Particulate matter (PM) in ambient air is an important risk factor for acute and long-term adverse effects related to pulmonary and cardiovascular diseases, cancer, and mortality (Pope and Dockery 2006). Traffic-related PM may be particularly relevant to these health effects, as indicated by studies on both acute and long-term effects (Hock et al. 2002; Peters et al. 2004). The ultrafine particle (UFP) fraction of PM with a diameter of <100 nm typically consists of “fresh” combustion emissions of which vehicle engines are the primary source in urban areas (Sioutas et al. 2005). For UFPs, the size, surface area, chemical composition, and ability to translocate through the epithelium of terminal bronchioles and alveoli are thought to be important in relation to adverse health effects (Delfino et al. 2005; Oberdörster et al. 2005). The mechanisms of action of PM are thought to involve inflammation and oxidative stress, with small particles being more potent than larger particles because of their higher surface area and reactivity (Borm et al. 2004; Knaapen et al. 2004). Experimental studies in animals and cell cultures indicate that DNA can be oxidized by both UFPs and larger (PM10; PM with aerodynamic diameter <10 µm) particle size modes (Knaapen et al. 2004; Risom et al. 2005). DNA damage has been studied mainly as DNA strand breaks (SBs) and guanine oxidation products. The oxidation of guanine studied is primarily 8-oxoguanine, which is mutagenic (Moriya 1993) and related to carcinogenesis (Loft and Møller 2006; Loft et al. 2006). Biomonitoring studies have shown associations between exposure to UFPs, PM2.5 (PM with aerodynamic diameter <2.5 µm), and transition metals (both mass and content) in urban air and the level of oxidized guanine in DNA of peripheral blood mononuclear cells (PBMCs) (Avogoe et al. 2005; Sørensen et al. 2003b, 2005b; Vinzenz et al. 2005). However, these studies did not address the time course of DNA oxidation during exposure, identify responsible size modes or sources, or control confounding from other air pollutants, including gases and volatile organic compounds.

Moreover, 8-oxoguanine is removed from DNA by 7,8-dihydro-8-oxoguanine-DNA glycosylase (OGG1), whereas the nucleoside diphosphate linked moiety X-type motif 1 (NUDT1) enzyme removes 8-oxo-2′-deoxyguanosine 5′-triphosphate (8-oxo-dGTP) from the nucleotide pool, preventing incorporation of 8-oxoguanine during repair processes or replication (Loft and Møller 2006). Experimental studies indicate that acute exposure to PM induces DNA oxidation in target organs, whereas long-term exposure appears to increase the OGG1 repair activity and the oxidative stress response and defense enzyme heme oxygenase-1 (HO-1); this new steady-state situation during continued exposure may be associated with unchanged levels of DNA damage because of increased repair activity (Risom et al. 2003, 2005). If this situation also occurs in human cells, the actual ongoing oxidative stress and detrimental effects of PM may be underestimated by the levels of oxidatively damaged DNA in PBMCs.

Our objective in this study was to use carefully controlled exposure of healthy adults to real-life ambient air particles to delineate the relationship between source-specific particle size modes and oxidation in DNA of PBMCs. Physical exercise was included in the study to mimic real-life exposure because it increases the dose by an increase in the ventilation rate (Daigle et al. 2003), whereas the deposition rates of particles (12–320 nm) may be underestimated by exercise (Löndahl et al. 2007). Chamber air was monitored continuously for size distribution, total particle numbers, and concentration of gases. DNA damage, assessed as SBs and oxidized guanines in PBMCs, and OGG1 repair activity were determined by the comet assay, whereas mRNA expression was measured by real-time reverse transcriptase–polymerase chain reaction (RT-PCR).

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Materials and Methods

Study population. We invited volunteers to participate by posting a notice in a local newspaper and on campus at the University of Copenhagen. After preliminary screening we recruited 30 nonsmoking, healthy volunteers with no personal history of cardiovascular disease. The sample size was based on prestudy considerations of statistical power. In our earlier studies we detected statistically significant associations between 24- and 48-hr cumulated personal exposure and levels of damaged DNA by repeated measurements in 15–50 volunteers (Sørensen et al. 2003a, 2003b; Vinzents et al. 2005).

Twenty-nine of the 30 volunteers completed the entire program. The participants consisted of 20 men and 9 women, 20–40 years of age (median age, 25 years), with normal lung function (baseline forced expiratory volume in 1 sec: 4.55 ± 0.8 L) and a mean body mass index of 23.0 [95% confidence interval (CI), 22–24]. Participants were taking no medications other than contraception (5 women) and vitamin/mineral supplements (10 participants).

The study was approved by the local ethics committee and in accordance with the Declaration of Helsinki. All participants gave written, informed consent before the study commenced.

Study design. We used a single blind two-factor cross-over study design with randomized exposure to particles and/or cycling scenarios. Each participant was his/her own control, which excluded confounding by factors that are stable within an individual over time but vary between participants. To avoid a diurnal effect, participants entered the exposure chamber at the same time of the morning on each visit at either 0700 or 0730 hours and stayed for the following 24 hr. The median 24-hr period outside the chamber for these visits was 99 min. Data on individual diet throughout the study were obtained from self-administered food frequency questionnaires. Blood was sampled after 6 and 24 hr of exposure. Eight blood samples were lost during the study.

Peripheral mononuclear blood cell separation. PBMCs were collected and isolated in Vacutainer Cell Preparation Tubes (CPT; Vacutainer Systems, Plymouth, UK) according to the manufacturer’s instructions and frozen at –80°C in a mixture containing 50% fetal bovine serum (FBS; GibcoBRL, Renfrewshire, UK), 40% culture medium (RPMI 1640; GibcoBRL), and 10% dimethylsulfoxide (DMSO).

DNA damage measured by the Comet assay. The levels of SBs and formamido-pyrimidine DNA glycolase (FPG) sites were detected by single cell gel electrophoresis (Comet assay), including incubation with buffer and FPG enzyme for detection of SBs and oxidized purines in PBMCs as previously described (Møller 2005; Vinzents et al. 2005). This assay has been validated in an interlaboratory trial [European Standards Committee on Oxidative DNA damage (ESCODD) 2003]. Coded samples from each participant were analyzed in the same batch along with a quality control PBMC sample. The score of 100 comets per slide with a five-class scoring system (arbitrary score range: 0–400) was present in both exposure scenarios and continuously monitored. Each exposure scenario included two episodes of 90-min physical exercise on an ergometer bicycle after an exposure time of 1 and 8 hr, respectively. The intensity was controlled by a heart rate monitor (Polar, S720; Polar Electro, ApS, Holte, Denmark) and participants worked at 65–75% of their maximal heart rate defined as 220 beats per minute minus age in years.

All measurements were completed within a 5-month period beginning in February 2005. The median interval between individual exposures for each participant was 12 days. Each volunteer was allowed to leave the chamber to visit the bathroom, kitchen, or for measurement of lung function (not reported here). The median 24-hr period outside the chamber for these visits was 99 min. Data on individual diet throughout the study were obtained from self-administered food frequency questionnaires. Blood was sampled after 6 and 24 hr of exposure. Eight blood samples were lost during the study.

Measurement of OGG1 activity. The repair activity of PBMCs was determined as the incision activity of substrate DNA treated with Ro19-8022/white light, which generates 8-oxoguanine (Collins 2004; Collins et al. 2001). We introduced oxidized bases into PBMC substrate nuclei by irradiating cells in phosphate-buffered saline solution with 1 µM Ro 19-8022 (Hoffman-La Roche, Basel, Switzerland) at 0°C. The cells were washed and resuspended in a mixture containing 50% fetal bovine serum, 40% culture medium, and 10% DMSO, (3 × 106 cells/mL) and frozen at –80°C.

For the preparation of PBMC extracts, the cells were centrifuged (300 × g, 5 min, 4°C), and the pellet was resuspended in buffer A (45 mM HEPES, 0.4 M KCl, 1 mM EDTA, 0.1 mM dithiothreitol, 10% glycerol, pH 7.8) at a volume of 20 µL per 106 cells. The resuspended cells were divided in aliquots of 50 µL to which 12 µL 1% Triton X-100 was added. The lysate was centrifuged (700 × g, 5 min, 4°C), and the supernatant was mixed with 200 µL buffer B (40 mM HEPES, 0.1 M KCl, 0.5 mM Na2EDTA, 0.2 mM bovine serum albumin, pH 8). Approximately 3 × 1012 substrate cells were embedded in agarose and lysed as described for the comet assay. Repair incisions were detected by incubation of the agarose-embedded nuclei with 60 µL PBMC extract or buffer B for 20 min at 37°C. The subsequent alkaline treatment and electrophoresis were identical to the conditions used to determine DNA damage using the comet assay (Vinzents et al. 2005). The level of repair incisions was obtained as the difference in scores of parallel slides incubated with and without PBMC extract. An assay control (PBMC) was included in each electrophoresis run.

Expression levels of HO-1, OGG1, and NUDT1 mRNA by real-time RT-PCR. The PBMCs were isolated in Vacutainer tubes and cryopreserved in TRIZol reagent (Invitrogen, A/S, Taastrup, Denmark) at –80°C. On the day of analysis the samples were thawed rapidly, and the RNA was extracted according to the manufacturer’s instructions. Approximately 0.4 µg RNA was used for cDNA synthesis in a reaction volume of 20 µL using the TaqMan GeneAmp RT-PCR kit as recommended by Applied Biosystems (Nærum, Denmark). Quantitative PCR reactions were carried out in ABI PRISM 7900HT (Applied Biosystems), using primers and cDNA-specific probes purchased from Applied Biosystems. We used as the reference gene 18S rRNA, which is commercially available as a probe and primer solution (Eukaryotic 18S rRNA Endogenous Control, 4352930E; Applied Biosystems). Below are probes and primers for the genes. Sequence accession ID numbers are from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/); accessed 12 February 2007): hHO-1: forward primer: 5′-CAT GAG GAA CTT TCA GAA GGG C-3′; reverse primer: GAT GTG GTA CAG GGA GCC CAT-3; TaqMan probe: 5′-6-FAM-TGA
CCC GAG ACG GCT TCA AGC AGC

Results of size distribution and NC of particles from 6–700 nm in diameter were fitted using four log-normal modes, NC12 (median diameter 11.7 nm, geometric width 1.24), NC23 (22.6 nm, 1.48), NC32 (57.1 nm, 1.96), and NC123 (212 nm, 1.72), where the functional dependence of the modes on \( d \) was defined by Equation 1:

\[
\exp \left\{ \frac{(d - d_m)^2}{2d^2} \right\}.
\]

The first three modes represent the characteristic traffic particle modes that have been observed in busy streets in Copenhagen (Wåhlin et al. 2004). The last mode represents secondary long-range transport and a large fraction of particle mass.

Finally, when volunteers were outside the chambers (median, 99 min/24 hr), each carried a Condensed Particle Counter (TSI 3007; TSI Inc., St. Paul, MN, USA). These instruments monitored NCtotal (10–700 nm), with total exposure concentrations adjusted for these periods.

**Statistical strategy.** We investigated the effect of exposure on the outcome variables SBs, FPG sites, mRNA expression of DNA repair genes, and OGG1 repair activity using mixed-effects models by the PROC MIXED procedure of SAS (version 8.2; SAS Institute Inc., Cary, NC, USA). Subject nested within gender was included as a random factor variable to account for interindividual variation. Exposure in terms of presence or absence of particle filter in the air inlet, length of exposure (6 and 24 hr), performance of exercise as well as use of contraceptive pills and consumption of multivitamin supplements were included as categorical explanatory variables. Consumption amounts of fruit and vegetables (grams per day) were included as continuous variables. Subsequently, we investigated dose–response relationships related to exposure in terms of the average NC of particles within each of the four size modes, with mean diameters of 12, 23, 57, and 212 nm. Effects of each of the four size modes were investigated by mutual adjustment with inclusion of all as continuous variables simultaneously in the model. Finally, we estimated effects of exposure adjusted for possible confounding by including length of exposure and exercise as categorical variables and \( O_3 \), CO, NO\(_x\), and age as continuous variables. The distributions of the exposure concentrations, the DNA damage, and mRNA expression were skewed; therefore all statistical analyses were performed on the natural logarithm of these data, with model estimates representing slopes on the logarithmic scale. Significant differences between concentrations of NC, and gaseous parameters \( O_3 \), NO, NO\(_x\), and CO according to the two exposure scenarios were determined by a \( t \)-test. In all analyses, \( p < 0.05 \) was considered to be statistically significant.

**Results**  Exposure characterization. Table 1 summarizes levels and size distribution of PM and levels of gases during the two different exposure scenarios and during the corresponding period at nearby monitoring stations at a busy street and in an urban background. The 24-hr total NC ranged from 91–542/cm\(^3\) and

| Table 1. Total and size mode allocated number concentrations (NC), surface area, and volume of particles (aerodynamic diameter, 6–700 nm) as well as gases.* |
|---------------------------------------------|
| **Exposure chamber** | **Outdoor monitoring stations** |
| **NC** | **NFAs** | **PFAs** | **Urban background** | **Busy urban street** |
| **No. /cm\(^3\)** | **No. /cm\(^3\)** | **No. /cm\(^3\)** | **No. /cm\(^3\)** | **No. /cm\(^3\)** |
| **Volume** | **Volume** | **Volume** | **Volume** | **Volume** |
| **\(\mu m^3/cm^3\)** | **\(\mu m^3/cm^3\)** | **\(\mu m^3/cm^3\)** | **\(\mu m^3/cm^3\)** | **\(\mu m^3/cm^3\)** |
| **Nucleation** | 10,067 (6,169–15,362) | 235 (91–542) | 6,571 (4,530–8,645) | 22,809 (13,499–31,977) |
| **NC12** | 1,187 (521–1,320) | 15 (5–91) | 191 (35–484) | 157 (99–279) |
| **NC23** | 2,981 (1,976–4,356) | 89 (25–134) | 931 (169–2,090) | 296 (104–626) |
| **NC32** | 6,136 (4,829–8,345) | 168 (107–314) | 4,516 (3,096–6,848) | 261 (249–541) |
| **NC123** | 226 (121–378) | 8 (6–14) | 177 (99–279) | 36 (24–541) |
| **Area\(_n\)** | 193 (153–308) | 7 (4–12) | 157 (99–279) | 157 (99–279) |
| **Area\(_d\)** | 0.56 (0.25–0.63) | 0.01 (0.00–0.04) | 0.09 (0.02–0.23) | 0.80 (0.37–1.41) |
| **Area\(_d\)** | 6.30 (4.31–9.49) | 0.19 (0.05–0.29) | 2.03 (0.73–4.55) | 16.91 (7.11–29.23) |
| **Area\(_d\)** | 156 (117–212) | 4.2 (2.7–8.0) | 115 (79–174) | 257 (171–380) |
| **Area\(_d\)** | 57 (31–95) | 2.0 (1.3–3.8) | 45 (19–91) | 75 (26–159) |
| **Volume\(_d\)** | 6 (5–11) | 0.3 (0.2–0.4) | 5 (3–11) | 12 (6–19) |
| **Volume\(_d\)** | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) | 0.00 (0.00–0.00) |
| **Volume\(_d\)** | 0.03 (0.02–0.05) | 0.00 (0.00–0.00) | 0.01 (0.00–0.02) | 0.09 (0.04–0.16) |
| **Volume\(_d\)** | 4.6 (3.5–6.3) | 0.13 (0.08–0.24) | 3.4 (2.3–5.5) | 7.6 (5.1–11.26) |
| **Volume\(_d\)** | 4.2 (2.3–7.0) | 0.15 (0.09–0.26) | 3.3 (1.6–8.7) | 5.5 (1.9–11.7) |
| **O\(_3\)** | 25 (13.01–49.56) | 28.03 (14.32–53.56) | 11.58 (4.73–18.38) | 59.52 (37.94–88.17) |
| **NO** | 3.24 (0.72–14.49) | 3.21 (0.72–17.42) | 1.22 (0.41–3.05) | 0.24 (0.12–0.29) |
| **CO** | 0.25 (0.05–0.29) | 0.21 (0.07–0.29) | 0.55 (0.39–0.76) | 0.25 (0.05–0.29) |
| **O\(_2\)** | 12.08 (5.68–18.59) | 4.29 (1.99–10.49) | 30.05 (22.24–35.27) | 19.52 (11.88–26.67) |

*Values are median (interquartile range) of 24-hr average exposure scenarios and outdoor monitoring data. **Nucleation mode of vehicle exhaust system sulfur compounds with low vapor pressure and a median aerodynamic diameter of 11.7 nm. **Nucleation mode of volatile organic compounds with a median aerodynamic diameter of 22.6 nm. **Size mode with a median aerodynamic diameter of 57.1 nm and found mainly in soot. **Secondary long-range transported particles with a median aerodynamic diameter of 212 nm.
6,169–15,362/cm³ for PFA and NFA, respectively. The filter effectively removed particles assessed by all variables (all \( p < 0.01 \), \( t \)-test). NO\(_x\) and NO were unaffected by removal of PM by filtering the air, whereas O\(_3\) was significantly (\( p < 0.01 \), \( t \)-test) reduced (possibly because of a reaction with the filter material) and CO significantly increased (\( p = 0.04 \), \( t \)-test). During the NFA scenario the levels of PM and gases in the chambers resembled the composition of urban background air with penetration and mixing with busy street air. The daily 24-hr average of NC\(_{\text{total}}\) was resolved in four size modes (Figure 1). NC\(_{77}\) was the most abundant and also represented the major part of the surface area in both indoor and outdoor (background and urban) air (Table 1). Finally, the chemical composition of air during the NFA exposure scenario (Table 2) shows that the PM\(_{2.5}\) fraction was rich in sulfur, which is consistent with substantial contributions from long-range transport. This fraction was also rich in metals and carried relatively high concentrations of transition metals (vanadium, chromium, iron, copper).

Biomarkers. A summary of the levels of DNA damage, OGG1 activity, and mRNA levels according to exposure, exercise, and length of exposure is presented in Table 3. The levels of SBs and FPG sites were significantly increased during NFA exposure compared with PFA exposure independent of the length of the exposure. Exercise had no significant effect, although the exposure-related difference between the median levels of SBs and FPG sites appeared higher during periods of exercise than during periods of rest (Table 3). There were no effects of exposure on the OGG1 activity or mRNA levels of OGG1, NUDT1, or HO-1. The effect estimates in the regression model of the relationships between SB and FPG sites and the exposure variables are presented in Table 4 and this association is shown graphically in Figure 2. The levels of SBs and FPG sites were significantly associated with the NC of all size modes when assessed individually. However, in the regression model, including all size modes, SBs were only significantly associated with NC\(_{77}\), whereas FPG sites were significantly associated with NC\(_{23}\) and NC\(_{77}\). Adjustment for gases, including O\(_3\), NO\(_x\), and CO (Table 4), fruit and vegetable intake, or use of multivitamin supplements or contraceptive pills (data not shown) had no significant effects of the predictive value of main exposure variables, which were not significantly associated with any of the biomarkers. There were no significant associations between exposure and either the OGG1 activity (\( p = 0.26 \)) or mRNA expression levels of repair enzymes (\( p > 0.13 \)) including HO-1, OGG1, and NUDT1.

Discussion

We found that controlled exposure to UFPs, especially the fraction with a median diameter of 57 nm, was associated with oxidative stress in terms of SBs and FPG sites in PBMCs, with possible minor effects of exercise during exposure. We found no sign of up-regulation of the oxidative stress response or DNA repair systems.

DNA damage is considered to be an important initial event in carcinogenesis. Moreover, oxidized DNA in PBMCs is an indicator of systemic oxidative stress relevant for cardiovascular and other outcomes (Li et al. 2003; Schins et al. 2004). The FPG enzyme used in this study recognizes mainly oxidized purines, primarily guanine, such as the premutagenic 8-oxoguanine lesion and the ring-opened formamidopyrimidine bases (Collins 2004). In a previous panel study of individuals living in Copenhagen, we found associations between oxidative stress in terms of 8-oxoguanine and oxidation products of proteins and lipids, respectively, and personal exposure to PM\(_{2.5}\) and gases.

Figure 1. Daily average number concentrations and size distributions of UFPs (6–700 nm in diameter) resolved into four size modes with median diameters 11.7, 22.6, 57.1, and 212 nm at an urban background monitoring station in Copenhagen from 15 May 2001–31 December 2004. Abbreviations: a, size mode; d, particle diameter. Vertical lines represent the median diameters on a logarithmic scale. Curved bold line is the measured size distribution and concentration of total particle numbers; dotted lines represent the modeled sum and individual modes (11.7, 22.6, 57.1, and 212 nm) concentration and size distribution.

Table 2. Particle mass [median (interquartile range)] and chemical composition (ng/m²) of particles in the exposure chamber air without filtering.

| Exposure chamber PM mass concentrations | Total mass (µg/m³) | Mass concentrations of elements |
|-----------------------------------------|-----------------|-------------------------------|
| PM\(_{10-2.5}\)                            | 12.6 (7.5–15.8) | Ti (6.26), V (0.43), Cr (0.49), Mn (1.43), Fe (88.11), Ni (0.45), Cu (4.91), Zr (0.59), Mo (0.34), K (51.82), Rb (0.17), Cs (205.52), Sr (4.15), Ba (1.85), Al (80.76), Zn (7.52), Ga (0.0), Sn (0.5), Pb (0.88), Si (153.93), S (79.05), As (0.0), Se (0.02), Sb (0.65), Cl (124.59), Br (1.30) |
| PM\(_{2.5}\)                                | 9.7 (7.0–11.6)  | Ti (3.81), V (4.81), Cr (4.18), Mn (1.78), Cu (7.43), Zr (0.61), Mo (0.59), K (59.12), Rb (0.18), Cs (124.59), Sr (6.02), Ba (4.50), Br (0.16), Zn (12.16), Ga (0.03), Sn (1.35), Pb (0.41), Si (85.46), S (466.39), As (0.09), Se (0.25), Sb (0.98), Cl (20.94), Br (1.51) |

Abbreviations: Al, aluminum; As, arsenic; Ba, barium; Ca, calcium; CI, chlorine; Cr, chromium; Cu, copper; Fe, iron; Ga, gallium; K, potassium; Mo, molybdenum; Mn, manganese; Ni, nickel; Pb, lead; Rb, rubidium; S, sulfur; Sn, antimony; Se, selenium; Si, silicon; Sn, tin; Sr, strontium; Ti, titanium; V, vanadium; Zn, zinc; Zr, zirconium.

Table 3. Median (interquartile range) of DNA damage, repair activity (OGG1), and mRNA levels according to exposure, physical activity, and length of exposure.

| Biomarker | All | Rest | Bicycling | 6-hr exposure | 24-hr exposure |
|-----------|-----|------|-----------|--------------|--------------|
| SBs/10⁶ bpa<sup>a</sup> | 0.24 (0.14–0.39) | 0.16 (0.09–0.25) | 0.23 (0.13–0.35) | 0.17 (0.09–0.24) | 0.25 (0.15–0.35) | 0.14 (0.08–0.25) | 0.24 (0.15–0.35) | 0.17 (0.09–0.24) | 0.24 (0.13–0.33) | 0.15 (0.08–0.23) |
| FPG/10⁶ bpa<sup>a</sup> | 0.53 (0.37–0.65) | 0.38 (0.31–0.53) | 0.52 (0.37–0.7) | 0.40 (0.32–0.53) | 0.53 (0.40–0.65) | 0.37 (0.27–0.53) | 0.52 (0.37–0.7) | 0.37 (0.30–0.51) | 0.53 (0.37–0.63) | 0.39 (0.31–0.55) |
| OGG1 activity (a.u.)<sup>b</sup> | 50.1 (36.8–64.1) | 47.0 (39.9–60.3) | 50.8 (39.3–64.6) | 48.1 (38.9–60.4) | 47.8 (38.3–61.9) | 46.1 (41.5–59.5) | 50.8 (39.3–64.6) | 46.4 (38.6–58.4) | 49.8 (37.0–65.4) | 47.9 (42.4–60.6) |
| OGG1 mRNA [x10⁶ copies] | 6.0 (1.9–20.1) | 5.7 (1.8–25.0) | 7.1 (2.1–41) | 4.3 (1.8–26) | 5.5 (1.9–14) | 8.1 (2.6–37) | 4.1 (1.8–19) | 4.3 (1.7–19) | 7.0 (2.4–35) | 7.9 (2.4–25) |
| NUDT1 mRNA [x10⁶ copies] | 2.14 (1.2–6.9) | 2.9 (1.0–7.0) | 2.8 (1.3–8.8) | 2.9 (0.95–7.3) | 2.1 (1.1–4.8) | 2.9 (1.3–7.0) | 2.0 (1.0–8.6) | 3.0 (1.3–4.4) | 2.5 (1.3–7.4) | 2.7 (1.0–6.4) |
| HO-1 mRNA [x10⁶ copies] | 7.35 (4–24) | 10.9 (4.5–33) | 8.3 (4.7–28) | 9.5 (4.7–33) | 7.5 (4.5–18) | 11 (4–44) | 7.5 (4.5–24) | 9.4 (4.5–31) | 6.5 (3.5–31) | 12 (4.2–31) |

<sup>a</sup>DNA strand breaks. <sup>b</sup>Oxidized purines as formamidopyrimidine DNA glycosylase sites. <sup>c</sup>Repair incision (arbitrary units). <sup>d</sup>mRNA expression of OGG1. <sup>e</sup>mRNA expression of NUDT1.
found in urban air is not of sufficient length and/or strength to cause a similar up-regulation of the defense systems in PBMCs despite the presence of significant damage. Thus, the levels of DNA damage are not obscured by changes in repair capacity in the present study.

Exposure chambers have been used previously to study mechanisms of effects of diesel exhaust, wood smoke, and concentrated ambient air particles, but the exposures have been much higher (200–250 µg/m³) than in our study (Behndig et al. 2006; Holgate et al. 2003; Lippmann et al. 2005; Mills et al. 2005; Sällsten et al. 2006). None of these studies have measured DNA damage or repair, and size modes of the UFP fraction have not been investigated. Within our exposure chambers we were able to study actual UFP levels encountered in streets with moderate traffic or in dwellings with ventilation to busy streets. The NC23- and NC57-size modes were associated with oxidative stress effects in terms of DNA damage. The NC57-size mode mainly represents carbonaceous soot from diesel engine exhaust and the largest fraction of surface area, whereas the NC23-size mode represents condensed semivolatile organic compounds from diesel vehicles. These size modes have high deposition fractions, which for hydrophobic UFPs with diameters of 12–64 nm were found to be above 50% in our participants (Löndahl et al. 2007). Although the extent of translocation of UFPs has been debated, the small aerodynamic diameter (e.g., 20–60 nm) is likely to be required (Kreyling et al. 2006; Wiebert et al. 2006). Particles in this size range readily induce cellular oxidative stress and DNA damage because of their large surface area and reactivity (Borm et al. 2004; Knäuper et al. 2004; Risom et al. 2005). Accordingly, systemic oxidative stress and DNA damage is biologically plausible in relation to these UFPs. Moreover, the consistent association between exposure to UFPs as number or PM2.5 mass and guanine oxidation in DNA of PBMCs, seen in the present and previous studies (Sørensen et al. 2003a; Avogbe et al. 2005; Vinzents et al. 2005), suggests that this is a highly sensitive biomarker of systemic exposure, even if translocation is marginal. Unchanged repair of oxidized guanine during exposure and very limited effects of diet and multivitamin supplement use or other variables (Sørensen et al. 2005a) are adjusted for age, exercise, and time of sampling. These three parameters were not significant predictors of SBs or FPG sites in PBMCs from participants the following morning, whereas FPG sites measured by the alkaline Comet assay represent frank breaks, alkaline labile sites, or transient repair breaks; they are normally rapidly repaired but can be regarded as a reliable biomarker of ongoing exposure to genotoxic effects in environmental and occupational settings (Collins 2004; Møller et al. 2000). Exposure to particles has been associated with elevated levels of SBs in cell culture systems and animal experimental models (Risom et al. 2005). In our previous study, we found no effect of biking in busy streets on the level of SBs the following morning, whereas FPG sites were elevated, which may be because of rapid repair of the former (Vinzents et al. 2005). The increased level of SBs in PBMCs of exposed participants in this study is consistent with an effect due to the continuous exposure until the time that blood was sampled. Similarly, high levels of SBs were associated with ongoing exposure to UFPs, benzene, and/or possibly other air pollutants among inhabitants in Benin (Avogbe et al. 2005). We observed no effect of exposure on the expression of the enzymes involved in repair of 8-oxoguanine in DNA and the nucleotide pool or in HO-1. In animals, exposure to diesel exhaust particles through inhalation of 20 mg/m³ for 4 days or in the diet for 3 weeks have caused up-regulation of HO-1 and OGG1 in lungs, liver, and colon (Dybdahl et al. 2003; Risom et al. 2003). Apparently, the 24-hr exposure to UFP levels

### Table 4

Effect estimates of the relationship between SB and FPG and exposure variables expressed as categorical and size mode allocated 24-hr average NC12, NC23, NC57, and NC212.**

| Outcome variable | DNA SBs/10⁶ bp | FPG sites/10⁶ bp |
|------------------|---------------|-----------------|
|                  | Single-size mode exposure model | Multiple-size mode exposure model with mutual adjustment |
|                  | Estimates (95% CI) | % increase | Estimates (95% CI) | % increase |
| DNA SBs/10⁶ bp   |               |               | FPG sites/10⁶ bp   |               |               |
| Category         |               |               | Category          |               |               |
| Categorical      |               |               | Categorical       |               |               |
| NC12             | 0.459 (0.34–0.56) | 5.7 | 0.267 (0.17–0.36) | 2.1 |
| NC23             | 0.080 (0.05–0.11) | 5.3 | 0.045 (0.03–0.06) | 3.1 |
| NC57             | 0.119 (0.09–0.15) | 5.9 | 0.070 (0.04–0.09) | 4.3 |
| NC12             | 0.102 (0.07–0.14) | 6.3 | 0.070 (0.04–0.09) | 4.7 |
| NC23             | 0.119 (0.09–0.15) | 5.9 | 0.070 (0.04–0.09) | 4.3 |
| NC57             | 0.065 (0.04–0.09) | 6.3 | 0.070 (0.04–0.09) | 4.7 |

CI, 95% confidence interval.

*We used mixed model regression regarding subject nested in gender as random factor. All model estimates in Table 4 are adjusted for age, exercise, and time of sampling. These three parameters were not significant predictors of SBs or FPG sites in any of the models. The natural logarithms of outcome variables were included and the predictive value (% increase) of estimates is expressed per doubling in exposure variable. Adjustment for gases included O₃, NOₓ, and CO as the natural logarithm of the average gas concentration, which were not significant predictors per se in any case.

Figure 2. (A) Relationship between SBs and 24-hr average exposure in terms of NC_{total} (6–70 nm). (B) Relationship between FPG sites and 24-hr average exposure in terms of NC_{total} (6–70 nm). Individual exposure gradients (NC_{HSi}/NC_{LH}) were on average 48-fold (range, 2- to 239-fold).
exposures indicate some specificity for UFPs in studies within individuals, whereas interindividual variation is substantial and specificity does not necessarily extend to cross-sectional studies (Løft and Møller 2006; Møller and Løft 2006). We were not able to study the chemical composition of the UFP size modes. The PM$_{1.5}$ fraction in the chamber showed high levels of sulfur and transition metals such as iron, chromium, copper, and vanadium, which have been associated with high levels of 8-oxoguanine in PBMCs (Sørensen et al. 2005b). However, sulfur and vanadium may be related to long-range contributions from the 212-nm-size mode, whereas the major part of copper in busy streets is in the form of larger (3 μm) brake-wear particles, with only a minor part from tail pipe emissions (Wählin et al. 2006).

Exercise may increase the internal dose of air pollutants because of enhanced ventilation (Daigle et al. 2003). A modest effect of exercise was expected because the deposition fraction of UFPs is not increased during exercise, and the actual increase in ventilation was limited to the two 90-min periods of cycling. The nominal difference between the median values of SBs and FPG sites during cycling. The nominal difference between the two 90-min periods of exercise was expected because the deposition fraction of UFPs is not increased during exercise (Daigle et al. 2003). A modest effect of exercise may have enhanced the effect of air pollutants because of enhanced ventilation (Daigle et al. 2003).

The irritant and oxidant gases O$_3$ and NO$_2$ are usually present in ambient air and may be potential confounders when studying effects of particles (Pereira et al. 2005). The chamber NO$_2$ levels were constant, and the relatively low O$_3$ levels decreased further during filtration of the inlet air, probably due to reaction with the filter material. None of the gases had any significant associations with the biomarkers and adjustment for their levels had only minor influence on the associations between UFP exposure and the biomarkers of DNA damage.

**Conclusion**

Controlled exposure to UFPs, especially related to the NC$_{27}$ mode, was significantly associated with oxidation of guanines and SBs in DNA of PBMCs, indicating systemic oxidative stress, although there was no sign of up-regulation of relevant defence genes. Exercise may have enhanced the effect of exposure, although this failed to reach statistical significance. The data support that UFPs, mainly from diesel vehicles, cause systemic oxidative stress at exposure levels encountered in streets or in dwellings near busy roads.

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