Dietary Intake of Antioxidants and Risk of Alzheimer Disease

Marianne J. Engelhart, MD, MSc
Mirjam I. Geerlings, PhD
Annemieke Ruitenberg, MD, PhD
John C. van Swieten, MD, PhD
Albert Hofman, MD, PhD
Jacqueline C. M. Witteman, PhD
Monique M. B. Breteler, MD, PhD

Context Laboratory findings have suggested that oxidative stress may contribute to the pathogenesis of Alzheimer disease. Therefore, the risk of Alzheimer disease might be reduced by intake of antioxidants that counteract the detrimental effects of oxidative stress.

Objective To determine whether dietary intake of antioxidants is related to risk of Alzheimer disease.

Design and Setting The Rotterdam Study, a population-based, prospective cohort study conducted in the Netherlands.

Participants A total of 5395 participants who, at baseline (1990-1993), were aged at least 55 years, free of dementia, and noninstitutionalized and had reliable dietary assessment. Participants were reexamined in 1993-1994 and 1997-1999 and were continuously monitored for incident dementia.

Main Outcome Measures Incidence of Alzheimer disease, based on *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition* (*DSM-III-R*) criteria and National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria, associated with dietary intake of beta carotene, flavonoids, vitamin C, and vitamin E.

Results After a mean follow-up of 6 years, 197 participants developed dementia, of whom 146 had Alzheimer disease. When adjustments were made for age, sex, baseline Mini-Mental State Examination score, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements, high intake of vitamin C and vitamin E was associated with lower risk of Alzheimer disease (rate ratios [RRs] per 1-SD increase in intake were 0.82 [95% confidence interval {CI}, 0.68-0.99] and 0.82 [95% CI, 0.66-1.00], respectively). Among current smokers, this relationship was most pronounced (RRs, 0.65 [95% CI, 0.37-1.14] and 0.58 [95% CI, 0.30-1.12], respectively) and also was present for intake of beta carotene (RR, 0.49 [95% CI, 0.27-0.92]) and flavonoids (RR, 0.54 [95% CI, 0.31-0.96]). The associations did not vary by education or apolipoprotein E genotype.

Conclusion High dietary intake of vitamin C and vitamin E may lower the risk of Alzheimer disease.

JAMA. 2002;287:3223-3229 www.jama.com

SEVERAL FINDINGS SUGGEST THAT oxidative stress may play an important role in the pathogenesis of Alzheimer disease. First, the brains of patients with Alzheimer disease contain lesions that are typically associated with exposure to free radicals.[1,2] In addition, oxidative stress in brains of Alzheimer patients is indicated by elevated cerebral levels of endogenous antioxidants that scavenge free radicals.[3] Moreover, in vitro studies suggest that exogenous antioxidants reduce the toxicity of β-amyloid in brains of Alzheimer patients.[1,2] Based on these findings, it has been hypothesized that antioxidants from food may reduce the risk of Alzheimer disease.

A previous randomized controlled trial[4] found that patients taking vitamin E supplement had a slower progression of Alzheimer disease than patients taking placebo. It is thus possible that high intake of antioxidants may also prevent the onset of dementia, because antioxidants may reduce neuronal loss due to oxidative damage.[1,2]

Two studies examined the longitudinal relationship between antioxidants from supplements and risk of Alzheimer disease.[5,6] These studies found conflicting results: vitamin C supplement use was related to lower risk of Alzheimer disease in one study,[5] whereas the other found no association for combined use of vitamin C and vitamin E supplements.[6] However, studies on supplement use are prone to bias, because people who use supplements may also have more health problems[7] and more health-seeking behavior.[8] In addition, use of supplements is generally of short duration.

To date, only 1 study has prospectively examined the association between dietary antioxidants and risk of dementia,[9] and found a reduced risk of dementia associated with increased intake of flavonoids. We investigated whether intake of a range of antioxi-

See also pp 3230 and 3261.

Author Affiliations: Department of Epidemiology and Biostatistics (Drs Engelhart, Geerlings, Ruitenberg, Hofman, Witteman, and Breteler), and Department of Neurology (Drs Ruitenberg and van Swieten), Erasmus Medical Center, Rotterdam, the Netherlands.
Corresponding Author and Reprints: Monique M. B. Breteler, MD, PhD, Department of Epidemiology and Biostatistics, Erasmus Medical Center, PO Box 1738, 3000 DR Rotterdam, the Netherlands (e-mail: breteler@epib.fgg.eur.nl).

©2002 American Medical Association. All rights reserved.

Downloaded from www.jama.com by guest on January 10, 2010

dants from food, namely beta carotene, flavonoids, vitamin C, and vitamin E, was associated with the risk of Alzheimer disease, using data from a population-based cohort study.

METHODS

The Rotterdam Study

The Rotterdam Study is a population-based, prospective cohort study of the frequency and determinants of neurological, cardiovascular, locomotor, and ophthalmologic diseases in elderly persons. The medical ethics committee of the Erasmus University Rotterdam approved the study. The eligible population comprised all inhabitants of a suburb in Rotterdam, the Netherlands, who were aged at least 55 years (n = 10 275). Of these, 7983 subjects (response rate, 78%) gave their written informed consent and participated in the study.[10]

During the baseline examination (1990-1993), a research assistant interviewed participants in their homes and obtained information on current and past health, medication, lifestyle, and risk factors for chronic diseases. In addition, participants visited the research center twice for baseline clinical examinations. Follow-up examinations took place in 1993-1994 and 1997-1999. The total cohort was continuously monitored for mortality and major morbidity.

Diagnosis of Dementia and Alzheimer Disease

Case-finding and diagnostic procedures for dementia and Alzheimer disease have been described in detail.[11] At baseline visit and both follow-up examinations, a 3-stage protocol was used. Participants were cognitively screened with the Mini-Mental State Examination (MMSE)[12] and the Geriatric Mental State schedule (GMS) organic level.[13] If subjects scored lower than 26 on the MMSE or higher than 0 on the GMS organic level, the Cambridge Examination of Mental Disorders in the Elderly (CAMDEX)[14] was administered. The CAMDEX also included an informant interview. Finally, participants in whom dementia was suspected were examined by a neurologist and neuropsychologist and, if possible, had magnetic resonance imaging of the brain. In addition, the total cohort was continuously monitored for incident dementia cases through computerized linkage between the study database and computerized medical records from general practitioners and the Regional Institute for Outpatient Mental Health Care.[11] The diagnoses of dementia and Alzheimer disease were based on *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition* (*DSM-III-R*)[15] criteria and the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria,[16] respectively, and were made by a panel of a neurologist (J.C.V.S.), neuropsychologist, and research physicians (M.J.E. and A.R.) who reviewed all existing information.[11] Follow-up with respect to dementia was virtually complete (99.9%).

Dietary Assessment

Dietary intake was assessed at baseline by means of a 2-stage protocol. Participants first indicated on a checklist all foods and drinks they had consumed at least twice a month during the preceding year. The checklist also contained questions on dietary habits, use of supplements, and prescribed diets. At the second baseline visit to the research center, the participants were interviewed on the basis of the completed checklist. This interview was performed by a dietitian, who used an extensive, validated semiquantitative food-frequency questionnaire (SFFQ).[17,18] The SFFQ data were converted to energy intake and nutrient intake using the computerized Dutch Food Composition Table.[19,20] We used data on intake of the antioxidants beta carotene, flavonoids, vitamin C, and vitamin E. Important sources of beta carotene are kale, carrots, broccoli, and spinach. Flavonoids are found in cranberries, green and black tea, and pulses. Vitamin C is mainly found in citrus fruits, kiwi, sprouts, broccoli, and cabbage. Important sources of vitamin E are grain, nuts, milk, and egg yolk. Daily dietary intake of the antioxidants from food was calculated in milligrams.

Other Variables

During the baseline home interview, participants were asked about their highest attained level of education and their smoking habits. At the visits to the research center, which were part of the baseline clinical examination, the MMSE was performed, height and weight were measured, and intake of alcohol, total energy, antioxidative supplements, total fat, and saturated fat were indicated on the SFFQ. Furthermore, ultrasonography of the carotid arteries was performed[21] and blood samples were drawn.

Level of education was categorized in 3 groups: low (primary education only); intermediate (lower vocational or general education); and high (intermediate or higher vocational or general education, college, or university). Smoking habits were categorized as never, former, and current smoking. For former and current smokers, the number of pack-years was defined as the number of years of smoking times the number of cigarettes smoked daily divided by 20. Alcohol intake was categorized in 5 groups: no alcohol intake, less than 1 drink per week, between 1 drink per week and 1 drink per day, between 1 and 4 drinks per day, and 4 drinks per day or more. Subjects who used beta carotene supplement, flavonoid supplement, vitamin C supplement, vitamin E supplement, or multivitamin supplement were classified as users of antioxidative supplements; all others were classified as nonusers. Intake of total and saturated fat was expressed in grams per day. Atherosclerotic plaques in the carotid arteries were defined as a focal widening relative to adjacent segments, with the protrusion into the lumen composed of either only calcified deposits or a combination of calcification and noncalcified material.[21] The presence of carotid plaques was assessed at 6 different locations: the common carotid artery, carotid bifurcation, and internal carotid artery at both left and right side.[21] Subsequently, according to the number of locations with plaques, 4 categories were made: plaques at 0, 1 to

 ©2002 American Medical Association. All rights reserved.

Downloaded from www.jama.com by guest on January 10, 2010

2, 3 to 4, and 5 to 6 locations. Apolipoprotein E (*APOE*) genotype was assessed on coded DNA samples using polymerase chain reaction without knowledge of the dementia diagnosis.[22] We dichotomized *APOE* genotype into presence or absence of the apolipoprotein E∗4 (*APOE∗4*) allele.

Study Sample

At the baseline clinical examination, 7525 participants of the Rotterdam Study were screened for dementia. Dementia was diagnosed in 482 participants, resulting in 7043 participants who were free of dementia at baseline. Of these, we excluded 602 participants from dietary assessment for 2 reasons. First, dietary intake was not assessed in 125 participants who had questionable cognitive status (<80 CAMDEX score), because the participants might provide unreliable answers regarding their food patterns. Second, we excluded nursing home residents (n=477), because their current diet may not reflect dietary habits in the past. Thus, 6441 participants were eligible for dietary assessment. Of these, reliable dietary data were missing in 1046 participants (16%) for several reasons. First, due to logical inconsistencies in dietary interviews, 212 participants were excluded. Second, because the SFFQ was administered at the second baseline visit to the research center, participants who did not complete the second visit did not have dietary assessment (n=192). Finally, 642 participants did not have dietary data due to logistic reasons. Thus, the sample comprised 5395 participants who had normal cognition, lived independently, and had reliable dietary assessment at baseline.

Eligible participants without dietary data were somewhat older (2.6 years) compared with participants with dietary data, a somewhat lower percentage (4%) were women and a higher percentage had only primary education (7%). Smoking habits and body mass index were similar across the 2 groups.

Data Analysis

To assess the relationship between intake of antioxidants from food and cognitive function at baseline, we performed linear regression analysis with antioxidant intake as the dependent variable and baseline MMSE score as independent variable. We adjusted for age, sex, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements.

To determine whether the incidences of Alzheimer disease differed between the sample and the eligible population with missing dietary data, we performed a Cox proportional hazards regression analysis with adjustment for age, sex, and education.

We evaluated the associations of daily dietary intake of antioxidants with risk of Alzheimer disease using Cox proportional hazards regression analysis. Intake of antioxidant was represented in the model either by a linear term or by 2 dummy variables representing the 2 highest tertiles. In the first case, the regression coefficient was expressed per SD increase. Standard deviations and tertiles of the respective intake of antioxidants were based on the distribution of the complete sample (N=5395). All analyses were initially adjusted for age and sex. Subsequently, additional adjustments were made for baseline MMSE score and alcohol intake, respectively. In another model, adjustments were simultaneously made for the following confounders: age, sex, baseline MMSE score, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements. Missing values were indicated by a missing indicator for categorical variables. For continuous variables, we replaced missing values by the mean or median of the study population, depending on the distribution. Age was used as the timescale in the model. Entry time was defined as age at study entry. Participants were followed up until onset of Alzheimer disease, onset of other types of dementia, death, or end of study, whichever came first. Age at onset of Alzheimer disease and other types of dementia was determined as the midpoint between the age of participant last known to be at risk of dementia and age at diagnosis of dementia.

To avoid confounding by supplement use, we also performed the analyses excluding users of antioxidative supplements (n=639). We investigated the combined effect of antioxidant intake from food and from supplements in an analysis in which users of an antioxidative supplement were added to the highest tertile of the corresponding antioxidant intake from food. For instance, users of beta carotene supplements were added to the highest tertile of beta carotene intake from food. Users of multivitamins were added to the highest tertile of each of the 4 antioxidant intakes from food, because multivitamins can contain more than 1 antioxidant and because multivitamins generally contain higher amounts of antioxidants than antioxidant intake from food.

All rate ratios (RRs) in the subsequent analyses were calculated in 1-SD increases in intake of antioxidants after adjustment for age, sex, baseline MMSE score, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements. Because low intake of total fat and saturated fat is related to both high intake of antioxidants[23] and risk of dementia,[24] the analyses were repeated with additional adjustments for total fat intake and saturated fat intake.

To examine possible effect modification by education, we performed stratified analysis by educational level. Furthermore, because smoking increases the load of free radicals and thus the extent of oxidative stress,[25] we also performed the analyses within strata of smoking habits. Because the *APOE∗4* allele is an important risk factor for Alzheimer disease[26] and is related to oxidative stress,[2] we also performed the analyses within strata of *APOE∗4* allele. In this latter analysis, 226 subjects, of whom no *APOE* genotype was available, were excluded.

©2002 American Medical Association. All rights reserved.

Downloaded from www.jama.com by guest on January 10, 2010

Table 1. Baseline Characteristics of the Sample (N = 5395)*

Characteristic	No. (%)
Age, mean (SD), y	67.7 (7.8)
Women	3183 (59)
Baseline MMSE score, median (interquartile range)	28 (27-29)
Alcohol intake	
None	1113 (21)
<1 drink/wk	1156 (21)
1 drink/wk – 1 drink/d	1518 (28)
1-4 drinks/d	1443 (27)
≥4 drinks/d	165 (3)
Educational level†‡	
Low	1854 (35)
Intermediate	1538 (28)
High	2003 (37)
Smoking‡	
Current	1257 (23)
Former	2305 (43)
Never	1808 (34)
Pack-years of smoking, median (interquartile range)	
Current	28 (14-42)
Former	18 (5-35)
Body mass index, mean (SD), kg/m^2	26.3 (3.7)
Intake, mean (SD)	
Total energy, cal/d	1975 (503)
Total fat, g/d	80.7 (26.5)
Saturated fat, g/d	34.4 (12.1)
Beta carotene, mg/d	1.53 (0.75)
Flavonoids, mg/d	28.5 (12.2)
Vitamin C, mg/d	121.6 (54.1)
Vitamin E, mg/d	13.8 (6.2)
Use of antioxidative supplements	639 (12)
No. of locations of carotid plaques‡	
0	1930 (43)
1-2	1551 (34)
3-4	782 (17)
5-6	284 (6)
Carrier of ≥1 *APOE**4* allele‡	1426 (28)

*MMSE indicates Mini-Mental State Examination; APOE, apolipoprotein E.
†Low education represents primary education only; intermediate education represents lower vocational or general education; high education represents intermediate or higher vocational or general education, college, or university.
‡Proportion is calculated on the basis of actual number of individuals with data on this variable.

Finally, to ensure that observed associations were not the result of changing dietary habits due to subclinical dementia, we excluded all subjects with less than 2 years of follow-up (n=212). Statistical interactions were tested by adding a product term to the unstratified model. Because the antioxidative effects of vitamin C and vitamin E might be synergistic,[27] statistical interaction was also tested between vitamin C and vitamin E intake. All data analyses were performed using SAS statistical software version 6.12 (SAS Institute Inc, Cary, NC). We used a significance level of .05 based on a 2-sided test.

RESULTS

TABLE 1 presents the baseline characteristics of the sample. At baseline, the mean age was 67.7 years, the majority (59%) were women (n=3183), 23% (n=1257) were current smokers, 12% (n=639) used antioxidative supplements, and 28% (n=1426) carried at least 1 *APOE**4* allele.

Intake of flavonoids was significantly greater with a higher baseline MMSE score; every point increase on the MMSE-score intake of flavonoids increased with 0.24 mg/d (regression coefficient, 0.24 [95% confidence interval {CI}, 0.031-0.45]). Intake of beta carotene, vitamin C, and vitamin E were not associated with baseline MMSE score (regression coefficient, 0.009; 95% CI, –0.004 to 0.022 for beta carotene; –0.19; 95% CI, –1.12 to 0.75 for vitamin C; and 0.059; 95% CI, –0.034 to 0.15 for vitamin E).

After baseline dietary assessment, participants were followed up for an average of 6 years (32341 person-years of follow-up). During this period, 197 participants developed dementia, of whom 146 had Alzheimer disease (134 without and 12 with cerebrovascular disease). The incidence of Alzheimer disease did not differ between the sample and the eligible population with missing data on dietary intake; when adjustments were made for age, sex, and education, the RR for subjects with dietary data compared with subjects without dietary data was 0.75 (95% CI, 0.54-1.05).

TABLE 2 shows the RRs of Alzheimer disease associated with intake of antioxidants per SD increase. When adjustments were made for age and sex; age, sex, and baseline MMSE score; or age, sex, and alcohol intake, intake of beta carotene, flavonoids, or vitamin E was not related to risk of Alzheimer disease. High intake of vitamin C had a borderline significant association with risk of Alzheimer disease in all models. When additional adjustments were made for education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements, high intake of vitamin C was significantly related to reduced risk of Alzheimer disease: the RR per SD increase was 0.82 (95% CI, 0.68-0.99). For vitamin E, the inverse relationship was of borderline significance (RR, 0.82; 95% CI, 0.66-1.00). The results for beta carotene and flavonoids did not change after extensive adjustment. Ex-

Table 2. Adjusted Rate Ratios of Alzheimer Disease per SD Increase in Dietary Antioxidant Intake*

		Adjusted Rate Ratio (95% Confidence Interval)				
Antioxidant	SD, mg/d	Age and Sex	Age, Sex, and Baseline MMSE Score	Age, Sex, and Alcohol Intake	Fully Adjusted Model†	Supplement Users Excluded (n = 4756) (128 Cases)‡
Beta carotene	0.75	0.88 (0.72-1.08)	0.88 (0.71-1.08)	0.88 (0.72-1.08)	0.87 (0.70-1.09)	0.81 (0.63-1.03)
Flavonoids	12.2	0.98 (0.83-1.16)	0.99 (0.83-1.17)	0.98 (0.82-1.15)	0.99 (0.83-1.18)	0.98 (0.81-1.19)
Vitamin C	54.1	0.85 (0.71-1.01)	0.83 (0.69-1.00)	0.85 (0.71-1.01)	0.82 (0.68-0.99)	0.83 (0.68-1.01)
Vitamin E	6.2	0.90 (0.76-1.08)	0.90 (0.75-1.07)	0.91 (0.76-1.08)	0.82 (0.66-1.00)	0.84 (0.68-1.05)

*MMSE indicates Mini-Mental State Examination.
†Adjusted for age, sex, baseline MMSE score, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements.
‡Adjusted for age, sex, baseline MMSE score, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, and presence of carotid plaques.

©2002 American Medical Association. All rights reserved.

Downloaded from www.jama.com by guest on January 10, 2010

clusion of supplement users did not substantially alter the results (Table 2).

TABLE 3 presents RRs of Alzheimer disease across tertiles of antioxidant intake. When adjustments were made for age and sex only, antioxidant intake was not related to Alzheimer disease. However, in the fully adjusted model, higher intake of vitamin E was significantly associated with lower risk of Alzheimer disease, and higher intake of vitamin C had a borderline significant association with lower risk of Alzheimer disease. For vitamin C, the RR of highest compared with lowest tertile was 0.66 (95% CI, 0.44-1.00) and for vitamin E, it was 0.57 (95% CI, 0.35-0.91). Beta carotene and flavonoids were not associated with Alzheimer disease across tertiles of intake. Adding supplement users to the highest tertile of dietary intake did not change the results for any of the 4 antioxidants. When we performed the analyses with additional adjustments for total fat intake or saturated fat intake, respectively, the results were similar.

TABLE 4 shows the relationship between antioxidant intake and risk of Alzheimer disease across strata of education. The risk of Alzheimer disease for beta carotene, flavonoids, and vitamin E did not significantly differ across strata of education and none of the statistical interaction terms was significant. High vitamin C intake was related to a lower risk of Alzheimer disease within participants with intermediate level of education. However, the statistical interaction was not significant.

TABLE 5 presents associations of antioxidants with risk of Alzheimer disease across strata of smoking habits. The risk of Alzheimer disease associated with higher intake of vitamin C and vitamin E was lower in current smokers compared with former and never smokers, but the respective statistical interaction terms were not significant. For beta carotene, statistical interaction with smoking habits was significant and of borderline significance for flavonoids: high intake of beta carotene and flavonoids was associated with reduced risk of Alzheimer disease in current smokers.

Table 3. Adjusted Rate Ratios of Alzheimer Disease Across Tertiles of Antioxidant Intake*

	Tertile of Antioxidant Intake		
	1	2	3
Beta Carotene			
Intake, mg/d	<1.22	1.22-1.67	>1.67
No. of cases	62	49	35
Model 1, RR (95% CI)	1.00	0.95 (0.66-1.39)	0.85 (0.56-1.30)
Model 2, RR (95% CI)	1.00	0.94 (0.64-1.38)	0.85 (0.55-1.30)
Flavonoids			
Intake, mg/d	<22.6	22.6-32.7	>32.7
No. of cases	47	44	55
Model 1, RR (95% CI)	1.00	0.83 (0.55-1.26)	1.00 (0.68-1.49)
Model 2, RR (95% CI)	1.00	0.84 (0.55-1.28)	1.03 (0.68-1.55)
Vitamin C			
Intake, mg/d	<95	95-133	>133
No. of cases	57	48	41
Model 1, RR (95% CI)	1.00	0.78 (0.53-1.15)	0.71 (0.48-1.07)
Model 2, RR (95% CI)	1.00	0.76 (0.51-1.12)	0.66 (0.44-1.00)
Vitamin E			
Intake, mg/d	<10.5	10.5-15.5	>15.5
No. of cases	56	55	35
Model 1, RR (95% CI)	1.00	1.15 (0.79-1.67)	0.70 (0.46-1.08)
Model 2, RR (95% CI)	1.00	1.03 (0.70-1.51)	0.57 (0.35-0.91)

*RR indicates rate ratio; CI, confidence interval. Model 1 was adjusted for age and sex. Model 2 was adjusted for age, sex, baseline Mini-Mental State Examination score, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements.

Table 4. Adjusted Rate Ratios of Alzheimer Disease per SD Increase in Dietary Antioxidant Intake Across Strata of Education*

		Education, Rate Ratio (95% Confidence Interval)†			
Antioxidant	SD, mg/d	Low (n = 1854) (82 Cases)	Intermediate (n = 1538) (26 Cases)	High (n = 2003) (38 Cases)	*P* Value‡
Beta carotene	0.75	0.89 (0.66-1.19)	0.98 (0.66-1.44)	0.70 (0.44-1.13)	.69
Flavonoids	12.2	1.07 (0.85-1.36)	0.91 (0.58-1.43)	0.91 (0.65-1.28)	.34
Vitamin C	54.1	0.92 (0.73-1.16)	0.50 (0.30-0.85)	0.80 (0.55-1.16)	.44
Vitamin E	6.2	0.81 (0.61-1.09)	0.86 (0.52-1.42)	0.76 (0.51-1.14)	.62

*Adjusted for age, sex, baseline Mini-Mental State Examination score, alcohol intake, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements.
†Low education represents primary education only; intermediate, lower vocational or general education; high, intermediate or higher vocational or general education, college, or university.
‡Statistical interaction of intake with education.

Table 5. Adjusted Rate Ratios of Alzheimer Disease per SD Increase in Dietary Antioxidant Intake Across Strata of Smoking Habits*

		Smokers, Rate Ratio (95% Confidence Interval)			
Antioxidant	SD, mg/d	Never (n = 1808) (64 Cases)	Former (n = 2305) (58 Cases)	Current (n = 1257) (22 Cases)	*P* Value†
Beta carotene	0.75	0.77 (0.53-1.11)	1.09 (0.87-1.38)	0.49 (0.27-0.92)	.03
Flavonoids	12.2	1.04 (0.80-1.36)	1.07 (0.81-1.42)	0.54 (0.31-0.96)	.05
Vitamin C	54.1	0.83 (0.62-1.10)	0.91 (0.69-1.19)	0.65 (0.37-1.14)	.35
Vitamin E	6.2	0.98 (0.71-1.35)	0.77 (0.56-1.06)	0.58 (0.30-1.12)	.21

*Adjusted for age, sex, baseline Mini-Mental State Examination score, alcohol intake, education, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements.
†Statistical interaction of intake with smoking habits.

©2002 American Medical Association. All rights reserved.

Downloaded from www.jama.com by guest on January 10, 2010

Table 6. Adjusted Rate Ratios of Alzheimer Disease per SD Increase in Dietary Antioxidant Intake According to *APOE* Genotype*

		APOE∗4* Allele, Rate Ratio (95% Confidence Interval)		
Antioxidant	SD, mg/d	None (n = 3743) (75 Cases)	At Least 1 (n = 1426) (68 Cases)	*P* Value†
Beta carotene	0.75	0.95 (0.72-1.25)	0.73 (0.49-1.09)	.62
Flavonoids	12.2	0.95 (0.73-1.23)	1.04 (0.81-1.34)	.61
Vitamin C	54.1	0.89 (0.69-1.15)	0.74 (0.55-1.00)	.90
Vitamin E	6.2	0.82 (0.61-1.09)	0.74 (0.54-1.02)	.82

*Adjusted for age, sex, baseline Mini-Mental State Examination score, alcohol intake, education, smoking habits, pack-years of smoking, body mass index, total energy intake, presence of carotid plaques, and use of antioxidative supplements.
†Statistical interaction of intake with *APOE* (apolipoprotein E) genotype.

TABLE 6 shows the relationship between intake of antioxidants and risk of Alzheimer disease across strata of *APOE*∗*4* allele. In participants with at least 1 *APOE*∗*4* allele, higher intake of 3 of the 4 antioxidants (except for flavonoids) was associated with somewhat lower risk of Alzheimer disease compared with the risk of Alzheimer disease in participants without an *APOE*∗*4* allele. However, statistical interactions of intake with *APOE* genotype were not significant.

We observed no statistical interaction between vitamin C intake and vitamin E intake with Alzheimer disease. Finally, restriction of the analyses to participants with at least 2 years of follow-up did not substantially change the results: RRs per SD increase in intake were 0.80 (95% CI, 0.61-1.06) for beta carotene, 0.95 (95% CI, 0.77-1.18) for flavonoids, 0.82 (95% CI, 0.66-1.03) for vitamin C, and 0.85 (95% CI, 0.66-1.08) for vitamin E.

COMMENT

We found that high intake of vitamin C and vitamin E from food may be associated with a lower incidence of Alzheimer disease after a mean follow-up period of 6 years. The risk reduction associated with intake of all 4 antioxidants was consistently largest for current smokers, although the differences in RRs for beta carotene and flavonoids between smokers and nonsmokers were of marginal statistical significance, while those for vitamin C and vitamin E were not significant. Nonetheless, these associations persisted after controlling for a number of potentially confounding variables, such as use of vitamin supplements, education, and alcohol use.

Before interpreting the results, some methodological issues should be considered. First, although we adjusted for a large number of potential confounding factors, such as age, sex, alcohol intake, education, smoking habits, and use of supplements, the possibility of residual confounding can never be completely excluded from an observational study. Second, we cannot completely exclude the possibility of subclinical dementia at time of dietary assessment, which may have led to changes in dietary reporting or dietary habits. To minimize this potential source of confounding, we excluded cognitively impaired subjects and adjusted for baseline MMSE score. In addition, we also recomputed the results after excluding the first 2 years of follow-up, which did not alter the results. Thus, we do not think that our results were affected by the presence of subclinical dementia. Third, because dietary assessment was performed only once, it may not have precisely reflected the participants' long-term dietary habits, which are more likely to influence disease risk. However, this may have led to dilution and thus an underestimation of the associations of antioxidants with risk of Alzheimer disease. Finally, we cannot completely rule out the possibility of confounding by use of dietary supplements. Although only a small number of participants reported supplement use, we do not have data on duration of use and dosage of the antioxidative supplements. Nonetheless, our results were unchanged after either excluding supplement users from the analysis or after controlling for supplement use, suggesting that our results are not confounded by supplement use.

The strengths of our study are its prospective design and the population-based setting. Another important feature is that follow-up with dementia diagnosis was virtually complete, and thus there was no resulting selection bias.

Several studies have examined the relationship between Alzheimer disease and intake of vitamin C and vitamin E from supplements.[4-6,28,29] A case-control study[28] and a prospective study in men[6] showed no association between supplement intake and Alzheimer disease. Another prospective study found that use of supplements, in particular vitamin C but not vitamin E, was associated with a lower risk of Alzheimer disease.[5] The only controlled trial of supplemented antioxidant intake and Alzheimer disease was performed within patients who were already diagnosed with Alzheimer disease.[4] This study reported that patients who took vitamin E supplements had a slower progression of the disease than patients who took placebo.

Results on supplement use and risk of dementia, however, may not be comparable with results on intake from food for several reasons. First, supplement users are generally a select group of persons with either health problems[7] or more health-seeking behaviors.[8] Therefore, associations between supplement use and Alzheimer disease may be biased. Second, intake of antioxidants from food reflects long-term intake, whereas supplement intake is generally of shorter duration. If duration of antioxidant intake is more important than the dose, high-lifetime intake from food would more likely be related to Alzheimer disease than short-term high intake by supplements. Finally, antioxidants from food are always simultaneously consumed with other nutrients in a certain proportion, whereas antioxidants from supplements are consumed in a very high dose either with or without other substances. This might lead to differences in absorption or biological activ-

©2002 American Medical Association. All rights reserved.

Downloaded from www.jama.com by guest on January 10, 2010

ity between antioxidants from food and antioxidants from supplements, though little is yet known on these issues.[30]

Previously, the relationship between intake of flavonoids from food and risk of dementia has been studied.[9] This prospective study found that high flavonoid intake was significantly associated with a lower risk of dementia. However, the response rate for dietary assessment was relatively low, only a small part of the study population underwent a detailed dietary assessment, and confounding or effect modification by smoking was not examined.

In our study, risk of Alzheimer disease associated with vitamin C and vitamin E was lowest in current smokers and beta carotene, and flavonoids seemed inversely related to Alzheimer disease in current smokers only. Because smoking itself is associated with increased risk of Alzheimer disease,[31] high antioxidant intake may partly counteract the excess risk of Alzheimer disease for smokers. This is supported by the finding of smokers' increased load of free radicals,[25] which may be reduced by antioxidants.

Several biological mechanisms could explain a possible relationship between antioxidants from food and Alzheimer disease. First, antioxidants may decrease the level of oxidative stress in the brain. Antioxidants may thereby reduce the amount of DNA damage, neuronal cell death, and the aggregation of β-amyloid within the brain.[1,2] These phenomena are all important neuropathological features in Alzheimer disease; by preventing the genesis of these features, the risk of dementia might be reduced. Second, because Alzheimer disease is associated with both cardiovascular risk factors and atherosclerosis,[32,33] and oxidative processes are involved in atherogenesis,[34] high intake of antioxidants could also decrease the risk of dementia by reducing the risk of atherosclerosis. However, because additional adjustment for carotid plaques as a measure of atherosclerosis did not change our results, we doubt that atherosclerosis is an important intermediary in the relationship between antioxidants and risk of Alzheimer disease.

In conclusion, our results suggest that higher intake of vitamin C and vitamin E from food may be associated with a lower risk of Alzheimer disease. Whether this reflects a causal association remains to be elucidated. Randomized controlled trials can help evaluate a possible causal relationship between antioxidant intake from supplements and risk of Alzheimer disease. However, the effect of short-term supplement use in clinical trials may not be comparable with long-term intake from dietary sources. Therefore, more cohort studies are needed to further investigate the relationship between dietary antioxidant intake and risk of Alzheimer disease.

Author Contributions: *Study concept and design:* Hofman, Breteler.
Acquisition of data: Engelhart, Ruitenberg, van Swieten, Breteler.
Analysis and interpretation of data: Engelhart, Geerlings, Witteman, Breteler.
Drafting of the manuscript: Engelhart, Geerlings.
Critical revision of the manuscript for important intellectual content: Engelhart, Geerlings, Ruitenberg, van Swieten, Hofman, Witteman, Breteler.
Statistical expertise: Engelhart, Geerlings, Witteman, Breteler.
Obtained funding: Hofman, Breteler.
Study supervision: Breteler.
Funding/Support: This study was supported by the Netherlands Organization for Scientific Research (NWO), the Health Research Development Council, the municipality of Rotterdam, and an unrestricted grant from Numico Research BV, the Netherlands. Dr Breteler is a fellow of the Royal Netherlands Academy of Arts and Sciences and is supported by Alzheimer Association grant NR IIRG-99-1534.

REFERENCES

1. Behl C. Amyloid beta-protein toxicity and oxidative stress in Alzheimer disease. *Cell Tissue Res.* 1997; 290:471-480.
2. Christen Y. Oxidative stress and Alzheimer disease. *Am J Clin Nutr.* 2000;71:621S-629S.
3. Grundman M. Vitamin E and Alzheimer disease. *Am J Clin Nutr.* 2000;71:630S-636S.
4. Sano M, Ernesto C, Thomas RG, et al. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer disease. *N Engl J Med.* 1997; 336:1216-1222.
5. Morris MC, Beckett LA, Scherr PA, et al. Vitamin E and vitamin C supplement use and risk of incident Alzheimer disease. *Alzheimer Dis Assoc Disord.* 1998; 12:121-126.
6. Masaki KH, Losonczy KG, Izmirlian G, et al. Association of vitamin E and C supplement use with cognitive function and dementia in elderly men. *Neurology.* 2000;54:1265-1272.
7. Bender MM, Levy AS, Schucker RE, Yetley EA. Trends in prevalence and magnitude of vitamin and mineral supplement usage and correlationship with health status. *J Am Diet Assoc.* 1992;92:1096-1101.
8. Kirk SF, Cade JE, Barrett JH, Conner M. Diet and lifestyle characteristics associated with dietary supplement use in women. *Public Health Nutr.* 1999;2:69-73.
9. Commenges D, Scotet V, Renaud S, et al. Intake of flavonoids and risk of dementia. *Eur J Epidemiol.* 2000;16:357-363.
10. Hofman A, Grobbee DE, de Jong PTVM, van den Ouweland FA. Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol.* 1991;7:403-422.
11. Ott A, Breteler MMB, van Harskamp F, Stijnen T, Hofman A. Incidence and risk of dementia: the Rotterdam Study. *Am J Epidemiol.* 1998;147:574-580.
12. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res.* 1975;12:189-198.
13. Copeland JRM, Kelleher MJ, Kellett JM, et al. A semi-structured clinical interview for the assessment of diagnosis and mental state in the elderly. *Psychol Med.* 1976; 6:439-449.
14. Roth M, Tym E, Mountjoy CQ, et al. CAMDEX: a standardised instrument for the diagnosis of mental disorder in the elderly with special reference to the early detection of dementia. *Br J Psychiatry.* 1986; 149:698-709.
15. *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition*. Washington, DC: American Psychiatric Association; 1987.
16. McKhann G, Drachman D, Folstein M, et al. Clinical diagnosis of Alzheimer disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer Disease. *Neurology.* 1984;34:939-944.
17. Goldbohm RA, van den Brandt PA, Brants HA, et al. Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr.* 1994;48:253-265.
18. Klipstein-Grobusch K, den Breeijen JH, Goldbohm RA, et al. Dietary assessment in the elderly. *Eur J Clin Nutr.* 1998;52:588-596.
19. Food and Nutrition Council. *Dutch Food Composition Table (NEVO).* The Hague, the Netherlands: Voorlichtingsbureau voor de Voeding [in Dutch]; 1993.
20. Klipstein-Grobusch K, Geleijnse JM, den Breeijen JH, et al. Dietary antioxidants and risk of myocardial infarction in the elderly. *Am J Clin Nutr.* 1999; 69:261-266.
21. Bots ML, van Swieten JC, Breteler MMB, et al. Cerebral white matter lesions and atherosclerosis in the Rotterdam Study. *Lancet.* 1993;341:1232-1237.
22. Slooter AJ, Cruts M, Kalmijn S, et al. Risk estimates of dementia by apolipoprotein E genotypes from a population-based incidence study: the Rotterdam Study. *Arch Neurol.* 1998;55:964-968.
23. Ness AR, Powles JW. Fruit and vegetables, and cardiovascular disease. *Int J Epidemiol.* 1997;26:1-13.
24. Kalmijn S, Launer LJ, Ott A, et al. Dietary fat intake and the risk of incident dementia in the Rotterdam Study. *Ann Neurol.* 1997;42:776-782.
25. Duthie GG, Arthur JR, Beattie JA, et al. Cigarette smoking, antioxidants, lipid peroxidation, and coronary heart disease. *Ann N Y Acad Sci.* 1993;686:120-129.
26. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer disease in late onset families. *Science.* 1993;261:921-923.
27. Meydani M. Vitamin E. *Lancet.* 1995;345:170-175.
28. Broe GA, Henderson AS, Creasey H, et al. A case-control study of Alzheimer disease in Australia. *Neurology.* 1990;40:1698-1707.
29. Launer LJ. Is there epidemiologic evidence that anti-oxidants protect against disorders in cognitive function? *J Nutr Health Aging.* 2000;4:197-201.
30. Bronner F. Nutrient bioavailability, with special reference to calcium. *J Nutr.* 1993;123:797-802.
31. Ott A, Slooter AJ, Hofman A, et al. Smoking and risk of dementia and Alzheimer disease in a population-based cohort study. *Lancet.* 1998;351:1840-1843.
32. Hofman A, Ott A, Breteler MMB, et al. Atherosclerosis, apolipoprotein E, and prevalence of dementia and Alzheimer disease in the Rotterdam Study. *Lancet.* 1997;349:151-154.
33. Breteler MMB. Vascular risk factors for Alzheimer disease. *Neurobiol Aging.* 2000;21:153-160.
34. Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest.* 1991; 88:1785-1792.

©2002 American Medical Association. All rights reserved.

Downloaded from www.jama.com by guest on January 10, 2010