The Risk of Developing Lung Cancer Associated with Antioxidants in the Blood: Ascorbic Acid, Carotenoids, α-Tocopherol, Selenium, and Total Peroxyl Radical Absorbing Capacity


Abstract

Lung cancer cases diagnosed during the period 1975 through 1993 and matched controls were identified in the rosters of Washington County, Maryland residents who had donated blood for a serum bank in 1974 or 1989. Plasma from participants in the 1989 project was assayed for ascorbic acid; serum or plasma was assayed for participants in either project for α- and β-carotene, cryptoxanthin, lutein/zeaxanthin, lycopene, α-tocopherol, selenium, and peroxyl radical absorption capacity. Among the total group of 258 cases and 515 controls, participants in either project for α- and β-carotene, cryptoxanthin, lutein/zeaxanthin, lycopene, α-tocopherol, selenium, and peroxyl radical absorption capacity. Among the total group of 258 cases and 515 controls, serum/plasma concentrations were significantly lower among cases than controls for cryptoxanthin, lutein/zeaxanthin, lycopene, α-tocopherol, selenium, and peroxyl radical absorption capacity. The results of previous studies and those from previous studies suggest that β-carotene is a marker for some protective factor(s) against lung cancer; that cryptoxanthin, α-carotene, and ascorbic acid need to be investigated further as potentially protective factors or associates of a protective factor; and that lycopene, α-tocopherol, selenium, and peroxyl radical absorption capacity are unlikely to be associated with lung cancer risk. Until specific preventive factors are identified, the best protection against lung cancer is still the avoidance of airborne carcinogens, especially tobacco smoke; second best is the consumption of a diet rich in fruits and vegetables.

Introduction

The generally accepted causes of lung cancer are inhalants: tobacco smoke; dusts or fumes containing carcinogens such as arsenic, asbestos, chloromethyl ether, and chromates; and gases such as radon (1–4). However, the fact that not all persons exposed to even high concentrations of these airborne pollutants develop cancer suggests that there are substances that can prevent or inhibit carcinogenesis. An appealing hypothesis involves the following simplified chain of events. Many carcinogens create free oxidative radicals that damage cells; damaged cells are prone to develop malignant changes; and antioxidants can neutralize free radicals, thereby preventing cell damage and the subsequent development of cancer (5–7). This hypothesis would be strengthened if it could be consistently demonstrated that persons who developed cancer had lower concentrations of antioxidant substances in their blood before they developed cancer than persons who had remained free of cancer.

An impressive number of observational studies has addressed this hypothesis with respect to the association of serum or plasma concentrations of several antioxidants and lung cancer (8, 9). The antioxidants assayed in these studies include retinol, total carotenoids, β-carotene, α-tocopherol, and selenium, the last being a surrogate for the selenium-containing enzyme, glutathione peroxidase. Serum retinol showed only a trivial and inconsistent association with the subsequent development of lung cancer. The results of the two studies that reported on total carotenoids were inconsistent. A larger number of studies dealing with α-tocopherol showed only weak associations; they were also inconsistent in the direction of the associations. The results of studies involving serum or toenail selenium were stronger but were also inconsistent. In contrast, the associations of β-carotene concentrations in the serum were remarkably consistent in showing a considerably lower risk among persons with higher serum concentrations.

Unfortunately, nearly all studies of serum antioxidants and lung cancer have been limited to men or have grouped men and women together in the analysis (9). In the few instances where the findings were stratified by sex, the results tended to be similar for men and women. Most studies that took smoking into account did so by adjusting the data to remove the effects of smoking, a procedure which makes it impossible to examine the extent to which the associations of serum components with lung cancer might differ according to smoking status. One
study that stratified the results for nonsmokers and current smokers found that the associations between lung cancer and retinol, β-carotene, and α-tocopherol were all stronger in a protective direction for nonsmokers than for current smokers (10). Two studies looked at the associations with selenium by smoking categories; both found them to be weak and nonsignificant for smokers and nonsmokers but in a protective direction (10, 11).

Although the results of observational studies strongly suggest that β-carotene might in some way protect against lung cancer, the findings in three controlled trials have demonstrated that this is not so, at least during treatment periods of 4–12 years (12–14). In Finland, the Alpha-Tocopherol Beta-Carotene Trial among smokers, and in the United States, the Beta-Carotene and Retinol Efficacy Trial among smokers and workers exposed to asbestos showed that persons assigned to take β-carotene had rates of lung cancer that were 18 and 28% higher, respectively, than the control rates (12, 13). A third trial in the United States, the Physicians’ Health Study, showed a nonsignificant reduction of 8% among the participants assigned to a β-carotene-containing regimen (14).

Only men were included in the Finnish trial and the U.S. trial among physicians and workers exposed to asbestos. The United States trial among heavy smokers included 6,289 women and found their risk associated with supplementation to be similar to that among men (13). The efficacy of β-carotene in preventing lung cancer among former smokers and persons who had never smoked could not be determined in the Finnish study because all participants were current smokers at the start of the trial (12). The United States study among asbestos-exposed workers and heavy smokers included only 132 persons who had never smoked but did identify 7,174 former smokers and 11,008 current smokers. Although the efficacy of β-carotene in preventing lung cancer among former heavy smokers was a nonsignificant 20%, β-carotene was associated with a significant 42% increase in lung cancer among current smokers (13). Among the United States physicians, the preventive efficacy of β-carotene was 22% for nonsmokers, 0% for former smokers, and 10% for current smokers. Again, because of the small numbers of cases, especially among nonsmokers, these estimates of efficacy could easily have occurred by chance (14).

A contrast is afforded by selenium. Although the observational studies for the most part showed only a weak association in a protective direction, a preliminary report of a controlled trial of selenium supplementation noted a statistically significant reduction in lung cancer mortality (15).

The present study was undertaken to add to the meager evidence on the relationships of serum β-carotene, α-tocopherol, and selenium with subsequent lung cancer risk among women and nonsmokers. It also offered an opportunity to assess similar associations with ascorbic acid, additional carotenoids, and a measure of total antioxidant capacity among both men and women as well as among smokers and nonsmokers.

Materials and Methods

In the autumn of 1974, a campaign (CLUE I) was conducted in Washington County, Maryland to collect blood for a serum bank, along with a brief history, which included information on education, smoking, and recent medications. A total of 25,802 persons participated, of whom 20,305 were identified as county residents in a private census done in the summer of 1975. Approximately 30% of the adult population took part in the program. Participation was best in the age group 45–64 years and was somewhat higher among women and better-educated individuals.

Blood was collected in 15-ml Vacutainers (Becton Dickinson, Rutherford, NJ). It was allowed to clot at room temperature for 30 min and was then kept at 4°C until the serum was separated, usually 3–4 h later. Serum from each participant was placed in two 5-ml Nunc tubes (Nunc, Roskilde, Denmark). Most of the sera had been kept frozen at −70°C until aliquots were removed for this study. Sixty-seven of the 258 case-control sets had been previously thawed and refrozen; in all such instances, sera from cases have been handled similarly to their matched controls.

A similar campaign (CLUE II) was conducted during the summer and autumn of 1989. A brief history was obtained, and blood was collected from 32,319 persons, of whom 24,655 gave Washington County addresses. Comparison of the participation with data from the 1990 Census showed that participation rates were similar to those in 1974, except that peak participation came at a slightly older age.

For the second program, blood was collected in 20-ml Vacutainers containing heparin to prevent clotting (Fisher Scientific, Pittsburgh, PA). The blood was refrigerated at 4°C until centrifugation 2–6 h later. For ascorbic acid assays, 0.7-ml of plasma was placed in 5-ml blue-capped Vanguard Cryogenic Vials (Sumitomo Bakelite, Neptune, NJ) containing 0.7-ml of a 10% solution of metaphosphoric acid. Equal aliquots of the remaining plasma were placed in two 5-ml Cryotubes with clear caps. The plasma,uffy coat, and 2-ml of packed RBCs were frozen at −70°C.

Lung cancer cases occurring among Washington County residents who donated blood samples for CLUE I or CLUE II were identified from death certificates of county residents and from discharge records of Washington County Hospital, the only general hospital in the county. The hospital has a well-equipped oncology center that tends to attract patients from surrounding areas. These two sources of information appear to give reasonably complete counts of lung cancer cases. At rates of lung cancer among whites in the Surveillance, Epidemiology, and End Results (SEER) cancer registries for 1986–1990, 245 cases of lung cancer would have been expected among Washington County residents for the 3-year period 1989–1991. (There are too few nonwhites in Washington County to allow reasonable estimates for them.) During this 3-year period, 267 lung cancer cases were observed, yielding an observed:expected ratio of 1.09. A similar calculation for the years 1974–1989 for participants in CLUE I gave an observed:expected ratio of 1.01. More direct comparisons are now possible with the institution of compulsory reporting to the Maryland Cancer Registry. For 1993, the state registry recorded 96 lung cancer cases among Washington County residents; our local registry recorded 95.

Among the Washington County residents who gave blood samples in one or both of the two programs were 258 persons who had been diagnosed with lung cancer after donating blood. For all of them, a hospital record was abstracted. There were 229 persons diagnosed during the years 1975–1993 among CLUE I participants who donated blood in 1974 and 29 diagnosed during 1990–1993 among persons who participated only in 1989 in CLUE II. For 257 cases, two matched controls were selected who were not known to have died or to have developed cancer other than nonmalignant skin cancer at the time the case was diagnosed. For one case, assay results were available for only one control. Matching characteristics were race, sex, age, date of blood donation, and participation in CLUE I and CLUE II. For age matching, the next youngest and the next
oldest persons who met the other matching criteria were selected from the list of participants. Because diet can change appreciably with season of the year, date of blood drawing for each control had to be within 2 weeks of the time the case was bled.

Serum specimens were arranged in sets of three, each set containing serum from a case and its two matched controls. (“Serum” will refer either to serum obtained in the 1974 program or to plasma from the 1989 program unless otherwise specified.) Sets were numbered sequentially with a terminal digit added to indicate case-control status. Interspersed throughout the sets were quality control sets, also in triplets, numbered in a way to make them indistinguishable from case-control sets. There were 14 quality control triplets for the ascorbic assays, 17 for the peroxyl radical-absorbing capacity assays, and 25 for the others. Each of the quality control triplets was made up of one specimen from a large pool (A) plus a pair of specimens (BB, CC, DD, etc.). As a result, specimen A was assayed 14–25 times and assays were also done on 14–25 different pairs of sera. Coefficients of variation were calculated for each of the 14–25 pairs, and the values were averaged.

Sera for ascorbic acid assays were sent to the laboratory without having been thawed. Other serum specimens were thawed in ice-water under dim yellow light. The necessary aliquots were quickly refrozen and shipped to the laboratories with dry ice in styrofoam containers.

Ascorbic acid was assayed using 2,4-dinitrophenylhydrazine as a chromogen (16–18). Carotenoids and α-tocopherol were assayed by reverse-phase high performance liquid chromatography (18, 19). Neutron activation analysis was used for the selenium assays (20). Oxygen radical absorption capacity assays for this study are limited to the serum collected in 1974. They measure the ability of the serum to protect the indicator protein B-phycoerythrin against oxidation by peroxyl radicals (21, 22). Peroxyl absorption capacity is expressed in ORAC units. One ORAC unit equals the net protection against peroxyl radicals afforded by 1 μM of 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid, a water-soluble analogue of vitamin E (21).

For all assays, laboratories were instructed to assay the specimens in each set on the same day and with the same reagents. The mean intra-pair coefficients of variation for the quality control specimens were 2.1% for ascorbic acid, 6.0% for α-carotene, 5.2% for β-carotene, 3.2% for cryptoxanthin, 4.6% for lutein and zeaxanthin combined, 3.7% for lycopene, 3.3% for α-tocopherol, 2.5% for selenium, and 5.6% for peroxyl radical-absorbing capacity.

Paired t tests were used to assess the statistical significance of differences in mean values of each matched pair (23). Relative risks of developing lung cancer were estimated by odds ratios obtained by conditional multiple logistic regression (24). Trend tests were performed by conditional logistic regression using median values of each interval (fifths or thirds) among the controls as independent variables. To give some idea of how closely the fitted points agreed with the regression lines, correlation coefficients between natural logarithms of the fitted odds ratios and median values of each interval were calculated. In assessing the role of chance in the tables for this paper, Ps are shown because they cause less “clutter” than confidence limits and hence are less likely to obscure the basic findings. Because of the large number of comparisons made in analyzing the results of this study, only Ps of 0.01 or less will be considered to be statistically significant.

In the analyses of the total material and also for each sex, adjustments were not made for smoking status because dietary intake of antioxidants differs considerably among smokers and nonsmokers (25, 26). As a consequence, adjusting for smoking is tantamount to partial adjustment for dietary intake. To examine the associations of antioxidants with lung cancer stratified by cigarette smoking status at baseline, the original matching had to be broken. Accordingly, unconditional multiple linear regression was used to estimate the differences in mean serum concentrations of the analysis for cases and controls, adjusted for the effects of sex, age, months in which blood was drawn, and program of participation (because of possible storage effects).

Results
Baseline characteristics of cases and controls are shown in Table 1 separately for males and females. The only statistically significant differences between cases and controls were smoking histories for each sex. Only 19 of the cases had never smoked, and 13 of them were females. The risk of lung cancer was strongly associated with the amount of cigarettes smoked per day at baseline.

The distribution of the study population by smoking status is presented in Table 2. Although this tabulation required ignoring the matching of cases and controls, the case and control distributions remained reasonably similar. Only with respect to years of school completed among former and current smokers did the case and control differences achieve statistical significance (P = 0.04 for both).

Histological cell types of the cases in this study are shown in Table 3. Among the total group, the most common cell type was adenocarcinoma (31.8%), followed by squamous cell carcinomas (24.4%) and small cell types (21.7%). Adenocarcinomas were the most common type among females, whereas squamous cell and adenocarcinoma were equally common among males. Limiting the comparisons to current smokers, small cell carcinomas were more common among females than males, whereas squamous cell carcinomas and adenocarcinomas were similar in frequency among each sex.

Prediagnostic serum concentrations are shown in Table 4. All of the antioxidant concentrations were lower among cases than controls, although the differences for lycopene, α-tocopherol, selenium, and total peroxyl-absorbing capacity were trivial and nonsignificant. All of the other case-control differences were in a protective direction and were 10.0% or greater. Those for cryptoxanthin and β-carotene were largest and least likely to have occurred by chance.

Odds ratios of lung cancer incidence are shown for each fifth of the distributions among controls in Table 5. Ascorbic acid is an exception. Because of the small number of cases for which this antioxidant could be assayed, the odds ratios are based on thirds of the control distribution. The likelihood that the observed trend according to median values of each fraction of the distribution of serum concentrations among the controls is compatible with a monotonic dose-response trend was assessed by Ps for trend, which are shown regardless of the degree of monotonic linearity. As an indication of how closely the values for each fraction of the distributions approached the calculated trend line, the correlation coefficients are also shown.

α- and β-carotene, cryptoxanthin, and lutein/zeaxanthin all have odds ratios less than 0.5 for the highest fifth of their distributions. The distributions of odds ratios for these antioxidants are consistent with a linear downward trend, with the trend for β-carotene most closely approaching linearity. How-
ever, the strength of the association appears to be strongest for cryptoxanthin.

Serum concentrations of the antioxidants for cases and controls are shown for each sex separately in Table 6, along with the case-control differences expressed as percentages. Female cases and controls had appreciably higher serum concentrations of ascorbic acid, α- and β-carotene, and cryptoxanthin than males. There were small differences between the sexes in the concentrations of the other antioxidants. Case-control differences of the various serum antioxidants were generally similar for both males and females and comparable to those for the total group. Cryptoxanthin was somewhat more strongly and consistently associated with decreased risk than the other antioxidants. The case-control differences for the carotenoids tended to be greater among females than males.

Adjusted prediagnostic serum concentrations of carotenoids among cases and controls are shown in Fig. 1 and those for the other four analytes are shown in Fig. 2 according to cigarette smoking history at baseline. Adjustments were done for sex, age, date when blood was drawn, and program participation. Carotenoid concentrations tended to be lowest among current smokers and highest among those who had never smoked, the difference being most marked for β-carotene. Concentrations among former smokers were intermediate except for lycopene. In general, cases had lower concentrations than controls, except for α- and β-carotene among never smokers, and for lycopene among each category of smoking. When stratified by smoking status, case-control differences expressed as percentages became less strongly negative and even shifted to the positive side for α- and β-carotene concentrations among persons who had never smoked.

A similar presentation is made for the other four analytes in Fig. 2. It should be noted that the concentrations in this figure are greater than those for the carotenoids by approximately two to three orders of magnitude. Among controls, smokers had the lowest concentrations of ascorbic acid and α-tocopherol, whereas persons who had never smoked cigarettes had the highest concentrations. For both of these analytes, cases had lower concentrations than controls in each of the three smoking categories. There were only trivial and inconsistent case-control differences in the concentrations of selenium and peroxyl radical-absorbing capacity. None of the case-control differences in these two figures was statistically significant.

It is not surprising that several of the serum antioxidants assayed in this study showed somewhat similar associations with the subsequent development of lung cancer. As can be seen in Table 7, there are some moderately high and highly significant correlations between the concentrations of ascorbic acid, α-tocopherol, and individual carotenoids. Most notable
Table 2  Percentage distribution of baseline characteristics of total study population by cigarette smoking status and case-control status. Washington County, MD. 1975-1993

<table>
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<tr>
<th>Baseline characteristic</th>
<th>Cases (n = 19)</th>
<th>Controls (n = 199)</th>
<th>Cases (n = 57)</th>
<th>Controls (n = 164)</th>
<th>Cases (n = 182)</th>
<th>Controls (n = 152)</th>
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<td>100.0</td>
<td>100.0</td>
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<td>73.7</td>
<td>79.3</td>
<td>83.5</td>
<td>84.2</td>
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<td>CLUE II (1989)</td>
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<td>19.3</td>
<td>17.1</td>
<td>8.8</td>
<td>7.9</td>
</tr>
<tr>
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<td>10.1</td>
<td>7.0</td>
<td>3.7</td>
<td>7.7</td>
<td>7.9</td>
</tr>
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<td></td>
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<td>Male</td>
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<td>80.7</td>
<td>78.7</td>
<td>57.7</td>
<td>65.8</td>
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<td>19.3</td>
<td>21.3</td>
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<td>34.2</td>
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<td>25-44</td>
<td>5.3</td>
<td>8.5</td>
<td>5.3</td>
<td>7.3</td>
<td>13.2</td>
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<td>67.1</td>
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<td>38.6</td>
<td>25.6</td>
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<td>10.5</td>
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<td>Years of school completed</td>
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<td>&lt;8</td>
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<td>29.1</td>
<td>29.8</td>
<td>21.3</td>
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<td>9-11</td>
<td>10.5</td>
<td>19.6</td>
<td>17.5</td>
<td>20.7</td>
<td>28.0</td>
<td>15.8</td>
</tr>
<tr>
<td>12</td>
<td>52.6</td>
<td>28.1</td>
<td>42.1</td>
<td>30.5</td>
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</tr>
<tr>
<td>13+</td>
<td>5.3</td>
<td>23.1</td>
<td>10.5</td>
<td>27.4</td>
<td>18.7</td>
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<td>Month blood drawn</td>
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<td>2.5</td>
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<td>8.5</td>
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<td>31.6</td>
<td>37.8</td>
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<td>57.9</td>
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<td>Hours since last meal</td>
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<td>0-1</td>
<td>21.1</td>
<td>27.1</td>
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<td>29.7</td>
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<td>2-4</td>
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<td>5-7</td>
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<td>7.0</td>
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<tr>
<td>8+</td>
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<tr>
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<td>1.8</td>
<td>1.2</td>
<td>0</td>
<td>0.7</td>
</tr>
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* NS, not significant.

Table 3  Percentage distribution of lung cancer cases by sex, cell type, and cigarette smoking history. Washington County, MD 1975-1993

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Males</th>
<th></th>
<th>Females</th>
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<tr>
<td></td>
<td>Never</td>
<td>Former</td>
<td>Current</td>
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<td></td>
<td>(n = 157)</td>
<td>(n = 6)*</td>
<td>(n = 46)</td>
<td>(n = 105)</td>
</tr>
<tr>
<td>Total percentage</td>
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<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
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<tr>
<td>Small cell</td>
<td>17.2</td>
<td>33.3</td>
<td>10.9</td>
<td>19.0</td>
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<tr>
<td>Squamous cell</td>
<td>29.3</td>
<td>33.3</td>
<td>37.0</td>
<td>25.7</td>
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<tr>
<td>Adenocarcinoma</td>
<td>28.7</td>
<td>33.3</td>
<td>28.3</td>
<td>28.6</td>
</tr>
<tr>
<td>Large cell</td>
<td>10.2</td>
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<td>8.7</td>
<td>11.4</td>
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<tr>
<td>Undetermined</td>
<td>14.6</td>
<td>0</td>
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* Includes two who smoked only pipes or cigars.

are the associations of β-carotene with α-carotene and cryptoxanthin.

Because of the possibility that the presence of an undiagnosed cancer might in some way be related to the serum concentrations of the antioxidant micronutrients, case-control differences among the 29 sets in which the case had been diagnosed within 2 years of blood donation were compared with these differences among the rest of the study population. Although there was a tendency for the later case-control differences to be slightly greater, in no instance were these differences statistically significant.

Discussion

Although there have been many studies of the relationships of dietary antioxidants and their serum concentrations to the risk of lung cancer, only about one-third have been cohort or nested case-control studies in which the exposure cannot have been influenced by the presence of disease (8, 9). Most have also been limited to a few antioxidants: retinol, β-carotene, α-tocopherol, and selenium. Although dietary studies of ascorbic acid are relatively numerous, only one study has reported the association of prediagnostic serum concentrations of ascorbic acid and subsequent lung cancer (27). The present study adds to the ascorbic acid story. It offers new evidence regarding the relationship of lung cancer risk to prediagnostic serum concentrations of the carotenoids cryptoxanthin, lutein/zeaxanthin, and lycopene and the capacity of the serum to absorb peroxyl radicals.

The study population has the major advantage of having been drawn from an ordinary general population, although it...
Table 4  Concentrations of selected antioxidants in prediagnostic sera of lung cancer cases and controls and percentage of differences between concentrations among cases and matched controls, Washington County, MD, 1975–1993

<table>
<thead>
<tr>
<th>Statistic</th>
<th>Ascorbic acid*</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>β-Cryptoxanthin</th>
<th>Lutein/zeaxanthin</th>
<th>Lycopene</th>
<th>α-Tocopherol</th>
<th>Selenium</th>
<th>Peroxyl-absorbing capacity*</th>
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<tbody>
<tr>
<td></td>
<td>(mg/dl)</td>
<td>(μg/dl)</td>
<td>(μg/dl)</td>
<td>(μg/dl)</td>
<td>(μg/dl)</td>
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<td>ORAC units</td>
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<td>TOTAL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cases/controls</td>
<td>44/86</td>
<td>233/490</td>
<td>257/514</td>
<td>258/515</td>
<td>258/515</td>
<td>256/514</td>
<td>258/515</td>
<td>258/515</td>
<td>228/451</td>
</tr>
<tr>
<td>Mean, cases</td>
<td>1.04</td>
<td>2.65</td>
<td>13.1</td>
<td>5.42</td>
<td>18.6</td>
<td>34.1</td>
<td>.755</td>
<td>.108</td>
<td>782</td>
</tr>
<tr>
<td>Mean, controls</td>
<td>1.23</td>
<td>3.05</td>
<td>15.8</td>
<td>7.28</td>
<td>20.7</td>
<td>34.5</td>
<td>.795</td>
<td>.110</td>
<td>783</td>
</tr>
<tr>
<td>% difference</td>
<td>-1.54</td>
<td>-13.1</td>
<td>-17.1</td>
<td>-25.5</td>
<td>-10.1</td>
<td>-1.2</td>
<td>-5.0</td>
<td>-1.8</td>
<td>-0.1</td>
</tr>
<tr>
<td>P difference*</td>
<td>0.07</td>
<td>0.04</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.003</td>
<td>0.76</td>
<td>0.04</td>
<td>0.09</td>
<td>0.58</td>
</tr>
</tbody>
</table>

* CLUE II participants only.
* CLUE I participants only.
* Paired t test.

Table 5  Risk of lung cancer (odds ratio) by fifths of distributions among controls for selected serum antioxidants, Washington County, MD, 1975–1993

<table>
<thead>
<tr>
<th>Fifths of control distribution</th>
<th>Ascorbic acid*</th>
<th>α-Carotene</th>
<th>β-Carotene</th>
<th>β-Cryptoxanthin</th>
<th>Lutein/zeaxanthin</th>
<th>Lycopene</th>
<th>α-Tocopherol</th>
<th>Selenium</th>
<th>Peroxyl-absorbing capacity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>2</td>
<td>0.40</td>
<td>0.80</td>
<td>0.68</td>
<td>0.76</td>
<td>1.12</td>
<td>0.88</td>
<td>1.35</td>
<td>0.68</td>
<td>1.15</td>
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<tr>
<td>3</td>
<td>0.44</td>
<td>0.47</td>
<td>0.68</td>
<td>0.29</td>
<td>0.83</td>
<td>0.82</td>
<td>1.31</td>
<td>0.53</td>
<td>1.16</td>
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<tr>
<td>4</td>
<td>NA*</td>
<td>0.75</td>
<td>0.54</td>
<td>0.49</td>
<td>0.76</td>
<td>0.98</td>
<td>0.83</td>
<td>0.76</td>
<td>0.99</td>
</tr>
<tr>
<td>High</td>
<td>NA*</td>
<td>0.48</td>
<td>0.44</td>
<td>0.29</td>
<td>0.41</td>
<td>1.01</td>
<td>0.77</td>
<td>0.65</td>
<td>0.93</td>
</tr>
<tr>
<td>P (trend)</td>
<td>0.09</td>
<td>0.01</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.99</td>
<td>0.06</td>
<td>0.08</td>
<td>0.64</td>
</tr>
<tr>
<td>r²</td>
<td>-0.85</td>
<td>-0.71</td>
<td>-0.92</td>
<td>-0.78</td>
<td>-0.91</td>
<td>0.27</td>
<td>-0.65</td>
<td>-0.58</td>
<td>-0.49</td>
</tr>
</tbody>
</table>

* Thirds for ascorbic acid.
* CLUE II participants only.
* CLUE I participants only.
* NA, not applicable.
* r, correlation coefficient.

Lung Cancer and Serum Antioxidant Micronutrients

suffers from the fact that only whites are adequately represented. It is the largest study of its kind to date, with 258 lung cancer cases, 101 of whom are females. It also has the largest number (70) of cases who were nonsmokers at baseline, although only 19, including 2 pipe or cigar smokers, had never smoked cigarettes. Even so, because of the low risk of lung cancer among nonsmokers, it was necessary to combine findings for males and females in each of the three categories of smoking history.

Only one previous study has been able to look at the associations of plasma ascorbic acid with subsequent cancer. Plasma vitamin C was assayed at baseline for all participants in the Prospective Basel Study (27). Its mean concentration at that time was less than 1% lower among the 68 men who died of lung cancer during a 12-year follow-up period than among the survivors. However, age-adjusted mean values in an earlier report showed a more substantial although still nonsignificant decrease of 7.4% among cases (28). On average, the dietary studies have shown a modest decrease in lung cancer risk associated with higher intakes of ascorbic acid but with considerable variability between studies (29–35). The 15% lower ascorbic acid concentrations in the sera of cases in this study is in keeping with the average difference observed in the dietary studies and also with the earlier serological study when its findings, like ours, are adjusted for age (28). Two prospective dietary studies reported findings for each sex, and another stratified its findings among men by current smoking status (31–33). Moderately strong protective associations were found for women and for nonsmokers. Protective associations (all nonsignificant) were also found in the present study, essentially the same for men and women, and strongest for persons who had never smoked, although this last result was based on only 4 cases and 34 controls.

With respect to β-carotene, the present findings are consistent in direction with those of previous prospective serological studies, but the association is not nearly as strong (27, 36–43). Only two studies, both dietary, have examined associations of other carotenoids with lung cancer in a prospective fashion (31, 35). A Finnish study among men found a moderately strong negative association with α-carotene; it and the Iowa Women’s Health Study found essentially no risk and a slight risk of lung cancer, respectively, associated with lutein and lycopene. In the present study, the association with α-carotene in the total study group was protective but not as strong as in the Finnish study. The results were similar among men and women, but the association was protective only among current smokers. With respect to lutein, the present findings differ from the two dietary studies in that the associations for all sex and smoking groups are in a protective direction. Although they are statistically significant in some instances, none of these associations is strong. As in the dietary studies, there is essentially no association with lycopene.

The association of cryptoxanthin with lung cancer has been examined previously in only two studies, both retrospective case-control studies of dietary intake (44, 45). In a group of Florida women who had never smoked, dietary consumption of cryptoxanthin was reported to be much lower among cases than controls. Only a slight protective association was noted among
men and women in Hawaii. In the latter study, the adjustment of results for the effects of smoking may have weakened the association, as it did in the present study (data not shown). The present findings contrast with those based on dietary histories. The difference in case-control serum concentrations was only slight among persons who had never smoked and considerably greater among women than men.

Previous studies of serum concentrations of α-tocopherol
have been almost equally divided between those that found slightly lower values among cases than controls and those that found the opposite, a balance that suggests that there is little if any real association of \( \alpha \)-tocopherol with lung cancer (27, 36, 37, 39, 42, 43, 46–48). The slight case-control difference in the present study does not contradict this conclusion. Only in two Finnish studies were the results stratified by sex or smoking status. There were no demonstrable differences between the sexes, but this was based on only eight females (48). For current smokers, there was a slight nonsignificant increase in risk with increasing serum concentrations in \( \alpha \)-tocopherol and a significant decrease in risk among current nonsmokers (43). In the present study, there was no demonstrable difference in the slight degree of protective association observed for both males and females nor in the slight protective associations observed for each of the three smoking categories. These slight negative associations were essentially unchanged when the findings were stratified by serum cholesterol concentrations at baseline (data not shown).

Prediagnostic concentrations of serum selenium and their associations with lung cancer have been reported in 11 studies (37, 43, 47, 49–56) and prediagnostic concentrations in toenail clippings in two others (57, 58). All but 3 of these 13 studies found that selenium concentrations were slightly lower in cases than in controls; the average case-control difference was 3.7%. The case-control difference found in the present study, \(-1.8\%\), is in keeping with the results of the earlier studies, as is its one-third reduction in lung cancer risk among persons with the highest concentrations of selenium. Only trivial differences in risk of lung cancer associated with selenium were noted between males and females and between smokers and nonsmokers in the earlier studies as well as the present one, in which the associations are somewhat weaker.

In the earlier study of lung cancer from Washington County, a strong interaction was reported between \( \alpha \)-tocopherol and selenium (37). No evidence of a similar interaction was found in the present data, in keeping with the lack of interaction noted in a similar study in Hawaii (51).

Adjusting case-control differences in serum concentrations for the effects of smoking made more than trivial changes only for the carotenoids other than lycopene (data not shown). For \( \alpha \)-carotene, \( \beta \)-carotene, and lutein/zeaxanthin, the smoking-adjusted differences were smaller than the unadjusted differences, and none of them varied meaningfully from zero. Although there was a considerable change for cryptoxanthin, the smoking-adjusted concentration among cases was still \( 1.0\% \) lower among cases than among controls.

In summary, several antioxidant micronutrients detectable in the serum are associated with the subsequent risk of lung cancer whereas others appear not to be associated. In this study, retinol was not included among the analytes because earlier studies had shown that serum levels in cases and controls were essentially the same (8, 9, 59). Findings that high serum concentrations of \( \beta \)-carotene are associated with protection against
lung cancer have been well-nigh universal, although recent controlled trials have demonstrated that \( \beta \)-carotene is not a protective factor against cancer promotion. Whether it protects against cancer initiation is not yet settled. Studies of the other carotenoids are too few to allow more than hints regarding their potential roles. \( \alpha \)-Carotene and cryptoxanthin could be protective, although their associations, like that with \( \beta \)-carotene, might merely reflect their correlation with some other potential causal factor. Also on the basis of sparse data, lutein/zeaxanthin controlled trials have demonstrated that \( \beta \)-carotene is not a protective agent that only some and not others have a protective association with lung cancer, and that cryptoxanthin, highly correlated with \( \beta \)-carotene, is the real protective factor in prospective observational studies (12-14). It might be that three controlled trials in which \( \beta \)-carotene failed to protect against lung cancer in spite of its consistent protective association with serum carotenoids assayed in this and similar studies are merely markers for some other potential protective substance.

The ability of serum to absorb (and neutralize) peroxyl radicals has not been studied previously in relation to the protective capacity of serum to protect against oxidative damage within cells? Are the antioxidants that protect against intracellular oxidative damage present in serum in such low concentrations that their contribution to peroxyl radical absorbing capacity is trivial compared to that of other antioxidants in the serum? A more general question is whether the antioxidant micronutrients assayed in this and similar studies are merely markers for some protective factor(s) that are not yet identified. The answer to this question may have been partially answered by the results of three controlled trials in which \( \beta \)-carotene failed to protect against lung cancer in spite of its consistent protective association in prospective observational studies (12-14). It might be that cryptoxanthin, highly correlated with \( \beta \)-carotene, is the real protective substance, although it would be foolishly hard to make such a claim on the basis of a single study. It is also possible that the protective association of a diet high in fruits and vegetables is due to the concomitant decrease in consumption of fat or iron-containing meats. However, if that were the case, one would expect to find lung cancer risk to be associated somewhat similarly with all of the serum carotenoids. The fact that only some and not others have a protective association points to the possibility that there is some other protective substance(s) in the major sources of these particular carotenoids, i.e., certain fruits and vegetables.

There is no quick and easy answer to identify the specific protective substance or substances in fruits and vegetables. Subsequent observational studies will continue to confirm or refute previously observed associations and to produce new ones. In some ways, replicated findings of no association are likely to be more useful than findings that an association exists. There can hardly be a causal relationship if there is no association. On the other hand, confirmed associations are most likely to be helpful as clues pointing to the real protective factor. Although the number and variety of antioxidants in fruits and vegetables is great, the more different clues we find that point toward the real protective factor, the easier it should be to identify it. In the meantime, we need to keep firmly in mind that smoking and a diet rich in fruits and vegetables can already do much in preventing cancer of the lung.

References


The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, alpha-tocopherol, selenium, and total peroxyl radical absorbing capacity.


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