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Vitamin E Intake and Risk of Amyotrophic Lateral Sclerosis: A Pooled Analysis of Data From 5 Prospective Cohort Studies

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The authors investigated whether vitamin E intake was associated with amyotrophic lateral sclerosis (ALS) in the Nurses’ Health Study (1976–2004), the Health Professionals Follow-up Study (1986–2004), the Cancer Prevention Study II Nutrition Cohort (1992–2004), the Multiethnic Cohort Study (1993–2005), and the National Institutes of Health-AARP Diet and Health Study (1995–2005). ALS deaths were identified through the National Death Index. In the Nurses’ Health Study and the Health Professionals Follow-up Study, confirmed nonfatal ALS cases were also included. Cohort-specific results were estimated using Cox proportional hazards models and pooled using random-effects models. Among 1,055,546 participants, 805 developed ALS. Overall, using vitamin E supplements was not associated with ALS. However, within cohorts with information on duration of vitamin E supplement use (231 cases), ALS rates declined with increasing years of use (P-trend = 0.01). Compared with nonusers, the multivariable-adjusted relative risk was 1.05 (95% confidence interval (CI): 0.60, 1.84) among users for ≤1 year (12 cases), 0.77 (95% CI: 0.33, 1.77) among users for 2–4 years (7 cases), and 0.64 (95% CI: 0.39, 1.04) among users for ≥5 years (18 cases). For dietary vitamin E intake, the multivariable-adjusted relative risk comparing the highest quartile with the lowest was 0.79 (95% CI: 0.61, 1.03); an inverse dose-response was evident in women (P-trend = 0.002) but not in men (P-trend = 0.71). In this large, pooled prospective study, long-term vitamin E supplement use was associated with lower ALS rates. A possible protective effect of vitamin E deserves further consideration.

alpha-tocopherol; amyotrophic lateral sclerosis; antioxidants; cohort studies; diet; vitamin E

Abbreviations: ALS, amyotrophic lateral sclerosis; CI, confidence interval; CPS-II, Cancer Prevention Study II; FFQ, food frequency questionnaire; HPFS, Health Professionals Follow-up Study; MEC, Multiethnic Cohort Study; NHS, Nurses’ Health Study; NIH, National Institutes of Health; RR, relative risk.

A role for oxidative stress in the pathogenesis of amyotrophic lateral sclerosis (ALS) is supported by the presence of biomarkers of oxidative damage in sporadic ALS patients (1–4) and by the occurrence of familial ALS among carriers of several distinct mutations in the copper/zinc superoxide dismutase gene (SOD1), a critical component of cellular antioxidant defense mechanisms (4–6). Vitamin E is an important cellular antioxidant that has been shown to delay the onset of clinical disease in transgenic mice expressing mutant copies of the gene coding for superoxide dismutase, an animal model of ALS (7). Although promising at the time, vitamin E supplementation was found to be ineffective in randomized trials of patients with ALS (8, 9). However, it remains possible that high vitamin E intake in apparently healthy persons could reduce disease risk or delay its onset. The results of previous epidemiologic studies on vitamin E and ALS risk have been inconsistent. No association was found in 2 early case-control studies (including 107 and 161 ALS patients) (10, 11). A significantly decreased risk of ALS was reported in a more recent case-control study in the Netherlands (including 132 ALS patients) among persons with higher dietary vitamin E intake (12) and in a prospective study in the United States (including 525 ALS deaths) among long-term users of vitamin E supplements.
Therefore, we conducted a larger prospective study to examine whether supplemental and dietary vitamin E were related to risk of ALS using data from 5 large ongoing cohort studies: the Nurses’ Health Study (NHS), the Health Professionals Follow-up Study (HPFS), the Cancer Prevention Study II Nutrition Cohort (CPS-II Nutrition), the Multiethnic Cohort Study (MEC), and the National Institutes of Health-AARP Diet and Health Study (NIH-AARP). Together these cohorts comprise over 1 million men and women, 805 of whom developed ALS during follow-up.

MATERIALS AND METHODS

Study population

The NHS cohort was established in 1976 when 121,700 female registered nurses aged 30–55 years in one of 11 US states responded to a mailed questionnaire about disease history and lifestyle (14). The HPFS began in 1986 when 51,529 male health professionals (dentists, optometrists, pharmacists, podiatrists, and veterinarians) aged 40–75 years answered a similar mailed questionnaire (15). Follow-up questionnaires were mailed to the participants in both studies every 2 years to update information on potential risk factors for chronic diseases and to ascertain whether major medical events had occurred. The CPS-II Nutrition Cohort is a subgroup of the CPS-II baseline cohort (over 1 million participants enrolled in 1982) (16) and comprises 86,404 men and 97,786 women aged 50–79 years from 21 states with population-based cancer registries who completed a mailed questionnaire in 1992 collecting detailed information on diet and lifestyle (17). Similar questionnaires were sent in 1997 and every 2 years thereafter to update exposure information and newly diagnosed diseases. MEC consists of 96,937 men and 118,843 women aged 45–75 years at baseline living in Hawaii and California (primarily Los Angeles), mainly from 5 self-reported racial/ethnic groups: African-American, Japanese-American, Latino, Native Hawaiian, and white. From 1993 to 1996, participants entered the cohort by completing a self-administered mailed questionnaire (18). Additional questionnaires were mailed to the participants at 5-year intervals. The NIH-AARP Diet and Health Study includes 340,148 men and 227,021 women aged 50–71 years residing in one of 6 US states or 2 metropolitan areas with high-quality cancer registries (19). The participants completed a mailed food frequency questionnaire (FFQ) at baseline in 1995–1996. Approximately two-thirds of participants completed a supplementary risk factor questionnaire in 1996. Each of the above studies was reviewed and approved by the institutional review board of the institution at which the study was conducted.

Exclusion criteria

In addition to criteria originally applied by the individual studies, we excluded participants if they had an extreme intake of energy, defined as a loge-transformed energy intake beyond 3 standard deviations from the study-specific loge-transformed mean energy intake of the baseline population. Overall, 34,337 (3.2%) participants were excluded because of extreme energy intake. In NIH-AARP, participants who reported at baseline that their general health was fair or poor (2%) or left the question unanswered (1.6%) were excluded to remove prevalent ALS from the analyses.

Ascertainment of ALS

In CPS-II Nutrition, MEC, and NIH-AARP, participants with ALS were found through automated linkage with the National Death Index. Persons with an International Classification of Diseases, Ninth Revision, code of 335.2 (motor neuron disease) listed as either the underlying cause of death or a contributing cause of death were considered to have had ALS. In a previous validation study, we found that ALS was the primary diagnosis in virtually all instances where code 335.2 was listed as a cause of death (20).

In NHS and HPFS, incident ALS was also documented. On each biennial follow-up questionnaire, participants were asked to report on a specific list of medically diagnosed conditions (initially not including ALS) and “any other major illness.” A specific question on a diagnosis of ALS was added to the list of conditions in 1992 in NHS and in 2000 in HPFS, and on each subsequent biennial questionnaire. For all participants who reported a diagnosis of ALS, either in response to the open question on major illnesses or by answering the specific question, we requested permission for release of relevant medical records. Because of the rapidly progressive nature of the disease, however, many participants with ALS died before we could send the request for release of medical records; therefore, the request was sent to the closest family member. After obtaining permission, we asked the treating neurologist to complete a questionnaire to confirm that the participant had ALS, to report the certainty of the diagnosis (definite, probable, or possible), and to send a copy of the medical records. Confirmation of the diagnosis was made by an in-house neurologist with experience in ALS diagnosis (G. L.) based on the review of medical records. We relied on the diagnosis made by the treating neurologist if the information in the medical record was insufficient or could not be obtained. When we were unable to obtain either a copy of the medical record or the treating neurologist’s questionnaire for incident self-reported ALS, we classified the diagnosis as “possible ALS.” The primary analyses included definite and probable ALS.

Assessment of dietary variables

Average food consumption over the previous year was assessed at baseline using a validated FFQ developed for each study population (14, 15, 17–19, 21). Intake of individual nutrients was calculated as the sum, across all foods, of the frequency at which each food was consumed multiplied by the nutrient content of the specified portion size. Each questionnaire contained detailed questions on use of multivitamins and vitamin E supplements, including frequency of use and dosage. In NHS, HPFS, and MEC, information on duration of use of supplemental vitamin E was also collected. Persons who reported using vitamin E supplements but did not provide data on dose (<2%) were assigned a dose of 400 IU per day, the most common


Vitamin E Intake and Risk of ALS

RESULTS

Among the 545,377 men and 510,169 women who formed the baseline population, 805 with ALS were identified during a follow-up period that ranged from 10 years to 18 years across the 5 cohorts. The proportion of participants taking vitamin E supplements at baseline, either in multivitamins or as a single supplement, ranged from 38% in NHS (1980) to 71% in NIH-AARP women (1995–1996) (Table 1). Median dietary vitamin E intake was lowest among women in NIH-AARP (8.8 IU/day) and highest among men in HPFS (14.6 IU/day).
Overall, there was no significant association between supplemental vitamin E intake and ALS risk; among persons who consumed at least 400 IU per day, the multivariable-adjusted pooled relative risk was 1.18 (95% confidence interval: 0.83, 1.67; \( P = 0.35 \)) compared with nonusers (Table 2). (See Web Table 1 [http://aje.oxfordjournals.org/] for sex-specific results.) The combined use of vitamin E and vitamin C supplements was also associated with ALS; the multivariable-adjusted relative risk for persons using both vitamin E and vitamin C supplements compared with those who used neither was 1.03 (95% CI: 0.86, 1.22; \( P = 0.79 \)). In analyses restricted to the cohorts with information on duration of use (231 cases), there was an inverse trend between number of years vitamin E supplements were used and ALS (Figure 1). Although the overall inverse trend was significant (\( P\text{-trend} = 0.01 \)), the numbers of cases were small in some categories (compared with nonusers at baseline, multivariable-adjusted relative risk (RR) = 1.05 (95% CI: 0.60, 1.84) among users for \( \leq 1 \) year (12 cases), RR = 0.77 (95% CI: 0.33, 1.77) among users for 2–4 years (7 cases), and RR = 0.64 (95% CI: 0.39, 1.04; \( P = 0.075 \)) among users for \( \geq 5 \) years (18 cases)).

Rates of ALS were lower among persons in the highest quartile of energy-adjusted dietary vitamin E intake compared with those in the lowest quartile (RR = 0.77, 95% CI: 0.59, 0.99; \( P = 0.04 \)) (Table 3). Multivariable adjustment did not materially change the results, although power was reduced and the \( P \) value was no longer significant. In women, there was a clear inverse dose-response with increasing dietary vitamin E intake (compared with persons in the lowest quartile of energy-adjusted dietary vitamin E intake, multivariable RR = 0.88 (95% CI: 0.64, 1.21) for the second quartile, RR = 0.71 (95% CI: 0.51, 0.99) for the third quartile, and RR = 0.57 (95% CI: 0.40, 0.80) for the fourth quartile; \( P\text{-trend} = 0.002 \) (Web Table 2). In analyses of dietary vitamin E restricted to nonusers of supplemental vitamin E, results were similar to those found among all participants (Table 4), although statistical significance did not persist, particularly among the women (Web Table 3).

Results were similar when the first 4 years of follow-up were excluded. The pooled relative risks were 1.42 (95% CI: 0.75, 2.68; \( P = 0.29 \)) comparing users of vitamin E supplements (\( \geq 400 \) IU/day) with never users and 0.80 (95% CI: 0.58, 1.10; \( P = 0.17 \)) comparing persons in the highest quartile of dietary vitamin E intake with those in the lowest.

There was no evidence of effect modification by smoking, sex, or vitamin C intake (\( P > 0.05 \)). Only in MEC was there some evidence that the association between dietary vitamin E and ALS was modified by age at baseline. Compared with persons in the lowest quartile of energy-adjusted dietary vitamin E intake, the multivariable adjusted relative risk of ALS among persons aged 65 years or above was 0.45 (95% CI: 0.22, 0.91; \( P = 0.03 \)) for the second quartile, 0.32 (95% CI: 0.15, 0.67; \( P = 0.003 \)) for the third quartile, and 0.21 (95% CI: 0.09, 0.48; \( P = 0.0002 \)) for the fourth quartile.

### Table 1. Characteristics of the Cohorts Included in a Pooled Analysis of Vitamin E Intake and Amyotrophic Lateral Sclerosis Risk, 1976–2005

<table>
<thead>
<tr>
<th>Study</th>
<th>Follow-up</th>
<th>No. of ALS Cases</th>
<th>Baseline Age Range, Years</th>
<th>Vitamin E Supplement Use, %</th>
<th>Dietary Vitamin E Intake, mg/day&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health Professionals Follow-up Study</td>
<td>1986–2004</td>
<td>56</td>
<td>39–79</td>
<td>45.6</td>
<td>9.8</td>
</tr>
<tr>
<td>CPS-II Nutrition Cohortc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1992–2004</td>
<td>106</td>
<td>41–93</td>
<td>39.1</td>
<td>8.7</td>
</tr>
<tr>
<td>Women</td>
<td>1992–2004</td>
<td>73</td>
<td>40–87</td>
<td>49.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Multietnic Cohort Study</td>
<td>1993–1997</td>
<td>66</td>
<td>41–78</td>
<td>50.4</td>
<td>8.7</td>
</tr>
<tr>
<td>Women</td>
<td>1993–1997</td>
<td>45</td>
<td>42–78</td>
<td>45.6</td>
<td>7.7</td>
</tr>
<tr>
<td>NIH-AARP Diet and Health Study</td>
<td>1995–1996</td>
<td>280</td>
<td>50–72</td>
<td>61.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Women</td>
<td>1995–1996</td>
<td>115</td>
<td>50–72</td>
<td>70.6</td>
<td>5.9</td>
</tr>
<tr>
<td>Total</td>
<td>1,055,546</td>
<td>805</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ALS, amyotrophic lateral sclerosis; CPS-II, Cancer Prevention Study II; NHS, Nurses’ Health Study; NIH, National Institutes of Health.

<sup>a</sup> Cohort size after applying study-specific exclusion criteria and then excluding participants with log-transformed energy intake values beyond 3 standard deviations from the study-specific mean.

<sup>b</sup> To convert mg to IU, multiply by 1.49.

<sup>c</sup> Forty-seven participants with ALS in the CPS-II Nutrition Cohort were included in our previous study of vitamin E supplement use and ALS (13) in the parent CPS-II baseline cohort (over 1 million participants enrolled in 1982).

<sup>d</sup> NHS (1980) and NHS (1990) were the same participants followed for ALS during different time periods. Therefore, the total number of participants does not include NHS (1990), which is a subset of NHS (1980).
DISCUSSION

In this large investigation including data from 5 prospective cohort studies, ALS risks were similar in users and nonusers of vitamin E supplements. However, among participants in cohorts with information on years of vitamin E supplement use, ALS risk declined with increasing duration of use. Dietary intake of vitamin E was inversely associated with the risk of ALS, although this association was only marginally significant.

To our knowledge, this is the largest study to date to have examined the association between dietary and supplemental vitamin E intake and ALS. By including several prospective cohort studies with validated dietary assessment instruments, we minimized the possibility of recall bias and misclassification. Together, these cohorts are likely to be representative of the whole spectrum of ALS patients, avoiding the selection bias that is often inherent when cases in case-control studies are recruited from ALS tertiary-care centers (30, 31). A limitation was the use of ALS mortality in CPS-II Nutrition, MEC, and NIH-AARP as a proxy for ALS incidence. We assume, however, that mortality is a reasonable surrogate for incidence, because median survival of ALS patients after diagnosis (1.5–3 years).
years) is relatively short (32–35), and death certificates have been estimated to accurately identify 70%–90% of cases of ALS or motor neuron diseases (36–39). Bias could also have resulted if some participants initiated use of vitamin E supplements because of symptoms related to ALS, but this seems unlikely, because the results of sensitivity analyses excluding the first 4 years of follow-up in each cohort did not differ from those obtained using the complete set of follow-up data. A further limitation was the use of baseline vitamin E data; in MEC and NIH-AARP, dietary information was collected at baseline only, so we had inadequate power to assess changes in vitamin E intake over time, whether from diet or supplements.

In our previous prospective study using the CPS-II baseline cohort, we observed a 42% lower risk of death from ALS among regular, long-term (>10 years) users of vitamin E supplements as compared with nonusers (13). In the current study, we found a similar inverse association with long-term (≥5 years) use of vitamin E supplements among participants in the 3 cohort studies that collected information on duration of use at baseline (231 cases). We were unable to further refine the duration variable because of the structure of the questions on the individual questionnaires.

Our finding that dietary vitamin E was associated with lower rates of ALS is similar to that reported in a case-control study from the Netherlands in which the odds of developing ALS were 50% lower among persons in the highest tertile of vitamin E intake from food compared with those in the lowest tertile (12), although 2 earlier case-control studies found no association, possibly because of inadequate power (10, 11). The discrepancy between the lower ALS risk associated with higher dietary vitamin E intake and the lack of association with overall supplemental vitamin E intake could be explained if diet reflected long-term vitamin E intake better than a single question on current supplement use. This interpretation is consistent with the significant inverse association we found between duration of use of supplemental vitamin E and ALS risk. Nevertheless, differences between the biologic effects of dietary and supplemental vitamin E should also be considered. Vitamin E from food is derived from 4 tocopherol compounds (α-, β-, γ-, δ-) and 4 tocotrienol (α-, β-, γ-, δ-) compounds,

<table>
<thead>
<tr>
<th>Table 3. Pooled Relative Risk of Amyotrophic Lateral Sclerosis by Dietary Vitamin E Intake in Data From 5 Cohort Studies, 1976–2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile of Dietary Vitamin E Intake</td>
</tr>
<tr>
<td>1 (lowest) 2 3 4 (highest)  P-trend</td>
</tr>
<tr>
<td>No. of cases 213 188 193 211</td>
</tr>
<tr>
<td>Age-adjusted RR (95% CI)a 1.00 0.75 (0.61, 0.92) 0.71 (0.58, 0.87) 0.77 (0.59, 0.99) 0.15</td>
</tr>
<tr>
<td>P value 0.005 0.001 0.04</td>
</tr>
<tr>
<td>P for heterogeneityb 0.86 0.86 0.24</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)c 1.00 0.77 (0.63, 0.94) 0.73 (0.60, 0.90) 0.79 (0.61, 1.03) 0.23</td>
</tr>
<tr>
<td>P value 0.01 0.003 0.08</td>
</tr>
<tr>
<td>P for heterogeneityb 0.85 0.89 0.23</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; RR, relative risk.

<table>
<thead>
<tr>
<th>Table 4. Pooled Relative Risk of Amyotrophic Lateral Sclerosis by Dietary Vitamin E Intake Among Nonusers of Supplemental Vitamin E in Data From 5 Cohort Studies, 1976–2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quartile of Dietary Vitamin E Intake</td>
</tr>
<tr>
<td>1 (lowest) 2 3 4 (highest)  P-trend</td>
</tr>
<tr>
<td>No. of cases 89 77 93 83</td>
</tr>
<tr>
<td>Age-adjusted RR (95% CI)a 1.00 0.72 (0.52, 0.98) 0.83 (0.62, 1.13) 0.73 (0.53, 1.01) 0.19</td>
</tr>
<tr>
<td>P value 0.04 0.24 0.06</td>
</tr>
<tr>
<td>P for heterogeneityb 0.88 0.99 0.40</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)c 1.00 0.75 (0.55, 1.04) 0.87 (0.64, 1.18) 0.77 (0.56, 1.06) 0.28</td>
</tr>
<tr>
<td>P value 0.08 0.38 0.11</td>
</tr>
<tr>
<td>P for heterogeneityb 0.83 0.99 0.40</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; RR, relative risk.

600  Wang et al.

whereas vitamin E supplements typically contain only \( \alpha \)-tocopherol. The biologic effects of \( \gamma \)-tocopherol, which is the most abundant form of vitamin E in food, could be different from those of \( \alpha \)-tocopherol (40). In addition, there are 8 isomeric forms of \( \alpha \)-tocopherol; natural \( \alpha \)-tocopherol (RRR-\( \alpha \)-tocopherol) is the only form present in foods, while synthetic \( \alpha \)-tocopherol, found in some supplements, includes all 8 isomers (all-rac-\( \alpha \)-tocopherol). All forms of vitamin E are absorbed; however, natural \( \alpha \)-tocopherol is preferentially retained because of its 100% affinity with the \( \alpha \)-tocopherol transfer protein (40). Lastly, we cannot rule out the possibility that the dietary vitamin E association observed could have resulted from unmeasured confounding by an unknown dietary predictor of ALS.

In summary, the results of this large longitudinal analysis including over 1 million persons from 5 prospective cohort studies suggest that long-term use of vitamin E supplements could be inversely associated with risk of ALS. A suggestive inverse association between dietary vitamin E intake and ALS warrants further study.

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