Personal Exposure to $PM_{2.5}$ and Biomarkers of DNA Damage[1]

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Abstract

Ambient particulate air pollution assessed as outdoor concentrations of particulate matter ≤ 2.5 μm in diameter ($PM_{2.5}$) has been associated with an increased cancer risk. However, outdoor $PM_{2.5}$ concentrations may not be the best measure of the individual particle exposure that is a sum of many sources besides outdoor particle levels, *e.g.*, environmental tobacco smoke and cooking. We measured personal $PM_{2.5}$ and black smoke exposure in 50 students four times over 1 year and analyzed for biomarkers of different types of DNA damages. Ambient $PM_{2.5}$ concentrations were also measured. Exposure was measured for 48 h, after which blood samples were collected and analyzed for DNA damage in lymphocytes in terms of 7-hydro-8-oxo-2′-deoxyguanosine (8-oxodG), strand breaks, endonuclease III- and fapyguanine glycosylase-sensitive sites, and polyaromatic hydrocarbon adducts. Twenty-four-h urine collections were analyzed for 8-oxodG and 1-hydroxypyrene. Personal $PM_{2.5}$ exposure was found to be a predictor of 8-oxodG in lymphocyte DNA with an 11% increase in 8-oxodG/10 $\mu g/m^3$ increase in personal $PM_{2.5}$ exposure ($P = 0.007$). No other associations between exposure markers and biomarkers could be distinguished. The genotype of glutathione *S*-transferase M1 (GSTM1), T1 (GSTT1), and P1 (GSTP1) and NADPH:quinone reductase was also determined, but there were no effects of genotype on DNA polyaromatic hydrocarbon adducts or oxidative damage. The results suggest that moderate exposure to concentrations of PM can induce oxidative DNA damage and that personal $PM_{2.5}$ exposure is more important in this aspect than is ambient $PM_{2.5}$ background concentration.

Introduction

Epidemiological studies have found particulate air pollution in the urban environment to be associated with an increase in cancer, especially lung cancer (1). Particles generated by combustion are composed of a carbon core to which compounds, such as metals and PAHs,[3] adhere. One hypothesis to the observed adverse health effects is that PM can induce oxidative stress mediated by a particle-induced inflammation causing macrophages to release ROS, by transition metals on the particle surface capable of generating ROS through the Fenton reaction or by quinones in the particles that produce ROS through redox cycling (2, 3). Several studies have demonstrated that diesel exhaust particles induce production of ROS both with (3, 4) and without (5) the presence of biological-activating systems. Oxidative damage to DNA is most widely assessed with 8-oxodG as a biomarker. Several experimental studies have found increased 8-oxodG levels after exposure to PM both *in vitro* (6, 7) and *in vivo* (8). The induction of oxidative stress by quinones is speculated to be modified by genotype of the Phase II enzyme NQO1, which catalyzes a detoxification of quinones (9).

PAHs are also hypothesized to be involved in the particle-induced carcinogenesis as several PAHs found in urban and indoor PM, *e.g.*, benzo[a]pyrene is classified as probably carcinogenic in humans (10). PAHs undergo metabolic activation by cytochrome P450 enzymes. This involves the formation of epoxides and diolepoxides capable of binding covalently to DNA, thereby potentially initiating the carcinogenic process (11). Additional metabolism results in deactivation through glutathione conjugation by GSTs, followed by excretion in urine. The biological response to PAHs in humans varies substantially. These interindividual differences, *e.g.*, measured by concentrations of PAH adducts in DNA have been associated with polymorphisms in several metabolism enzymes (12, 13).

In most studies assessing the effect of particulate air pollution on health, either occupationally exposed groups have been studied or outdoor monitoring of urban background $PM_{2.5}$ or PM_{10} concentrations have been used as exposure estimate. However, people spent ~90% of their time indoors, and several indoor PM sources have been identified (14, 15). This associates risk assessment based mainly on outdoor measurements of air pollutants with some uncertainty, because the outdoor PM concentrations used in the health studies to estimate exposure may not reflect true population exposures to PM. Monitoring personal exposure to PM and relevant biological effects may be difficult. However, by means of biomarkers mechanistically

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[3] The abbreviations used are: PAH, polyaromatic hydrocarbon; PM, particulate matter; PM_{10}, particulate matter ≤ 10 μm in diameter; $PM_{2.5}$, particulate matter ≤ 2.5 μm in diameter; 8-oxodG, 7-hydro-8-oxo-2′-deoxyguanosine; GST, glutathione *S*-transferase; NQO1, NADPH:quinone reductase; 1-HP, 1-hydroxypyrene; ROS, reactive oxygen species; SB, strand break; FPG, fapyguanine glycosylase; ENDO, endonuclease III; HPLC, high-performance liquid chromatography; ETS, environmental tobacco smoke.

related to relevant health effects, it may be possible to assess relevant exposure to PM and the involved sources.

The aim of this study was to examine the link between personal exposure to fine PM and biomarkers related to an increased risk of cancer. Personal $PM_{2.5}$ exposure was estimated for 50 students living in central Copenhagen four times in 1 year. Collected blood and urine samples were analyzed for PAH adducts, 1-HP excretion, markers of oxidative DNA damage, and genotypes of the susceptibility genes *GSTT1, GSTM1, GSTP1,* and *NQO1*.

Materials and Methods

Experimental Design. The personal exposure to $PM_{2.5}$ and black smoke were measured in 50 students living and studying in central Copenhagen. To account for seasonal variation, the measurements were repeated four times in 1 year. Measuring campaigns of 5 weeks were conducted in November 1999 (autumn), January through February 2000 (winter), April through May 2000 (spring), and August 2000 (summer). Measurements were conducted over 2-day periods for each subject, with 5 subjects monitored from Monday morning to Wednesday morning and 5 other subjects from Wednesday morning to Friday morning. In each 2-day monitoring period, urban background concentrations of $PM_{2.5}$ were measured on the rooftop of a building (20 meters above ground) on the Copenhagen University campus. At the same rooftop, outdoor temperature was registered on an hourly basis throughout the 48-h measuring periods and averaged for each 2-day measuring period.

The subjects were recruited through a notice in a university newsletter. All participants were nonsmokers, living, and studying in central parts of Copenhagen. They were 20–33 years of age, with a median age of 24 years, and there was an even distribution of males and females. Not all of the 50 subjects could participate in all four campaigns, and new subjects were recruited so that in each campaign, 50 subjects participated. In all, 68 subjects participated of whom 31 subjects participated in all four campaigns (corresponding to 124 measurements), 12 subjects participated in three campaigns (corresponding to 36 measurements), 10 subjects participated in two campaigns (corresponding to 20 measurements), and 15 subjects participated in only one campaign (corresponding to 15 measurements). All together, 195 measurements were collected which were all included in the subsequent statistical analysis. The subjects filled in questionnaires, which included registration of how long they spent at the same location as smokers (exposure to ETS). Morning blood samples were collected at the end of each 2-day campaign, and 24-h urine samples were collected at the 2nd day of the measuring campaign. The local ethics committee approved the study protocol, and subjects gave written informed consent before entry into the study.

Air Sampling and Analysis. The particles were sampled using a system from the International Gravity Bureau (Toulouse, France) (16), a KTL $PM_{2.5}$ cyclone developed for the European EXPOLIS study (17), a International Gravity Bureau 400 pump (flow 4 liters/min), and a battery for 48-h operation. The samplers were in operation for 48 h to collect sufficient $PM_{2.5}$ material for accurate measurement of mass. The equipment for personal sampling was placed in a backpack, which the subjects carried or placed nearby when they were indoors. Sampling was done on 37-mm Teflon filters (Biotech Line, Lynge, Denmark). Before and after sampling, the filters were weighted on a Micro weight MT5 from Mettler-Toledo (Glostrup, Denmark) after conditioning for 24 h in the laboratory. The detection limit was determined to ~26 μg and defined as three times the SD on blank filters. On the basis of eight parallel measurements, repeated six times, the coefficient of variation was calculated to 14.4%.

The reflectance level (black smoke) of the $PM_{2.5}$ filters was measured by a Model 43 Smokestain Reflectometer (Diffusion Systems LTD, London, United Kingdom). On each filter, the reflectance was measured with triple determinations in five different spots. The 15 measurements were averaged and transformed into the absorption coefficient (a, m^{-1}) using the following formula: $a = (A/2V) \times \ln(R_0/R)$ (18). A is the area of the stain on the filter paper (m^2), V is the volume sampled (m^3), R is the intensity of reflected light from the exposed filter, and R_0 is the intensity of reflected light from a clean filter. The coefficient of variation was 22.2%, calculated on the basis of eight parallel measurements repeated five times. Three black smoke measurements were below detection limit of 0.01×10^{-6} m^{-1}. To include these measurements in a logarithmic model, they were given the value of 0.007×10^{-6} m^{-1} estimated according to the formula: detection limit/square root 2, suggested by Hornung and Reed (19).

Analysis of 8-oxodG. After venous puncture, blood samples were collected in 2 × 10-ml sodium heparin tubes (Termo Venoject glass tubes, Leuven, Belgium). Immediately, lymphocytes from the 20-ml whole blood were isolated by centrifugation for 30 min at 350 × *g* after diluting the blood 1:1 with 0.9% NaCl and adding 4 ml of Lymphoprep (Nycomed Pharma, Oslo, Norway) to the bottom of 5 ml of the blood/NaCl solution. The lymphocytes were then collected, washed in 0.9% NaCl, and centrifuged 10 min at 350 × *g*. The pellet was subsequently washed in 2 ml of lysis buffer [0.32 M sucrose, 5 mM $MgCl_2$, 10 mM Tris, 0.1 mM desferoxamine mesylate, and 1% Triton X (pH 7.5)] and centrifuged at 1500 × *g* for 10 min. The DNA isolation procedure and the HPLC with electrochemical detection were performed immediately as described previously (20). Storage problems preclude the use of lymphocytes for quality control. However, with rat tissues used for quality control, the interassay coefficient of variation was <18%. The urinary concentration of 8-oxodG was measured by column switching HPLC-electrochemical detection as described previously (21).

Single-cell Gel Electrophoresis (Comet Assay). Lymphocytes were isolated by adding 60 μl of whole blood collected in sodium heparin tubes (Termo Venoject glass tubes) to 1 ml of RPMI + 10% FCS, followed by incubation on ice for 30 min. Then, 200 μl of Lymphoprep were added, and the solution was centrifuged for 6 min at 300 × *g*. The lymphocytes were collected and washed with 1 ml of PBS. After centrifugation, the pellet was resuspended in 60 μl of PBS. Single-cell gel electrophoresis and measurement of SBs, as well as FPG- and ENDO-sensitive sites, were done as described previously (22). FPG- and ENDO-sensitive sites reflect oxidized purine and pyrimidines in DNA, respectively (23). The cells were visually scored according to five classes (0–4) and summed, giving a total score between 0 and 400 (23).

PAH Adducts and 1-HP. PAH adducts were measured in lymphocyte DNA by ^{32}P-postlabelling using butanol enrichment as described previously (12). An internal standard was used to correct for assay variability, and each sample was measured in at least two separate analyses. The interassay coefficient of variation was <10%. After measuring all 186 samples, further quality control was used as we repeated the measurement of 16 samples selected from different runs and seasons. The coefficient of variation between the separated

Table 1 Median concentrations of the measured biomarkers and exposure markers overall and split into seasons

	All		Autumn		Winter		Spring		Summer		Difference
	Median (Q25–Q75)	*n*	Median (Q25–Q75)	*n*	Median (Q25–Q75)	*n*	Median (Q25–Q75)	*n*	Median (Q25–Q75)	*n*	*P*
8-oxodG in lymphocytes (per 10^5 dG)	0.52 (0.33–0.68)	121	0.55 (0.34–0.76)	40	0.27 (0.15–0.33)	26	0.62 (0.52–0.78)	38	0.58 (0.47–0.70)	17	<0.0001
8-oxodG in urine (nmol secreted/kg body weight)	0.23 (0.18–0.30)	193	0.26 (0.20–0.39)	49	0.22 (0.19–0.28)	49	0.24 (0.16–0.29)	47	0.21 (0.16–0.27)	47	0.055
SBs (comet score)[b]	4.5 (2–10)	186	5 (2–9)	47	3 (1–5)	47	3 (1–5)	45	11 (5–21)	47	<0.0001
Endonuclease sensitive sites (comet score)[b]	0.5 (−2–3)	184	0 (−2–3)	47	0 (−1–1)	45	1 (−1–3)	45	2 (−2–6)	47	0.42
FPG-sensitive sites (comet score)[b]	2.0 (−1–5)	182	2 (−2–5)	47	1 (−1–4)	45	1 (−1–5)	45	3 (−1–10)	47	0.98
PAH adducts (fmol/μg DNA)	0.26 (0.14–0.49)	186	0.09 (0.06–0.14)	48	0.30 (0.20–0.48)	48	0.40 (0.26–0.60)	46	0.37 (0.23–0.59)	43	<0.0001
1-HP (fmol secreted/kg BW)	9.2 (6.3–14.2)	191	9.7 (7.5–12.2)	49	13.1 (8.2–19.1)	47	9.3 (6.3–12.5)	47	7.8 (4.2–10.7)	47	<0.0001
Personal $PM_{2.5}$ exposure ($\mu g/m^3$)	16.1 (10.0–24.5)	180	20.7 (13.1–27.6)	44	15.7 (10.5–24.2)	43	20.6 (12.6–28.2)	46	12.7 (8.3–21.4)	46	0.011
Urban background $PM_{2.5}$ ($\mu g/m^3$)	9.2 (5.3–14.8)	157	10.2 (5.3–10.6)	34	7.4 (6.2–9.2)	31	15.3 (12.9–21.8)	43	6.7 (5.0–12.6)	48	<0.0001
Personal black smoke exposure (10^{-6} m^{-1})	8.1 (5.0–13.2)	177	12.6 (6.5–17.2)	42	8.2 (5.1–13.3)	46	7.1 (5.4–10.4)	46	6.8 (3.4–9.0)	47	0.050

[a] Mixed model regression, regarding subject levels as a random factor, was used to test for differences according to the season. The natural logarithm of the biomarkers and exposure markers was set as an outcome variable in 10 separate models, where the season was included as predictor.
[b] For analysis, 20 was added to the FPG and ENDO score and 1 to the SB score to obtain positive values for transformation to the natural logarithm.

analytical periods was 12.6%. 1-HP was measured by HPLC with fluorescence detection as described previously (24).

Determination of GSTT1, GSTM1, GSTP1, and NQO1 Genotypes. DNA was isolated from lymphocytes using standard phenol extraction procedures. The genotypes of GSTT1, GSTM1, GSTP1, and NQO were determined by PCR-based assays as described previously (25).

Statistics. All statistical analyses were carried out using SAS software (version 8e). Mixed model repeated measures analysis (Proc mixed) was used to describe concentrations of the biomarkers (8-oxodG in lymphocytes, urine, comet scores, PAH adducts, and 1-HP) as a function of various predictors. As predictors, we included season, gender, average outdoor temperature, and, in four separate models for each dependent variable, the exposure markers: (*a*) personal $PM_{2.5}$ exposure; (*b*) personal black smoke exposure; (*c*) background $PM_{2.5}$ concentration; and (*d*) percentage of time exposed to ETS. The season was included to account for systematic errors in exposure sampling and analysis of the biomarkers. In the PAH adduct models, the genotypes of GSTM1, GSTT1, and GSTP1 were included as predictors. In the models with biomarkers of oxidative stress (8-oxodG-, ENDO-, and FPG-sensitive sites), the NQO1 genotype was included as a predictor. Subject nested in gender was included as a random factor to account for factors that could possibly lead to an (within the subject) inherent basis level in the dependent variable that was not included in the model. A backward selection was applied, and in the final model, only significant factors were included. The dependent variables were transformed by the natural logarithm to obtain variance homogeneity and normal distribution of the residuals. The models are therefore not linear in original scale, and model estimates represent slopes in the logarithmic analysis. To calculate the predictive value of an X unit increase in one of the predictors, the following formula was used: [exp(model estimate $\times$ X) $-$ 1] $\times$ 100.

Similar models were used to test for associations between the exposure markers, with the natural logarithm of personal $PM_{2.5}$ exposure as dependent variable and personal black smoke exposure, background $PM_{2.5}$ concentration, and exposure to ETS as predictors. Subject was included as a random factor.

Results

Descriptive Data. Results on biomarkers and exposure markers according to the four seasons are presented in Table 1. For all of the markers, except ENDO- and FPG-sensitive sites, the season was found to be a significant predictor (Table 1). The 68 participating subjects showed the following genotype distribution: (*a*) for GSTM1, 53% were 1/1 or 1/2, and 47% were 2/2; (*b*) for GSTT1, 86% were 1/1 or 1/2, and 14% were 2 of 2; (*c*) for GSTP1, 44% were a/a, 50% were a/b, and 6% were b/b; and (*d*) for NQO1, 71% were C/C, 26% were C/T, and 3% were T/T.

Relationship between the Exposure Markers and Biomarkers. The personal $PM_{2.5}$ exposure was a predictor of 8-oxodG in lymphocyte DNA with an 11% increase in 8-oxodG/10 $\mu g/m^3$ increase in personal $PM_{2.5}$ exposure ($P = 0.007$; Table 2; Fig. 1). Season was found to be a significant predictor ($P < 0.0001$), whereas average outdoor temperature, gender, and NQO1 were excluded in a backward selection. The inclusion of subject nested in gender as a random factor in the model corresponds to specifying the responses observed on the same subject to be correlated. However, this correlation was estimated to zero, indicating no unexplained similarity between observations on the same subject. The residual variance was 0.18, corresponding to a coefficient of variation on 42% for unexplained interindividual variation. Personal exposure to black smoke and background $PM_{2.5}$ concentrations showed no significant relationship to 8-oxodG in lymphocytes (Table 2), and neither did exposure to ETS. For 8-oxodG in urine, the only significant predictors were season and gender.

There were no significant associations between the exposure markers and PAH adducts (Table 2) and no effects of the GST genotypes or average outdoor temperature. Season was found to be a significant predictor with highest concentrations

Table 2 Associations between the exposure markers and biomarkers

Mixed model regression regarding subject nested in gender as a random factor was used to test for associations between the biomarkers and exposure markers measured. The natural logarithm of the biomarker in question was included as an outcome variable. Exposure marker (personal exposure to $PM_{2.5}$ or black carbon or background $PM_{2.5}$ concentration), gender, season, average outdoor temperature, and several genotypes were set as explanatory variables in 21 separate models. Except for the models with 8-oxodG in urine as outcome variable, gender was excluded from all models because of insignificance. Season was excluded from the models where 8-oxodG in urine and ENDO- and FPG-sensitive sites were set as outcome variable. Average outdoor temperature was only a significant predictor in models with SBs as outcome variables. The genotypes were excluded from all models.

Outcome variables	Personal exposure to $PM_{2.5}$ ($\mu g/m^3$)		Personal exposure to carbon black (10^{-6} m^{-1})		Background concentration of $PM_{2.5}$ ($\mu g/m^3$)	
	Model estimate	*P*	Model estimate	*P*	Model estimate	*P*
8-oxodG in lymphocytes (per 10^5 dG)	0.010	0.007*	−0.0021	0.79	0.0076	0.43
8-oxodG in urine (nmol secreted/kg body weight)	−0.0007	0.75	0.0002	0.94	0.0109	0.08
SBs (comet score)[a]	−0.0024	0.60	−0.0075	0.27	−0.0011	0.93
ENDO-sensitive sites (comet score)[a]	0.0025	0.30	0.0049	0.16	−0.0024	0.70
FPG-sensitive sites (comet score)[a]	0.0014	0.50	0.0033	0.26	0.0024	0.70
PAH adducts (fmol/μg DNA)	−0.0035	0.31	−0.0012	0.80	0.0117	0.21
1-HP (fmol secreted/kg BW)	0.0050	0.17	0.0048	0.35	0.0134	0.23

[a] For analysis, 20 was added to the FPG and ENDO score and 1 to the SB score to obtain positive values for transformation to the natural logarithm.

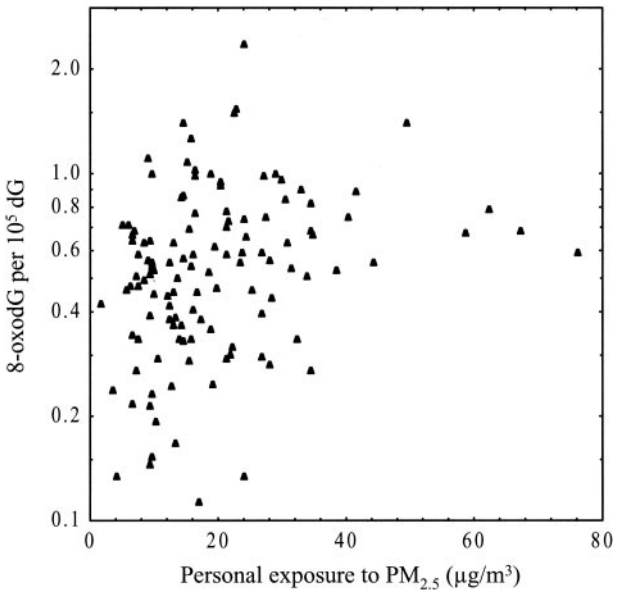

Fig. 1. Relationship between oxidative DNA damage assessed as 8-oxodG in lymphocyte DNA and personal exposure to $PM_{2.5}$ assessed over 48 h in 68 subjects with up to four samples each collected in each of the four seasons.

during the summer ($P < 0.0001$). If season was removed from the model, outdoor average temperature was found to be a significant predictor causing a 3% increase in PAH adducts per 1°C increase in average outdoor temperature ($P = 0.01$). For 1-HP excretion, season was found to be the only significant predictor with highest excretion during the winter ($P < 0.0001$).

The comet assay measurements of SB, as well as FPG- and ENDO-sensitive sites, showed no significant associations to any of the exposure markers (Table 2). The season was a significant predictor of SB level ($P < 0.001$), with the summer levels being higher than the other three seasons. In addition, average outdoor temperature was a significant predictor in the model causing a 5% increase in SBs per 1°C increase in average outdoor temperature ($P = 0.03$). NQO1 and gender were excluded as predictors because of lack of significance. For all three measurements, the intraindividual variance was estimated to zero, indicating no unexplained similarity between observations on the same subject.

Relationship between the External Exposure Markers. The urban background $PM_{2.5}$ concentration was a predictor of personal $PM_{2.5}$ exposure ($P = 0.03$), with an increase of 12% in personal exposure/10 $\mu g/m^3$ increase in background $PM_{2.5}$. The personal black smoke exposure was also a predictor of personal $PM_{2.5}$ ($P < 0.0001$), with an increase of 30% in personal $PM_{2.5}$ exposure per $1 \times 10^{-5} m^{-1}$ increase in personal black smoke exposure. In addition, exposure to ETS predicted personal $PM_{2.5}$ exposure ($P = 0.0005$), with an increase of 4% in personal exposure to $PM_{2.5}$/1 h increase in time exposed to ETS. In three of the seasons, we also measured personal exposure to nitrogen dioxide, which showed no significant correlations with the markers of $PM_{2.5}$ exposure or the biomarkers (data not shown).

Relationship between the Biomarkers. There were no significant associations between any pair of biomarkers of genotoxicity, including 8-oxodG in lymphocytes or urine and SB-, FPG-, or ENDO-sensitive sites in lymphocytes or PAH adducts. Similarly, there were no significant associations between any of these biomarkers and 1-HP excretion ($P > 0.2$).

Discussion

Exposure to PM has been associated with an increase in lung cancer (1). During the last decade, several studies have examined the relationship between personal exposure and indoor–outdoor monitoring of $PM_{2.5}$ or PM_{10}, and most of these studies, including the present, find only weak or no relationship between personal and outdoor concentrations (15, 26). In addition, a recent *in vitro* study on alveolar macrophages indicated that indoor-generated PM was more bioactive than outdoor PM (27). This indicates that PM included in the personal exposure may contain fractions capable of inducing DNA damage that is not included in the background $PM_{2.5}$ concentration and that personal exposure is likely to be more directly related to such biomarkers.

Induction of 8-oxodG after exposure to PM has been shown to correlate significantly with induction of lung tumors in mice, suggesting that 8-oxodG could be a premutagenic lesion in PM-induced lung cancer (28). We found personal $PM_{2.5}$ exposure to be a predictor of 8-oxodG concentrations in lymphocyte DNA with an 11% increase in 8-oxodG/10 $\mu g/m^3$ in personal $PM_{2.5}$ exposure. This indicates that exposure to

moderate particle concentrations can result in an increased oxidative stress. Experimental studies have found increased concentrations of 8-oxodG after exposure to PM doses many times higher than the exposures seen in this study both *in vitro* (7) and *in vivo* (8). In addition, increased urinary excretion of 8-oxodG and increased levels of PAH adducts in lymphocyte DNA were found in Copenhagen bus drivers from the central area as compared with drivers from the rural and suburban area (29). The concentration of 8-oxodG in lymphocytes was not measured in that study (29). In the present study, we found no relationships between these biomarkers and PM exposure. A possible explanation is that the urban bus drivers were exposed to higher concentrations of diesel-emitted PM than the subjects in this study, which could lead to a more extensive PAH exposure and oxidative stress. A recent study similar to the present investigated personal $PM_{2.5}$ and PAH exposure in 194 nonsmoking students living either in the city of Athens or in the region of Halkida in rural surroundings with a minimal burden of urban air pollution (30). Surprisingly, significantly higher concentrations of PAH adducts were found in the Halkida subjects compared with the subjects from Athens, although the Athens subjects were exposed to significantly higher concentrations of particle-bound PAHs (30). Moreover, the exposure gradient in terms of $PM_{2.5}$ mass was much larger in the Greek study than the present study (31). The conclusion of the Greek study was that for cohorts with moderate to low particle-bound PAHs, no simple correlation with biomarkers of genotoxicity could be detected, possibly attributable to contributions to the overall genotoxic burden by additional factors, such as exposure to ETS (31). However, exposure to ETS was not a predictor of any biomarker in the present study. Other studies of personal exposure to $PM_{2.5}$ by mass and in terms of black smoke have demonstrated both larger and smaller exposure gradients than in the present study (26, 32). However, none of these studies have included biomarkers of oxidative DNA damage. It remains to be studied whether stronger correlations can be shown between external exposure and such biomarkers with a larger exposure gradient.

The experimental part of this study spanned 1 year and included one measuring campaign in each season. For most of the biomarkers and external exposure markers, significant differences between the seasons were found. In Copenhagen, the sources of ambient $PM_{2.5}$ are partly long-range transport of particles composed of ammonium sulfate and nitrate salts and partly emission from combustion engines, in particular, diesel vehicles, whereas heating is a very minor source. The higher levels in the spring could be attributable to weather conditions favoring long-range transport. In addition, behavioral patterns are known to vary through the seasons, *e.g.*, people spent more time indoors during winter when they are more subjected to indoor sources, such as cooking and burning of candles, and less subjected to outdoor air pollution. Other components of ambient air pollution in terms of nitrogen dioxide showed no correlations with measures of $PM_{2.5}$ or the biomarkers. For 8-oxodG in lymphocytes, the winter season showed the lowest concentrations. Similarly, season was a significant predictor of SBs, with average outdoor temperature as an additional significant predictor. This is similar to a previous study from Copenhagen that found a seasonal effect with increased SB concentrations related to solar flux (33). A recent review of intervention studies conclude that diet or antioxidant consumption has limited influence on oxidative DNA damage and is unlikely to be responsible in the seasonal variation in 8-oxodG in the present study (34). Season was also a significant predictor of the PAH adducts with the highest concentrations of PAH adducts during spring and summer. In contrast, the study from Greece found the highest concentrations of DNA adducts during wintertime (30). If we removed season from our model, outdoor average temperature was a significant predictor, causing a 3% increase in PAH adducts per 1°C increase in average outdoor temperature ($P = 0.01$). This suggests that temperature or a related component could influence the level of DNA PAH adducts.

For both 8-oxodG in lymphocytes and the comet data, the intraindividual variance was estimated to zero, strongly suggesting that there was no inherent damage level from campaign to campaign in the same subject. Although FPG-sensitive sites in principle should reflect lesions, including 8-oxodG in DNA and partly 8-oxodG in urine, we found no correlation between these three biomarkers in agreement with other studies (35–37). However, correlations may not be expected because 8-oxodG concentration in nuclear cell DNA reflects the balance between formation and repair, whereas the urinary excretion supposedly reflects the summed rates of formation and turnover of 8-oxodG in nuclear and mitochondrial DNA, as well as the nucleotide pool in the whole body (21). Generally, the 8-oxodG concentration determined by HPLC is $\geq$10 times higher than the concentration determined as FPG-sensitive sites for yet unknown reasons (38).

We found no relationship between personal exposure or ambient PM concentrations and excretion of 1-HP. Only a few studies have investigated this relationship in nonoccupationally exposed populations (39, 40). One study examined the 1-HP excretion in nonsmoking inhabitants from two Polish cities that showed marked differences in ambient PM_{10} concentrations (39). The inhabitants from the city showing the highest concentrations of PM_{10} (>120 $\mu g/m^3$) excreted significantly higher concentrations of 1-HP than inhabitants from the less polluted city (<70 $\mu g/m^3$), suggesting that 1-HP might be useful as an exposure marker of high concentrations of PAH-loaded particles (39). However, other studies find no significant differences in 1-HP concentrations when comparing subjects from urban and suburban areas (31, 40). Nevertheless, we found significantly increased concentrations of 1-HP during wintertime as compared with summertime, suggesting that increased PAH exposure with little impact on $PM_{2.5}$ exposure in terms of mass may occur. Cooking procedures, in particular charcoal broiling, which may be an important contributor to 1-HP excretion, are unlikely to explain the high values during wintertime, although we have no specific information on that subject.

The current study suggests that exposure to $PM_{2.5}$ at modest levels can induce oxidative DNA damage in terms of 8-oxodG in lymphocytes. If similar damage is induced in lung cells, as suggested by experimental work, it may be related to an increased risk in lung cancer. The association to oxidative DNA damage was confined to the personal exposure, whereas the ambient background concentrations showed no significant association.

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