Background: The generation of oxygen free radicals is involved in the pathogenesis of Alzheimer disease (AD).

Objective: To determine whether the intake of antioxidant vitamins decreases the risk of AD.

Methods: We investigated the relationship between AD and the intake of carotenes, vitamin C, and vitamin E in 980 elderly subjects in the Washington Heights-Inwood Columbia Aging Project who were free of dementia at baseline and were followed for a mean time of 4 years. Semiquantitative food frequency questionnaires were administered between baseline and the first follow-up visit. Cox proportional hazards regression models were conducted with quartiles of each vitamin intake as the exposure of interest and incident AD as the outcome, adjusted for age, level of education, sex, APOE ε4 status, ethnicity, and smoking.

Results: There were 242 incident cases of AD in 4023 person-years of follow-up (6 per 100 person-years). Intake of carotenes and vitamin C, or vitamin E in supplemental or dietary (nonsupplemental) form or in both forms, was not related to a decreased risk of AD. Trend tests for the association between quartiles of total intake of vitamins C and E also were not significant.

Conclusion: Neither dietary, supplemental, nor total intake of carotenes and vitamins C and E was associated with a decreased risk of AD in this study.

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Alzheimer disease (AD) affects nearly 50% of people older than 85 years. Although current treatments for AD have had modest success, measures to prevent or modify its development are needed. Measures that could delay the onset of AD by 5 years could halve its prevalence. This disease is a neurodegenerative process characterized by the deposition of amyloid β peptide in diffuse and neuritic plaques. The generation of reactive oxygen species may increase the damage that deposition of amyloid β peptide exerts on the brain. Antioxidants available in foods or in supplement form may prevent these processes. In light of the potential protective effect of antioxidant vitamins against AD, the press has promoted their use to enhance cognitive function and prevent cognitive decline. Moreover, physicians may also be prescribing vitamins to patients on the basis of their putative protective effect against neurodegenerative diseases. We therefore analyzed data from the Washington Heights-Inwood Columbia Aging Project (WHICAP) to test the hypothesis that dietary intake of antioxidant vitamins is associated with a lower risk of AD.

METHODS

STUDY POPULATION

Participants in the WHICAP cohort were obtained through random sampling of healthy Medicare beneficiaries aged 65 years or older residing within a geographically defined area of northern Manhattan (New York, NY). At entry, each subject underwent a structured in-person interview including an assessment of health and function, a standard medical history, physical and neurological examinations, and a neuropsychological battery. Subjects were recruited between 1991 and 1996 and received follow-up annually, repeating the baseline examination at each follow-up visit. Individuals who completed at least 1 year of follow-up were included in the analysis. A food frequency questionnaire was completed by 1422 individuals between baseline and the first follow-up examination. Of these, 230 were excluded because of prevalent dementia, 210 because of loss to follow-up, and 2 because of missing data for antioxidant vitamin intake. Thus, the analytic sample comprised 980 subjects.
DIAGNOSIS OF DEMENTIA AND COGNITIVE IMPAIRMENT

Diagnosis of dementia and assignment of specific cause were made by the consensus of a group of neurologists, psychiatrists, and neuropsychologists based on the information gathered at the initial and follow-up visits. The diagnosis of dementia was based on criteria from the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition11 and required evidence of cognitive deficits on the neuropsychological test battery as well as evidence of impairment in social or occupational function (score ≥0.5 on the Clinical Dementia Rating Scale12). Diagnosis of AD was based on criteria from the National Institute of Neurological and Communicative Disorders and Stroke–Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA).12 These criteria and diagnostic methods have been used extensively in the analysis of data in this cohort.8

DIETARY DATA

Dietary data were obtained using a 61-item version of the semiquantitative food frequency questionnaire developed by Willett et al13 (Channing Laboratory, Cambridge, Mass). This questionnaire was administered by telephone between the baseline and first follow-up examinations by trained interviewers in English or Spanish. Intakes of carotenes and vitamins C and E were classified as supplemental, nonsupplemental, and total (supplemental plus nonsupplemental). Intakes of vitamin C were measured in grams per day, and carotenes and vitamin E were measured in international units per day. Intakes were transformed using natural logarithm (vitamin E) or square root transformation (calories, carotenes, and vitamin C) to achieve a more normal distribution. Nonsupplemental intakes were adjusted for total caloric intake as recommended by Willett14 by calculating the residuals from linear regression models (nutrient intake regression on total caloric intake using transformed values for both nutrients and calories) and adding a constant (mean nutrient intake). Nutrient intakes from supplements were not adjusted for total caloric intake. The total nutrient intake was calculated by addition of the caloric-adjusted nonsupplemental intake and the non–calorie-adjusted supplemental nutrient intake. Semiquantitative food frequency questionnaires have been used and validated for the determination of nutrient intake in elderly people.15-19 The validity of the food frequency questionnaire used in the WHICAP cohort was assessed in a subsample of 78 individuals using two 7-day food records as the criterion. The intraclass correlations for energy-adjusted nutrients were 0.32 for vitamin A (carotenes and retinol), 0.57 for vitamin C, and 0.45 for vitamin E (M. Siddiqui, MPH, written communication, December 7, 2000). Intakes of carotenes and vitamin A were strongly correlated (r=0.91; P<.001); 86% of dietary vitamin A intake was represented by carotenes, and only carotene intake is considered in our analyses.

DEFINITION OF COVARIATES

Ethnic group was based on self-report using the format of the 1990 census.20 Individuals were also asked if they were of Hispanic origin. Participants were then assigned to 1 of 3 groups: African American (non-Hispanic), Hispanic, or white (non-Hispanic). Data on years of education and smoking status (current, ever, or never) were obtained by self-report. Smoking was included as a covariate because it has been reported to increase oxidative stress and may decrease the effect of antioxidants.21,22 APOE genotyping was determined using the method of Hixson and Vernier.23 Participants were classified as positive for the APOE ε4 allele genotype if they had 1 or 2 ε4 alleles.

RESULTS

We used χ² tests to compare the 2 highest vs the 2 lowest quartiles of vitamin intake with respect to sex, APOE ε4 status, and smoking. We used t tests to compare these groups with respect to mean age and number of years of education. Cox proportional hazards regression was used for multivariate analyses with the time-to-event variable in the models specified as time from baseline examination to the onset of dementia. Individuals with dementia not caused by AD were censored at the time of dementia onset. In light of the association reported between education and vitamin intake,24 the final model was stratified according to number of years of education using the STRATA statement from the SAS procedure PROC PHREG.25 Dietary intakes and other covariates were treated as baseline time constant covariates. Trend tests were conducted using Cox proportional hazards regression with each dietary intake variable, categorized into quartiles of intake; the P value for the coefficient of the dietary intake variable was used for the trend test.26 SAS version 7 statistical software (SAS Institute Inc, Cary, NC) for Windows was used for all analyses.

The mean age of the sample was 75.3±5.8 years; 67% were women, 25% were white, 43% were Hispanic, and 32% were African American. The median level of education was 9 years. Of the cohort, 28% were homozygous or heterozygous for the APOE ε4 allele, 16% were current smokers, and 33% were past smokers. There were 242 cases of incident AD and 18 cases of dementia associated with stroke during 4023 person-years of observation (6 cases per 100 person-years). The mean ± SD period of observation was 4.0±1.5 years.

No intake of carotene supplements was reported; 36% of the subjects reported a regular intake of vitamin C supplements (mean supplemental dose, 302 mg/d), and 34% reported a regular intake of vitamin E supplements (mean supplemental dose, 187 IU/d). The mean ± SD daily intake of carotenes was 121±88 IU. The mean ± SD daily intakes of nonsupplemental and total vitamins were 141±78 mg and 251±261 mg for vitamin C, and 6±4 IU and 70±152 IU for vitamin E, respectively.

Users of vitamin C supplements had more years of education than nonusers. Users of vitamin E supplements also reported more years of education, and compared with nonusers, this group had a lower proportion of Hispanic subjects. (Table 1).

Individuals in the highest 2 quartiles of carotene intake were more likely to be women (Table 2). The highest 2 quartiles of vitamin C intake had a higher proportion of women and a lower proportion of current smokers. The highest 2 quartiles of nonsupplemental vitamin E intake had a higher proportion of women, more years of education, a higher proportion of African American subjects, and a lower proportion of Hispanic subjects compared with individuals in the lowest 2 quartiles of vitamin E intake. Demographic differences were also present between the highest and lowest 2 quartiles of total (nonsupplemental plus supplemental) vitamin intake (Table 3).

The hazard ratios for intake of vitamin C (hazard ratio=0.75; 95% confidence interval [CI], 0.57-0.98) supplement adjusted for age and sex as compared with individuals not taking that supplement were related to a
decreased risk of AD. This association was attenuated and became nonsignificant after adjustment for age, sex, education, presence of the APOE ε4 allele, ethnicity, and smoking status (hazard ratio = 0.85; 95% CI, 0.64-1.13). Vitamin E supplement use was not associated with the risk of AD (hazard ratio = 0.91; 95% CI, 0.68-1.22). Analyses examining the cumulative effect of vitamin supplement intake revealed that the use of any combination of vitamin supplements was not related to a decreased risk of AD as compared with individuals not taking supplements. Analysis of calorie-adjusted dietary intake of antioxidant vitamins without supplements showed weaker and nonsignificant associations between higher intake and a lower risk of AD as compared with the lowest quartiles of intake (Table 4).

Analyses of total vitamin intake (supplement plus calorie-adjusted dietary intake) for vitamins C and E showed that the adjusted hazard ratios for AD in subjects in the highest quartiles of total intake were not significant (Table 5) compared with subjects in the lowest quartiles of intake of each vitamin. The adjusted hazard ratio for AD for individuals in the quartile of highest intake of total vitamin C as compared with individuals in the lowest quartile of intake was 0.71 and was close to statistical significance (95% CI, 0.71-1.04). However, the result of a trend test for the relationship between quartiles of total vitamin C intake and AD was not significant.

We repeated all analyses stratifying by APOE ε4 status, and the results were essentially the same as in the main analyses.

Our analyses of 242 cases of incident AD in 4023 person-years of observation found that the risk of AD was not associated with supplemental, dietary, or total intake of carotenoids, vitamin C, or vitamin E.

Several mechanisms by which oxidative stress contributes to neuronal degeneration in AD have been pos-

### Table 1. Comparison of Characteristics Between Users and Nonusers of Vitamin Supplements: Washington Heights–Inwood Columbia Aging Project, New York City, 1991-1996*

<table>
<thead>
<tr>
<th>Vitamin C</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplement (n = 355)</td>
<td>No Supplement (n = 625)</td>
</tr>
<tr>
<td>Female sex</td>
<td>68.7</td>
</tr>
<tr>
<td>White</td>
<td>28.2</td>
</tr>
<tr>
<td>African American</td>
<td>31.6</td>
</tr>
<tr>
<td>Hispanic</td>
<td>40.3</td>
</tr>
<tr>
<td>APOE ε4 allele</td>
<td>30.4</td>
</tr>
<tr>
<td>Current smoking</td>
<td>14.1</td>
</tr>
<tr>
<td>Age, mean, y</td>
<td>74.8</td>
</tr>
<tr>
<td>Education, median, y</td>
<td>10</td>
</tr>
<tr>
<td>Incidence of Alzheimer disease per 100 person-years</td>
<td>6.51</td>
</tr>
</tbody>
</table>

*Data are presented as percentage unless otherwise indicated.
†P < .05.
‡P < .01.

### Table 2. Comparison of Characteristics Between the Lowest 2 and Highest 2 Quartiles of Nonsupplemental Intake of Carotenoids and Vitamins C and E: Washington Heights–Inwood Columbia Aging Project, New York City, 1991-1996*

<table>
<thead>
<tr>
<th>Carotenoids</th>
<th>Vitamin C</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest Quartiles</td>
<td>Highest Quartiles</td>
<td>Lowest Quartiles</td>
</tr>
<tr>
<td>Age, mean, y</td>
<td>75.1</td>
<td>75.6</td>
</tr>
<tr>
<td>Female sex</td>
<td>63.9</td>
<td>70.4†</td>
</tr>
<tr>
<td>Education, median, y</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>African American</td>
<td>30.4</td>
<td>34.5</td>
</tr>
<tr>
<td>Hispanic</td>
<td>43.4</td>
<td>42.5</td>
</tr>
<tr>
<td>White</td>
<td>26.3</td>
<td>23.1</td>
</tr>
<tr>
<td>APOE ε4 allele</td>
<td>25.6</td>
<td>31.1</td>
</tr>
<tr>
<td>Smoking</td>
<td>17.5</td>
<td>13.9</td>
</tr>
<tr>
<td>Incidence of Alzheimer disease per 100 person-years</td>
<td>5.76</td>
<td>6.27</td>
</tr>
</tbody>
</table>

*Data are presented as percentage unless otherwise indicated.
†P < .05.
‡P < .01.
§P < .001.
tulated: protein oxidation, DNA oxidation, and lipid peroxidation. A key step in the pathogenesis of AD is the deposition of amyloid/β-42 in neuronal cells. Experimental evidence suggests that accumulation of amyloid β protein generates reactive oxygen species that are toxic to neurons. Oxidative stress has also been shown to promote the intracellular accumulation of amyloid β protein by enhancement of the amyloidogenic pathway. Thus, the intake of antioxidants in vitamin supplements and food could potentially modify the deposition or toxicity of amyloid β protein. Antioxidants that could potentially have an effect on AD and are widely available in food and supplements include vitamin E (α-tocopherol), vitamin C (ascorbic acid), and carotenes. Vitamin E protects against lipid peroxidation, vitamin C blocks the creation of nitrosamines by reducing nitrates, and carotenes interfere with lipid peroxidation.

There are conflicting cross-sectional data about the relationship between antioxidant vitamin intake and risk of AD. Data from longitudinal studies have been conflicting as well. One study involving 633 people found a lower risk of incident AD in users of vitamin C and E supplements, whereas another found a protective effect against vascular dementia but not AD. The Rotterdam Study recently reported that higher dietary intakes of vitamins C and E were associated with a lower risk of AD.

### Table 3. Comparison of Characteristics Between the Lowest 2 and Highest 2 Quartiles of Total Vitamin C and E Intake (Supplemental Plus Calorie-Adjusted Nonsupplemental Intake): Washington Heights-Inwood Columbia Aging Project, New York City, 1991-1996*

<table>
<thead>
<tr>
<th>Vitamin C</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowest Quartiles</td>
<td>Highest Quartiles</td>
</tr>
<tr>
<td>Lowest Quartiles</td>
<td>Highest Quartiles</td>
</tr>
</tbody>
</table>

*Data are presented as percentage unless otherwise indicated.
†P < .05
‡P < .001.
§P < .01.

### Table 4. Hazard Ratios and 95% Confidence Intervals for Alzheimer Disease for Subjects in Each Quartile of Calorie-Adjusted Intake of Carotenoids and Vitamins C and E, Using the Lowest Quartile as a Reference*

<table>
<thead>
<tr>
<th>Carotenoids</th>
<th>Vitamin C</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile of Intake</td>
<td>Quartile, Mean, IU</td>
<td>Adjusted for Age and Sex Full Model</td>
</tr>
<tr>
<td>First</td>
<td>65</td>
<td>1.00</td>
</tr>
<tr>
<td>Second</td>
<td>86</td>
<td>1.06 (0.73-1.53)</td>
</tr>
<tr>
<td>Third</td>
<td>110</td>
<td>1.13 (0.79-1.62)</td>
</tr>
<tr>
<td>Fourth</td>
<td>152</td>
<td>0.95 (0.66-1.38)</td>
</tr>
</tbody>
</table>

P for trend . . . . . . .88 . . . . . . .71 . . . . . . .83

*The full model is adjusted for age, sex, APOE ε4 allele presence, smoking status, and years of education. Ellipses indicate not applicable.

### Table 5. Hazard Ratios and 95% Confidence Intervals for Alzheimer Disease for Subjects in Each Quartile of Total (Supplemental and Dietary) Intake of Vitamins C and E, Using the Lowest Quartile as a Reference*

<table>
<thead>
<tr>
<th>Vitamin C</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile of Intake</td>
<td>Quartile, Mean, mg</td>
</tr>
<tr>
<td>First</td>
<td>79</td>
</tr>
<tr>
<td>Second</td>
<td>141</td>
</tr>
<tr>
<td>Third</td>
<td>203</td>
</tr>
<tr>
<td>Fourth</td>
<td>580</td>
</tr>
</tbody>
</table>

P for trend . . . . . . .38 . . . . . . .38

*The full model is adjusted for age, sex, APOE ε4 allele presence, smoking status, and years of education. Ellipses indicate not applicable.
and the Chicago Health and Aging Project (CHAP) reported an association between a higher intake of dietary vitamin E and a lower risk of AD but no association between total (dietary and supplemental) vitamin E intake and AD.42 Our study had an older cohort (>65 years vs >55 years), a smaller sample (980 vs 5395 subjects), and a shorter follow-up period (4 vs 6 years) compared with the Rotterdam Study. Consistent with that study, we found no association between beta carotene intake and incident dementia. It is possible that the discrepancy between our results and those of the Rotterdam Study regarding vitamins C and E is because the latter examined a period of exposure in which the prevention of disease was more likely than in an older cohort, and the longer follow-up time may also have allowed for a better opportunity to find an association. The CHAP study had a similar age group and follow-up period to our study, and the sample was slightly smaller (980 vs 815 subjects). Our results are consistent with the CHAP study in finding no association between vitamin C and beta carotene intake and incident AD. Compared with the CHAP study, our correlation coefficient for vitamin E intake was more modest (0.45 vs 0.63). It is possible that higher measurement error for the intake of vitamin E in our study biased the results to the finding of no association. Although the consistency of the results of the CHAP and Rotterdam studies regarding vitamin E is compelling, the findings were more different than they were alike. The CHAP study did not find an association between vitamin C intake and AD. Stratified analyses in the CHAP study revealed that the association between vitamin E intake and AD was restricted to individuals without the APOE ε4 allele, whereas the Rotterdam Study reported no effect modification. In addition, the results of the CHAP seem to be inconsistent with the original hypothesis relating antioxidant vitamin intake to AD. Those studies and ours attempted to aggregate and separate the effects of dietary and supplemental antioxidants. If the effects of antioxidants were specific to the vitamins studied, one would expect to find an effect for total intake (supplemental and dietary intake) if an effect were found for dietary intake. In fact, our study showed that vitamin E intake from supplements was significantly higher than intake from dietary sources. These discrepancies raise the possibility that some findings may be due to chance.43 It is also important to put the results of these studies in the context of past experience. Observational studies have reported associations of vitamin C and vitamin E intake with better cardiovascular outcomes not replicated in clinical trials.44 Randomized trials of the primary prevention of AD with antioxidants are needed. The largest randomized trial of antioxidants and AD involved secondary prevention in subjects with AD; subjects were randomized to selegiline, vitamin E, both, or placebo. There were no differences between the treatment groups in scores on cognitive scales, but there were delays in the loss of ability to perform activities of daily living, institutional placement, severe dementia, or death in the selegiline, vitamin E, and combined treatment groups compared with placebo.45 Measurement imprecision for the dietary variables may have affected our results. The finding of no association between intake of carotenoids, vitamin C, or vitamin E and risk of AD for the whole sample could be due to measurement imprecision or to a true lack of association with AD risk. The validity studies of semiquantitative food frequency questionnaires showed modest to moderate correlations with more quantitative recall methods. This imprecision in dietary assessment was probably greater for dietary than for supplemental vitamin intake. It is also possible that there are threshold effects and that some individuals in the cohort did not take the quantity of antioxidant needed to obtain an effect. The dose and duration of antioxidant treatment in the primary prevention of AD is unknown.46 According to the year 2000 dietary intake references issued by the Institute of Medicine, the recommended daily dietary intakes for individuals older than 70 years are 90 mg of vitamin C for men, 75 mg for women, and 15 mg of vitamin E for both men and women47; 84% of men and 65% of women had intakes of vitamin C at or higher than the recommended daily dietary intake, whereas 31% of individuals had daily intakes of vitamin E at or higher than the recommended values. The only reported randomized trial of vitamin E used a much higher dose than the intakes used in our study (2000 mg/d),48 and we cannot address whether intakes of antioxidants in this range may be associated with a lower risk of AD in individuals without dementia.

Our study has several notable strengths. The main purpose of the WHICAP cohort was the longitudinal study of dementia and determination of its risk factors, and all measurements were made prospectively with that intention. The vitamin intake data included supplemental as well as dietary sources and were obtained using an instrument with measured validity. Although the accuracy of this instrument for the dietary intake of vitamins appears to be limited, it has been validated previously and used widely in epidemiological studies.13,15-19 The determination of dementia was made in a standardized fashion according to widely accepted criteria. In addition, measures were available for other established risk factors for AD, including level of education and APOE ε4 status. The main limitation of our study pertains to the measurement of antioxidant vitamin intake. The food frequency questionnaire is a measure of habitual intake during 1 year and does not account for day-to-day variation or longer-term periods of intake. As previously discussed, this measure has limited accuracy. To the extent that this measurement error is random (ie, not related to AD incidence or covariates used to adjust the multivariate risk estimates for AD), this would reduce the statistical power and bias the magnitude of the observed protective effects toward the null.20 All subjects with even mild levels of dementia were excluded from this analysis, and therefore, differential measurement error is unlikely.

Higher intake of antioxidant vitamins at the usual levels, reported in our sample of elderly individuals without dementia at baseline, was not associated with a decrease in the risk of incident AD.

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Author contributions: Study concept and design (Drs Luchsinger and Mayeux); acquisition of data (Drs Tang and Mayeux); analysis and interpretation of data (Drs...
REFERENCES


