Environmental Tobacco Smoke in the Workplace Induces Oxidative Stress in Employees, Including Increased Production of 8-Hydroxy-2′-Deoxyguanosine[1]

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Abstract

Environmental tobacco smoke (ETS) is a pervasive contaminant in the workplace. Our objective was to determine the oxidative stress effects of ETS on employees who are exposed. The results provide information that is useful to the resolution of risk assessment questions associated with ETS. We analyzed two blood draws from volunteers in our control and exposed groups. The level of exposure to ETS was determined through plasma cotinine measurements, which showed a 65% increase from the control group to the exposed group. Exposure to ETS resulted in a statistically significant increase of 63% of the oxidative DNA mutagen 8-hydroxy-2′-deoxyguanosine in the blood of exposed subjects. This oxidative DNA damage has been linked to an increased risk of developing several degenerative chronic diseases, including coronary heart disease and cancer. The exposed subjects also had increased levels of superoxide dismutase, catalase, glutathione peroxidase (GPOX), and glutathione reductase. However, these increases were only statistically significant in catalase and GPOX. Catalase levels were 13% higher in the exposed group, and GPOX levels were 37% higher in exposed volunteers. The biochemical evidence suggests that exposure to ETS causes oxidative stress, resulting in DNA damage that may increase the risk of certain diseases.

Introduction

In 1986, two landmark reports, one by the Surgeon General of the United States and the other by the National Academy of Sciences, were released that independently reached similar conclusions regarding the adverse health effects of exposure to ETS[3] (1, 2). The Surgeon General's report, based on epidemiological evidence, asserted a direct link between exposure of nonsmokers to ETS and disease, most notably, lung cancer. In February 1997, the California Environmental Protection Agency released the final draft of a report detailing the known health effects of exposure to ETS (3). This report estimates that there are between 35,000 and 62,000 ischemic heart disease deaths in the United States every year that result from ETS exposure. In addition, 3000 lung cancer deaths and over 2000 childhood deaths from bronchitis and sudden infant death syndrome have also been linked to ETS. ETS's potent impact on human health is due, in part, to widespread exposure. The recent publication of a nationally representative cross-sectional survey reported that, in the United States, 37.4% of the adult nonsmokers reported exposure to ETS at home or in the workplace and that 25% of nonsmoking adults reported exposure to ETS in the workplace (4). The conclusions from these reports have intensified the debate over smoking and catalyzed an entirely new discussion regarding ETS's health impact on nonsmokers. This controversy covers medical, social, and legal battlefields, with particular emphasis on smoking in the workplace and public buildings. Despite the epidemiological evidence, the health risks associated with the exposure to ETS have been difficult to establish. The risks are confounded by a variety of factors inherent in epidemiological studies, particularly those found in retrospective studies. Previous work performed by this laboratory has shown an increase in oxidative stress and oxidative DNA damage in mouse tissues resulting from short-term exposure to sidestream smoke, a major component of ETS. Additional studies, such as that presented here, that look at ETS's impact on the biochemical processes linked to disease are required to help resolve the debate.

We set out to determine whether exposure to ETS in the workplace would result in an increase in cellular oxidative damage. Oxidative damage occurs in a cell when the production of ROS overwhelms the cell's natural antioxidant defenses. Exposure to chemicals such as those found in ETS can lead to the generation of a large number of ROS during their metabolism by the cell. When the balance of ROS supersedes the antioxidant capacity of the cell, the ROS can damage cellular constituents, such as membrane lipids, proteins, DNA, and RNA. This damage can lead to cell mutation or death. The process of cellular oxidative damage has been linked to the etiology of a number of chronic degenerative conditions, including cancer and coronary heart disease, both closely associated with smoking and exposure to ETS (5–12). Oxidative

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[3] The abbreviations used are: ETS, environmental tobacco smoke; ROS, reactive oxygen species; SOD, superoxide dismutase; GPOX, glutathione peroxidase; GR, glutathione reductase; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; HPLC, high-performance liquid chromatography; Hb, hemoglobin; dG, deoxyguanosine; TBARS, thiobarbituric acid assay; BHT, butylated hydroxytoluene.

stress is thought to play a role in carcinogenesis by oxidative damage to DNA (8).

We evaluated the activity of the enzymes SOD, catalase, GPOX, and GR because their activities increase with exposure to ROS in blood. The antioxidant vitamins C and E and β-carotene were also analyzed because they protect against oxidative damage. Finally, the DNA adduct 8-OHdG and lipid peroxidation, both markers of physiological oxidative damage, were analyzed. 8-OHdG, the oxidized form of the nucleoside 2′-deoxyguanosine, is an excellent marker of DNA damage because it is capable of reflecting extremely low levels of oxidative damage. It is one of the most abundantly formed oxidative DNA products (13) and can be detected by HPLC-electrochemical methods in the femtomole range. Increases in 8-OHdG levels are induced by several carcinogens (14, 15) and are similarly present in higher concentrations in the DNA of malignant cells (16). 8-OHdG has also been shown to increase in the leukocytes of smokers (17). It is important to remember that, although 8-OHdG is a marker of oxidative stress, it is itself a mutagen, linked with several disease states (5, 11) and able to participate in at least two types of transcriptional errors (18).

To quantify the exposure to ETS experienced by our volunteers, we analyzed the level of plasma cotinine in our control and exposed groups. Cotinine is a specific breakdown product of nicotine and, as such, is an excellent indicator of tobacco smoke exposure. Compared to nicotine, cotinine has a much longer half-life in the body (~17 h), which makes it more reliable than nicotine as a biological marker of ETS exposure (19).

Materials and Methods

Blood Specimens. Volunteers for the study were recruited through an article in a local newspaper detailing the study. Criteria for volunteer participation included the following: each volunteer was required to be a nonsmoker; none of the volunteers could be exposed to ETS at home; and none of the volunteers were allowed to supplement their diet with vitamins either during or for a period of 6 weeks prior to the study. The participants were then separated into two groups, those who were exposed to ETS at work and those who were not.

Following approval for participation, two 20-ml blood samples were drawn from each participant. Duplication was performed to minimize variations that might result from a single sampling. Blood samples taken by venous puncture were drawn within 12 h of the subjects' last work shift to obtain an accurate blood cotinine value. The two draws were also scheduled within 2 weeks of one another. Before blood was taken, each volunteer filled out a short questionnaire and signed a study participation consent form.

Once drawn, the blood was kept at 4°C in a low-light environment. Aliquots were then distributed for the following analyses.

SOD. One ml of whole blood was centrifuged at 3000 rpm for 15 min at 4°C. The plasma was removed, and the packed cells were gently resuspended in an equal volume of PBS. The samples were washed three times. Finally, the washed pellet was resuspended in an equal volume of PBS, and the cells were lysed by sonication (two 5-s bursts). The resultant hemolysate was used for analysis of SOD, catalase, GPOX, and GR.

SOD was assayed by the procedure of McCord and Fridovich (20), as modified by Oberley and Spitz (21). Briefly, a working buffer was prepared, consisting of 50 mM potassium phosphate (pH 7.8), 1 mM diethylenetriamine pentaacetic acid, 1 mM xanthine, 0.056 mM nitroblue tetrazolium, and 1 unit/ml catalase. Xanthine oxidase was used to establish a rate of superoxide radical anion production, and known amounts of SOD were added to inhibit the reaction and generate a standard curve. These reactions were monitored for 2 min at 560 nm. The inhibition of this reaction rate is the basis for the SOD activity determination, both in the standards and the samples. SOD activity in the samples was based on the external standard curve and expressed in units of SOD/g protein.

Catalase. Catalase activity was measured from the same blood hemolysate preparation described above. The method used for catalase determination follows that described by Aebi (22). Briefly, hydrogen peroxide (H_2O_2) was added to a 50 mM potassium phosphate buffer until the $A_{240\ nm}$ of the buffer plus H_2O_2 was between 0.50 and 0.53 *versus* a blank buffer alone. The sample was diluted 1:150 in water, and 5 μl were added to the buffer. The reaction was monitored at 240 nm for 2 min. Catalase activity is expressed as μmol of H_2O_2 decomposed/min/mg of protein.

GR. GR activity was measured from the same blood hemolysate preparation described above. The method used for GR determination follows that described by Racker (23), with modifications for use in a Bio-Tek Instruments EL-340 (Winooski, VT) microplate reader. In this method, a 1 M potassium phosphate buffer (pH 7.6), with NADPH, BSA, and glutathione disulfide, was prepared. Two hundred ninety μl of this buffer were dispensed into a microtiter plate well, and 10 μl of a 1:5 dilution of the hemolysate were added. The reaction was allowed to proceed for 2 min, and the loss of NADPH was monitored by the change in $A_{340\ nm}$/min. GR is expressed as μmol of NADPH oxidized/min/g of Hb.

GPOX. GPOX activity was measured in the same blood hemolysate preparation described above. GPOX was determined through the use of a method described by Strauss *et al.* (24). Again, modifications were required for the adaptation of the method to a microplate reader. A 50 mM potassium phosphate buffer (pH 7.0), with EDTA, NADPH, glutathione, and sodium azide, was prepared. Two hundred eighty μl of this buffer were added to each well, in addition to 10 μl of 2.2 mM H_2O_2 and 10 μl of a 1:5 dilution of the hemolysate. The reaction was run for 0.5 min, and the loss of NADPH was monitored by the change in $A_{340\ nm}$/min. GPOX is expressed as μmol of NADPH oxidized/min/g of Hb.

8-OHdG. DNA was extracted from 1 ml of whole blood using the Wako DNA Extraction WB Kit (Richmond, VA). Following the extraction process, the DNA was digested to component nucleosides using a method described by Shigenaga *et al.* (25). Briefly, the DNA pellets were suspended in 200 μl of a 1 mM desferoxamine mesylate (DFAM)-20 mM sodium acetate solution (pH 5.0). Once the DNA was in solution, it was hydrolyzed to nucleotides through the addition of 4 μl of 3.0 mg/ml nuclease P1 (Sigma Chemical Co., St. Louis, MO) in 20 mM sodium acetate (pH 5.0). The digestion took place in a water bath at 65°C for 10 min.

The nucleotide solution was then adjusted to pH 8.5 through the addition of 20 liters of 1 M Tris-HCl (pH 8.5), followed by 4 μl of 1 unit/liter calf intestine alkaline phosphatase. The nucleotide solutions were incubated for 1 h at 37°C to convert the nucleotides to their corresponding nucleosides. Following conversion, the pH was adjusted by the addition of 20 μl of 3 M sodium acetate (pH 5.0) and 20 μl of 50 mM EDTA-10 mM DFAM prepared in HPLC-grade water. The samples were then filtered through a 0.45 μm/3 mm MSI (Westboro, MA) membrane in preparation for analysis by HPLC.

The DNA samples were analyzed by HPLC using an Alltech Absorbosphere C-18 3U MF-Plus column (Deerfield, IL; 150 mm × 4.6 mm) with a mobile phase consisting of 100 mM sodium acetate (pH 5.2)-4% methanol and a flow rate of 1 ml/min. The 8-OHdG and dG were detected using an ESA Coulochem II electrochemical detector (Guard Cell = 200 mV; E1 = 325 mV), in line with a UV detector (262 nm), as described by Shigenaga *et al.* (25). Daily standards of 8-OHdG and dG were run to verify the initial calibration curve.

The levels of 8-OHdG in the blood were quantitated against external standards. The dG was acquired from Sigma, and the 8-OHdG was from Wako.

Cotinine Analysis. Cotinine levels were determined from 1 ml of plasma using a method described by Perkins (26). Briefly, 1 ml of plasma was run through a rinsed C-18 cartridge. The cartridge was then rinsed again, after which the cotinine was eluted with chloroform:isopropanol (95:5). The eluant was then dried using a vacuum centrifuge. The residue, containing the cotinine, was resuspended with 200 liters of water. The sample was then filtered through a 0.45 μm/3 mm MSI (Westboro, MA) filter and analyzed by HPLC-UV.

The mobile phase for this method was 30 mM sodium citrate, 30 mM potassium phosphate (pH 6.0), and 6% acetonitrile. The flow rate was 1 ml/min. The column was a Supelcosil LC-18-DB column, and the detector was a Spectra Physics Focus (262 nm; San Jose, CA). Quantitation was based on extracted spiked samples, but daily standards were run to confirm instrument performance.

Lipid Peroxidation. Lipid peroxidation was determined by the TBARS, as described by Buege and Aust (27). Reagent, containing trichloroacetic acid, hydrochloric acid, and thiobarbituric acid, was added to the plasma. The sample was then heated at 90°C for 30 min, centrifuged at 3000 rpm for 5 min, and read spectrophotometrically at 535 nm. The number of malondialdehyde equivalents formed was determined from the absorbance of the sample.

Vitamin C. Plasma vitamin C levels were analyzed spectrophotometrically using a method described previously by Zannoni *et al.* (28). Briefly, 100 μl of plasma were extracted with trichloroacetic acid through the addition of 100 μl of 10% trichloroacetic acid, followed by vortexing and centrifugation. Twenty μl of a solution containing dinitrophenylhydrazine, thiourea, and copper sulfate were added to the supernatant and incubated for 2 h at 37°C. One hundred fifty μl of ice-cold 65% sulfuric acid was then added to the sample, which was placed on ice. The samples were subsequently incubated at room temperature, in a low-light environment, for 1 h. Vitamin C levels were determined using a microplate reader set to 515 nm.

Vitamin E. Vitamin E was measured as α-tocopherol, which was extracted from 500 μl of plasma through the addition of 500 μl of cold methanol-0.125% BHT, followed by a 1-min vortex and the addition of 1500 μl of cold heptane-0.125% BHT. The samples were then vortexed for 2 min and centrifuged for 15 min at 3000 rpm. One ml of the organic layer was removed and evaporated in a vacuum centrifuge, purged with nitrogen, and placed in a freezer at −70°C, until the samples were analyzed by HPLC.

The samples were resuspended to a volume of 100 μl with methanol-0.125% BHT. The HPLC mobile phase was methanol:water (93:7), at a flow rate of 1 ml/min. The samples were analyzed using a Perkin-Elmer model 250 equipped with a Beckman Ultrasphere C-18 ODS 5-μm column (Fullerton, CA; 15 mm × 4.6 mm) and a Perkin-Elmer LC 95 UV/VWAS detector set at 292 nm. An initial calibration curve was performed prior to the analyses of each batch of samples. This method follows the procedure described by Kahlon *et al.* (29).

Table 1 Composite of study group parameters

Parameter	Control group[a]	Exposed group[a]
Men *(n)*	17	15
Women *(n)*	19	23
Average age (yr)	40.1 ± 1.6	44.0 ± 1.4
Self-perceived health status[b]	3.11 ± 0.12	3.21 ± 0.14
Ethanol consumption (drinks/week)[c]	1.72 ± 0.40	2.29 ± 0.45
Self-reported exposure (h/day)	0	6.6 ± 1.6
Cotinine (ng/ml)[d]	2.72 ± 0.52	4.50 ± 0.56

[a] Variance is expressed in SE for all results.
[b] Based on a rating of 1–4 (1, least healthy compared to others their age; 2, less healthy; 3, as healthy; 4, more healthy).
[c] Self-reported.
[d] Cotinine was extracted using C-18 solid-phase extraction cartridges and analyzed by HPLC-UV (13).

Blood Protein. Blood protein was determined using a bicinchoninic acid Protein Assay kit (kit 23225 H) purchased from Pierce (Rockford, IL). Briefly, 10 μl of a 1:100 dilution of blood hemolysate were placed into wells, followed by 200 μl of the Pierce bicinchoninic acid reagent. The sample was automatically incubated and analyzed at 562 nm in the plate reader. A standard curve was generated for each batch of samples.

Hb. Hb levels were determined using Drabkin's reagent in the Hb kit (model 525-A) from Sigma. Sigma Drabkin's solution (2.5 ml) was added to 10 liters of blood hemolysate. The mixture was allowed to stand for 15 min. The sample was read spectrophotometrically at 540 nm. A standard curve was generated for each batch of samples.

β-Carotene. β-Carotene levels were determined through extraction and HPLC analysis. β-Carotene was extracted from 1 ml of plasma through the addition of 500 μl of cold methanol-0.125% BHT, followed by a 1-min vortex and the addition of 2 ml of hexane-0.125% BHT. The sample was vortexed for 3 min and then centrifuged for 10 min. One ml of the supernatant was removed from the sample and evaporated in the vacuum centrifuge. The sample was resuspended in 200 μl of acetonitrile:methylene chloride:methanol (50:20:30), which also served as the mobile phase. The flow rate was 1 ml/min. The samples were analyzed using a Perkin-Elmer model 250 equipped with a Beckman Ultrasphere C-18 ODS 5-μm column (15 mm × 4.6 mm) and a Perkin-Elmer LC 95 UV/VWAS detector set at 450 nm. An initial calibration curve was performed prior to the analyses of each batch of samples. This method follows the procedure described by Hatam and Kayden (30).

Results

Profile of Study Volunteers. The characteristics of the control and exposed volunteers are shown in Table 1. A total of 74 volunteers were accepted into the study. Thirty-eight of those were placed in the exposed group, and the remaining 36 were placed in the control group. All exposed respondents were placed in the exposed group regardless of the length of exposure. The average ETS exposure time at work for the subjects in our study was 6.6 h/day (Table 1), as determined from the study questionnaire. The study questionnaire also provided us with information on age, ethanol consumption, and self-perceived health status. Statistical analysis using Student's *t* test indicated no significant difference for these three parameters.

Table 2 Summary of study results

Parameter	Control group[a]	Exposed group[a]	% difference[b]
Enzyme activity			
SOD (units/μg)	6.23 ± 0.41 (33)[c]	6.70 ± 0.42 (37)	+8%
Catalase (units/mg)	391 ± 10 (35)	442 ± 18 (37)	+13%
GPOX (NADPH consumed/min/mg)	9.38 ± 0.27 (34)	10.3 ± 0.40 (37)[d]	+10%
GR (NADPH consumed/min/mg)	2.23 ± 0.08 (34)	2.31 ± 0.09 (37)[d]	+4%
Vitamin status			
Vitamin C (μg/100 μl)	0.64 ± 0.04 (35)	0.64 ± 0.03 (37)	0%
α-Tocopherol (μg/ml)	12.2 ± 0.6 (35)	14.9 ± 0.6 (37)[d]	+22%
β-Carotene (μg/ml)	0.616 ± 0.079 (35)	0.781 ± 0.117 (37)	+27%
Physiological damage			
Lipid peroxidation (TBARS eq)	10.2 ± 0.5 (35)	9.2 ± 0.5 (37)	−10%
8-OHdG (pg/μg)	17.2 ± 2.3 (27)	28.0 ± 3.6 (29)[d]	+63%

[a] Values represent average ± SE.
[b] Values represent the % difference between the exposed group average and the control group average.
[c] Values in parentheses represent *n* for the determination.
[d] Represents values that are significantly different from respective control group values by Student's *t* test ($P < 0.05$).

Student's *t* test was used for statistical comparison between the control group and the exposed group for all analytes.

Exposure. To gauge exposure to ETS, we measured the levels of cotinine in the plasma of the subjects. Cotinine levels were 65% greater in the exposed group than they were in the control group. This increase was statistically significant ($P < 0.05$, Student's *t* test). The level of cotinine seen in the exposed group is far below the levels seen in smokers (>275 ng/ml; Ref. 31). These values are expressed in Table 1.

Enzyme Induction. SOD activity was greater in the exposed subjects than it was in the control subjects (Table 2). The 7.5% increase in SOD activity was not statistically significant but does suggest that the exposed group was experiencing greater oxidative stress than was the control group. Catalase activity was increased by 13% in the group exposed to ETS (Table 2), and this increase was statistically significant by Student's *t* test ($P < 0.05$). This increase supports the SOD results as a reflection of increased oxidative stress in the exposed group. GPOX levels in the exposed group were higher than those in the control group (Table 2). Like the catalase increase, this 10% rise in GPOX activity was statistically significant ($P < 0.05$) and indicates increased oxidative stress in the exposed group, as compared to the control group. The GR activities (Table 2) were consistent with the pattern for the other enzyme activities. Although not statistically significant, there was a 4% increase in the exposed group over the control group. Taken together, the increases in activity for all four antioxidant enzymes clearly reflect a greater level of oxidative stress in the exposed group.

Vitamin Levels. Vitamin C levels in the control group did not vary from those of our exposed group, whereas the exposed subjects had a vitamin E average higher than did the control subjects (Table 2). This represented a 22% increase above the control levels, a statistically significant increase ($P < 0.05$). This increase in the exposed group average is contrary to what we expected to see in a population undergoing oxidative stress. Similarly, the exposed subjects possessed levels of β-carotene that were 27% higher than those of control plasma (Table 2). This difference was not statistically significant by Student's *t* test.

Physiological Impact. The level of lipid peroxidation for the control subjects was 10% lower than the exposed group, a difference that was not statistically significant (Table 2). Like the increase in vitamin E, this decrease in lipid peroxidation ran counter to our expectations. Oxidative DNA damage induced by exposure to ETS, however, was clearly evident by the much higher levels of 8-OHdG found in the exposed group (Table 2). This 63% increase was statistically significant ($P < 0.05$).

Discussion

The availability of a suitable ETS-exposed population in the Reno/Sparks (Nevada) area was critical to the execution of this study. The tourism-based industries in this region provide a large population base of employees who are exposed to ETS during the performance of their jobs. Study subjects were recruited through an article published in a local newspaper. Volunteers were not paid and were required to satisfy three conditions: they were nonsmokers; they did not live with a smoker and therefore were not exposed to ETS at home; and they had not used vitamin supplements for a period of at least 6 weeks prior to the start of the study. All respondents satisfying these conditions were allowed to participate in our study.

A variety of methods can be used for the detection of cotinine in human samples, including RIA, HPLC, gas chromatagraphy, and gas chromotagraphy-mass spectroscopy (31). Cotinine values obtained by the different methods of analysis have produced varying baseline values, which can be related to each other for comparison, as described by Benowitz (31). For reasons of cost and ease of analysis, we chose HPLC analysis. The values for the two groups in our study were consistent with those obtained for exposed and unexposed individuals in other studies, in which cotinine levels were determined by HPLC (31, 32).

The chemical composition of ETS is extremely complex. Over 3800 compounds have been identified in cigarette smoke (33), including more than 200 semivolatile phenols and a number of quinones, aldehydes, ketones, and polynuclear aromatic hydrocarbons, many of which are capable of generating ROS during metabolism. An elaborate biological defense system is present in eukaryotic cells to defend against the toxic reactions of these oxygen species. A series of inducible antioxidant enzymes, including SOD, catalase, GPOX, and GR, can respond to a xenobiotic-induced oxidative insult. The induction of any of these enzymes is considered evidence of an increased production of ROS or oxidative stress. When the production of ROS exceeds the antioxidant capacity of the cell, oxidative damage occurs. This oxidative damage can take the form of protein, lipid, or nucleic acid oxidation. If this excessive production of ROS persists, such that oxidative damage continues

to accumulate and/or is not properly repaired, cytotoxic or mutagenic events can result. These events have been correlated with the initiation and progression of a number of chronic diseases (5, 11). The results of this study provide strong evidence supporting the hypothesis that exposure to ETS in the workplace results in increased cumulative oxidative damage.

The results of the antioxidant enzyme activity studies demonstrate increased enzyme activities for all four enzymes tested. Although SOD and GR activities were only marginally increased and not statistically significant, both of the enzymes with peroxidase activities, catalase and GPOX, were significantly increased (13 and 37%, respectively). From these studies, however, it is not possible to determine with certainty whether these increases were due to induction of enzyme levels or an increase in enzymatic activities, although it seems that the increases would most likely occur due to an induction of the enzymes.

Vitamins C and E have antioxidant activity and are considered important antioxidant compounds in the protection against oxidative stress. Vitamin C, which is a first line of defense against oxidative stress, was unchanged in the ETS-exposed group, relative to the unexposed group. α-Tocopherol, on the other hand, was 22% greater in the exposed group than it was in the unexposed group. One would expect both vitamin C and E levels to be decreased in the exposed group due to the increased oxidative stress from the ETS. Vitamin E, however, can be mobilized from liver stores to the plasma following periods of depletion. It is quite possible that the routine exposure of these workers to ETS has resulted in a mobilization of vitamin E from their liver stores to maintain plasma vitamin E levels. During periods of mobilization, it is common for the biological response to overcompensate. This could easily account for the excess vitamin E levels in the exposed group. These exposed subjects would have a decreased total body burden of vitamin E, which would not be detected by our analysis of plasma vitamin E.

The increased plasma vitamin E levels are consistent with the lipid peroxidation data we observed. Interestingly, the plasma lipid peroxidation studies showed a decrease in lipid peroxidation in the ETS-exposed group, as compared to the nonexposed group. Higher plasma vitamin E levels, which protect membranes from lipid peroxidation, are, therefore, consistent with a lower plasma lipid peroxidation.

The marked increase in oxidative DNA damage in the ETS-exposed group is the most compelling result of this study. 8-OHdG levels were 63% higher in the exposed group than they were in the control group. This difference was statistically significant ($P < 0.05$). 8-OHdG levels have been shown to increase with both age and alcohol consumption. Our subject questionnaire addressed each of those questions. The ages and alcohol consumptions for each group are presented in Table 1 and show no statistical difference between the groups. The slight differences in age and alcohol consumption between the groups are not enough to account for any alteration of 8-OHdG levels (34). DNA represents a much different target for ROS than do membrane lipids. Although membrane lipids are protected against oxidation by compounds such as vitamins C and E, as well as some enzymes, DNA is not as well protected against ROS. The formation and detection of 8-OHdG appears to be more sensitive to certain types of oxidative stress than is the TBARS response in lipid peroxidation (35). 8-OHdG has also been shown to be a mutagen. Accumulation of 8-OHdG in the DNA would increase the risk of a stable mutation occurring, thereby increasing the risk for development of a disease state.

These results demonstrate that exposure to ETS in the workplace causes an increase in oxidative stress, resulting in an increase in oxidative DNA damage. This DNA damage is reflective of cellular oxidative damage and is correlated with a higher overall exposure to ETS, as demonstrated by the cotinine results. Increasing cellular DNA oxidation poses heightened risk for the development of a number of diseases. Consequently, these results provide strong evidence that routine exposure to ETS presents a risk factor for the development of disease.

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