Oxidative Stress Induced by Environmental Tobacco Smoke in the Workplace Is Mitigated by Antioxidant Supplementation

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Abstract
Environmental tobacco smoke (ETS) is a pervasive contaminant in the workplace. Previous studies by this laboratory have shown that exposure to workplace ETS results in increased oxidative stress and damage, as measured by increased levels of the antioxidant enzymes superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase. 8-Hydroxy-2-deoxyguanosine, a marker of oxidative DNA damage, was also 63% greater in the exposed group compared with controls. Subjects in the previous study who reported workplace exposure to ETS were given a 60-day supply of an over-the-counter antioxidant formulation consisting of 3000 µg of β-carotene, 60 mg of vitamin C, 30 I. U. of α-tocopherol, 40 mg of zinc, 40 µg of selenium, and 2 mg of copper. After the 60-day supplementation period, blood samples were again drawn, and the results were compared with the presupplementation values. A 62% decrease in 8-hydroxy-2-deoxyguanosine was observed after supplementation. Lipid peroxidation levels were also decreased, as were the antioxidant enzyme activities. The biochemical evidence suggests that exposure to ETS in the workplace increases oxidative stress and that antioxidant supplementation may provide some protection.

Introduction
A report released by the California Environmental Protection Agency in September of 1997 attributed 35,000—62,000 ischemic heart disease deaths every year in the United States to ETS. Additionally, over 2,000 childhood deaths from both bronchitis and Sudden Infant Death Syndrome as well as 3,000 lung cancer deaths have been linked to ETS (1). Support for the California Environmental Protection Agency values comes from two earlier studies promulgated by the Surgeon General of the United States and the National Academy of Sciences (2, 3). Both of these studies reached similar conclusions about the adverse health effects of ETS. The Surgeon General’s report went so far as to assert a link between exposure of nonsmokers to ETS and disease states such as lung cancer. A nationally representative survey reported that 37.4% of adult nonsmokers report exposure to ETS at home or in the workplace, and 25% of nonsmoking adults report exposure at work (4). The significant health impact reported in the California Environmental Protection Agency report is not surprising, considering the widespread exposure to ETS.

The conclusions reached by these reports have intensified the debate over smoking. Given the current polarization between smokers and nonsmokers, it is important to conduct studies that rationally address the risks, or lack thereof, associated with ETS in public places, including the workplace. It is anticipated that these studies will help elucidate the health risks associated with ETS in the workplace. Previous work performed by this laboratory has shown an increase in oxidative stress, in terms of increased antioxidant enzyme activity and DNA damage, in nonsmokers exposed to ETS at work (5). Specifically, our initial study determined that exposure to ETS in the workplace resulted in an increase in cellular oxidative damage. This oxidative damage occurs any time the production of reactive oxygen species in a cell exceeds the cell’s natural antioxidant defenses. Tobacco smoke has been reported to contain more than 10^14 radicals/puff in the gas phase (6). Furthermore, the tar phase contains compounds capable of redox cycling to produce even more ROS (7). When the balance of ROS exceeds the cell’s antioxidant capacity, injury can occur to cellular constituents such as membrane lipids, proteins, DNA, and RNA, leading to cell damage or death. The process of cellular oxidative damage has been linked to the etiology of several chronic degenerative conditions, including cancer and coronary heart disease, both closely associated with smoking and exposure to ETS (8–16). Oxidative stress is also thought to play a role in carcinogenesis by oxidative damage to DNA (12).

A cell may defend itself against oxidative stress through the use of antioxidants such as the antioxidant vitamins (ascorbate, α-tocopherol, and β-carotene) and minerals (copper, zinc, and selenium). The antioxidant mechanisms by which these compounds work have been extensively studied, and deficiencies have been correlated to increased risks of oxidative damage and disease (17). Vitamin E is a well-known scavenger of peroxide radicals in cellular lipid membranes (16). Vitamin C is also a free radical scavenger, neutralizing ROS such as superoxide, hydrogen peroxide, and hypochlorous acid. β-Carotene is a quencher of singlet oxygen in vitro. Zinc, copper, and selenium are also important antioxidants. Copper and zinc are incorporated into the cytosolic form of SOD, whereas selenium is part of the antioxidant enzyme GPOX.
Selenium is also thought to provide antioxidant protection through other mechanisms aside from its role in GPOX (18). The important role of these metals as prosthetic groups in antioxidant metalloenzymes makes them critical in the prevention of oxidative stress. As one would expect, an increase in the antioxidant enzymes has also been shown to reduce levels of oxidative stress (19, 20).

The purpose of this study was to determine the effect of antioxidant supplementation on the oxidative stress induced by ETS exposure at work. To that end, we evaluated the activity of the antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase, as well as the antioxidant vitamins C, E, and β-carotene. Finally, the DNA adduct 8-OHdG and lipid peroxidation, both markers of physiological oxidative damage, were analyzed. All of these markers were analyzed both before and after supplementation. 8-OHdG, the oxidized form of the nucleoside 2'-deoxyguanosine, is an excellent marker of DNA damage because of its capability of reflecting extremely low levels of oxidative damage. It is one of the most abundantly formed oxidative DNA products (21) and can be detected by HPLC-electrochemical methods in the femtomolar range. Increases in 8-OHdG levels are induced by several carcinogens (22, 23) and have been shown to be present in higher concentrations in the DNA of malignant cells (24). 8-OHdG has also been shown to increase in the leukocytes of smokers (25). 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 (8, 14) and able to participate in at least two types of transcriptional errors (26).

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. As opposed 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 (27).

Materials and Methods

Blood Specimens. Volunteers for the study were recruited through an article in a local newspaper detailing the study. The laboratory number was provided in the article and calls were taken over a period of 2 weeks. All callers who satisfied the following criteria for volunteer participation were included in the study: (a) each volunteer was required to be a nonsmoker; (b) none of the volunteers could be exposed to ETS at home; and (c) 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. This length of time was selected because plasma levels of β-carotene and α-tocopherol both drop to presupplementation levels within 4 weeks of withdrawal of supplements (28). The participants were then separated into two groups, those who were exposed to ETS at work and those who were not. No volunteers were removed from the study for any reason other than the three criteria listed above.

Before blood was taken, each volunteer filled out a short questionnaire and signed a study participation consent form. After approval for participation, two 20 ml of 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 shifts to obtain an accurate blood cotinine value. The two draws were also scheduled within 2 weeks of one another. Once drawn, the blood was kept at 4°C and in a low light environment. Aliquots were then distributed for the various analyses. The participants in the group who were exposed to ETS in the workplace were subsequently requested to participate in the second phase of the study (see below).

Supplementation. Each member of the exposed group was asked to participate in the second phase of the study. In this way, paired pre- and postsupplementation values were obtained. Study subjects exposed to ETS in the workplace were provided with 60 tablets of an over-the-counter antioxidant supplement containing 3000 µg of β-carotene, 60 mg of vitamin C, 30 IU of α-tocopherol, 40 mg of zinc, 40 µg of selenium, and 2 mg of copper. The subjects were directed to take 1 tablet/day. Blood was drawn from the volunteers at 49 and 56 days (±1 day). At the 56-day blood draw, the remaining tablets were collected to determine the number of missed days. More than 50% of the subjects had a perfect record of supplementation, and no single volunteer missed >10% of the supplements. The values obtained after supplementation were compared with the values obtained from the same subjects before supplementation.

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, glutathione peroxidase, and glutathione reductase.

SOD was assayed by the procedure of McCord and Fridovich (29) as modified by Oberley and Spitz (30). Briefly, a working buffer was prepared consisting of 50 mM potassium phosphate (pH 7.8), 1 mM DETAPAC, 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/µg of protein.

Catalase. Catalase activity was measured from the same blood hemolsate preparation described for SOD. The method used for catalase determination follows that described by Aebi (31). Briefly, H₂O₂ was added to a 50 mM potassium phosphate buffer until the absorbance of the buffer plus H₂O₂ was between 0.50 and 0.53 absorbance units at 240 nm 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 H₂O₂ decomposed/min/mg of protein.

GR. GR activity was measured from the same blood hemolsate preparation described for SOD. The method used for GR determination follows that described by Racker (32), with modifications for use in a Bio-Tek Instruments EL-340 (Winooski, VT) microplate reader. In this method, a 1 mM 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 absorbance/min at 340 nm. GR is expressed as µmol of NADPH oxidized/min/g of hemoglobin.
GPOX. GPOX activity was measured in the same blood hemolysate preparation described for SOD. GPOX was determined through the use of a method described by Strauss (33). Again, modifications were required for adaptation 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 H2O2 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 absorbance/min at 340 nm. GPOX is expressed as μmol of NADPH oxidized/min/g of hemoglobin.

8-OHdG. DNA was extracted from 1 ml of whole blood using the Wako DNA Extraction WB kit (Richmond, VA). After the extraction process, the DNA was digested to component nucleosides using a method described by Shigenaga (34). Briefly, the DNA pellets were suspended in 200 μl of a 1 mM DFAM/20 mM sodium acetate solution (pH 5.0). The DNA in solution was hydrolyzed to nucleotides through the addition of 4 μl of 3.0 mg/ml Sigma Nuclease P1 (St. Louis, MO) in 20 mM Tris-HCl (pH 8.5). By following 4 μl of 1 unit/liter of calf intestine alkaline phosphatase. The nucleotide solutions were incubated for 1 h at 37°C to convert the nucleotides to their corresponding nucleosides. After conversion, the pH was adjusted by the addition of 20 μl of 3 mM sodium acetate (pH 5.0) and 20 μl of 10 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 x 4.6 mm) with a mobile phase consisting of 100 mM of 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; High Efficiency; 150 x 4.6 mm) with a mobile phase of sodium acetate (pH 5.0) and 20 μl of 50 mM sodium citrate, 30 mM of potassium phosphate (pH 6.0), and 6% acetonitrile. The flow rate was 1 ml/min. The column was a Supelcosil LC-18-DB (Fullerton, CA; 15 x 4.6 mm), 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 verify the initial calibration curve. The samples were analyzed using a Perkin-Elmer model 250 equipped with a Beckman Ultrasphere C-18 ODS 5 μm column (Fullerton, CA; 15 x 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. (38).

Blood Protein. Blood protein was determined using a BCA Protein Assay kit 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 BCA 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.

Hemoglobin. Hemoglobin levels were determined using Drabkins reagent in the Hemoglobin kit from Sigma. Sigma Drabkin 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 suspended 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 x 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 analysis of the samples.
Effect of Antioxidants on Workplace ETS

Cotinine was extracted using C-18 solid-phase extraction cartridges and analyzed by HPLC-UV (13).

Values represent average ± SE for all results.

Based on a rating of 1–4 (1, least healthy compared with others their age; 2, less healthy; 3, as healthy; 4, more healthy).

Self-reported.

Cotinine was extracted using C-18 solid-phase extraction cartridges and analyzed by HPLC-UV (13).

Table 1 Composite of study group parameters

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Exposed group</th>
<th>Supplemented group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>17</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Women</td>
<td>19</td>
<td>22</td>
<td>19</td>
</tr>
<tr>
<td>Average age</td>
<td>40.1 ± 1.6</td>
<td>44.0 ± 1.4</td>
<td>43.1 ± 1.5</td>
</tr>
<tr>
<td>Self-perceived health statusa</td>
<td>3.11 ± 0.12</td>
<td>3.21 ± 0.14</td>
<td>3.30 ± 0.16</td>
</tr>
<tr>
<td>Ethanol consumption (drinks/week)b</td>
<td>1.72 ± 0.40</td>
<td>2.29 ± 0.45</td>
<td>2.19 ± 0.40</td>
</tr>
<tr>
<td>Self-reported exposure (h/day)</td>
<td>0</td>
<td>6.6 ± 0.25</td>
<td>6.6 ± 0.25</td>
</tr>
<tr>
<td>Cotinine (ng/ml)c</td>
<td>2.72 ± 0.52</td>
<td>4.55 ± 0.60</td>
<td>4.63 ± 0.64</td>
</tr>
</tbody>
</table>

a Variance expressed in SE for all results.
b Based on a rating of 1–4 (1, least healthy compared with others their age; 2, less healthy; 3, as healthy; 4, more healthy).
c Self-reported.

t-Test was used for all statistical comparison between the exposed group and the supplemented group.

Table 2 Summary of study results

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Exposed group</th>
<th>Supplemented group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (units/µg)</td>
<td>6.23 ± 0.41 (33)d</td>
<td>6.70 ± 0.42 (37)</td>
<td>8%</td>
</tr>
<tr>
<td>Catalase (units/mg)</td>
<td>391 ± 10.35</td>
<td>442 ± 18.37 (37)</td>
<td>13%</td>
</tr>
<tr>
<td>GPOX (µmol NADPH consumed/min/g)</td>
<td>9.38 ± 0.27 (34)</td>
<td>10.4 ± 0.40 (37)</td>
<td>10%</td>
</tr>
<tr>
<td>GR (µmol NADPH consumed/min/g)</td>
<td>2.23 ± 0.08 (34)</td>
<td>2.31 ± 0.09 (37)</td>
<td>4%</td>
</tr>
<tr>
<td>Vitamin status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C (µg/100 µl)</td>
<td>0.64 ± 0.04 (35)</td>
<td>0.64 ± 0.03 (37)</td>
<td>0%</td>
</tr>
<tr>
<td>α-Tocopherol (µg/ml)</td>
<td>12.2 ± 0.6 (35)</td>
<td>14.9 ± 0.6 (37)</td>
<td>22%</td>
</tr>
<tr>
<td>β-Carotene (µg/ml)</td>
<td>0.616 ± 0.079 (35)</td>
<td>0.781 ± 0.117 (37)</td>
<td>27%</td>
</tr>
<tr>
<td>Physiological damage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid peroxidation (TBARS Eq.)</td>
<td>10.2 ± 0.5 (35)</td>
<td>9.2 ± 0.5 (37)</td>
<td>-10%</td>
</tr>
<tr>
<td>8-OHdG (pg/µg)</td>
<td>17.2 ± 2.3 (27)</td>
<td>28.0 ± 3.6 (29)d</td>
<td>63%</td>
</tr>
</tbody>
</table>

Values represent average ± SE.

Values represent the percentage difference between the exposed group average and the control group average.

Values represent the n for the determination.

Represents values that are significantly different from respective control group values by Student’s unpaired t test (P < 0.05).

Results

Profile of Study Volunteers. The characteristics of the control and ETS-exposed volunteers are shown in Table 1. There were a total of 73 volunteers accepted into the study. Thirty-seven of those were placed in the exposed group, whereas the remaining 36 were placed in the control group. All exposed respondents were placed in the exposed group regardless of the length of time of exposure. Every exposed volunteer was given the opportunity to remain in the study for the supplementation phase; 30 of them did. The average ETS exposure time at work was 6.6 h/day for both the exposed and supplemented subjects (Table 1), as determined from the study questionnaire. The 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 between any of the groups. Student’s unpaired t test was used for all statistical comparison between the control group and the exposed group for all analytes, whereas Student’s paired t test was used for all statistical comparison between the exposed group and the supplemented group. Seven exposed study subjects did not participate in the supplementation phase of the study; therefore, those analyte values were removed in the exposed/supplementation paired comparison.

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 the control group. This increase was statistically significant (P < 0.05, Student’s t test). The supplemented group had cotinine levels that were 3% greater than the exposed group. The levels of cotinine seen in the exposed and supplemented groups are far below the levels seen in smokers (>275 ng/ml; Ref. 40). These values are expressed in Table 1.

Enzyme Activity. SOD activity was greater in the exposed subjects than 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 the control group. The supplemented group had an SOD activity level that was 18% lower than that of the exposed group (Table 3), and this increase was statistically significant by Student’s paired t test (P < 0.05). Catalase activity was increased by 13% in the group exposed to ETS (Table 2), and this increase was statistically significant by Student’s unpaired t test (P < 0.05). The exposed group catalase activity was also 7% greater than that of the supplemented group, a difference that was not statistically significant. This increase in the exposed group and subsequent decrease in the supplemented group supports the SOD results as a reflection of increased oxidative stress in the exposed group, followed by reduction of oxidative stress in the supplemented 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 compared with the control group. The level of GPOX activity...
in the supplemented group was 1% lower than that in the exposed group (Table 3). This difference was not statistically significant; however, it was consistent with the pattern seen for SOD and catalase. The glutathione reductase activities (Table 2) were consistent with the pattern for the other enzyme activities, as well. Although not statistically significant, there was a 4% increase in the exposed group over the control group. The supplemented group’s GR values were 8% below those of the exposed group (Table 3), which was not statistically significant. Taken together, the greater antioxidant activity in the ETS exposed group for all four antioxidant enzymes clearly reflects a higher level of oxidative stress in the exposed group. Similarly, the subsequent decrease in the antioxidant activity in the ETS exposed group after supplementation is indicative of a reduced level of oxidative stress.

**Vitamin Levels.** Vitamin C levels in the control group did not vary from those of our exposed group. As expected, the supplemented group’s vitamin C levels were significantly greater (22%) than the exposed group (Table 3). The exposed subjects had a vitamin E average higher than 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. The supplemented subjects had a vitamin E level comparable with the exposed group (Table 3). β-Carotene followed an identical pattern. The exposed subjects possessed levels of β-carotene 27% higher than those of control plasma (Table 2), whereas the supplemented group was comparable with the exposed group. Neither of the β-carotene differences was statistically significant by Student’s $t$ test.

**Physiological Impact.** The level of lipid peroxidation for the control subjects was 10% higher 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. The supplemented group had an average that was 4% lower than the exposed group, a difference that was also not statistically significant. 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 ($P < 0.05$). The supplemented group had an average 8-OHdG level 62% below that of the exposed group, which was also a statistically significant change. The 8-OHdG levels in the supplemented ETS exposed group actually fell to levels below ($P < 0.05$) those in the control group.

**Discussion**

One of the most important features of this study was the availability of nonsmokers who were exposed to ETS at their places of employment. The existence of this population is due largely to the tourism-based industry in the Reno-Sparks area. Recruitment of these subjects was made possible by an article published in a local paper. To participate, volunteers were required to satisfy three conditions: (a) they were nonsmokers; (b) they did not live with a smoker and were therefore not exposed to ETS at home; and (c) 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. The volunteers were not paid.

As mentioned earlier, ETS contains not only radicals in the gas phase but also a quinone-hydroquinone-semiquinone system in the tar that is capable of redox cycling, reducing oxygen to superoxide in the process (8). In fact, over 3800 compounds have been identified in cigarette smoke (41), 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. Fortunately, eukaryotic cells possess an elaborate biological defense system to protect themselves from the toxic reactions of these oxygen species. This defense includes the inducible antioxidant enzymes SOD, catalase, GPOX, and GR, which can respond to a xenobiotic-induced oxidative insult. Because of the inducible nature of these enzymes, an increase in enzymatic activity is considered evidence of an increased production of ROS or oxidative stress. Similarly, a decrease in enzymatic activity can indicate a reduction in oxidative stress.

If the antioxidant capacity of the cell is exceeded by oxidative stress, then oxidative damage occurs. Although any cellular component may be oxidized during oxidative damage, including protein, lipid, or nucleic acid, our study looked specifically at DNA and lipid 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 (8, 16). The results of this study provide strong evidence

**Table 3** Summary of study results

<table>
<thead>
<tr>
<th>Enzyme activity</th>
<th>Exposed group*</th>
<th>Supplemented group*</th>
<th>% Difference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (units/µg)</td>
<td>6.80 ± 0.46 (30)</td>
<td>5.57 ± 0.34 (25)</td>
<td>-18%</td>
</tr>
<tr>
<td>Catalase (units/mg)</td>
<td>450 ± 20 (30)</td>
<td>419 ± 8 (30)</td>
<td>-7%</td>
</tr>
<tr>
<td>GPOX (µmol NADPH consumed/min/g)</td>
<td>10.3 ± 0.42 (30)</td>
<td>10.2 ± 0.34 (29)</td>
<td>-1%</td>
</tr>
<tr>
<td>GR (µmol NADPH consumed/min/g)</td>
<td>2.33 ± 0.10 (30)</td>
<td>2.15 ± 0.08 (30)</td>
<td>-8%</td>
</tr>
<tr>
<td>Vitamin status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C (µg/100 µl)</td>
<td>0.62 ± 0.04 (30)</td>
<td>0.78 ± 0.04 (30)</td>
<td>26%</td>
</tr>
<tr>
<td>α-Tocopherol (µg/ml)</td>
<td>14.8 ± 0.6 (30)</td>
<td>14.3 ± 0.5 (30)</td>
<td>-3%</td>
</tr>
<tr>
<td>β-Carotene (µg/ml)</td>
<td>0.711 ± 0.113 (30)</td>
<td>0.740 ± 0.085 (30)</td>
<td>4%</td>
</tr>
<tr>
<td>Physiological damage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid peroxidation (TBARS Eq.)</td>
<td>8.89 ± 0.5 (30)</td>
<td>8.50 ± 0.6 (30)</td>
<td>-4%</td>
</tr>
<tr>
<td>8-OHdG (pg/µg)</td>
<td>29.4 ± 4.2 (23)</td>
<td>11.1 ± 0.9 (19)</td>
<td>-62%</td>
</tr>
</tbody>
</table>

*Values represent average ± SE.

*Values represent the percentage difference between the exposed group average and the supplemented group average.

*Values represent the $n$ for the determination.

*Represents values that are significantly different from respective exposed group values by Student’s paired $t$ test ($P < 0.05$).
Effect of Antioxidants on Workplace ETS

Cotinine is an excellent marker of this exposure, but there are damage induced is the determination of ETS exposure levels. Prevented through antioxidant supplementation. Place results in increased oxidative damage that may be largely demonstrated increased enzyme activities for all four enzymes subjects exposed at work recently received further support from a study performed in an Atlantic City casino by the National Institute of Occupational Health and Safety. 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, 13%, and glutathione peroxidase, 10%) were significantly increased. 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 appears that the increases would most likely occur due to an induction of the enzymes. The results for the supplemented group also exhibit a particular pattern. Specifically, all four enzyme activities decreased after the antioxidant treatment. The decrease was statistically significant for SOD (P < 0.05). Notably, SOD and GR dropped down below control values. Supplementation with moderate doses of antioxidant vitamins, in combination with trace elements, has been shown to increase the activity of GPOX and SOD (44). However, these studies were performed in subjects not exposed to oxidative stress. In supplemented rats exposed to exercise-induced oxidative stress, the levels of SOD and GPOX remained below the nonsupplemented rats exposed to oxidative stress (45). This indicates that the antioxidant supplementation of these rats provided a substantial prophylactic effect against oxidative stress. Likewise, our data indicate a decrease in the activity of catalase, SOD, GPOX, and GR after supplementation, most likely due to a reduction in the overall level of oxidative stress. There is also evidence that suggests that certain ROS may behave as signal transduction messengers. With this in mind, it is reasonable to believe that a cellular oxidant load that has been reduced through antioxidant supplementation might lead to a decrease in the transcription of antioxidant enzymes. The results of our enzyme assays support this hypothesis.

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, proved unchanged in the ETS exposed group relative to the unexposed group. a-Tocopherol, on the other hand, was 22% greater in the exposed group than 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 after 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. Bronchoalveolar cells of smokers show increased levels of vitamin E and beta-carotene when compared with nonsmokers (46), consistent with the excess vitamin E and beta-carotene levels found in our exposed group. These exposed subjects would presumably have a decreased total body burden of vitamin E, which would not be detected by our analysis of plasma vitamin E.

Previous studies have related supplementation to increased plasma levels of vitamin C, vitamin E, and beta-carotene. We witnessed a statistically significant increase in plasma vitamin C after supplementation. However, the beta-carotene and vitamin E plasma levels were not altered significantly by supplementation. If the initial increase in vitamin E is linked to an oxidative stress response, it follows that a decrease in that stress would result in a decrease in mobilization. We believe that supplementation may reduce the oxidative stress levels to the extent that liver mobilization is reduced.

The increased plasma vitamin E levels are consistent with the lipid peroxidation studies in the ETS-exposed group compared with the nonexposed group. Higher plasma vitamin E levels, which protect membranes from lipid peroxidation, are therefore consistent with a lower plasma lipid peroxidation. The supplementation data provide further support for the conclusion that the increased oxidative stress from ETS exposure is reduced by supplementation. A 4% decrease in plasma vitamin E would ordinarily be accompanied by an increase in lipid peroxidation. However, our data reflect a 7% decrease in lipid peroxidation after supplementation, as measured by TBARS.

The marked increase in oxidative DNA damage in the ETS-exposed group was the most compelling result of the first phase of this study. Similarly, the marked reduction in oxidative DNA damage was the most compelling result of the supplementation phase of this study. The initial comparison between the control and exposed groups indicated that 8-OHdG levels were 63% higher in the exposed group than in the control group, a statistically significant (P < 0.05) difference. 8-OHdG levels have been shown to increase with both age and alcohol consumption, which were addressed in our subject questionnaire. The age and alcohol consumption for each group is presented in Table 1 and shows 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 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 appear to be more sensitive to certain types of oxidative stress than 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.

It appears that supplementation with moderate levels of antioxidants were able to reduce the cellular accumulation of this DNA mutagen. After supplementation, the levels of 8-OHdG were 62% lower than in the exposed group. The reduction of oxidative DNA damage in the supplemented group lends strong support to the enzymatic, vitamin, and lipid peroxidation indications of reduced oxidative stress. This is espe-
cially true considering the role of 8-OHdG as a more sensitive marker of oxidative DNA damage than the other markers measured.

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. The second phase of this study clearly suggests that the increased oxidative stress and damage measured in the first phase of the study can be reduced by supplementation with antioxidant vitamins and trace minerals. However, it must be understood that the control group values were taken from un-supplemented subjects. Any comparison of values between the ETS exposed but supplemented group and the control group must be made with the understanding that the control group was not taking any supplements. Supplementation of the control group would have most likely reduced that group’s level of oxidative stress and damage as well.

The results of this study suggest that persons exposed to ETS in the workplace have an increased amount of oxidative stress and damage than those who had not been exposed to ETS. The study also suggests that for those workers exposed to ETS in the workplace, supplementation with antioxidant vitamins and trace minerals may provide some protection against this increased oxidative stress and damage, which may reduce the risk of disease associated with these increases.

References
Oxidative stress induced by environmental tobacco smoke in the workplace is mitigated by antioxidant supplementation.
