinary 8-OHdG levels in the exposed group and the years of employment ($p = 0.032$) in summer; however, it was not significant in the adjusted model ($p = 0.314$). No associations between 8-OHdG and age, smoking, or BMI, in either the crude or adjusted model, were observed. The control samples did not show a positive association between 8-OHdG and years of employment, age, smoking and BMI in winter and summer (Table III). A significant relationship was observed between the creatinine concentrations and the urinary 8-OHdG levels ($n = 245$, $R = 0.829$, $P < 0.0001$).

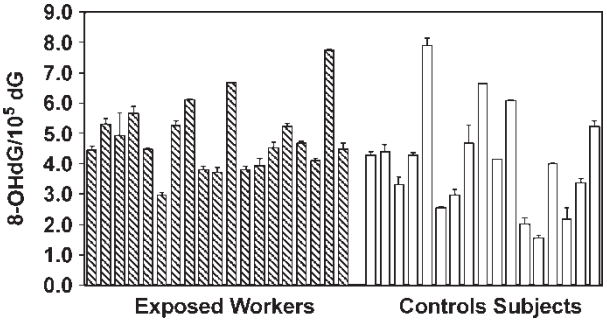

Figure 4. Interindividual variations of mean (± SD) 8-OHdG/10^5 dG levels analyzed in the duplicate samples of mononuclear cell DNA of the exposed workers ($n = 19$) and control subjects ($n = 17$). Samples were collected in the summer and measured by an amperometric HPLC-ECD method.

Discussion

Diesel exhaust is a mixture of several components containing gases, vapors and particles of variable sizes and compositions with organic compounds adsorbed to the particles [6,11]. Airborne particles, as well as diesel exhaust, have been shown to generate ROS, resulting in consequences such as oxidative stress, cellular damage, chronic diseases and cancer, although the critical component(s) in environmental and occupational settings still remain to be identified [3–5,12–15,19–23,40–43].

We used seasonal urine samples and DNA 8-OHdG as biomarkers to investigate whether the oxidative DNA damage in outdoor and indoor environments is

Table III. Summary of the significance coefficient (p) obtained from the linear mixed regression model between urinary 8-OHdG levels obtained from the pre- and post-workshift samples and years of employment, age, ex- or non-smoking status, BMI, PAH air concentrations, and urinary monohydroxy-metabolites.

	Exposed workers		Control persons	
Variables	Crude (p)	Adjusted (p)	Crude (p)	Adjusted (p)
	Winter		Winter	
Employment (years)	0.472	0.347	0.657	0.657
Age (years)	0.638	0.229	0.423	0.509
Ex-/Non-smoking*	0.431	0.507	0.629	0.956
Body mass index (kg/m^2)	0.790	0.475	0.340	0.494
Air PAH concentrations†	0.826	0.618		
PAH monohydroxy metabolites†	0.219	0.106		
	Summer		Summer	
Employment (years)	0.032	0.314	0.562	0.294
Age (years)	0.088	0.645	0.945	0.269
Ex-/Non-smoking*	0.918	0.890	0.469	0.556
Body mass index (kg/m^2)	0.163	0.241	0.645	0.789
Air PAH concentrations†	0.679	0.688		
PAH monohydroxy metabolites†	0.817	0.777		

*Three smokers in winter and 4 smokers in summer were excluded from the variables. †Statistical analyses were carried out only on PAH data obtained from the personal air monitors and urinary hydroxy-metabolites of the exposed workers.

associated with diesel exhaust exposure. Between the exposed workers and the controls and between the pre- and post-workshift samples no significant differences in urinary 8-OHdG were observed. In the summer, the total mean 8-OHdG in the post-workshift urine samples of the exposed workers was elevated in comparison to with those collected in the winter; however, a significant difference was missing. Levels of urinary 8-OHdG were similar, as analyzed in workers exposed to quartz, or asbestos, as well as those in rubber work, or roofing [1,21,40,41]; however, about 3.5-fold higher levels than those present in our study were obtained for boilermakers, taxi drivers, and toll station workers [14,42,43]. The possible reasons for the low levels of 8-OHdG in our study could be that the post-shift sampling was presumably not from a recent peak exposure, but rather instead represented an accumulation of a constant background excretion level. The urine samples were stored for two years before they were analyzed, and although the 8-OHdG might have degraded during this time, the control samples revealed no significant difference between the summer and winter samples (difference of six months storage in -20°C). Previous studies in our laboratory showed a low air PAH exposure and consequently low levels of urinary PAH monohydroxy-metabolites analyzed in the same samples [33–35]. Assuming that the formation of oxidative DNA damage is only correlated with the PAH compounds, the low PAH exposure would be expected to induce correspondingly low levels of 8-OHdG. Based on the urine spot samples, we cannot entirely conclude that the 8-OHdG is dependent on the PAH exposure during the period prior to the collection of urine. In all of the workplaces where diesel exposure was measured, the air concentration of B[a]P exposure was several orders of magnitude lower than the OEL8 h value (10 μg/m^3) set for B[a]P in Finland. One reason for the small differences between the diesel exposed and non-exposed subjects, in general, could also be the low number of diesel-powered vehicles in Finland and the reformulated diesel fuel used. Furthermore, a recent epidemiological study reported that the risk for lung cancer among Finnish workers occupationally exposed to diesel (and gasoline) engine exhaust is not elevated [44].

A significant difference in the DNA 8-OHdG levels between asbestos-exposed workers and controls was reported [13]; however, the 8-OHdG levels analyzed of the total white blood cells of the coke oven and graphite-electrode workers were similar to those of the control persons [22,23]. These studies are in agreement with our study, which found no association between 8-OHdG levels and external PAH exposure and PAH monohydroxy-metabolites. The 8-OHdG levels in DNA analyzed here and in several studies suggest a cautious interpretation of the results, since many technical (cell type DNA, storage of samples, sampling period and DNA hydrolysis) and other confounding factors (diet, smoking, alcohol consumption, sex, age, specificity of repair enzymes) needs to be better optimized [4,27,45]. Air PAH sampling indicates an exposure during the daily work-shift, whereas urinary PAH metabolites possess different half-lives, and thus reflect exposure from the last work-shift to some days before the work-shift [22,23]. Furthermore, biomarkers of an effective dose, such as DNA 8-OHdG, and an internal dose, such as PAH urinary metabolites, are indicators of exposure; however, in this study the diesel exhaust-derived PAH were probably too low to induce a measurable dose-response related effect.

The group of exposed workers and controls in our study was relatively homogeneous, in terms of years of employment, age and BMI. The differences in years of employment and age were larger in winter than in summer, which we considered to have an effect; however, in an adjusted model no significant difference in the 8-OHdG levels between the control and exposed groups was observed (Table III). Smoking has also been reported to be an important factor contributing to high 8-OHdG concentrations [46]. We had only 3 and 4 smokers in winter and summer, respectively, which were too few to have a statistically significant influence on the 8-OHdG levels. Smoking has been reported to increase the 8-OHdG levels significantly among taxi drivers and engine room personnel [41,42], but not among boilermakers, coke-oven workers, graphite-electrode plant workers and highway toll station workers [14,22,23,43]. No correlation was obtained between 8-OHdG and age, in contrast to the results of some studies [14,41], that showed that age is an important predictor (adjusted regression model) of increased levels of 8-OHdG, but in agreement with other studies that found no effect of age [22,23,40,46].

The mice model has shown that exposure to carbon particles without the organic chemicals on their surfaces, or the presence of carbon particles in the lung tissues of lung cancer patients elevated the formation of 8-OHdG, and thus it was concluded that the particles themselves, not the mutagenic and carcinogenic chemicals, induced the oxidative damage [12]. In the experimental cell cultures, however, the role of particle-derived chemicals has been demonstrated to be relevant in inducing apoptosis, mitochondrial damages and allergic inflammation [47,48]. Li et al. have compared the effects on macrophages and bronchial epithelial cells in response to chemicals containing aliphatic, aromatic and polar fractions deriving from the extracts of diesel particles [49]. The extracted chemicals induced oxidative stress and cytotoxicity in bronchial epithelial cells, and were positively correlated with makers of these effects. It has also been shown that P450 CYP1A1/1B1 and human dihydrodiol dehydrogenase compete in the metabolic activation of benzo[a]pyrene-7,8-diol (BPD) to form anti-BPDE or through the auto-oxidation quinones (BP-7,8-diones), which then generate reactive oxygen species [50]. Thus, in addition to diesel exhaust particles the effects of PAH compounds on ROS generation should be considered. In our study, we measured 16 PAH compounds in particle extracts obtained from the personal monitors equipped with a 2 μm pore size filters and from the XAD-trapped volatile PAH fraction. Nearly 300-fold higher levels of volatile PAH, consisting mainly of naphthalene and a 2-fold lower amount of phenanthrene, were detected in comparison with those measured in particle extracts [33,34]. Kim et al. suggested that more ROS are generated by high- than low molecular weight PAH, and also that the inhalation exposure of naphthalene may cause less oxidative damage than that adsorbed through the skin and gastrointestinal tract [51]. The high naphthalene levels, and high volatile PAH compounds, could be one explanation for the low 8-OHdG values in our study. Furthermore, a more sensitive marker such as the analysis of heme oxygenase expression, which is used as an indicator for low exposure to diesel particles, rather than 8-OHdG, could have been used [49]. In this occupational study, the impact of the exposure to diesel exhaust, with its multi-components, would not only influence the dose and bioavailability of the inhaled exhaust particles, but also the ROS derived from particles and the repair processes [18,47–49]. Thus, urinary 8-OHdG levels could be due to several induced and/or inhibited metabolic pathways and repair processes.

In conclusions, we showed that low occupational exposure to diesel exhaust did not generate higher 8-OHdG levels among the exposed workers in comparison with the control subjects. There were no positive associations found between the 8-OHdG levels and the air PAH concentrations and the urinary PAH monohydroxy-metabolites after adjusting for years of employment, age, ex- and non-smoking and BMI. Whether PAH derived from the diesel exhausts, or the particles themselves, have a crucial effect on the oxidative damage at the cellular level, more sensitive markers are needed to understand the consequences of low exposure to diesel exhaust particles.

Acknowledgements

The Academy of Finland and the Japan Society for the Promotion of Science is acknowledged for the financial support. Ms Nina Tamminen and Ms Sayumi Yamasaki are thanked for the skilful assistance and Helsinki City Transport and Metropolitan Council in Finland are thanked for their contributions to this study.

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