Influence of environmental tobacco smoke on vitamin C status in children

Alan M Preston, Cindy Rodriguez, Cynthia E Rivera, and Hardeo Sahai

ABSTRACT

Background: It is known that vitamin C status is compromised in smokers. The vitamin C status of nonsmokers who are exposed to environmental tobacco smoke (ETS) is now being elucidated.

Objective: We assessed vitamin C status in children who were either exposed or not exposed to ETS, and we sought to associate changes in vitamin C status with the amount of ETS exposure.

Design: The study group included 512 children aged 2–12 y; 50% of them were exposed to ETS in the home because their parents smoked. Dietary intake of vitamin C, obtained with a 24-h recall questionnaire, and blood ascorbate concentrations were compared in the exposed and unexposed groups. Smoke exposure was assessed by measuring a biomarker, urinary cotinine. Age, sex, and body mass index were examined as potential correlates of vitamin C status in each exposure category.

Results: Plasma ascorbate concentrations were lower, by 3.2 μmol/L on average, in ETS-exposed children than in unexposed children who consumed equivalent amounts of vitamin C; this was a highly significant difference (P = 0.002). This reduction in plasma ascorbate occurred even with very low exposure to ETS.

Conclusions: ETS can reduce concentrations of ascorbate, an important blood antioxidant, even when the amount of smoke exposure is minimal. Children exposed to ETS should be encouraged to consume increased amounts of foods rich in vitamin C or be given the equivalent amount of this vitamin as a supplement.

KEY WORDS Environmental tobacco smoke, passive smoking, ascorbic acid, vitamin C, cigarette smoking, cigarettes, children, dietary recall

INTRODUCTION

Health risks linked to environmental tobacco smoke (ETS) exposure are being documented more frequently in nonsmoking populations. After years of denial by the tobacco industry (1), it is now being recognized that ETS is a risk factor for cardiovascular disease, cancer, and pulmonary diseases, maladies that were previously attributed only to the long-term effects of active smoking (2–4).

The mechanism most often cited as the cause of smoking-related disorders is oxidant damage from free radicals generated in cigarette smoke and from reactive oxidants created by smoke-induced activation of the inflammatory immune system (5). Vitamin C (ascorbic acid) is an effective free radical scavenger and is among the strongest determinants of plasma total antioxidant defense (6, 7). It is well established that cigarette use compromises vitamin C status in active smokers. The observation that vitamin C excretion in smokers was lower than in nonsmokers was first made over 60 y ago (8), and it is now known that metabolic turnover of vitamin C in smokers is approximately double that in nonsmokers (9, 10). Furthermore, there is ample evidence that smoking is also associated with unhealthy patterns of nutrient intake (11, 12). The consumption of vitamin C–rich foods, such as fruits and vegetables, by smokers is well below that of nonsmokers (13).

A very relevant and greatly understudied issue is the effect of ETS on vitamin C status in nonsmoking populations. In this article, we present information on the plasma ascorbate concentrations and vitamin C intakes of children either exposed or not exposed to ETS in the home because of parental smoking. In previous studies, we identified factors that influence smoke exposure by administering a questionnaire and then validating the results by measuring urinary cotinine, a sensitive biomarker of smoke exposure (14, 15). The determinants of smoke exposure were found to be 1) the presence of parental smoke, 2) the number of cigarettes smoked, and 3) the age of the child, with younger children (aged 2–4 y) exhibiting more exposure than older children (aged 5–12 y).

In the current study, we also evaluated the effects of sex and body mass index (BMI) on vitamin C status. A previous study found that the plasma ascorbate concentration attained after a given dose of dietary vitamin C depends on body weight (16). We also compared our results with data on vitamin C status in populations of adult smokers (11) and adult nonsmokers who were either chronically or acutely exposed to ETS (17–20).
SUBJECTS AND METHODS

Our study group included 512 healthy children aged 2–12 y who routinely visited the Pediatric Care Clinic of the Cataño Health Center, a satellite program of the University of Puerto Rico Pediatrics Department. Cataño is an industrial city of ~42,000 inhabitants that is located across the harbor from San Juan. Blue collar workers make up the bulk of the population. The city is highly homogeneous in terms of socioeconomic factors, and children visiting the clinic are representative of children residing in the community as a whole. However, the method of subject selection was basically by convenience, constituting a nonprobability sample, and therefore no inferences should be drawn regarding the generalizability of the findings to a broader population.

Data collection: smoking and dietary questionnaires

The smoking and dietary questionnaires were administered during the period from August 1993 through November 1996. When mothers (n = 709) arrived at the clinic with their children, they were given an informed consent form for participation in the study. Almost everyone who was approached agreed to participate, but failure to adhere to the study protocol reduced the response rate to ~75% (n = 532). The reasons why subjects did not adhere to the protocol included failure to appear for the interview or to provide a urine sample and inability to have a blood sample collected. The rates of nonresponse were rather evenly distributed across age, sex, and BMI and should not result in any appreciable bias in the study. Persons who agreed to participate completed a smoking questionnaire (a copy is available in English or Spanish from the first author), which was designed to identify children exposed to ETS, to determine the sources of the ETS, and to assess the relative importance of each source. Questions were also included about the number of cigarette smokers in each household and exposure outside the home. The study protocol was approved by the Institutional Review Board of the University of Puerto Rico, Medical Sciences Campus.

Dietary data were obtained by using a 24-h-recall method (21). Mothers supplied this information for the youngest children, and children aged ≥8 y also contributed some information. Detailed descriptions of all foods and beverages consumed, including cooking methods and brand names, were recorded by the interviewers. Use of vitamin and mineral supplements was also noted. Because the study focused on food sources of vitamin C, those persons taking vitamin C supplements were excluded from the analysis. Only 20 children (11 exposed and 9 not exposed to ETS) fell into this category. The remaining 512 children were included in the statistical analysis.

Clinical and laboratory procedures: blood and urine samples

Eligible children provided fasting blood and urine samples, which were collected from 0800 to 1000 at the clinic immediately before administration of the 24-h dietary recall. Urine samples were refrigerated and cotinine concentrations were determined within 48 h by using an enzyme-linked immunosorbent assay (Solar Care Technologies, Bethesda, PA). In a previous study, this assay was verified against gas-liquid chromatography and was found accurate for measuring cotinine concentrations ≥3 ng/mL (22). To adjust for urine dilution, urinary cotinine concentrations were standardized to creatinine concentrations and were expressed as ratios of cotinine to creatinine. Creatinine was measured colorimetrically by using picric acid in an alkaline environment (23). Blood samples were obtained by venipuncture and were drawn into EDTA-coated tubes. Plasma was separated by centrifugation at 2000 × g for 20 min at 4 °C. Supernatant fluids were analyzed for ascorbic acid content by derivatization with 2,4-dinitrophenyl hydrazine (24) within 6 h of collection. This colorimetric procedure was selected so that ascorbate values could be compared conveniently with those obtained in the second National Health and Nutrition Examination Survey, which also utilized the dinitrophenyl hydrazine assay (25). The results are expressed in μmol/L (to convert to mg/dL, divide μmol/L by 57).

Determination of body mass index

The heights and weights of the children were obtained according to published assessment methods (21). The BMI, also called Quetelet’s index, was calculated as weight in kg divided by the square of the height in m (kg/m²); BMI was used as a measure of obesity. Weight is a determining factor in plasma ascorbate concentrations because weight is inversely related to vitamin C concentration independent of the amount of smoke exposure (16).

Determination of dietary ascorbate

Vitamin C intakes were estimated from the 24-h dietary recall interviews. To determine the vitamin C contents of the foods consumed, we used the Minnesota Nutrition Data System 32, which contains >6000 brand-name foods, fast foods, and >16,000 other foods. In addition, it is a comprehensive nutrient database including data derived from the US Department of Agriculture tables, food manufacturers, the scientific literature, and foreign food consumption tables; hence, it contains many ethnic foods that are commonly eaten in Puerto Rico.

Statistical analysis

Descriptive statistics, such as frequencies and percentages, were analyzed with a chi-square test. Means and medians of continuous variables were compared by using Student’s t test or the Wilcoxon-Mann-Whitney test (26). To test for differences between >2 means or medians, either parametric analysis of variance or the Kruskal-Wallis test, as appropriate, was used (26).

The distribution of urinary cotinine concentrations was highly skewed, therefore the natural logarithm was used in any parametric analysis to correct for skewness and kurtosis of the variable (27). Pearson’s product-moment correlation coefficients were used to test the associations between dietary intake of vitamin C and plasma vitamin C. Analysis of covariance was used to adjust mean values of plasma vitamin C for age, sex, BMI and dietary vitamin C intake. Analysis of covariance was also used to adjust the mean values for dietary vitamin C intake for age, sex, and BMI. Multiple regression analysis was performed with the dependent variable as plasma vitamin C and age, sex, and BMI as predictors (26). A P value <0.05 was considered statistically significant, and all tests were two-tailed. All statistical analyses were performed with SAS, version 6.12 (28).

RESULTS

The demographic characteristics and BMI of the study subjects are shown in Table 1. There were no significant differences in the percentage of children exposed to ETS when subjects were compared by age, sex, or BMI. It should be emphasized that the category labeled household exposure represents exposure to parental smoke only. In a previous study, we found that mean...
TABLE 1
Demographic characteristics and BMI of children exposed or not exposed to environmental tobacco smoke

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unexposed</th>
<th>Exposed</th>
<th>Total</th>
<th>Household exposure</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–4</td>
<td>25.7 [69]</td>
<td>25.1 [61]</td>
<td>25.4 [130]</td>
<td>46.9</td>
<td>0.650</td>
</tr>
<tr>
<td>5–8</td>
<td>39.0 [105]</td>
<td>35.8 [87]</td>
<td>37.5 [192]</td>
<td>45.3</td>
<td>0.877</td>
</tr>
<tr>
<td>9–12</td>
<td>35.3 [95]</td>
<td>39.1 [95]</td>
<td>37.1 [190]</td>
<td>50.0</td>
<td>0.066</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>48.7 [131]</td>
<td>49.4 [120]</td>
<td>49.0 [251]</td>
<td>47.8</td>
<td>0.777</td>
</tr>
<tr>
<td>Female</td>
<td>51.3 [138]</td>
<td>50.6 [123]</td>
<td>51.0 [261]</td>
<td>47.1</td>
<td>0.066</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 22</td>
<td>54.6 [147]</td>
<td>46.5 [113]</td>
<td>50.8 [260]</td>
<td>43.5</td>
<td>0.003</td>
</tr>
<tr>
<td>≥ 22</td>
<td>45.4 [122]</td>
<td>53.5 [130]</td>
<td>49.2 [252]</td>
<td>51.6</td>
<td>0.032</td>
</tr>
</tbody>
</table>

1 n in brackets.
2 All subjects (exposed plus unexposed groups).
3 Exposed subjects divided by total subjects.
4 Chi-square test of homogeneity of proportions between exposed and unexposed groups.

urinary cotinine concentrations in children exposed to smoke from non-parental sources were not significantly different from those of children who had no reported exposure to ETS (15). The overall rate of exposure to parental smoke was ≈50%, which is similar to the rate found in other inner city, low-income populations (2, 29, 30). Note that the midpoint value for BMI in our study population was 22 (Table 1). We compared the vitamin C status of subjects with BMI < 22 and ≥ 22. Our midpoint for BMI is not necessarily the same as that of other pediatric or adult populations because age, sex, ethnicity, and maturation stage can all independently contribute to the percentage body fat (31).

Amounts of exposure to ETS were quantified by using the biomarker cotinine; these results are shown in Table 2. For all age groups and BMI groups and for both sexes, children exposed to ETS had significantly higher concentrations of the biomarker in their urine than did the unexposed children. The mean values of 4.8–7.9 ng/mg for cotinine:creatinine in our unexposed children are similar to published values of 4.0–6.1 ng/mg reported for other unexposed populations (32, 33). There were no significant differences between the different age or BMI groups or the sexes.

As previously reported elsewhere, there is an inverse relation between age and cotinine excretion in children residing in households where someone smokes (14). The mean values of 19.2–38.2 ng/mg that we found in our exposed children are quite low when compared with values found in smokers and nonsmoking adults exposed to ETS. Active smokers (> 10 cigarettes/d) sustain urinary cotinine concentrations of > 1000 ng/mg (34), and nonsmokers exposed to ETS have cutoffs of ≈80 ng/mg (35). The low values of the biomarker that we found in Puerto Rican children are most likely a result of our open tropical environment, which allows maximum ventilation and dilution of cigarette smoke. However, there were highly significant differences between the different age groups, with the median for the youngest age group being more than twice that of the other 2 age groups.

Before we conducted separate analyses for different age, sex, and exposure groups, a multifactor analysis of variance was performed to examine the role of interaction effects. With the exception of sex × exposure for plasma ascorbate (P = 0.032), all other second- and third-order interactions were found to be negligible and not significant (Table 3).

Pearson’s product-moment correlation coefficients showed that both plasma vitamin C concentration and dietary intake of vitamin C tended to be inversely related to cotinine:creatinine; however, both of the r values were not statistically significant (r = −0.003, P = 0.9347 and r = −0.007, P = 0.8789, respectively).

TABLE 2
Ratio of cotinine to creatinine in urine of children exposed or not exposed to environmental tobacco smoke

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Unexposed</th>
<th>Exposed</th>
<th>95% CI</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Mean</td>
<td>Median</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–4</td>
<td>69</td>
<td>7.9</td>
<td>5.0</td>
<td>(5.9, 10.0)</td>
</tr>
<tr>
<td>5–8</td>
<td>105</td>
<td>5.2</td>
<td>4.0</td>
<td>(4.2, 6.3)</td>
</tr>
<tr>
<td>9–12</td>
<td>95</td>
<td>4.8</td>
<td>4.0</td>
<td>(4.0, 5.7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>131</td>
<td>5.7</td>
<td>4.0</td>
<td>(4.7, 6.7)</td>
</tr>
<tr>
<td>Female</td>
<td>138</td>
<td>5.9</td>
<td>5.0</td>
<td>(4.8, 7.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 22</td>
<td>147</td>
<td>4.9</td>
<td>4.0</td>
<td>(4.2, 5.6)</td>
</tr>
<tr>
<td>≥ 22</td>
<td>122</td>
<td>6.5</td>
<td>5.0</td>
<td>(5.3, 7.7)</td>
</tr>
</tbody>
</table>

1 In the multfactorial ANOVA, the ratio of cotinine to creatinine differed significantly between the exposed and unexposed groups, but the interaction between exposure and each of the other variables (age, sex, and BMI) was not significant.
The results of the analysis of covariance for variables associated with plasma and dietary vitamin C are shown in Table 3. For the overall population, vitamin C intakes did not differ significantly between the ETS-exposed and unexposed groups, but plasma vitamin C concentrations were lower by 3.2 µmol/L on average in ETS-exposed than in unexposed children, a highly significant decrease (\( P = 0.002 \)). Vitamin C intakes of the different age, sex, and BMI groups showed mean values that were substantially higher (95% CI: 71, 108 mg/d) than the recommended dietary allowance for children ages 2–12 (45–60 mg/d) (36). The mean plasma concentration for the entire combined sample was 50.0 µmol/L (95% CI: 46, 55), which is considered desirable according to a recent meta-analysis regarding plasma vitamin C (37). There were no significant effects of age or BMI on dietary intake of vitamin C. Girls had intakes that were 11% lower than boys’ intakes, but this difference was not significant. In addition, plasma values were not related to ETS exposure in boys but were 11.6% lower in girls with ETS exposure compared with values in unexposed girls (\( P < 0.001 \)). In the multiple regression analysis, exposure to ETS was the most important predictor of plasma vitamin C in girls, explaining more of the total variation than was explained by either BMI or age.

Regression lines plotting plasma vitamin C against vitamin C intake in children either exposed or not exposed to ETS are shown in Figure 1. Tests for equality showed that there were no significant differences between exposure groups in the intercepts (\( P = 0.265 \)) and slopes (\( P = 0.517 \)) of these equations. Curves relating vitamin C intake and blood vitamin C were published for adults (12, 38). Information gained from this research was used to estimate the additional recommended dietary allowance for vitamin C in smokers (36).

**DISCUSSION**

Although the subjects’ values for vitamin C intake were within the normal ranges reported by other investigators (37), it should be recognized that the 24-h recall method most often underestimates intakes and it may not be representative of a typical day. However, the purpose of this study was to compare group means in a large population, and therefore the 24-h recall method was appropriate because it can provide adequate estimates of intakes of individual nutrients in this type of study (39).

Exposure of children to ETS is a major concern because of its long-term consequences in terms of increased disease risk and morbidity.
This situation is even more distressing when exposure occurs in very young children who have little mobility and thus are unable to avoid the exposure; young children are also unaware of the health risks. The current investigation addressed the question of whether vitamin C status is compromised in smoke-exposed children.

Vitamin C intake did not differ among the age, sex, BMI, or ETS-exposure categories. It is well known that smokers typically consume a less healthy diet than do nonsmokers (11, 13), so one might suspect that the same foods consumed by smokers would be offered to children in their households. However, children are not entirely dependent on home-prepared meals and have the freedom to choose foods high in vitamin C.

This study showed that blood ascorbate concentrations of ETS-exposed children were below those of children not exposed to ETS. Although the magnitude of this difference was small (3.2 μmol/L) in the current study, the overall effect may be substantial. Comparisons of plasma ascorbate concentrations in adult smokers and nonsmokers show that the former have concentrations that are ~11.4 μmol/L (0.2 mg/dL) lower (37, 38, 41). Therefore, the magnitude of the reduction in plasma ascorbate concentrations in the ETS-exposed children was ~28% of the reduction found previously in active smokers, as compared with nonsmoking, unexposed individuals.

When we analyzed the data separately for boys and girls, there was no significant difference in plasma vitamin C concentrations between exposed and unexposed boys, but girls with ETS exposure had significantly lower ascorbate concentrations than unexposed girls. This discrepancy between the sexes is difficult to explain. One might expect that girls spend more time at home and closer to their parents, which could lead to more ETS exposure if parents smoke. However, the values of the biomarker (urinary cotinine) did not differ significantly between the sexes, and so this issue remains unresolved.

Our results also merit comparison with 5 studies on ETS exposure in nonsmoking adults. The effects of chronic exposure were described by Tribble et al (17), who found that plasma ascorbate concentrations in nonsmokers exposed to ETS were intermediate between those of active smokers and those of nonsmokers not exposed to ETS, even when dietary intakes were similar. In fact, hypovitaminosis <23 μmol/L was observed in 12% of nonsmokers exposed to ETS. These findings were confirmed in a recent study by Farchi et al (20), who reported an inverse dose-response relation between plasma ascorbate in nonsmoking Italian women and the intensity of exposure to their husbands’ cigarette smoke, after controlling for dietary vitamin C intake. Further evidence of impaired ascorbate status was presented by Ayaori et al (18), who found that nonsmoking soldiers exposed to ETS had higher ratios of oxidized ascorbate to total ascorbate in the plasma than did nonsmoking, unexposed soldiers; no group differences in vitamin C intake were found.

The effects of acute exposure to ETS on blood ascorbate concentrations are even more dramatic. Valkonen and Kuusi (19) reported on the effects of passive smoking in 10 healthy adults who spent 30 min in a room where 16 cigarettes were smoked by active smokers. Passive smoking caused an acute decrease in blood ascorbate 1.5 h after exposure, with a resultant breakdown of the plasma antioxidant defense system as evaluated in terms of lipid peroxidation. In a subsequent study by Valkonen and Kuusi (42), a similar group of nonsmokers was supplemented with 3 g vitamin C, and this prevented the drop in plasma ascorbate that had occurred after ETS exposure in the previous study.

The magnitude of the adverse effect of ETS exposure on vitamin C status is largely dependent on the amount of smoke exposure, according to the studies of adults and our work in children. Obviously, the greater the concentration of ambient smoke, the greater its effect will be on vitamin C status. In the current study, very low levels of the biomarker appeared in the urine of our exposed children. Only 2 of the 5 previously cited studies in adults provided biomarker measurements. Ayaori et al (18) reported that blood thiocyanate concentrations are greater in smoke-exposed nonsmokers than in nonsmokers without smoke exposure. Tribble et al (17) mentioned that the plasma cotinine concentrations in nonsmokers exposed to ETS were below detectable quantities when analyzed with an HPLC method. Valkonen and Kuusi (19, 42) did not report any biomarker information. Consequently, the current study is the first to show a reduction in plasma vitamin C in children with minimal exposure to ETS, as confirmed by urinary cotinine values.

Data from our study also support the theory that persons exposed to ETS require more dietary vitamin C than do persons with no ETS exposure. It has been estimated that smokers require an additional 35 mg vitamin C/d over the amount needed by nonsmokers (10, 25, 43). In the current study, the children exposed to ETS had lower plasma ascorbate concentrations at a given intake of vitamin C than did the children not exposed to ETS. Although these data are not sufficient to allow estimation of a specific vitamin C requirement for children regularly exposed to ETS, these children should be urged to eat more vitamin C–rich foods or they should take supplemental vitamin C.

Among all the predictors in our model, with the exception of dietary vitamin C intake, smoke exposure had the greatest effect on plasma ascorbate concentrations. Although the decrease was modest, it was statistically significant (P = 0.002). This finding raises the question of whether a small, but statistically significant, decrease in plasma ascorbate resulting from exposure to ETS has clinical importance. The present study cannot provide a definitive answer to this question because long-term health effects were not measured. Yet it is reasonable to assume that reduced antioxidant concentrations could be meaningful in vulnerable populations. It is unlikely that smoke-exposed children ingesting high amounts of vitamin C would experience significant reductions in plasma concentrations. It is known that active smokers taking supplemental vitamin C maintain blood ascorbate concentrations similar to or above those of nonsupplemented nonsmokers (44). However, in children ingesting marginal amounts of vitamin C (ie, <40 mg/d), even a minimal reduction in circulating antioxidants could potentiate the risks associated with oxidative damage.

In summary, we have confirmed and extended previous studies of vitamin C status in ETS-exposed populations. Blood ascorbate concentrations were lower in smoke-exposed children than in unexposed children consuming equal amounts of dietary vitamin C. It is rather remarkable that this reduction in blood ascorbate occurs at such a minimal amount of smoke exposure as indicated by biomarker concentrations in these children. Tropical homes are primarily ventilated through open windows and ceiling fans, and therefore cigarette smoke does not become concentrated in the home. Yet even in this very open environment, ETS was a factor in reducing blood vitamin C concentrations, with the subsequent risk that antioxidant protection may be suboptimal.

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