*Short Communication*

# Active and Passive Smoking and Lifestyle Determinants of 8-Oxo-7,8-Dihydro-2′-Deoxyguanosine Levels in Human Leukocyte DNA

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## Abstract

**We investigated the effects of smoking and exposure to environmental tobacco smoke (ETS) on oxidative DNA damage by measuring 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) levels in DNA of leukocytes of healthy donors (30 smokers, 29 nonsmokers, and 28 ETS-exposed subjects). Nonsmokers had lower 8-oxodGuo levels compared with smokers ($5.94 \pm 0.87 \times 10^{-6}$ and $19.85 \pm 4.75 \times 10^{-6}$ 2-deoxyguanosine, respectively, means ± SE, $P = 0.00007$). Subjects exposed to ETS had higher mean value of 8-oxodGuo compared with nonsmokers ($9.18 \pm 1.53 \times 10^{-6}$ 2-deoxyguanosine, mean ± SE), nonsignificant by univariate analysis ($P = 0.074$). Multiregression analysis indicated that the increase of 8-oxodGuo levels induced by ETS was significant ($P = 0.045$) and that coffee and tea consumption reduced DNA oxidation ($P = 0.0053$). Oxidative leukocyte DNA damage was positively correlated with plasma cotinine levels in ETS-exposed subjects ($r = 0.47$, $P < 0.01$, $n = 28$) and was increased by age in nonsmokers and ETS-exposed subjects ($P = 0.049$). The results seem to confirm that ETS exposure is capable of inducing some oxidative DNA damage in circulating leukocytes and that coffee and tea consumption might partially protect against smoking-induced oxidation damage.** (Cancer Epidemiol Biomarkers Prev 2005;14(12):2975–7)

## Introduction

Reactive oxygen species, formed by endogenous oxygen metabolism and by various xenobiotics, induce cell damage through oxidation of DNA, RNA, proteins, and lipids (1). Among the many oxidation products described in DNA (2), 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo) has been extensively used as a marker of oxidation damage because it can be measured with sensitive methods and induces mutations in various genes (3), including tumor-related genes (4, 5). Increased 8-oxodGuo levels were found in human cells (5) and in target organs of animals exposed to various carcinogens (6); higher values of 8-oxodGuo have also been observed in some human cancers (7, 8). A number of studies have also documented increased 8-oxodGuo levels in leukocytes from patients with degenerative diseases (9, 10). Moreover, lifestyle factors, such as level of physical activity (11), smoking (12, 13), and tea or coffee drinking (14), can modify oxidative DNA damage in humans.

Tobacco smoke contains numerous compounds that generate reactive oxygen species, which can damage DNA directly or indirectly via increased inflammation, thus promoting carcinogenesis in smokers (15). However, data regarding the effects of exposure to environmental tobacco smoke (ETS) on nonsmokers are still limited and controversial (16), particularly due to the presence of many strong confounding factors in epidemiologic studies investigating ETS effects. In a previous study from our group (13), we documented a higher 8-oxodGuo level in leukocyte DNA of smokers (>10 cigarettes/d) relative to nonsmokers but data on ETS exposure of nonsmokers were then not available.

Therefore, we decided to evaluate the effect of ETS on circulating human leukocytes and to estimate the correlation of DNA oxidative damage with exposure. To quantify ETS exposure, we measured plasma cotinine, a good indicator of exposure to cigarette smoke (17). Because lifestyle factors can also influence oxidative DNA damage, we also investigated the effect of physical activity, body mass index, alcohol, and tea and coffee consumption.

## Materials and Methods

**Human Subjects.** Healthy male volunteers, afferent to the Transfusion Unit of the Mayer Hospital, Florence, Italy, were enrolled in this study ($n = 87$; age: 18-60 years, 30 smokers, 29 nonsmokers, and 28 ETS exposed). Written informed consent, approved by the local hospital ethical committee, was obtained from all subjects. We also asked each donor to answer a questionnaire regarding age, body mass index, and habits such as physical activity, smoking (number of cigarette smoked/d), and alcohol, tea, and coffee consumption. In the same day (from 7 a.m. to 9 a.m.), we collected buffy coats and blood samples for 8-oxodGuo and cotinine analyses. To assess ETS exposure in nonsmokers, beside measuring plasma cotinine levels, we used a questionnaire, which included information about smoking habits of spouses or other family members, number of hours spent at home in the presence of smokers, estimated number of cigarettes smoked at home or at work, and space ventilation. Subjects were divided into three groups according their smoking status: smokers, ETS-exposed subjects, and nonsmokers. Smokers

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**Table 1. Parameters of the subjects in the study groups**

| | Nonsmokers | ETS-exposed subjects | Smokers |
|---|---|---|---|
| No. subjects ($n$) | 29 | 28 | 30 |
| Average age (y) | 40.3 ± 2.1 | 37.5 ± 1.5 | 41 ± 2.2 |
| Body mass index (kg/m$^2$) | 24.8 ± 0.7 | 24.7 ± 0.5 | 25.2 ± 0.7 |
| Smoked cigarettes/d | — | — | 13.5 ± 1.8 |
| Self-reported exposure to ETS (h/d) | 0 | 5.8 ± 0.45 | — |
| Self-reported exposure to ETS (cigarettes/d)* | 0 | 44.3 ± 14.2 | — |
| Plasma cotinine levels (ng/mL) | 2.4 ± 0.7 | 3.3 ± 0.77 | 79 ± 15.8 |

NOTE: Data are means ± SE.
*Estimated number of cigarettes smoked around ETS-exposed subjects each day.

were classified as subjects who smoked >3 cigarettes/d for over 1 year; ETS-exposed subjects were nonsmokers who were exposed to ETS in poorly ventilated rooms at home and/or at work or elsewhere for a period >3 h/d in the year preceding the enrollment. Nonsmokers were subjects who had never smoked and were not exposed to ETS. Average age, body mass index, self-reported exposure to ETS, number of estimated cigarettes smoked in proximity of the ETS-exposed subjects, and plasma cotinine levels are reported in Table 1.

**Isolation of Human Leukocytes.** WBC isolated as ''buffy coat'' (13) were supplied by the Blood Transfusion Unit of the Meyer Paediatric Hospital, Florence, Italy. Buffy coat cells were frozen at −80°C until 8-oxodGuo and 2-deoxyguanosine levels were determined as previously described (13).

**Analysis of 8-OxodGuo.** Isolation of cell DNA was done using TRIzol (Invitrogen Corporation, Paisley, Scotland, United Kingdom) following Chomczynski's method (18). DNA (~100 μg) was dried with a nitrogen stream, dissolved in 10 mmol/L Tris-HCl (pH 7.3), and hydrolyzed with nuclease P1 (15 IU) and alkaline phosphatase (10 IU). Levels of 8-oxodGuo were detected as previously reported (13) and expressed as 8-oxodGuo × $10^{-6}$ 2-deoxyguanosine.

**Plasma Cotinine Measurements.** Cotinine levels were determined from plasma, using the method of Perkins et al. (19). A calibration curve, constructed using spiked samples, was used for cotinine quantification. Extraction recovery rate was 98.2 ± 10.7% (mean ± SD). Each sample was measured in duplicate and the intra-assay precision was ~8.5%.

**Statistical Analysis.** The normality of 8-oxodGuo distribution values was checked using the Shapiro-Wilks test. A log transformation of 8-oxodGuo values, which were normally distributed, was used in the analysis. Drinkers were defined as subjects who drank >15 g ethanol/d, corresponding to ~1 glass of wine/d; habitual consumers of coffee or/and tea were subjects who drank =3 cups/d; moderate exercise was defined as engaging in physical activity >3 h/wk; subjects with more intense physical activity (> 9 h/wk) were excluded.

*Univariate Analysis.* For categorical variables, a test of log(8-oxodGuo) in each group was done; $P$ values for the differences were calculated with ANOVA using the STATA statistical package (STATA, College Station, TX). For continuous variables, the association between the factor variable and log 8-oxodGuo was assessed by Pearson's correlation coefficient. To evaluate the association between 8-oxodGuo and lifestyle (smoking, coffee and tea consumption, age, and physical activity), a multiregression analysis of log(8-oxodGuo) against lifestyle factors was done, where independent variables were tested against each lifestyle factor using the STATA statistical package with a 0.15 entry level.

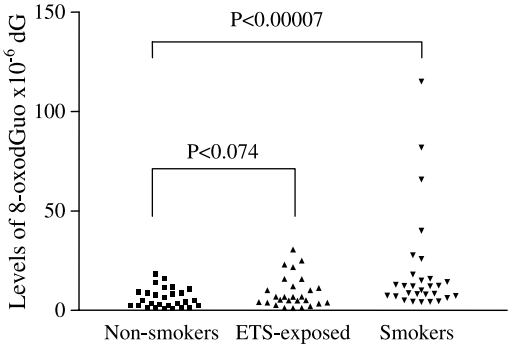


**Figure 1.** Levels of 8-oxodGuo in leukocytes from nonsmokers ($n$ = 29), ETS-exposed ($n$ = 28), and smokers ($n$ = 30).

## Results

The characteristics of subjects in the different experimental groups are shown in Table 1. The average self-reported ETS exposure of our subjects was 5.8 h/d, calculated from questionnaire data; the mean plasma cotinine level in ETS-exposed subjects was 3.3 ng/mL (slightly lower than that reported in ref. 16—6.6 h/d self-reported ETS exposure and cotinine levels of 4.5 ng/mL). ETS-exposed subjects had slightly higher plasma cotinine levels than nonsmokers (Table 1) but this difference was nonsignificant with univariate analysis ($P$ = 0.196). Cotinine plasma levels were not correlated with ETS exposure, calculated as exposure time or as total number of cigarettes smoked in the close proximity of ETS-exposed subjects (data not shown). The daily average cigarette consumption by smokers was 13.5, ranging from 3 to 35; plasma cotinine levels of smokers were ~24-fold higher than in ETS-exposed subjects (Table 1) as reported by others (20).

Regarding 8-oxodGuo levels, a wide interindividual variation was found in smokers (range 4.2 × $10^{-6}$-115 × $10^{-6}$ 2-deoxyguanosine) and in ETS-exposed subjects (range 1.3 × $10^{-6}$-30.7 × $10^{-6}$ 2-deoxyguanosine; Fig. 1). Univariate analysis showed that 8-oxodGuo levels in smokers were significantly higher compared with nonsmokers ($P$ < 0.00007); on the contrary, the difference between ETS-exposed and controls (nonsmokers) was not significant with this type of analysis ($P$ = 0.074; Fig. 1). Univariate analysis also showed significantly lower levels of 8-oxodGuo in subjects engaging in moderate physical activity ($P$ = 0.055) but no direct effect of coffee, tea, alcohol, body mass index, and age (data not shown).

**Table 2. Multiple regression analysis of log(8-oxodGuo) levels against lifestyle factors**

| Variable | Coefficient | SE | $P$ |
|---|---|---|---|
| All subjects | | | |
| Smoking status | 0.591 | 0.124 | 0.00001 |
| Coffee and tea consumption | −0.432 | 0.207 | 0.041 |
| Age | 0.0169 | 0.009 | 0.088 |
| Physical activity | −0.298 | 0.211 | 0.162 |
| Nonsmokers and ETS-exposed subjects | | | |
| ETS exposure | 0.517 | 0.251 | 0.045 |
| Coffee and tea consumption | −0.767 | 0.262 | 0.0053 |
| Age | 0.0253 | 0.0128 | 0.049 |
| Physical activity | −0.207 | 0.249 | 0.411 |

A positive, although modest, correlation ($r = 0.456$, $n = 58$, $P < 0.01$) was found between plasma cotinine levels and 8-oxodGuo levels in smokers and ETS-exposed subjects; in smokers, no correlation was observed between number of cigarettes smoked/d and 8-oxodGuo levels ($r = 0.144$, $n = 30$, $P = 0.18$).

Four independent variables were selected for multivariate analysis and their effect on log(8-oxodGuo) levels was investigated, as specified in the statistical methods. With this approach, smoking status increased 8-oxodGuo levels ($P = 0.00001$), whereas coffee and tea consumption had a significant protective effect ($P = 0.041$; Table 2, *top*). Analyzing only controls and ETS-exposed subjects (Table 2, *bottom*), a significant effect of ETS exposure was detected ($P = 0.045$), with a protective effect ($P = 0.0053$) of coffee and tea consumption. Moderate physical activity had no effect, whereas age increased 8-oxodGuo levels ($P = 0.049$).

## Discussion

In this study, we investigated the variations of 8-oxodGuo levels in leukocyte DNA from healthy adults with different exposure to passive and active cigarette smoke. DNA oxidation data were obtained following an improved method suggested by the European Standards Committee on Oxidative DNA Damage (21), which produces lower values of 8-oxodGuo levels, when compared with most previous methods (13).

ETS exposure, evaluated from questionnaire data considering the reported duration of exposure (h/d) or the number of cigarettes smoked in closed proximity of each subject, was not correlated with cotinine plasma levels. It is possible ETS exposure calculated with questionnaire methods does not accurately represent indoor concentration of tobacco-smoke pollutants, as suggested by Kemmeren et al. (22).

As previously observed (13), smokers had significantly higher 8-oxodGuo levels in leukocyte DNA compared with nonsmokers; however, only four smokers had very high out of range values (~10-20-fold) compared with nonsmokers. These subjects smoked >10 cigarettes/d but other heavy smokers had 8-oxodGuo values in the range of nonsmokers. This suggests that the susceptibility to DNA oxidation damage from smoking shows a large individual variation, possibly linked to different metabolic capability, repair activity, or antioxidant defenses (23).

Multivariate analysis showed that oxidative DNA damage was significantly increased by ETS exposure ($P = 0.045$), whereas univariate analysis did not detect a significant increase, possibly because of sample variability or other confounding factors. A significant effect of ETS on oxidation damage confirms previous observations by Howard et al. (16). We also observed that oxidative DNA damage was related to age in nonsmokers and in ETS-exposed subjects but not in smokers, possibly due to the strong effect of smoking itself and a protective effect of coffee and tea consumption. A similar observation was reported by Klaunig et al. (24) with green tea, whereas coffee was reported to have an opposite effect by others (14). We did not find significant effects of moderate physical activity, in agreement with the data of Kasai et al. (11) on urinary 8-oxodGuo levels and no effect of moderate alcohol consumption. Similar results on alcohol have been reported by others (11, 14).

In conclusion, our results seem to support the hypothesis that ETS exposure, as well as active smoking, is associated with some level of increased oxidative stress. Oxidative stress has a large individual variability and tends to be correlated with plasma cotinine levels, a marker of exposure to cigarette smoke. The DNA oxidation-inducing effects of cigarette smoke seem to be mitigated by coffee and tea consumption, possibly because of the relatively high concentrations of antioxidant compounds in these beverages (25).

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