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Differences in DNA-damage in non-smoking men and women exposed to environmental tobacco smoke (ETS)

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Abstract

There is much data implicating environmental tobacco smoke (ETS) in the development and progression of disease, notably cancer, yet the mechanisms for this remain unclear. As ETS is both a pro-oxidant stressor and carcinogen, we investigated the relationship of ETS exposure to intracellular and serum levels of DNA-damage, both oxidative 8-hydroxy-2-deoxyguanosine (8OHdG) and general, in non-smokers from non-smoking households, occupationally exposed to ETS. General DNA-damage consisting of single and double strand breaks, alkali-labile sites and incomplete base-excision repair, increased significantly in a dose-dependent manner with ETS exposure in men ($P=0.015$, $n=32$, Pearson) but not women ($P=0.736$, $n=17$). Intracellular 8OHdG-DNA-damage and general DNA-damage were both greater in men than women ($P=0.0005$ and 0.016, respectively) but 8OHdG serum levels did not differ between the genders. Neither 8OHdG-DNA-damage nor serum levels correlated with increasing ETS exposure. This is the first study to demonstrate dose-dependent increases in DNA-damage from workplace ETS exposure. Perhaps most interesting was that despite equivalent ETS exposure, significantly greater DNA-damage occurred in men than women. These data may begin to provide a mechanistic rationale for the generally higher incidence of some diseases in males due to tobacco smoke and/or other genotoxic stressors.

Keywords: Environmental tobacco smoke; DNA-damage; DNA repair; 8-Hydroxy-2-deoxyguanosine

Abbreviations: 8OHdG, 8-hydroxy-2-deoxyguanosine; ELISA, enzyme-limited immuno sorbent assay; ETS, environmental tobacco smoke; Fpg, formamidopyrimidine DNA glycosylase; HPLC/ECD, high pressure liquid chromatography with electro coulometric detection; hOGG1, human 8-oxoguanine DNA glycosylase

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1. Introduction

Recently, non-smoking areas in bars and sports clubs have been shown to provide little or no protection for patrons (Cains et al., 2004), corroborating anecdotal medical reports and previous studies contending that legislation is the most effective tool to reduce environmental tobacco smoke (ETS) exposure and smoking behaviour (Heloma et al., 2001). While ETS exposure has been linked to a number of disorders such as asthma, intra-uterine growth restriction, sudden infant death syndrome and coronary heart disease, it has been most consistently and strongly implicated in the initiation and progression of cancer (Anon, 1996). Despite this, the temporal relationships and mechanisms by which cigarette smoke causes initiation and progression of disease remains relatively unknown. Furthermore, although social ETS exposure is relatively controllable (an individual may choose not to enter premises where smokers are present), employees have little or no influence over ETS and may be exposed for a large part of their working day.

Having previously identified significantly higher 8-hydroxy-2-deoxyguanosine (8OHdG) levels in non-smoking workers occupationally exposed to ETS compared to non-smoking workers who are not exposed to ETS (Howard et al., 1998), the goal of this study was to assess a quantitative relationship between DNA-damage and workplace ETS exposure, and to investigate the contribution of the oxidative DNA lesion 8-hydroxy-2-deoxyguanosine (8OHdG) to total ETS-induced DNA-damage in the blood of non-smokers. Differences in DNA-damage with respect to 8OHdG were also assessed in relation to ETS exposure and demographic variables.

2. Methods

2.1. Participants and recruitment

Non-smokers, living in non-smoking households who work at casinos and bars in Reno and Las Vegas, NV were recruited for the Nevada Environmental Tobacco Smoke Study (NETSS). Fifty baseline blood samples from NETSS were randomly selected for the current study. All participants provided informed consent and the study were approved by the Biomedical Institutional Review Board at the University of Nevada, Reno. Participant demographics and baseline characteristics are shown in Table 1.

2.2. Cotinine analysis

Cotinine levels were determined to verify self-reported non-smoking status and to quantitate ETS exposure. Plasma samples, 1 mL from NETSS filtered through Oasis® MCX (Waters, Milford, MA) cation-exchange solid phase extraction cartridges and cotinine eluted prior to GC/MS analysis, as described in the Product Insert. Briefly, acidified plasma samples (0.05 meq HCl) were filtered through separate prepared cation-exchange cartridges, washed with 2 mL 0.1 M HCl then 2 mL methanol and cotinine eluted with 1.5 mL ammonium hydroxide (5% in methanol). Eluates were vacuumed to dryness, 60 °C (1 h), redissolved in 0.2 mL ethyl acetate and filtered through 0.45 μm membrane filters into autosampler vials. Samples were chromatographed on an Agilent 6890 Plus gas chromatograph (San Fernando, CA) and detected with an Agilent 5973N mass spectrometer operating in selected ion mode (EI: 70 eV). An aliquot (2 μL) of each sample was injected onto a 30 × 0.25 mm (i.d.) DB5-MS fused silica open tubular column (J&W Scientific) and chromatograms developed using the following GC/MSD program: 60 °C (1 min), then 25 °/min to 300 °C (hold 1 min); a post-run hold of 10 min at 300 °C was imposed after each sample (total run time = 21.6 min). Clean solvent injections were made between standard and sample injections to minimize carry over of the cotinine analyte. The fragment at $m/z = 98.1$ was used for quantitation, and retention time for the cotinine analyte was 7.68 min. Average recovery from clean, spiked plasma (0.3–4 ng/mL) was 100.7 ± 1.6% with a CV of 8.0 ± 4.4%. The concentration range for the standard curves was 1–80 ng/mL with limits of detection and quantitation of 0.1 ng/mL and 0.3 ng/mL, respectively.

2.3. Single cell gel electrophoresis (Comet) assay

DNA-damage was assessed by the Comet assay in whole blood samples with or without inclusion of human 8-oxoguanine glycosylase (hOGG1) enzyme, which cleaves 8OHdG-DNA lesions and allows them to run under electrophoresis and thus be detected. This

Table 1
Baseline demographics of study participants for the whole study population and for male and female participants

	Total population (N = 50)	Males (N = 33)	Females (N = 17)
Males	33	n/a	n/a
Females	17	n/a	n/a
Mean age ± standard deviation (range)	48.8 ± 9.5 (25–64)	48.2 ± 10.2 (25–64)	51.1 ± 8.4 (29–62)
Mean body mass index ± standard deviation (range)	28.9 ± 4.3 (20.4–37.8)	29.6 ± 4.0 (23.3–37.8)	28.0 ± 4.9 (20.4–37.7)
Ethnicity			
Caucasian	26 (52%)	19 (57.6%)	7 (41.2%)
Latino	11 (22%)	7 (21.2%)	4 (23.5%)
Asian	2 (4%)	1 (3.0%)	1 (5.9%)
Black/African American	3 (6%)	2 (6.0%)	1 (5.9%)
American Indian or Alaska native	4 (8%)	3 (9.1%)	1 (5.9%)
Native Hawaiian or other Pacific Islander	2 (4%)	0	2 (11.8%)
Other	2 (4%)	2 (6.0%)	0
Education			
Some high school (9–11 years)	1 (2%)	1 (3.0%)	0
High School diploma or G.E.D.	13 (26%)	6 (18.1%)	7 (41.2%)
Vocational or training school after high school graduation	6 (12%)	3 (9.1%)	3 (17.6%)
Some college or associate degree	22 (44%)	18 (54.5%)	4 (23.5%)
College graduate or Baccalaureate degree	6 (12%)	4 (12.1%)	2 (11.8%)
Some college or professional school after college graduation	1 (2%)	0	1 (5.9%)
Master's degree	1 (2%)	1 (3%)	0
Marital status			
Never married	8 (16%)	5 (15.2%)	3 (17.6%)
Divorced or separated	12 (24%)	8 (24.2%)	4 (23.5%)
Widowed	0	0	0
Presently married	24 (48%)	15 (45.5%)	9 (52.9%)
Living in a marriage-like relationship	6 (12%)	5 (15.2%)	1 (5.9%)

No significant differences in baseline characteristics of age, BMI, ethnic background, education level and marital status were observed between male and female participants.

was performed using a commercially available Flare™ assay kit (Trevigen, Gaithersburg, MD) as per the manufacturer's instructions. Briefly, cells were plated in low melting point agarose, slides were lysed and DNA unwinding occurred for 1 h at pH >13. Slides were electrophoresed for 30 min (0.85 V/cm) and dried down before staining with SYBR green™ and visualization using a fluorescent microscope at 400× magnification. Three blood samples per person were assessed and 50 cells were scored randomly by eye in each sample, on a scale of 0–4, based on fluorescence beyond the nucleus and were previously described by Kobayashi et al. (1995). The scale used was as follows: 0 = no cometing; 1 = Comet < 0.5 times the width of nucleus; 2 = Comet equal to width of nucleus; 3 = Comet greater than width of nucleus; 4 = Comet > two times the width of the nucleus. Scoring cells in this manner has been shown to be as accurate and precise as using computer image analysis (Kobayashi et al., 1995). The individual scoring of the slides was blinded to any demographic or biochemical aspects of the blood samples. General DNA-damage was calculated from the samples with no hOGG1 enzyme added, while specific 8OHdG-DNA-damage was calculated by subtracting general DNA-damage from damage in corresponding samples containing hOGG1. Variability caused specifically by the Comet assay procedure was controlled by running one loading control sample for every participant. Loading controls were not lysed and unwound, but were electrophoresed with experimental samples and developed with ethidium bromide. Every loading control was checked visually on a microscope and a picture was taken with a Geldoc

(Biorad, Hercules, CA) to ensure loading of a similar number of white blood cells and thereby control for retardation in DNA migration due to over-loading. Samples where white blood cells were overloaded were repeated. Loading controls also served to measure levels of artifactual DNA-damage caused by electrophoresis alone.

2.4. Determination of 8OHdG concentrations in serum

Concentrations of 8OHdG were determined in serum samples drawn from participants at the same time as plasma and whole blood samples. Blood was drawn into a SST vacutainer, allowed to clot at room temperature for 45 min–1 h and centrifuged at 1000 rpm for 10 min. Serum was aliquoted into cryovials and stored at −70 until use. Before 8OHdG concentrations were determined in serum, samples were thawed, applied to spin filters (10,000 Mw cut-off, Millipore, Billerica, MA) and centrifuged at 12,000 × *g* in a benchtop centrifuge for 30 min. Flow-through was applied to OXYcheck high sensitivity competitive ELISA plates (Genox, Baltimore, MD) and quantitation proceeded as per the manufacturer's instructions.

2.5. Statistical analyses and data manipulation

All analyses were performed with GraphPad Prism 3.0 (GraphPad Software, San Diego, CA). Data analysis showed that DNA-damage results approximated a Gaussian distribution; thus; unpaired Student's *t*-tests with Welch's correction for unequal variances where appropriate, linear regression and Pearson's correlation and one way ANOVA with Tukey's post test were used as detailed in Figs. 1 and 2 and Table 2. Statistical analysis was performed in a post hoc manner after all biochemical analyses were completed.

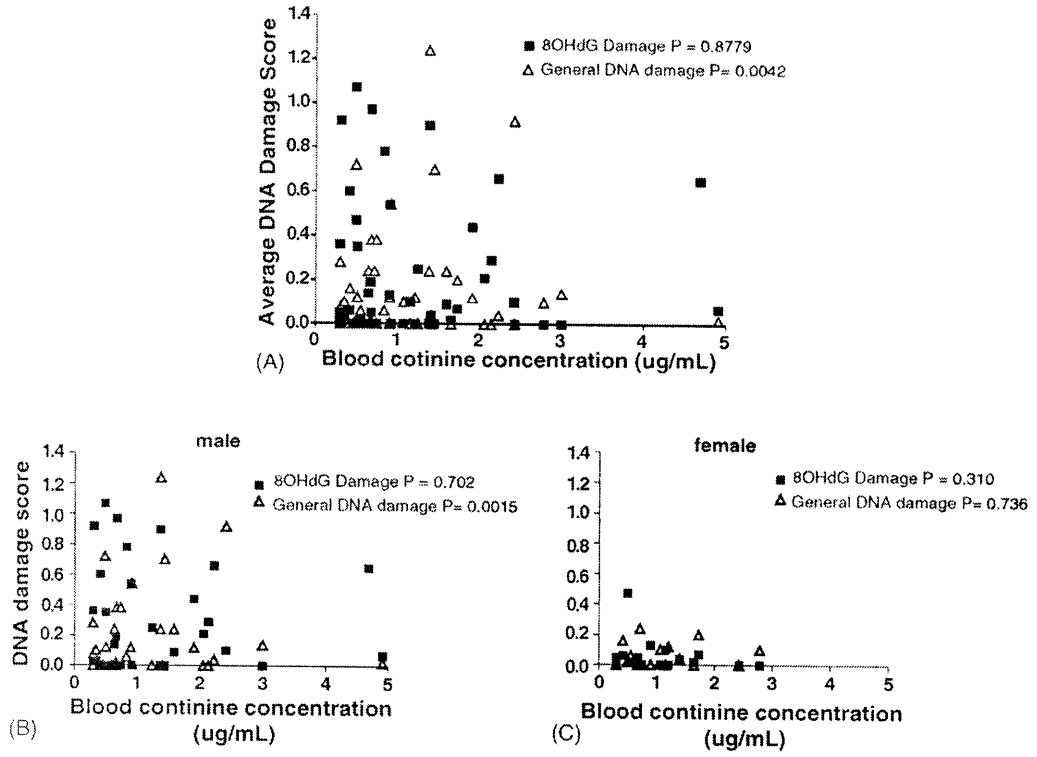

Fig. 1. General but not 8OHdG-DNA-damage is significantly correlated to workplace ETS exposure in the blood of male non-smokers. (A) Oxidative 8OHdG (■) and general DNA-damage (Δ) in the study population ($n = 49$). (B) Oxidative 8OHdG lesions (■) and total DNA-damage (Δ) in males only ($n = 32$). (C) Oxidative 8OHdG lesions (■) and total DNA-damage (Δ) in females only ($n = 17$). *P*-values presented are Pearson's correlation coefficients.

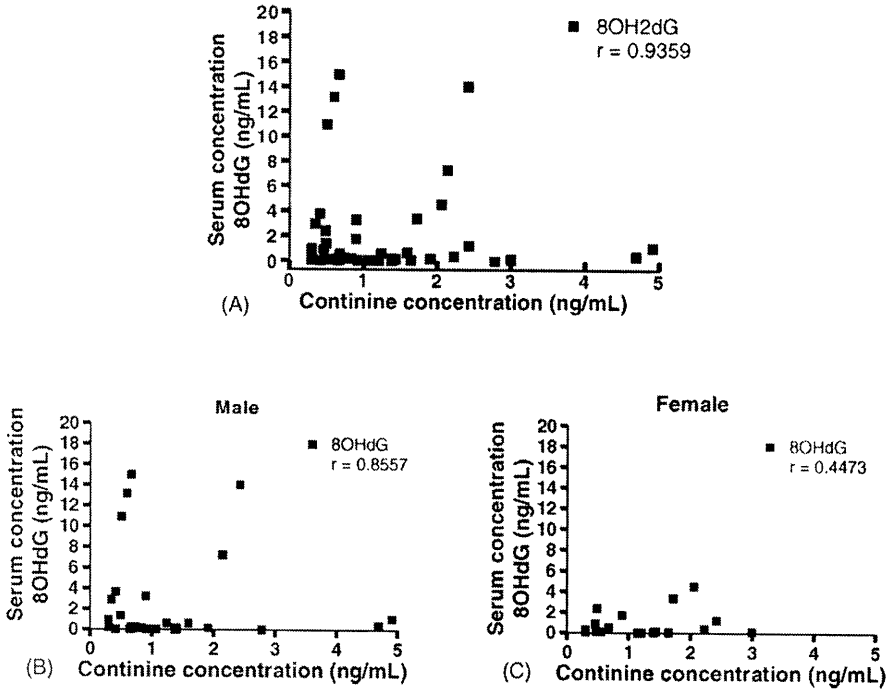

Fig. 2. 8OHdG levels in the serum of non-smokers are not correlated to workplace ETS exposure. (A) Serum 8OHdG concentrations (■) in the whole study population ($n = 49$). (B) Serum 8OHdG concentrations (■) in males only ($n = 32$). (C) Serum 8OHdG concentrations (■) in females only ($n = 17$).

3. Results

One participant was excluded because its cotinine value (137 ng/mL) represented an active smoker; subsequent statistical analysis was performed with 49 individuals (32 men and 17 women). Analysis of the remaining participants indicated that cotinine levels did not differ significantly between or within any demographic sub-group (Table 2).

General DNA-damage in the Comet assay correlated significantly with increasing cotinine levels in the whole population ($P = 0.0042$, Pearson, Fig. 1A) but 8OHdG damage did not. Further sub-group analyses showed that general DNA-damage increased

Table 2
Relationships of general DNA-damage, 8OHdG-DNA-damage, serum 8OHdG concentrations and cotinine to demographic sub-groups in the study population

	Gender (*t*-test)	Caucasian vs. other (*t*-test)	Latino vs. other (*t*-test)	BMI (Pearson)	Age (Pearson)	Marital status (ANOVA)	Education (ANOVA)
Non-oxidative DNA-damage	0.0163**	0.7531	0.5282	0.6477	0.8587	0.9203	0.6221
8OHdG-DNA-damage	0.0005**	0.7467	0.9872	0.5282	0.9506	0.4398	0.8512
8OHdG serum concentration	0.0707	0.1138	0.6102	0.8092	0.0289*	0.6002	0.4846
Cotinine	0.4230	0.3091	0.7028	0.9276	0.5602	0.6863	0.3977

Note: the lack of significance of cotinine levels between or within any demographic parameters, indicating equivalent ETS exposure in this population.

* $P < 0.05$.

** $P < 0.01$.

significantly with ETS exposure in males but not females ($P=0.015$, $n=32$, Fig. 1B and $P=0.736$, $n=17$, Fig. 1C respectively). The average general DNA-damage score for males (0.27 ± 0.47) was significantly higher at than in females (0.06 ± 0.08, $P=0.016$, t-test). In contrast, 8OHdG-DNA-damage did not correlate to increasing ETS exposure in the whole population nor in either gender ($P=0.79$, male; $P=0.31$ female, Fig. 1A–C). Despite this, the average damage scores for 8OHdG-DNA-damage were significantly higher in males (0.31 ± 0.34) than females (0.059 ± 0.11, respectively, $P=0.0005$, t-test).

Concentrations of 8OHdG in serum did not correlate to ETS exposure (Fig. 2A–C) and did not differ significantly between men than women ($P=0.0756$). The 8OHdG levels in serum did correlate significantly with age ($P=0.0289$) but did not differ with any other demographic variable (Table 2). No other demographics showed significant differences or correlations with DNA-damage (oxidative or otherwise), 8OHdG serum concentrations or cotinine levels (Table 2).

To eliminate confounding of our results caused by baseline mismatching, analysis of baseline demographics (age, body mass index, education level, marital status, and ethnic background) was performed. There were no statistically significant differences in baseline demographics between male and female participants (Table 1), strengthening the apparent gender-specific differences in DNA-damage. Furthermore, although no sample was thawed more than once prior to these experiments, we assessed whether or not the number of freeze–thaw cycles affected the average DNA-damage score from the Comet assay. No significant relation between the numbers of freeze–thaw cycles and general DNA-damage or 8OHdG-DNA-damage was observed ($P=0.778$ and $P=0.97$, respectively, Pearson). In addition, although actual numbers were not recorded, DNA-damage attributed to electrophoresis in the control slides was checked at random and was very low with few cells exhibiting any cometing (2–3 Comets/50 cells).

4. Discussion

General DNA-damage in the blood of non-smoking men increased in a dose-dependent manner with exposure to ETS in the workplace ($P=0.015$) and was significantly higher than in women ($P=0.0163$). In contrast, neither oxidative 8OHdG-DNA-damage nor 8OHdG concentrations in serum correlated with ETS exposure. In addition, although 8OHdG-DNA-damage levels were significantly higher in men than women ($P=0.0005$), serum concentrations of 8OHdG did not differ between genders.

One of the most important aspects of this study is that cotinine levels were the same between males and females (Table 2). Cotinine also did not differ between or within any other demographic group or stratification, which is further evidence for equivalent ETS exposure among all the participants. Different cotinine levels may be ascribed to differential times since exposure and/or genetic polymorphism in CYP2A6, although this polymorphism does not exist at significant levels in the population sampled (Yang et al., 2001). In addition, confounding variables for food-related nicotine levels in experimental participants have been previously investigated and cauliflower, eggplant, tomatoes, potatoes and black tea contain measurable levels of cotinine (Benowitz, 1996). While it has been suggested that the dietary intake of nicotine through these sources could confound studies of ETS, especially with respect to gender because of fruit and vegetables intake (Glynn et al., 2005); using pharmacokinetic analysis and typical American vegetable consumption data, Benowitz (1996) places the average dietary nicotine intake at around to 0.7 μg daily (Benowitz, 1996). Additionally, there are no other sex-related differences in cotinine clearances with gender (Gries et al., 1996). Up to 80% of a dose of nicotine is converted to cotinine via a C-oxidation pathway, and the resulting cotinine metabolite has a half-life of 15–17 h. In comparison, nicotine has a much shorter half-life of 2.6 h and has different clearance rates in smokers and non-smokers (Benowitz and Jacob, 1993; Kyerematen et al., 1990). Hence, due to its longer half-life, cotinine is readily identified in samples, which may have been drawn relatively long periods of time after ETS exposure and the methods used to detect and quantify it (generally GC/MS or LC/MS) are rapid, sensitive, precise and accurate (Tricker, 2003).

Another issue relating to the timing of procuring blood samples is the rate of 8OHdG-DNA lesion repair. The kinetics of hOGG1 for 8OH2dG follow Michaelis–Menten kinetics and have a V_{max} of

0.51 ± 0.17 nM/min 8OH2dG (Asagoshi et al., 2000). Thus, at high 8OH2dG levels, the enzyme may become saturated, indicating that the length of time from ETS exposure to DNA repair may also be dependent on the level of exposure. Despite this, the k_{cat} for hOGG1 with respect to 8OH2dG is 0.034 min, which is relatively fast (Asagoshi et al., 2000). This is complicated by a distinct sequence affinity of hOGG1 for 8OH2dG in certain portions of the genetic code, whereby sometimes the rate and efficiency of hOGG1 for 8OHdG is altered by the position on the lesion. The same is true for the other enzyme that removes 8OH2dG, formamidopyrimidine DNA glycosylase (Fpg). This enzyme has a V_{max} of 2.7 ± 0.7 nM and a k_{cat} of 1.8 min (resulting in similar intrinsic clearances to hOGG1) and also demonstrates a DNA-sequence specific affinity for lesions (Asagoshi et al., 2000).

This study was designed to determine whether a dose-dependent relationship between DNA-damage and increasing occupational ETS exposure exists. Previous research from our laboratory has demonstrated significantly higher 8OHdG levels in non-smoking workers occupationally exposed to ETS compared to non-smoking workers who are not exposed (Howard et al., 1998), and we wished to follow on from this finding and investigate dose–response relationships between levels of ETS exposure and levels of DNA-damage. It has also been shown that self-reported ETS exposure significantly underestimates the actual exposure of study participants to ETS (DeLorenze et al., 2002) and that ethnicity, marital status, education and age (Iribarren et al., 2004; Kurtz et al., 2003; Reynolds et al., 2004; Soliman et al., 2004) can affect exposure levels. In order to address this, the current study used blood cotinine levels as a marker of ETS exposure not self-reporting, and the data presented in Table 2 demonstrate that no significant differences in cotinine levels were observed between or within these demographic subgroups. This indicates that our findings are not skewed by mismatching between males and females and further, that the measured ETS exposure is likely to comprise almost exclusively of workplace ETS exposure and not exposure through the home (often correlated with marital status (Kurtz et al. 2003; Reynolds et al. 2004)) or social exposure (often correlated with age (Reynolds et al., 2004)).

Although previous studies have reported higher 8OHdG in smokers than non-smokers (Loft et al., 1992) and for workers in smoking compared to smoke-free workplaces (Howard et al., 1998), dose-dependent effects of ETS on either 8OHdG lesions or other forms of DNA-damage have not been reported in humans. It has been shown by HPLC with electro-chemical detection method that 8OHdG-DNA lesions are significantly higher in pooled male blood samples than pooled female blood samples, and that large geographical differences in damage levels occur (Collins et al., 1998). Our study is in agreement with these findings regarding the gender-differences in 8OHdG-DNA adducts, although all of our samples were from a similar geographic region. Because we employed a Comet assay approach, individual blood samples could be assessed (blood volume is the limiting factor for HPLC/ECD analysis), thereby increasing the power of the experiment. The advantages of using the Comet assay include the small number of cells required, the high sensitivity of the procedure (DNA-damage caused by <5 cGy gamma rays can be observed) and the economical, fast and simple nature of the assay (Rojas et al., 1999). The disadvantages of the Comet assay as it was used here mostly relate to technical variability in scoring cells and interpretation of results and the sample sizes per assay, which have been criticised for causing sampling bias. These concerns are somewhat mitigated by having all of the cell scoring performed by one person and scoring large numbers of cells (50 cells per determination, in triplicate) (Kobayashi et al., 1995; Rojas et al., 1999). In addition, sampling bias relates more to solid tissues where results may be skewed by region-specific differences in DNA-damage. In a circulating tissue such as blood, regio-specificity in DNA-damage is less likely to be observed and unlikely to have been a factor in this study. Finally, our manner of scoring cells using the 0–4 scale and assessing damage by eye has been shown by previous investigators to be as accurate, more sensitive and faster than computer image analysis and to correlate almost exactly with computerized analyses of ratio (DNA% in tail) and tail moment (product of ratio and tail distance) when graded concentrations of known DNA damaging agents were tested and scored by double blinded investigators (Kobayashi et al., 1995).

Since most of the damage caused by cigarette smoke is believed to occur through DNA adducts, the power of the Comet assay in detecting DNA-damage caused by smoking and/or ETS has been questioned due to the

fact that DNA cross links and adducts retard the migration in the assay (Sram et al., 1998). These properties of DNA migration under electrophoresis are likely to be responsible, at least in part, for many conflicting reports on DNA-damage caused by cigarette smoke when the Comet assay was used as the only form of analysis and may be accountable for the results of the lone study where Comet assays reported less DNA-damage in smokers than non-smokers (Speit et al., 2003). The pH of DNA unwinding and electrophoresis and the length of time unwinding and electrophoresis are performed are also critical to the amount of cometing observed. For example, as pH increases so do the amount and types of DNA-damage detected. Therefore, differences in DNA-damage reported by different laboratories could be due to variance in the pH of DNA unwinding and/or running buffers. In the present studies, as well as using an enzyme to cleave DNA at 8OHdG-adducts sites and comparing these results to assays in the same participants where enzyme was not added, unwinding was carried out at pH >13 where the assay detects the most possible forms of DNA-damage. Additionally, as discussed above, our use of loading and running controls enabled changes in DNA migration due to the number of cells loaded and cometing caused by the method itself to be eliminated as variables. Moreover, because SYBR green™ was used as the detector fluorophore, the assay is extremely sensitive. This is important as a recent investigation of differences in DNA-damage between smokers and non-smokers using ethidium bromide as a fluorophore reported that detection of smoke-induced DNA-damage was limited in the Comet assay (Sram et al., 1998). When comparing fluorophores, ethidium bromide shows 30-fold fluorescence enhancement upon binding to DNA while SYBR green™ exhibits 1000-fold fluorescence enhancement (Molecular Probes, 2004).

Despite equivalent workplace ETS exposure, both intracellular 8OHdG and general DNA-damage were greater in males than females, but there were no significant differences between men and women in serum levels of extracellular (repaired) 8OHdG. Some caution is needed in interpreting these results because it is possible that if these experiments were expanded to a larger population, differences in extracellular 8OHdG serum concentrations between the men and women would become significant, as the *P*-value between men and women in the current study was low (0.07). In addition, when discussing aspects of DNA repair in this study, it is important to note that DNA repair was not measured directly, thus attributing differences between DNA-bound 8OHdG lesions and free plasma 8OHdG to repair remains a hypothesis meaning that concrete conclusions regarding gender-related differences in DNA repair are difficult to substantiate within the size and power of this study. Despite this, these data may indicate gender-differences in DNA-damage and repair capacity and speculatively provide a rationale for the higher incidences of some syndromes (notably most cancers and heart disease) in males compared to females (Manton, 2000). Although women are more susceptible to certain forms of cancer such as cancers of the breast, these cancers are predominantly hormonal and not mechanistically genotoxic (Wingo et al., 2003). In addition, although women have overtaken men with respect to incidence of lung cancers since the 1980s, this has largely been attributed to behavioural and social factors not biochemical or cellular events (Levi et al., 2004; Wingo et al., 2003). For cancers where initiation is predominantly through genotoxic and mutagenic means, such as liver, gastro-intestinal, bladder and colo-rectal cancers, epidemiological studies have uniformly reported greater incidences in men than women in both Europe and USA over the last century (Levi et al., 2004; Manton, 2000 p. 11; Wingo et al., 2003). Recent research has demonstrated a constitutive polymorphism of the *hOGG1* gene that results in reduced ability to repair 8OHdG lesions. This polymorphism is also associated with higher lung cancer risk (Park et al., 2004). Also, Gackowski et al. (2003) have reported that levels of 8OHdG in DNA isolated blood of lung cancer patients was significantly higher than that in DNA isolated from healthy smokers and non-smokers (Gackowski et al., 2003). It is, therefore, possible that data presented showing higher 8OHdG lesions in the blood of men exposed to ETS may be associated with lung cancer risk.

The distinction between excised bases and intracellular, bound DNA-damage is particularly important in terms of long-term affects on homeostasis, cell cycling and the development of disease. For example, incomplete base-excision repair sites, which are detected in nuclear DNA by the Comet assay and likely make up some of the general DNA-damage observed here, frequently leads to inappropriate transcription, cell cycling and metastasis (Gantt et al., 1986). It is

difficult to say precisely how detrimental the general DNA-damage detected herein will be in the long-term because double and single strand breaks, incomplete base-excision repair sites and alkali-labile sites are all detected by the Comet assay, but their relative frequencies in the present study are unknown. In contrast, a direct measure of the amount of 8OHdG-DNA-damage was measured and the amount of 8OHdG in serum (which is more indicative of DNA repair capacity) was also measured providing a direct gauge of repair capacity.

ETS is known to be a carcinogen, pro-oxidant stressor and genotoxic agent. Here we have shown a significant, positive correlation of greater DNA-damage with increasing ETS exposure. A direct correlation between ETS exposure and non-8OHdG damage would be even more conclusive in the presence of either a control group (such as non-smokers not exposed to ETS) showing a lack of such correlation or in a longitudinal group showing increasing DNA-damage levels with time. However, perhaps the most interesting finding was the gender-specific differences in DNA-damage and potential differences in repair observed. These results provide a "snapshot" of the interplay between DNA-damage and ETS exposure, and along with other recent findings, further evidence that gene–environment interactions of DNA repair enzymes may be important in cancer risk and mutagen sensitivity (Ito et al., 2004; Tuimala et al., 2002). The dose-dependency observed for general DNA-damage and ETS, suggests a dynamic equilibrium between damage and repair with respect to ETS exposure over time, while discrepancies between higher levels of intracellular DNA-damage (both general and 8OHdG) in men than women but comparable amounts of (repaired) serum 8OHdG, may imply gender disparities in the expression and activity of DNA repair enzymes. This study may provide insight into the genotoxic, mutagenic and carcinogenic mechanisms of ETS and also may also help explain gender-differences in the susceptibility to cancer and other diseases caused or exacerbated by tobacco smoke.

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References

Anon, 1996. Cigarette smoking and health. Am. J. Respir. Crit. Care Med. 153, 861–865.

Asagoshi, K., Yamada, T., Terato, H., Ohyama, Y., Monden, Y., Arai, T., Nishimura, S., Aburatani, H., Lindahl, T., Ide, H., 2000. Distinct repair activities of human 7,8-dihydro-8-oxoguanine DNA glycosylase and formamidopyrimidine DNA glycosylase for formamidopyrimidine and 7,8-dihydro-8-oxoguanine. J. Biol. Chem. 275, 4956–4964.

Benowitz, N., 1996. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol. Rev. 18, 188–204.

Benowitz, N., Jacob, P., 1993. Nicotine and cotinine elimination pharmacokinetics in smokers and non-smokers. Clin. Pharmacol. Ther., 53.

Cains, T., Cannata, S., Poulos, R., Ferson, M., Stewart, B., 2004. Designated "no smoking" areas provide from partial to no protection from environmental tobacco smoke. Tob. Control 13, 17–22.

Collins, A., Gedik, C., Olmedilla, B., Southon, S., Bellizzi, M., 1998. Oxidative DNA-damage measured in human lymphocytes: large differences between sexes and between countries, and correlations with heart disease mortality rates. Federation of Soc. Exp. Biol. J. 12, 1397–1400.

DeLorenze, G., Kharrazi, M., Kaufman, F., Eskenazi, B., Bernert, J., 2002. Exposure to environmental tobacco smoke in pregnant women: the association between self-report and serum cotinine. Environ. Res. 90, 21–32.

Gackowski, D., Speina, E., Zielinska, M., Kowalewski, J., Rozalski, R., Siomek, A., Paciorek, T., Tudek, B., Olinski, R., 2003. Products of oxidative DNA-damage and repair as possible biomarkers of susceptibility to lung cancer. Cancer Res. 63, 4899–4902.

Gantt, R., Parshad, R., Price, F., Sanford, K., 1986. Biochemical evidence for deficient DNA repair leading to enhanced G2 chromatid radiosensitivity and susceptibility to cancer. Radiat. Res. 108, 117–126.

Glynn, L., Emmett, P., Rogers, I., 2005. ALSPAC Study Team Food and nutrient intakes of a population sample of 7-year-old children in the south-west of England in 1999/2000—what difference does gender make? J. Hum. Nutr. Diet 18, 7–19.

Gries, J., Benowitz, N., Verotta, D., 1996. Chronopharmacokinetics of nicotine. Clin. Pharmacol. Ther. 60, 389–395.

Heloma, A., Jaakkola, M., Kahkonen, E., Reijula, K., 2001. The short term impact of national smoke-free workplace legislation on passive smoking and tobacco use. Am. J. Public Health 91, 1416–1418.

Howard, D., Ota, R., Briggs, L., Hampton, M., Pritsos, C., 1998. Environmental tobacco smoke in the workplace induces oxidative stress in employees, including increased production of 8-hydroxy-2′-deoxyguanosine. Cancer Epidemiol. Biomarkers Prev. 72, 141–146.

Iribarren, C., Darbinian, J., Klatsky, A., Friedman, G., 2004. Cohort study of exposure to environmental tobacco smoke and risk of first ischemic stroke and transient ischemic attack. Neuroepidemiology 23, 38–44.

Ito, H., Matsuo, K., Hamajima, N., Mitsudomi, T., Sugiura, T., Saito, T., Yasue, T., Lee, K.-M., Kang, D., Yoo, K.-Y., Sato, S., Ueda,

R., Tajima, K., 2004. Gene-environment interactions between the smoking habit and polymorphisms in the DNA repair genes, *APE1 Asp148Glu* and *XRCC1 Arg399Gln*, in Japanese lung cancer risk. Carcinogenesis 25, 1395–1401.

Kobayashi, H., Sugiyama, C., Morikawa, Y., Hayashi, M., Sofuni, T., 1995. A comparison between manual and microscopic analysis and computerized image analysis in single cell electrophoresis assay. MMS Commun. 3, 103–115.

Kurtz, M., Kurtz, J., Contreras, D., Booth, C., 2003. Knowledge and attitudes of economically disadvantaged women regarding exposure to environmental tobacco smoke: a Michigan, USA study. Eur. J. Public Health 13, 171–176.

Kyerematen, G., Morgan, M., Chattopadhyay, B., de Bethizy, J., Vesell, E., 1990. Disposition of nicotine and eight metabolites in smokers and non-smokers: identification in smokers of two metabolites that are longer lived than cotinine. Clin. Pharmacol. Ther. 48, 641–651.

Levi, F., Lucchini, F., Negri, E., Vecchia, C.L., 2004. Trends in mortality from major cancers in the European Union, including acceding countries, in 2004. Cancer 101, 2843–2850.

Loft, S., Vistisen, K., Ewertz, M., Tjonneland, A., Overvad, K., Poulsen, H., 1992. Oxidative DNA-damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 13, 2241–2247.

Manton, K., 2000. Gender-differences in the cross-sectional and cohort age dependence of cause-specific mortality: the United States, 1962–1995. J. Gend. Specif. Med. 3, 47–54.

Molecular Probes, 2004. Nucleic Acid Detection and Quantitation in Electrophoretic Gels and Capillaries. Molecular Probes Incorporated, Foster City, CA.

Park, J., Chen, L., Tockman, M., Elahi, A., Lazarus, P., 2004. The human 8-oxoguanine DNA *N*-glycosylase 1 (hOGG1) DNA repair enzyme and its association with lung cancer risk. Pharmacogenetics 14, 103–109.

Reynolds, P., Goldberg, D., Hurley, S., 2004. Prevalence and patterns of environmental tobacco smoke exposures among California teachers; California Teachers Study Steering Committee. Am. J. Health Promot. 18, 358–365.

Rojas, E., Lopez, M., Valverde, M., 1999. Single cell gel electrophoresis. Methodology and applications. J. Chromatogr. B 1–2, 225–254.

Soliman, S., Pollack, H., Warner, K., 2004. Decrease in the prevalence of environmental tobacco smoke exposure in the home during the 1990s in families with children. Am. J. Public Health 94, 314–320.

Speit, G., Witton-Davies, T., Heepchantree, W., Trenz, K., Hoffmann, H., 2003. Investigations on the effect of cigarette smoking in the Comet assay. Mut. Res. 542, 33–42.

Sram, R., Rossner, P., Peltonen, K., Podrazilova, K., Mrackova, G., Demopoulos, N., Stephanou, G., Vlachodimitropoulos, D., Darroudi, F., Tates, A., 1998. Chromosomal aberrations, sister-chromatid exchanges, cells with high frequency of SCE, micronuclei and Comet assay parameters in 1,3-butadiene-exposed workers. Mut. Res. 419, 145–154.

Tricker, A., 2003. Nicotine metabolism, human drug metabolism polymorphisms and smoking behaviour. Toxicology 183, 151–153.

Tuimala, J., Szekely, G., Gundy, S., Hirvonen, A., Norppa, H., 2002. Genetic polymorphisms of DNA repair and xenobiotic-metabolizing enzymes: role in mutagen sensitivity. Carcinogenesis 23, 1003–1008.

Wingo, P., Cardinez, C., Landis, S., Greenlee, R., Ries, L., Anderson, R., Thun, M., 2003. Long-term trends in cancer mortality in the United States, 1930–1998. Cancer 97, 3133–3275.

Yang, M., Kunugita, N., Kitagawa, K., Kang, S., Coles, B., Kadlubar, F., Katoh, T., Matsuno, K., Kawamoto, T., 2001. Individual differences in urinary cotinine levels in Japanese smokers: relation to genetic polymorphism of drug-metabolizing enzymes. Cancer Epidemiol. Biomarkers Prev. 10, 589–593.