Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase γ-tocopherol in vivo after adjustment for dietary antioxidant intakes1–3

Marion Dietrich, Gladys Block, Edward P Norkus, Mark Hudes, Maret G Traber, Carroll E Cross, and Lester Packer

ABSTRACT

Background: Free radicals in cigarette smoke may cause oxidative damage to macromolecules, contributing to cardiovascular diseases and cancer. Decreased plasma antioxidant concentrations may indicate cigarette smoke–related oxidative stress.

Objective: We compared the effects on plasma antioxidant concentrations in cotinine–confirmed active and passive smokers with those in nonsmokers, independent of differences in dietary intakes and other covariates.

Design: Plasma samples from 83 smokers, 40 passive smokers, and 36 nonsmokers were analyzed for total ascorbic acid, α- and γ-tocopherols, 5 carotenoids, retinol, and cotinine. Groups were compared by using analysis of variance with adjustment for sex, age, race, body mass index, alcohol intake, triacylglycerol concentration, fruit and vegetable intakes, and dietary antioxidants.

Results: After adjustment for dietary antioxidant intakes and other covariates, smokers and passive smokers had significantly lower plasma β-carotene concentrations than did nonsmokers (0.15, 0.17, and 0.24 μmol/L, respectively) and significantly higher γ-tocopherol concentrations (7.8, 7.8, and 6.5 μmol/L, respectively). Smokers had significantly lower plasma ascorbic acid and β-cryptoxanthin concentrations than did nonsmokers and passive smokers (ascorbic acid: 43.6, 54.5, and 54.6 μmol/L, respectively; β-cryptoxanthin: 0.12, 0.16, and 0.16 μmol/L, respectively) and significantly lower concentrations of lutein and zeaxanthin than did nonsmokers (0.33 compared with 0.41 μmol/L). The P values for all the differences described above were < 0.05. No significant differences in plasma concentrations of α-tocopherol, α-carotene, total carotenoids, lycopene, or retinol were observed.

Conclusions: These results indicate that cigarette smokers and nonsmokers exposed to cigarette smoke have a significantly lower plasma antioxidant status than do unexposed nonsmokers, independent of differences in dietary antioxidant intakes. Further research is required to explain why plasma γ-tocopherol concentrations were significantly higher in smokers and passive smokers than in nonsmokers. Am J Clin Nutr 2003;77:160–6.

KEY WORDS Hydrophilic antioxidants, lipophilic antioxidants, γ-tocopherol, smokers, passive smokers, environmental tobacco smoke, body mass index, dietary micronutrients

INTRODUCTION

Active and passive smokers are exposed to reactive free radicals that are present in cigarette smoke (CS) (1). Because free radicals cause oxidative damage to macromolecules such as lipids, proteins, and DNA, they are believed to be involved in the pathogenesis of cardiovascular diseases and cancer (2–5). Free radicals in CS deplete some plasma antioxidants in vitro (4, 6), and several studies found lower plasma antioxidant concentrations in smokers in vivo (7–13). Less information is available on the effect of CS exposure on plasma antioxidant concentrations in passive smokers (14–18).

Smoking and passive smoking decrease plasma antioxidants and increase γ-tocopherol concentrations (7.8, 7.8, and 6.5 μmol/L, respectively). Smokers had significantly lower plasma ascorbic acid and β-cryptoxanthin concentrations than did nonsmokers and passive smokers (ascorbic acid: 43.6, 54.5, and 54.6 μmol/L, respectively; β-cryptoxanthin: 0.12, 0.16, and 0.16 μmol/L, respectively) and significantly lower concentrations of lutein and zeaxanthin than did nonsmokers (0.33 compared with 0.41 μmol/L). The P values for all the differences described above were < 0.05. No significant differences in plasma concentrations of α-tocopherol, α-carotene, total carotenoids, lycopene, or retinol were observed.

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examine the effect of active and passive smoking on several plasma antioxidants, unconfounded by other factors. We tested the hypothesis that after adjustment for dietary antioxidant intakes, smokers have lower plasma antioxidant concentrations than do nonsmokers, and passive smokers have antioxidant concentrations that are between those of smokers and nonsmokers.

SUBJECTS AND METHODS

Subjects were recruited through letters to members of the Kaiser Permanente Health Plan, as well as through fliers and advertisements to the general public, and were screened over the telephone. The protocol was approved by the Committee for the Protection of Human Subjects, University of California, Berkeley, and by the Kaiser Permanente Institutional Review Board, Oakland, CA. Written consent was obtained from all enrolled participants.

For the present study, we examined the baseline data from subjects who were recruited for an antioxidant intervention study of smokers (28). Blood samples were obtained from the subjects after they fasted overnight for 12 h.

The eligibility criteria regarding smoking status were as follows: smokers were eligible if they smoked ≥15 cigarettes/d, and passive smokers were eligible if they had not smoked cigarettes for ≥1 y and were exposed indoors to the smoke of ≥1 cigarette/d on ≥5 d/wk. Nonsmokers were eligible if they had not smoked cigarettes for ≥1 y and were not exposed to CS at all. For the present analyses of baseline antioxidants, subjects who were taking ascorbic acid, α-tocopherol, or multivitamin supplements before participating in the study were excluded. Exclusion criteria for participation in the study were reported consumption of ≥2 servings of fruit and vegetables/d; intake of ≥2 alcoholic drinks/d; a history of alcohol problems <1 y ago; pregnancy; use of blood-thinning drugs; a history of hemochromatosis, kidney stones or any other kidney problems, hepatitis, diabetes, or HIV infection; and cancer, stroke, or heart attack within the past 5 y. Three subjects reported having asthma, and 4 subjects reported smoking cigars in addition to cigarettes; exclusion of these subjects did not alter the conclusions. The detailed design of the antioxidant intervention study on smokers is described elsewhere (28).

On the basis of cotinine concentrations, 2 nonsmokers were excluded because of a measurable cotinine concentration in their plasma. 1 passive smoker was recategorized as a smoker, and 2 additional passive smokers were excluded because they could not be clearly categorized. In summary, after exclusions on the basis of prior supplement use, cotinine concentration, and missing data, the baseline plasma data of 159 subjects (36 nonsmokers, 40 passive smokers, and 83 smokers) were used.

Clinical protocol

Blood samples were obtained from all participants after an overnight 12-h fast. Clinic visits of smokers, passive smokers, and nonsmokers occurred throughout the time period of June 1998–June 1999. Smokers refrained from smoking for ≥1 h before their clinic visit. All participants completed the 1998 version of the Block food-frequency questionnaire, which allowed estimation of their intakes of macronutrients and micronutrients including dietary carotenoids and γ-tocopherol (29, 30). Extensive other data were obtained; this data included the number of cigarettes that subjects smoked or were exposed to per day and the numbers of years that subjects smoked or were exposed to CS. The smoke exposure data of the passive smokers were collected by using an environmental tobacco smoke questionnaire provided by Neal Benowitz.

Blood drawing and processing

Venous blood was drawn into EDTA-coated evacuated tubes and centrifuged at 5°C for 10 min at 1200 × g. The plasma was immediately removed from the blood cells and transferred to cryovials. Plasma aliquots for ascorbic acid measurement were mixed 1:1 with 10% (wt:vol) meta-phosphoric acid to stabilize ascorbic acid. All blood and plasma samples were kept on ice and protected from light throughout sample processing. The time between blood collection and freezer storage of the plasma aliquots (−70°C) did not exceed 1 h.

Laboratory measurements

Plasma from all participants was analyzed for total ascorbic acid, α-tocopherol, γ-tocopherol, α-carotene, β-carotene, β-cryptoxanthin, lutein and zeaxanthin, total carotenoids, retinol, C-reactive protein, total cholesterol, triacylglycerol, transferrin saturation, and cotinine. Total ascorbic acid was measured spectrophotometrically as chromogen by using 2,4-dinitrophenylhydrazine (31). The laboratory’s accuracy for measurement of total ascorbic acid in these samples, which was determined by using both internally and externally prepared quality control specimens, was 6%, and the laboratory’s day-to-day CV for the assay was <0.02.

Tocopherol and carotenoid concentrations were measured by reversed-phase HPLC (32). Cotinine concentrations were measured by gas chromatography with nitrogen-phosphorus detection (limit of quantitation: 57 nmol/L; used for smokers’ plasma) and by liquid chromatography atmospheric pressure ionization tandem mass spectrometry (limit of quantitation: 0.57 nmol/L; used for the plasma of passive smokers and nonsmokers) (33, 34) in the laboratory of Neal Benowitz at the University of California, San Francisco. C-reactive protein concentrations were measured by radial immunodiffusion assay (The Binding Site Ltd, San Diego), and total cholesterol and triacylglycerol concentrations were measured by endpoint spectrophotometry (Sigma-Aldrich, St Louis). Transferrin saturation was analyzed by a clinical laboratory (SmithKline Beecham Clinical Laboratories, Norristown, PA).

Dietary data analysis

The 1998 version of the Block food-frequency questionnaire was self-administered by respondents and edited for errors and completeness. The questionnaire contains questions on ~100 food items identified in the third National Health and Nutrition Examination Survey (NHANES III) as the major nutrient contributors in the US diet (35–37). Respondents reported their frequency of consumption of most food items in 9 frequency categories ranging from never to every day. Portion sizes for all foods were assessed by using food-portion photos. Nutrients were calculated as the product of frequency × portion size × nutrient content and were summed over all foods. Nutrient content values from the USDA Nutrient Database for Standard Reference, release 12, were used (30). γ-Tocopherol values were based on published data (29, 30). Earlier versions of this food-frequency questionnaire were validated (38, 39) and found to correlate well with reference data.

Statistical analysis

Demographic data, fruit and vegetable intake data, and plasma total ascorbic acid data are presented untransformed because the
distribution of those data was approximately normal. All other dietary and plasma antioxidant data were log transformed or square-root transformed to better approximate normality. Thus, the data on dietary and plasma antioxidants are presented as back-transformed means. All crude data are presented as means with 95% CIs, and adjusted plasma antioxidant data are presented as least-squares means. Subject groups were compared by analysis of variance techniques. Tukey-Kramer’s test with a 5% procedure-wise error rate was used as a follow-up technique to present significant differences between the 3 study groups. Statistical analyses were conducted by using SAS version 6.12 (SAS Institute Inc, Cary, NC).

The covariates that were examined included fruit and vegetable servings, the respective dietary antioxidant for each plasma antioxidant, sex, age, race, BMI, alcohol intake, lipids including plasma cholesterol and triacylglycerol, C-reactive protein, and transferrin saturation. For consistency, any covariate found to be statistically significant for any plasma antioxidant was included in all models. In addition, potential effect modification, including possible modification by age and BMI, was examined, but none was found.

RESULTS

Of the 159 subjects included in this analysis, 63 were men and 96 were women. The characteristics of the study subjects are shown in Table 1. The 3 study groups did not differ significantly in sex, age, or BMI but differed significantly in race (P < 0.001). The duration of cigarette smoking or of exposure to cigarette smoke was not significantly different between active and passive smokers. Plasma cotinine concentrations in the smokers were ≈400-fold those in the passive smokers (P < 0.0003). All of the nonsmokers that were included in this analysis had a plasma cotinine concentration below the limit of quantitation (ie, <0.57 nmol/L).

Several differences in dietary intakes were seen (Table 2). The 3 study groups differed significantly in their intakes of fruit and fruit juice, ascorbic acid, and α-carotene. The study groups did not differ significantly in their intakes of vegetables, α-tocopherol, γ-tocopherol, β-carotene, β-cryptoxanthin, lycopene, lutein, and retinol. These results did not change materially after adjustment for age and sex (data not shown).

The unadjusted plasma antioxidant concentrations in the smokers, passive smokers, and nonsmokers are shown in Table 2. Significant differences between the study groups were found for 8 of the 10 antioxidants. Adjustment of the lipid-soluble antioxidants for plasma triacylglycerol did not affect these results (data not shown).

Plasma antioxidant concentrations in the nonsmokers, passive smokers, and active smokers after adjustment for each respective dietary antioxidant, race, age, sex, BMI, alcohol intake, fruit and vegetable intakes, and triacylglycerol (for lipid-soluble antioxidants only) are shown in Table 3. The differences between the groups remained significant for 5 antioxidants: γ-tocopherol, total ascorbic acid, β-carotene, β-cryptoxanthin, lutein and zeaxanthin.

The smokers had significantly lower concentrations of total ascorbic acid, β-carotene, β-cryptoxanthin, and lutein and zeaxanthin and significantly higher concentrations of γ-tocopherol than did the nonsmokers. There were no significant differences between the 3 groups in concentrations of α-tocopherol, α-carotene, total carotenoids, lycopene, and retinol.

As shown in Table 3, plasma β-carotene concentrations in the passive smokers (0.15 μmol/L) were significantly lower [P < 0.05 in pairwise comparisons (Tukey’s test corrected)] than those in the nonsmokers (0.24 μmol/L). The results for γ-tocopherol also indicate an effect of passive smoke exposure on plasma concentrations, in this case resulting in significantly higher concentrations in the passive smokers than in the nonsmokers. The data in Table 3 indicate no effect of passive smoke exposure on plasma concentrations of α-tocopherol, total ascorbic acid, α-carotene, β-cryptoxanthin, lycopene, and retinol. Only for total carotenoids and lutein and zeaxanthin did the passive smokers achieve significance in sex, age, or BMI but differed significantly in race (P < 0.001). The duration of cigarette smoking or of exposure to cigarette smoke was not significantly different between active and passive smokers. Plasma cotinine concentrations in the smokers were ≈400-fold those in the passive smokers (P < 0.0003). All of the nonsmokers that were included in this analysis had a plasma cotinine concentration below the limit of quantitation (ie, <0.57 nmol/L).

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The smokers had significantly lower concentrations of total ascorbic acid, β-carotene, β-cryptoxanthin, and lutein and zeaxanthin and significantly higher concentrations of γ-tocopherol than did the nonsmokers. There were no significant differences between the 3 groups in concentrations of α-tocopherol, α-carotene, total carotenoids, lycopene, and retinol.

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### Table 1

| Characteristics of the 159 subjects by study group | Nonsmokers (n = 36) | Passive smokers (n = 40) | Smokers (n = 83) | P
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n)</td>
<td></td>
<td></td>
<td></td>
<td>0.104</td>
</tr>
<tr>
<td>M</td>
<td>13</td>
<td>11</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>23</td>
<td>29</td>
<td>44</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>22</td>
<td>18</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>3</td>
<td>11</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>11</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>45.7 (40.5, 50.9)</td>
<td>41.8 (37.0, 46.5)</td>
<td>42.7 (40.3, 45.1)</td>
<td>0.387</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 (25.1, 28.7)</td>
<td>29.7 (27.6, 31.8)</td>
<td>27.9 (26.5, 29.3)</td>
<td>0.125</td>
</tr>
<tr>
<td>Alcohol intake (drinks/wk)</td>
<td>2.1 (1.1, 3.1)</td>
<td>1.3 (0.6, 2.0)</td>
<td>2.3 (1.7, 3.0)</td>
<td>0.153</td>
</tr>
<tr>
<td>Years of smoking or of passive exposure to smoke (y)</td>
<td>22.9 (18.4, 27.5)</td>
<td>23.3 (21.0, 25.6)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Cigarettes smoked or exposed to per day (no.)</td>
<td>10.9 (6.7, 15.2)</td>
<td>22.9 (21.1, 24.7)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Plasma cotinine (nmol/L)</td>
<td>ND</td>
<td>3.5 (2.1, 4.8)</td>
<td>1469.3 (1328.4, 1609.7)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

1 Chi-square test or ANOVA.
2 Arithmetic ±; 95% CI in parentheses.
3 The limit of quantitation in the plasma of nonsmokers and passive smokers with the use of liquid chromatography atmospheric pressure ionization tandem mass spectrometry was 0.57 nmol/L; the limit of quantitation in the plasma of smokers with the use of gas chromatography with nitrogen-phosphorus detection was 57 nmol/L.

4 ND, none detected (<0.57 nmol/L).
have concentrations that were intermediate between those of the smokers and nonsmokers, but in neither case were the concentrations in the passive smoking group significantly different from those in either of the other groups. In summary, after adjustment for race, age, sex, BMI, alcohol intake, and triacylglycerol (for lipid-soluble antioxidants only), the smokers had significantly lower plasma concentrations than did the nonsmokers. The smokers and passive smokers had higher plasma α-tocopherol concentrations than did the nonsmokers. All of these comparisons were significant with the use of Tukey-Kramer’s correction with a 5% procedure-wise error rate.

The importance of other covariates on plasma antioxidant concentrations is also notable. BMI was highly significant for several carotenoids (data not shown), consistent with earlier findings on the importance of body weight to plasma ascorbic acid status (40).

**TABLE 3**

Plasma antioxidant concentrations in the 159 subjects by study group after adjustment for race, age, sex, body mass index, alcohol intake, fruit and vegetable intake, the respective dietary antioxidant, and triacylglycerol concentration

<table>
<thead>
<tr>
<th></th>
<th>Nonsmokers (n = 36)</th>
<th>Passive smokers (n = 40)</th>
<th>Smokers (n = 83)</th>
<th>P1</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Tocopherol (μmol/L)</td>
<td>30.0</td>
<td>30.4</td>
<td>29.7</td>
<td>0.933</td>
</tr>
<tr>
<td>γ-Tocopherol (μmol/L)</td>
<td>6.5a</td>
<td>7.8b</td>
<td>7.8b</td>
<td>0.032</td>
</tr>
<tr>
<td>Ascorbic acid (μmol/L)</td>
<td>54.5b</td>
<td>54.6b</td>
<td>43.6a</td>
<td>0.014</td>
</tr>
<tr>
<td>α-Carotene (μmol/L)</td>
<td>0.05</td>
<td>0.04</td>
<td>0.04</td>
<td>0.210</td>
</tr>
<tr>
<td>β-Carotene (μmol/L)</td>
<td>0.24a</td>
<td>0.15a</td>
<td>0.17a</td>
<td>0.026</td>
</tr>
<tr>
<td>Lutein and zeaxanthin (μmol/L)</td>
<td>0.180</td>
<td>1.60</td>
<td>1.53</td>
<td>0.141</td>
</tr>
<tr>
<td>Lycopene (μmol/L)</td>
<td>0.41b</td>
<td>0.36ab</td>
<td>0.33a</td>
<td>0.047</td>
</tr>
<tr>
<td>Retinol (μmol/L)</td>
<td>2.15</td>
<td>2.15</td>
<td>2.26</td>
<td>0.549</td>
</tr>
</tbody>
</table>

1Adjustment for triacylglycerol concentration was performed for lipid-soluble antioxidants only. Values in the same row with different superscript letters are significantly different at a 5% procedure-wise error rate.

2Overall general linear model.
Fruit and vegetable intakes also had significant independent effects on antioxidant status, even with the corresponding antioxidant nutrient intake also in the model.

**DISCUSSION**

To our knowledge, this is the first study to examine the effect of cigarette smoke exposure on plasma antioxidant concentrations in both smokers and passive smokers with control for dietary antioxidant intakes, fruit and vegetable intakes, BMI, and other covariates. These data permitted an analysis of the effect of smoke exposure unconfounded by differences between the 3 groups in dietary intakes and other covariates. Possible misclassification due to the use of self-reported smoking status was eliminated in our sample because plasma cotinine concentrations in all volunteers were measured (33, 34). None of our nonsmokers had detectable plasma cotinine concentrations, showing that the subjects were free from exposure to environmental cigarette smoke. In addition, all subjects with prior vitamin supplement use were excluded from this analysis. These steps eliminated many of the potentially confounding variables that may have influenced the results of other published studies.

These analyses indicate that, independent of differences in dietary intakes or other demographic factors, active smoking affects plasma concentrations of total ascorbic acid, several carotenoids, and γ-tocopherol. In addition, even passive smoke exposure altered the plasma concentrations of 2 of these antioxidants: β-carotene and γ-tocopherol. We also found dietary differences between the study groups.

Note that smoke exposure was moderate in our sample (Table 1). The data collection took place in California after the implementation of a state law restricting smoking in restaurants and other public places. Our results may not reflect passive smoke effects in other environments such as casinos, bars, and other places where smoking is common in other states or countries. However, our results do reflect passive smoke exposure in the typical daily environment in California.

Five earlier studies reported lower plasma ascorbic acid concentrations in smokers than in nonsmokers (7–11). The results of the present study confirm that finding.

In the present study, plasma total ascorbic acid concentrations in the passive smokers did not differ significantly from those in the nonsmokers (Table 3). Trend analyses with increasing daily exposure to cigarettes or with increasing plasma cotinine concentrations were also not significant (P for trend > 0.5). Three earlier studies observed significantly lower plasma ascorbic acid concentrations in passive smokers than in nonsmokers (14, 15, 17), which is in disagreement with our results. Tribble et al (15) did not exclude prior vitamin supplement users in their study of 141 women; nor did they adjust for this covariate. Thus, it is possible that the lower concentrations in their passive smokers were a result of different dietary and supplement habits. Farchi et al (17), who did adjust for dietary intakes of ascorbic acid, found a significant inverse dose-response relation between smoke exposure and plasma ascorbic acid. Valkonen and Kusi (14) exposed 10 nonsmokers to smoke from 16 cigarettes in 30 min, and although they found an acute decrease in plasma ascorbic acid concentrations, their results are not relevant to the exposure levels in our study. On the other hand, Ayaori et al (18) did not observe significant differences in plasma ascorbic acid concentrations between passive smokers and nonsmokers, which is consistent with our results.

In summary, these data suggest that passive exposure to cigarette smoke at the concentrations experienced in our study (mean: 11 cigarettes/d) may not have detectable effects on plasma ascorbic acid concentrations once dietary differences are controlled for.

Plasma α-tocopherol concentrations were not significantly different between the smokers, passive smokers, and nonsmokers. The dietary intake of α-tocopherol was also not significantly different between the 3 groups. Several other studies compared plasma α-tocopherol concentrations in smokers with those in nonsmokers. Three studies (7, 10, 11) did not find lower plasma α-tocopherol concentrations in smokers than in nonsmokers. Stryker et al (13) found a weak inverse (r = −0.11) relation only in women and concluded that cigarette smoking had no effect on plasma α-tocopherol concentrations. Three studies (14, 16, 17) did not observe any effect of passive smoke exposure on plasma α-tocopherol concentrations.

Plasma γ-tocopherol concentrations were significantly higher in both the smokers and the passive smokers than in the nonsmokers. This result was adjusted for dietary γ-tocopherol. No significant differences in dietary γ-tocopherol intakes were observed between the study groups. Thus, diet does not provide an explanation of the higher γ-tocopherol concentrations in smokers and passive smokers in this study. Lykkesfeldt et al (10) also reported significantly higher plasma γ-tocopherol concentrations in smokers than in nonsmokers, but their data were not adjusted for dietary intake. Our study is the first study to investigate γ-tocopherol effects of passive smoke exposure. Further research is needed to investigate the possible role of cigarette smoke exposure in elevated plasma γ-tocopherol concentrations.

Both the smokers and the passive smokers had lower plasma β-carotene concentrations than did the nonsmokers. The effect in smokers is consistent with that noted in other reports on this topic (9, 13, 41–43). However, the literature on passive smokers is inconsistent. Alberg et al (16) found lower β-carotene concentrations in passive smokers than in nonsmokers, but only in males, and the results were unadjusted for dietary β-carotene. After adjustment for dietary β-carotene, Farchi et al (17) found a significant inverse relation between smoke exposure and plasma β-carotene. Intense short-term smoke exposure did not affect nonsmokers’ plasma β-carotene concentrations (n = 10) in the study by Valkonen and Kusi (14).

Concentrations of lutein and zeaxanthin and β-cryptoxanthin were significantly lower in the smokers than in the nonsmokers. This observation is consistent with most of the data in the literature (13, 42, 44, 45). Maragon et al (9), however, found no significant effect of smoking on lutein and zeaxanthin but a trend for β-cryptoxanthin to decrease in heavy smokers. In the present study, concentrations of lutein and zeaxanthin and β-cryptoxanthin in the passive smokers were not significantly lower than those in the nonsmokers. No other reports of passive smoke exposure effects on this antioxidant were found. No significant differences between the study groups in plasma lycopene and retinol concentrations were observed, which is consistent with data from the literature (9, 42, 44–47).

The present study shows the importance of adjusting for dietary intakes of antioxidants and for other covariates when investigating the effect of active and passive smoking on plasma antioxidants. The 3 groups differed not only in the intakes and food sources of antioxidants, but also in other important factors, such as BMI, that can influence plasma antioxidant concentrations (40). The significantly lower β-carotene concentrations in passive smokers than in unexposed nonsmokers—independent of dietary β-carotene
intake—indicates that second-hand smoke decreases the human antioxidant defense system.

The higher γ-tocopherol concentrations in active and passive smokers suggest additional smoke effects on the redox or metabolic systems. This is a novel finding and requires further research.

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REFERENCES

36. Block G, Dresser CM, Hartman AM, Carroll MD. Nutrient sources


