

# Reduced Risk of Alzheimer Disease in Users of Antioxidant Vitamin Supplements

## The Cache County Study

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**Background:** Antioxidants may protect the aging brain against oxidative damage associated with pathological changes of Alzheimer disease (AD).

**Objective:** To examine the relationship between antioxidant supplement use and risk of AD.

**Design:** Cross-sectional and prospective study of dementia. Elderly (65 years or older) county residents were assessed in 1995 to 1997 for prevalent dementia and AD, and again in 1998 to 2000 for incident illness. Supplement use was ascertained at the first contact.

**Setting:** Cache County, Utah.

**Participants:** Among 4740 respondents (93%) with data sufficient to determine cognitive status at the initial assessment, we identified 200 prevalent cases of AD. Among 3227 survivors at risk, we identified 104 incident AD cases at follow-up.

**Main Outcome Measure:** Diagnosis of AD by means of multistage assessment procedures.

**Results:** Analyses of prevalent and incident AD yielded similar results. Use of vitamin E and C (ascorbic acid) supplements in combination was associated with reduced AD prevalence (adjusted odds ratio, 0.22; 95% confidence interval, 0.05-0.60) and incidence (adjusted hazard ratio, 0.36; 95% confidence interval, 0.09-0.99). A trend toward lower AD risk was also evident in users of vitamin E and multivitamins containing vitamin C, but we saw no evidence of a protective effect with use of vitamin E or vitamin C supplements alone, with multivitamins alone, or with vitamin B-complex supplements.

**Conclusions:** Use of vitamin E and vitamin C supplements in combination is associated with reduced prevalence and incidence of AD. Antioxidant supplements merit further study as agents for the primary prevention of AD.

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**T**HE PUBLIC HEALTH THREAT of Alzheimer disease (AD) will grow as people live longer.<sup>1</sup> Consequently, strategies for the prevention of AD are important. Because judicious doses of antioxidant vitamin supplements are relatively nontoxic and may have wide-ranging health benefits, antioxidants may offer an attractive prevention strategy.

Antioxidants scavenge free radicals and other reactive oxygen species that damage cellular membranes, organelles, and macromolecules. Accumulation of reactive oxygen species may overwhelm the protective reserves of antioxidants in cells (oxidative stress). In neurons, which are especially vulnerable to free radical-mediated damage, these processes may be important in aging of the brain and the pathogenesis of AD.<sup>2</sup> Thus, intake of antioxidants in the diet or, more power-

fully, in nutritional supplements may be neuroprotective.<sup>3,4</sup>

Antioxidants may mitigate age-related cognitive decline,<sup>5-12</sup> and a randomized trial showed that selegiline hydrochloride or vitamin E may slow the progression of AD.<sup>13</sup> However, few epidemiologic studies have examined whether antioxidants may delay AD onset. Three prospective studies found lower risks of dementia or AD in participants consuming more dietary antioxidants.<sup>14-16</sup> Another study of 633 participants found no incident AD cases during 4 years among individuals who reported use of vitamin E or vitamin C (ascorbic acid) supplements at baseline,<sup>17</sup> while an investigation of 3385 men found reduced prevalence of vascular and mixed dementias, but not AD, among users of both vitamin E and C supplements.<sup>18</sup> These results contrast with a recent study showing no associa-

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tion between AD and antioxidant vitamin consumption in either dietary or supplement form.<sup>19</sup>

To extend these findings, we examined data from the Cache County Study, a large, population-based investigation of the prevalence and incidence of AD and other dementias in relation to genetic and environmental antecedents. Using both prevalence and incidence data, we analyzed the association of antioxidant supplement use and occurrence of AD.

## METHODS

### STUDY POPULATION

In 1995 the study enrolled 5092 elderly residents of Cache County, Utah (90% those aged 65 years and older). We obtained buccal DNA for determination of genotype at the gene for apolipoprotein E (APOE) from 97% of these respondents and used a multistage screening and assessment protocol to identify and diagnose prevalent cases of dementia (wave I). Three years later, between 1998 and 2000, we used similar procedures to identify and diagnose incident cases of dementia (wave II). The institutional review board of each collaborating site approved the study, and all participants and/or their collateral informants provided informed consent.

The assessment procedures for both waves have been detailed elsewhere.<sup>20,21</sup> Briefly, screening began with an interview that included the modified Mini-Mental State Examination (3MS)<sup>22,23</sup> or, for those unable to participate, a questionnaire administered to a collateral informant.<sup>24</sup> Individuals who did poorly on this screen (eg, scoring <87 on the 3MS at wave I) were examined further by telephone interview with a collateral informant by means of the Dementia Questionnaire (DQ).<sup>25</sup> All those older than 90 years plus a weighted stratified subsample of 19% of all participants were also studied with the DQ, regardless of their initial screening results. Participants with DQ scores of 4 or more at wave I or 3 or more at wave II, as well as all members of the 19% subsample, were then examined at their place of residence. The examination included a medical history, a chronologic history of cognitive symptoms, and a structured neurologic examination, all administered by specially trained nurses, and a 1-hour battery of neuropsychological tests administered by psychometric technicians. A geriatric psychiatrist (J.C.S.B.) and neuropsychologist (J.T.T.) reviewed these data and assigned working diagnoses of dementia (*Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition*<sup>26</sup>) or other cognitive syndromes. Participants with working diagnoses of prevalent or incident dementia were further examined by a geriatric psychiatrist (J.C.S.B.) and were referred for laboratory studies including neuroimaging. These and other participants with apparent cognitive compromise received an identical clinical assessment 18 months later to assess longitudinal course. A consensus panel of experts in neurology, geriatric psychiatry, neuropsychology, and cognitive neuroscience then reviewed all available data and assigned final differential diagnoses. Diagnoses of AD used standard criteria,<sup>27</sup> while diagnoses of other dementing illnesses were also made according to current research practice.<sup>20,21</sup>

Data from the fully examined 19% subsample suggested an overall sensitivity of 93% for the study's screening and examination protocol for detection of prevalent dementia<sup>28</sup> and 89% for the detection of incident dementia.<sup>29</sup> A comparison of dementia diagnoses with neuropathologic findings in 54 individuals suggested that the accuracy of prevalent and incident AD diagnoses was comparable to typical rates reported from university AD clinics (eg, positive predictive value of 90%; un-

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### PREVALENCE DATA

Among the initial 5092 wave I respondents, we identified 355 individuals with prevalent dementia. Of these, 200 received diagnoses of AD and no other dementing illness. Of the remaining respondents, 4540 individuals completed study procedures sufficiently to demonstrate that they were free of dementia at the completion of the wave I assessments. Of these, 719 were members of the fully examined 19% subsample, while 3821 others showed no evidence of dementia on screening and assessment measures and therefore received no further evaluation at wave I.

### INCIDENCE DATA

At the end of wave II assessments, we identified 185 participants with incident dementia, of whom 104 received diagnoses of AD and no other dementing illness. Another 3123 wave II participants completed study procedures sufficiently to show that they remained free of dementia. Of these, 394 were members of the 19% subsample and were thus examined directly, while 2729 others showed no evidence of dementia on screening measures.

During the course of the study, 1429 individuals were lost to follow-up. Of these, 627 (43.9%) died, while the other 802 (56.1%) refused to complete the protocol, moved out of the area, or could not be located. Individuals among the latter group were older ( $t = -5.92, P < .001$ ) and less educated ( $t = 5.29, P < .001$ ) and had performed less well on their cognitive screen with the 3MS ( $t = 6.21, P < .001$ ) than those who completed the protocol.

### EXPOSURE ASSESSMENT

At the initial visit of wave I, we administered a standardized interview covering suspected risk factors for dementia. The interview included sections on sociodemographic factors including education, occupational history, medical history, tobacco and alcohol use, and medication use. The section on medications asked participants about use during the preceding 2 weeks of any prescription or over-the-counter medications, including vitamin supplements. Interviewers then asked to see the containers of these treatments, and recorded information about the use of each.

We categorized participants according to their use of vitamin supplements, counting individuals as vitamin E users if they reported taking an individual supplement of vitamin E or a multivitamin preparation that contained more than 400 IU of vitamin E. Similarly, vitamin C users reported taking vitamin C supplements or multivitamin preparations containing at least 500 mg of ascorbic acid. Individuals classified as multivitamin users reported similar use of a multivitamin preparation containing lower doses of vitamin E or C. Finally, we classified individuals as users of vitamin B formulations if they took a supplement preparation that specifically contained a complex of multi-B vitamins (riboflavin, pyridoxine, cyanocobalamin, etc). We examined B vitamins and multivitamins as "control" exposures to assess the specificity of any association between use of higher doses of antioxidant supplements and risk of AD, and to differentiate this association from a tendency toward supplement use in general.

### STATISTICAL ANALYSES

We compared the sociodemographic characteristics of supplement users vs nonusers with  $\chi^2$  tests for categorical

**Table 1. Characteristics of the 4740 Elderly Participants in the Prevalence Data According to Vitamin Supplement Use at the Baseline (Wave I) Visit**

	Vitamin E or C*	Multivitamins	No Supplements†	Missing Data
No. (%)	824 (17.4)	967 (20.4)	2828 (59.7)	121 (2.6)
Women, No. (%)	500 (60.7)‡	619 (64.0)‡	1517 (53.6)	76 (62.8)
Mean (SD) age, y	74.2 (6.5)‡	75.9 (6.9)	75.4 (7.2)	76.6 (7.4)
Mean (SD) years of education, y	13.5 (2.9)‡	13.3 (2.8)	13.1 (2.9)	13.3 (2.8)
Fair or poor health, No. (%)	153 (18.6)‡	271 (28.0)‡	698 (24.7)	39 (32.2)
Current smoker, No. (%)	17 (2.1)	19 (2.0)	74 (2.6)	2 (1.7)
No. of $\epsilon 4$ alleles, No. (%)				
0	535 (64.9)	654 (67.6)	1894 (67.0)	77 (63.6)
1	259 (31.4)	263 (27.2)	803 (28.4)	36 (29.8)
2	21 (2.5)	31 (3.2)	73 (2.6)	2 (1.7)
Missing	9 (1.1)	19 (2.0)	58 (2.1)	6 (5.0)

\*The characteristics of vitamin E and vitamin C (ascorbic acid) users were very similar; we therefore combined them into one group for this comparison.

†No use of multivitamins, vitamin E, or vitamin C.

‡Difference compared with nonusers of a vitamin supplement significant at  $P < .05$ .

variables and 2-sample  $t$  tests for continuous variables. We then compared the odds of prevalent AD among current users vs nonusers of the various vitamin supplements by means of multiple logistic regression, building on a “base” model for AD prevalence that had previously been developed.<sup>20</sup> This model included terms for age, the squared deviation of age from the population mean, sex, education, dummy-coded terms for the presence of 1 and 2  $APOE \epsilon 4$  alleles, and interactions between age and the dummy-coded  $APOE \epsilon 4$  terms. We also added an indicator term for “fair” or “poor” health (self-rated at interview) that proved to be inversely associated with antioxidant supplement use (**Table 1**). We analyzed the incidence data by means of discrete-time survival analysis<sup>30</sup> to compare annual risks of developing AD during the wave I–wave II interval related to supplement use reported at baseline. The discrete-time approach considered each person-year of observation, with participants entering the analytic pool at the age of their initial wave I interview. Observations were considered year by year thereafter until participants either developed AD or underwent wave II screening without evidence of dementia. Adjusted hazard ratios with various exposures were estimated by means of discrete-time multiple logistic regression models, again relying on a base model developed previously<sup>21</sup> and containing covariates identical to the prevalence model. All logistic models were fitted using SAS version 8.0 or 8.1 software (SAS Institute Inc, Cary, NC). Parameter estimates are reported with 95% profile likelihood confidence intervals.

## RESULTS

More than 97% of participants provided sufficient exposure data to classify their supplement use (Table 1). The remainder tended to be female, older, and in poorer health. Of those who provided data, approximately 17% reported taking vitamin E or C supplements. Compared with nonusers, these individuals were significantly more likely to be female, younger, and better educated, and to report better general health. Another 20% of the participants reported use of multivitamins without high-dose vitamin E or C. These multivitamin users were also more likely to be female, but reported poorer general health. There were few current smokers overall and little difference in rates of smoking between supplement users and nonusers.

## PREVALENCE ANALYSES

In unadjusted analyses, use of vitamin E, vitamin C, and multivitamins were all inversely associated with prevalent AD (**Table 2**). However, after adjustment for age, sex, years of education, number of  $APOE \epsilon 4$  alleles,  $APOE \times$  age interaction terms, and general health status, the inverse association remained significant only for use of vitamin E and multivitamins. There was no association between use of B-complex vitamins and AD prevalence, either before or after adjusting for the covariates in the base model.

Of the participants who reported current use of vitamin E, vitamin C, or multivitamin supplements, more than a third took 2 or more of these in combination. We therefore examined the relationship between AD prevalence and the use of these supplements alone and in combination. After adjusting for the covariates in the base model, there was no appreciable association with the use of vitamin C alone, vitamin E alone, or vitamin C and multivitamins in combination. There was, however, a significant, if relatively modest, inverse association with the use of multivitamins alone, and a suggestion of a stronger inverse association with the combination of vitamin E and multivitamins (the broad confidence interval presumably reflecting relatively small numbers). By far, the strongest inverse association with AD prevalence was observed with use of both vitamin E and vitamin C, with or without concomitant use of multivitamins (multivariable adjusted odds ratio, 0.22; 95% confidence interval, 0.05–0.60).

## INCIDENCE ANALYSES

Any baseline (wave I) use of vitamin E was associated with a reduced AD incidence that fell just short of statistical significance (**Table 3**), either before or after adjustment for the covariates in the base model. However, the adjusted point estimate for risk was similar to the estimate obtained with prevalence data (adjusted hazard ratio, 0.53 vs 0.44). The incidence of AD was not associated with use of vitamin C, B-complex, or (in contrast to the prevalence analyses) multivitamins. When we ex-

**Table 2. Association Between Vitamin Supplement Use and Prevalent Alzheimer Disease**

	No. With AD/Total No.*	Unadjusted OR (95% CI)	Adjusted OR† (95% CI)
Any vitamin E			
No	186/4127	1.0	1.0
Yes	8/492	0.35 (0.15-0.67)	0.44 (0.19-0.86)
Any vitamin C (ascorbic acid)			
No	175/3951	1.0	1.0
Yes	19/668	0.63 (0.38-0.99)	0.80 (0.46-1.30)
Any multivitamins			
No	152/3219	1.0	1.0
Yes	42/1400	0.62 (0.44-0.88)	0.63 (0.43-0.90)
Any B-complex vitamins			
No	188/4430	1.0	1.0
Yes	6/189	0.74 (0.29-1.55)	1.05 (0.39-2.35)
Supplements in combination			
No vitamin E, C, or multivitamins	138/2828	1.0	1.0
Multivitamins, no vitamin E or C	32/967	0.67 (0.44-0.97)	0.60 (0.39-0.91)
Vitamin C, no vitamin E or multivitamins	9/146	1.28 (0.60-2.43)	1.47 (0.63-3.08)
Vitamin E, no vitamin C or multivitamins	4/73	1.13 (0.34-2.78)	1.15 (0.32-3.21)
Vitamin C and multivitamins, no vitamin E	7/186	0.76 (0.32-1.54)	0.99 (0.39-2.15)
Vitamin E and multivitamins, no vitamin C	1/83	0.24 (0.01-1.08)	0.34 (0.02-1.64)
Vitamins E and C‡	3/336	0.18 (0.04-0.47)	0.22 (0.05-0.60)

Abbreviations: AD, Alzheimer disease; CI, confidence interval; OR, odds ratio (estimated from logistic regression models).

\*Total does not include 121 participants in the prevalence sample who did not provide data on supplement use.

†Adjusted for age, the squared deviation of age from the population median, sex, education, dummy-coded terms for the presence of 1 and 2 apolipoprotein E  $\epsilon$ 4 alleles, interactions between age and the dummy-coded apolipoprotein E  $\epsilon$ 4 terms, and an indicator term for general health status.

‡Includes 164 individuals who also reported multivitamin use.

**Table 3. Association Between Vitamin Supplement Use and Incident Alzheimer Disease**

	No. With AD/Total Person-Years*	Unadjusted HR (95% CI)	Adjusted HR† (95% CI)
Any vitamin E			
No	93/8778	1.0	1.0
Yes	6/1172	0.48 (0.19-1.02)	0.53 (0.20-1.12)
Any vitamin C (ascorbic acid)			
No	88/8411	1.0	1.0
Yes	11/1539	0.68 (0.34-1.23)	0.74 (0.37-1.35)
Any multivitamins			
No	70/6818	1.0	1.0
Yes	29/3132	0.90 (0.58-1.38)	0.79 (0.50-1.22)
Any B-complex vitamins			
No	95/9490	1.0	1.0
Yes	4/460	0.87 (0.27-2.09)	0.94 (0.28-2.29)
Supplements in combination			
No vitamin E or C or multivitamins	64/5928	1.0	1.0
Multivitamins, no vitamin E or C	21/2127	0.91 (0.55-1.47)	0.77 (0.45-1.27)
Vitamin C, no vitamin E or multivitamins	3/312	0.89 (0.22-2.42)	1.25 (0.30-3.52)
Vitamin E, no vitamin C or multivitamins	2/159	1.17 (0.19-3.77)	1.20 (0.19-4.13)
Vitamin C and multivitamins, no vitamin E	5/411	1.13 (0.39-2.55)	0.94 (0.32-2.20)
Vitamin E and multivitamins, no vitamin C	1/197	0.47 (0.03-2.14)	0.47 (0.03-2.22)
Vitamins E and C‡	3/816	0.34 (0.08-0.92)	0.36 (0.09-0.99)

Abbreviations: AD, Alzheimer disease; CI, confidence interval; HR, hazard ratio (estimated from discrete-time survival analysis).

\*Total person-years does not include contributions from 75 participants in the incidence sample who did not provide supplement use data.

†Adjusted for age, the squared deviation of age from the population median, sex, education, dummy-coded terms for the presence of 1 and 2 apolipoprotein E  $\epsilon$ 4 alleles, interactions between age and the dummy-coded apolipoprotein E  $\epsilon$ 4 terms, and an indicator term for general health status.

‡Includes 125 individuals (397 person-years) who also reported multivitamin use.

amined the risks with respect to vitamin E, vitamin C, and multivitamin use alone or in combination, the results were again similar to those from the prevalence analyses. There was a trend toward reduced AD incidence with vitamin E and multivitamins in combination, and a greater apparent reduction with use of vitamin E and vitamin C

together (adjusted hazard ratio, 0.36; 95% confidence interval, 0.09-0.99). Use of multivitamins alone was not notably related to AD risk. We found no difference in degree of association between supplement use and AD risk across different strata of age, sex, or APOE genotype (data not shown).

These analyses from the Cache County Study examined the degree to which use of vitamin supplements, especially vitamins E and C, was associated with occurrence of AD. Both prevalence and incidence analyses suggested that use of vitamin E supplements is associated with reduced occurrence of AD. This inverse association with vitamin E appears attributable almost entirely to the use of vitamin E and C supplements in combination (the latter in single-agent supplements or in multivitamins). There was no notable reduction in risk of incident AD with vitamin E or vitamin C alone or with multivitamins. There was also no association between AD risk and use of B-complex vitamins.

The current Institute of Medicine recommended daily allowance for vitamin E is 22 IU (15 mg), and for vitamin C (ascorbic acid), 75 to 90 mg.<sup>31</sup> Multivitamin preparations typically contain these approximate quantities of both vitamins E and C (more vitamin C in some instances), while individual supplements typically contain doses up to 1000 IU of vitamin E and 500 to 1000 mg or more of vitamin C (ascorbic acid). Our findings suggest that vitamins E and C may offer protection against AD when taken together in the higher doses available from individual supplements. There may also be some protective effect with vitamin E combined with the lower doses of vitamin C typically in multivitamins, although small numbers of such users in this sample (n=197) limit the inference. Vitamin E, a lipid-soluble molecule, is one of the strongest nutritional antioxidants. Sufficient levels of vitamin E may reduce the oxidative stress-related damage associated with pathological changes of AD.<sup>2</sup> Because vitamin C is water soluble and rapidly excreted after ingestion,<sup>32</sup> its effect may be limited to the reduction of lipid-soluble vitamin E after the latter has been oxidized.<sup>33</sup> There is, therefore, some biological rationale for benefit from combining vitamin E and C dosage, as observed herein.

Two previous observational studies have examined the relationship between dementia and use of vitamin E and C supplements. The East Boston Study<sup>17</sup> found no incident AD among participants who reported use of vitamin E or vitamin C at baseline, but the adjusted risk reduction was statistically significant only for vitamin C use. However, more than half of those who used vitamin C also reported taking vitamin E. The East Boston results are therefore not inconsistent with our current findings. Our findings are also reminiscent of those from the Honolulu-Asia Aging Study, although that study observed a relationship of combined vitamin E and C use with vascular dementia but not AD.<sup>18</sup>

Four previous prospective studies<sup>14-16,19</sup> have considered the relationship between dementia and dietary antioxidant intake. Dietary sources typically provide much lower levels of antioxidants than individual supplements. Nonetheless, 3 of these studies found evidence suggesting reduced risk of dementia or AD with high dietary intake of antioxidants. One report noted inverse association of dementia with dietary intake of flavonoids (mostly from red wine).<sup>14</sup> Because few of our participants drank wine or took supplements with flavonoids, we could not assess this association.

A prospective study from the Chicago Health and Aging Project (CHAP) found reduced risk of AD associated with dietary intake of vitamin E, but not vitamin C.<sup>16</sup> Curiously, when vitamin E intake from supplement sources was considered in that study, the inverse association with AD disappeared. The authors noted a dramatic increase in vitamin E supplement use during the study's course. It is therefore possible that these supplements had not been used long enough to afford protection, or perhaps they were initiated in response to cognitive difficulties (reflecting recent reports of a beneficial effect<sup>13</sup>). By contrast, more than 97% of vitamin E and C users in Cache County reported use of these supplements for 2 or more years (such long-term use of antioxidants by Cache County participants precluded any meaningful analyses of the relationship between duration of supplement use and AD risk). The CHAP found no statistical interaction between vitamin E and C intake, modeled as continuous variables. Because our data suggest effect modification only with *supplement* levels of vitamin E and C, an interaction between the 2 might not be apparent with continuous intake estimates including lower dietary amounts. Finally, the CHAP suggested an inverse association with vitamin E only among participants lacking an *APOE ε4* allele. We have no explanation for our inability to reproduce this finding.

The Rotterdam Study<sup>15</sup> found dietary intake of vitamin E and, to a lesser extent, vitamin C to be inversely associated with AD, after controlling for antioxidant supplement use. Like the CHAP, this study saw no interaction between vitamin E and vitamin C use. It is unclear, however, whether such interaction was tested by means of continuous variable modeling, as discussed earlier. The greatest AD risk reductions in the Rotterdam Study appeared among individuals in the highest tertiles of vitamin E and C intake, and it is therefore possible that these groups were similar to our categories of vitamin E and C supplement users. Because there were few smokers in Cache County, we were unable to test the Rotterdam finding of accentuated inverse association of AD and antioxidant vitamin intake among current smokers.

The newest prospective study of antioxidant vitamins, from the Washington Heights-Inwood Columbia Aging Project,<sup>19</sup> did not find a significant reduction in AD risk with use of vitamin E or C from either supplemental or dietary sources, although the relative risk estimates associated with the highest levels of these vitamins were not inconsistent with previous reports. The authors of the Washington Heights-Inwood Columbia Aging Project report did not test for interaction between intake of vitamins E and C, although they did examine the cumulative effects of various vitamin supplements alone and in combination, reporting no reduction in AD risk with combined use. Unfortunately, no details of these analyses (undertaken post hoc) were provided, so it is difficult to find explanations for the difference between their findings and ours.

Our study has several strengths (eg, population-based, large sample, prospective design), but there are also potential limitations. The prevalence data were cross-sectional, and the incidence data covered only a rela-

### Other Cache County Study Investigators Involved in the Project

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tively short (3-year) period of follow-up. Consequently, our findings could represent spuriously reduced report of antioxidant vitamin use by participants with AD at the prevalence wave, or by those with prodromal cognitive difficulties that later “converted” to incident AD. To explore this potential problem, we reanalyzed the incidence data while controlling for baseline 3MS score, but we found no notable changes in the associations between supplement use and AD incidence. We therefore doubt that incipient cognitive difficulties at baseline provide a likely explanation for our findings.

Invariably, observational studies cannot exclude the possibility that inverse associations observed with AD simply reflect some circumstance, characteristic, or condition associated with vitamin use in general. For example, Cache County antioxidant supplement users were younger, more educated, and in better general health (all potentially associated with reduced AD risk) than nonusers. However, statistical control on these characteristics failed to attenuate the association between use of vitamin E and C in combination and AD risk reduction. Such control may still fail, however, to consider other, unmeasured confounders such as a tendency toward a healthy lifestyle among supplement users. We doubt that such unsuspected confounding fully explains our findings because (1) our relatively objective method of exposure classification should afford some protection against selective underreporting of use; (2) we saw little evidence of such confounding when we considered nutritional supplements such as multivitamins, calcium supplements,<sup>34</sup> and B-vitamin formulations; and (3) our relatively consistent (and biologically plausible) finding of strongest association with vitamins E and C combined appears to argue against a simple artifact as the source of our results.

In summary, our findings using both prevalence and incidence data from the large, population-based Cache County study suggest that antioxidant vitamins, specifically the combination of vitamin E and C supplements, may prevent AD. As is widely appreciated, formal proof of such an effect can come only from randomized prevention trials. If proven efficacious in such trials, antioxidant vitamins (believed to offer other health benefits<sup>35</sup>) would offer an attractive prevention strategy for AD. Formal demonstration of their efficacy would therefore have significant public health implications, and we suggest that prevention trials are warranted.

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**Author contributions:** Study concept and design (Drs Anthony, Welsh-Bohmer, and Breitner); acquisition of data (Drs Anthony, Tschanz, Norton, Welsh-Bohmer, and Breitner); analysis and interpretation of data (Drs Zandi, Anthony, Khachaturian, Stone, Gustafson, Welsh-Bohmer, and Breitner); drafting of the manuscript (Drs Zandi, Anthony, Khachaturian, Welsh-Bohmer, and Breitner); critical revision of the manuscript for important intellectual content (Drs Anthony, Khachaturian, Stone, Gustafson, Tschanz, Norton, Welsh-Bohmer, and Breitner); statistical expertise (Drs Zandi, Anthony, Khachaturian, Stone, and Breitner); obtained funding (Drs Anthony, Welsh-Bohmer, and Breitner); administrative, technical, and material support (Drs Anthony, Gustafson, Tschanz, and Norton); study supervision (Drs Anthony, Welsh-Bohmer, and Breitner).

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### REFERENCES

1. Brookmeyer R, Gray S, Kawas C. Projections of Alzheimer's disease in the United States and the public health impact of delaying disease onset. *Am J Public Health.* 1998;88:1337-1342.
2. Behl C. Alzheimer's disease and oxidative stress: implications for novel therapeutic approaches. *Prog Neurobiol.* 1999;57:301-323.

3. Henderson AS. The risk factors for Alzheimer's disease: a review and a hypothesis. *Acta Psychiatr Scand*. 1988;78:257-275.
4. Grundman M. Vitamin E and Alzheimer disease: the basis for additional clinical trials. *Am J Clin Nutr*. 2000;71(2, pt 2):630S-636S.
5. Goodwin JS, Goodwin JM, Garry PJ. Association between nutritional status and cognitive functioning in a healthy elderly population. *JAMA*. 1983;249:2917-2921.
6. Jama JW, Launer LJ, Witteman JC, et al. Dietary antioxidants and cognitive function in a population-based sample of older persons: the Rotterdam Study. *Am J Epidemiol*. 1996;144:275-280.
7. La Rue A, Koehler KM, Wayne SJ, Chiulli SJ, Haaland KY, Garry PJ. Nutritional status and cognitive functioning in a normally aging sample: a 6-y reassessment. *Am J Clin Nutr*. 1997;65:20-29.
8. Perrig WJ, Perrig P, Stahelin HB. The relation between antioxidants and memory performance in the old and very old. *J Am Geriatr Soc*. 1997;45:718-724.
9. Berr C, Richard MJ, Rousset AM, Bonithon-Kopp C. Systemic oxidative stress and cognitive performance in the population-based EVA study: Etude du Vieilissement Arteriel. *Free Radic Biol Med*. 1998;24:1202-1208.
10. Mendelsohn AB, Belle SH, Stoehr GP, Ganguli M. Use of antioxidant supplements and its association with cognitive function in a rural elderly cohort: the MoVIES Project: Monongahela Valley Independent Elders Survey. *Am J Epidemiol*. 1998;148:38-44.
11. Paleologos M, Cumming RG, Lazarus R. Cohort study of vitamin C intake and cognitive impairment. *Am J Epidemiol*. 1998;148:45-50.
12. Perkins AJ, Hendrie HC, Callahan CM, et al. Association of antioxidants with memory in a multiethnic elderly sample using the Third National Health and Nutrition Examination Survey. *Am J Epidemiol*. 1999;150:37-44.
13. Sano M, Ernesto C, Thomas RG, et al. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease: the Alzheimer's Disease Cooperative Study. *N Engl J Med*. 1997;336:1216-1222.
14. Commenges D, Scotet V, Renaud S, Jacqmin-Gadda H, Barberger-Gateau P, Dartigues JF. Intake of flavonoids and risk of dementia. *Eur J Epidemiol*. 2000;16:357-363.
15. Engelhart MJ, Geerlings MI, Ruitenberg A, et al. Dietary intake of antioxidants and risk of Alzheimer disease. *JAMA*. 2002;287:3223-3229.
16. Morris MC, Evans DA, Bienias JL, et al. Dietary intake of antioxidant nutrients and the risk of incident Alzheimer disease in a biracial community study. *JAMA*. 2002;287:3230-3237.
17. 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.
18. 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.
19. Luchsinger JA, Tang MX, Shea S, Mayeux R. Antioxidant vitamin intake and risk of Alzheimer disease. *Arch Neurol*. 2003;60:203-208.
20. Breitner JC, Wyse BW, Anthony JC, et al. *APOE-ε4* count predicts age when prevalence of AD increases, then declines: the Cache County Study [published correction appears in *Neurology*. 2000;55:161-162]. *Neurology*. 1999;53:321-331.
21. Miech RA, Breitner JC, Zandi PP, Khachaturian AS, Anthony JC, Mayer L. Incidence of AD may decline in the early 90s for men, later for women: the Cache County Study. *Neurology*. 2002;58:209-218.
22. Teng EL, Chui HC. The Modified Mini-Mental State (3MS) examination. *J Clin Psychiatry*. 1987;48:314-318.
23. Tschanz JT, Welsh-Bohmer KA, Plassman BL, Norton MC, Wyse BW, Breitner JC. An adaptation of the modified mini-mental state examination: analysis of demographic influences and normative data: the Cache County Study. *Neuropsychiatry Neuropsychol Behav Neurol*. 2002;15:28-38.
24. Jorm AF, Jacomb PA. The Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE): socio-demographic correlates, reliability, validity and some norms. *Psychol Med*. 1989;19:1015-1022.
25. Silverman JM, Breitner JC, Mohs RC, Davis KL. Reliability of the family history method in genetic studies of Alzheimer's disease and related dementias. *Am J Psychiatry*. 1986;143:1279-1282.
26. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, Revised Third Edition*. Washington, DC: American Psychiatric Association; 1987.
27. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology*. 1984;34:939-944.
28. Khachaturian AS, Gallo JJ, Breitner JC. Performance characteristics of a two-stage dementia screen in a population sample. *J Clin Epidemiol*. 2000;53:531-540.
29. Hayden KM, Khachaturian A, Tschanz JT, Corcoran C, Norton MC, Breitner JC. Characteristics of a two-stage screen for incident dementia: the Cache County Study. *J Clin Epidemiol*. In press.
30. Allison P. *Event History Analysis: Regression for Longitudinal Event Data*. Beverly Hills, Calif: Sage Publications; 1984.
31. Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*. Washington, DC: National Academy Press; 2000.
32. Haas EM. *Staying Healthy With Nutrition: The Complete Guide to Diet and Nutritional Medicine*. Berkeley, Calif: Celestial Arts; 2000.
33. Harats D, Chevion S, Nahir M, Norman Y, Sagee O, Berry EM. Citrus fruit supplementation reduces lipoprotein oxidation in young men ingesting a diet high in saturated fat: presumptive evidence for an interaction between vitamins C and E in vivo. *Am J Clin Nutr*. 1998;67:240-245.
34. Zandi PP, Carlson MC, Plassman BL, et al. Hormone replacement therapy and incidence of Alzheimer disease in older women: the Cache County Study. *JAMA*. 2002;288:2123-2129.
35. Gey KF. Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. *Br Med Bull*. 1993;49:679-699.