Carotenoids, vitamin A, vitamin C, vitamin E, and folate and risk of self-reported hearing loss in women

Sharon G Curhan, Konstantina M Stankovic, Roland D Eavey, Molin Wang, Meir J Stampfer, and Gary C Curhan

ABSTRACT

Background: Higher intake of certain vitamins may protect against cochlear damage from vascular compromise and oxidative stress, thereby reducing risk of acquired hearing loss, but data are limited.

Objective: We prospectively examined the relation between carotenoids, vitamin A, vitamin C, vitamin E, and folate intake and risk of self-reported hearing loss in women.

Design: This prospective cohort study followed 65,521 women in the Nurses’ Health Study II from 1991 to 2009. Baseline and updated information obtained from validated biennial questionnaires was used in Cox proportional hazards regression models to examine independent associations between nutrient intake and self-reported hearing loss.

Results: After 1,084,598 person-years of follow-up, 12,789 cases of incident hearing loss were reported. After multivariable adjustment, we observed modest but statistically significant inverse associations between higher intake of β-carotene and β-cryptoxanthin and risk of hearing loss. In comparison with women in the lowest quintile of intake, the multivariable-adjusted risk of hearing loss among women in the highest quintile was 0.88 (95% CI: 0.81, 0.94; P-trend < 0.001) for β-carotene and 0.90 (95% CI: 0.84, 0.96; P-trend < 0.001) for β-cryptoxanthin. In comparison with women with folate intake 200–399 μg/d, very low folate intake (<200 μg/d) was associated with higher risk (RR: 1.19; 95% CI: 1.01, 1.41), and higher intake tended to be associated with lower risk (P-trend = 0.04). No significant associations were observed for intakes of other carotenoids or vitamin A. Higher vitamin C intake was associated with higher risk; in comparison with women with intake <75 mg/d, the RR among women with vitamin C intake ≥1000 mg/d (mainly supplemental) was 1.22 (95% CI: 1.06, 1.42; P-trend = 0.02). There was no significant trend between intake of vitamin E intake and risk.

Conclusion: Higher intakes of β-carotene, β-cryptoxanthin, and folate, whether total or from diet, are associated with lower risk of hearing loss, whereas higher vitamin C intake is associated with higher risk.

Keywords: aging, carotenoids, epidemiology, hearing loss, vitamins, folate

INTRODUCTION

Hearing impairment is a highly prevalent sensory deficit, affecting approximately 48 million individuals in the United States in at least one ear (1). Approximately 360 million (5.3%) individuals worldwide have hearing impairment that is disabling by WHO criteria (2). The risk of acquired hearing loss increases considerably with age (3), and the prevalence is expected to grow along with the aging population; therefore, identifying modifiable risk factors could lead to preventive interventions.

Vascular compromise and oxidative stress contribute to the development of acquired hearing loss (4–6). Therefore, it has been proposed that higher intake of foods or nutrients that can provide vascular or antioxidative benefits (e.g., carotenoids, vitamin A, vitamin C, vitamin E, and folate) may be protective. A recent metabolomic and network analysis of pharmacotherapies for sensorineural hearing loss identified the retinoic acid pathway as a promising target for the development of prevention and treatment strategies (7). In animal models, β-carotene and vitamins A, C, and E have been protective against hearing loss (4, 8, 9), but cross-sectional studies in humans have produced conflicting results (10–12), and prospective data are limited (10, 13). In an Australian study of older individuals, no prospective associations were observed between dietary intake of β-carotene, vitamin A, vitamin C, or vitamin E and 5-y incidence of audiometrically assessed hearing loss, but case numbers were limited, and follow-up was relatively short in duration (10). In an 18-y prospective US study among 26,000 older men, we observed no associations
between intakes of β-carotene, vitamin A, vitamin C, or vitamin E and risk of self-reported hearing loss, but higher folate intake was inversely associated with risk in men aged ≥60 y (13). Cross-sectional associations between low intake and low plasma red blood cell concentrations of folate and higher prevalence of hearing loss have been observed (14, 15). A randomized clinical trial in the Netherlands showed that daily oral folic acid supplementation was inversely associated with hearing decline over 3 y (16). However, the trial was performed in a subset of individuals with very high plasma homocysteine concentrations and in a country without folate fortification of the food supply. Thus, the generalizability of these results is uncertain. Because the relation between vitamin intake and risk of hearing loss remains unclear, we prospectively examined the associations between intake of carotenoids, vitamin A, vitamin C, and vitamin E and folate and risk of hearing loss in the Nurses’ Health Study II, a cohort study of 65,521 women with repeated dietary assessments and long-term follow-up from 1991 to 2009.

METHODS

Study population

The Conservation of Hearing Study examines risk factors for hearing loss among participants in the Nurses’ Health Study II, an ongoing cohort study of 116,430 female registered nurses in the United States aged 25–42 y at cohort inception in 1989. Participants have been followed by biennial mailed questionnaires that elicit updated information on diet, lifestyle, incident disease, and various health outcomes; the follow-up rate over 22 y exceeds 90% of eligible person-time. Beginning in 1991, detailed information on diet and nutrient intake from foods and supplements has been obtained every 4 y; thus, 1991 served as our study baseline. The 2009 questionnaire asked women whether they have a hearing problem and, if so, at what age a change in hearing was first noticed. Of the 90,488 women who answered that 2009 questionnaire, 12,160 were excluded because they had not answered the 1991 baseline diet questionnaire. Women who reported a hearing problem that began before 1991 (n = 2584), reported a hearing problem but did not report a date of onset (n = 173), did not answer the hearing questions (n = 9191), or reported a history of cancer other than nonmelanoma skin cancer (because of possible exposure to ototoxic chemotherapeutic agents; n = 859) were also excluded, leaving 65,521 women included in the analysis. The 1991 baseline characteristics of participants who did and did not answer the 2009 questionnaire did not differ appreciably (data not shown). The study protocol was approved by the Institutional Review Board of the Partners Health Care System.

Ascertainment of dietary intake

Intake of carotenoids; vitamins A, C, and E; and folate was assessed in 1991, 1995, 1999, 2003, and 2007 with a detailed validated semiquantitative food-frequency questionnaire (SFFQ) that included >130 items. For each food, a commonly used unit or portion size was specified, and participants were asked how often, on average, they had consumed each type of food or beverage during the previous year. Nine possible response options were provided that ranged from “never or less than one per month” to “6 or more times per day.” Intakes of the nutrients of interest were calculated by multiplying the portion size of a single serving of each food by its reported frequency of intake, multiplying the total amount consumed by the nutrient content of the food, and then summing the nutrient contributions of all food items, using USDA food composition data (17–19). Vitamin supplement use was assessed by collecting information on use of multiple vitamins (specific brand and usual number of tablets taken per week) and on use of specific supplements, including vitamin A, β-carotene, vitamin E, vitamin C, and folic acid (dose of tablet and the usual number of tablets taken per week). Vitamin A intake was assessed as both retinol (preformed vitamin A from animal sources, supplements, and fortified foods) and total vitamin A (retinol activity equivalents, described in the section on statistical analysis). All computed nutrient intakes were adjusted for total energy intake. Energy adjustment reduces variation introduced by questionnaire responses that underreport or overreport intake and improves the accuracy of nutrient measurements (20).

Nutrient intakes for the individual carotenoids were computed by using the USDA’s carotenoid database. Lutein and zeaxanthin intake were presented together because the analytic procedures did not permit the individual quantification of these carotenoids in foods. The carotenoid content of tomato-based food products was updated with values from the USDA. In our data, the foods providing the greatest contribution to the total absolute nutrient intake of the specific carotenoids were carrots for α-carotene; carrots, spinach, and tomato products for β-carotene; tomato products for lycopene; oranges, oranges juice, and peaches for β-cryptoxanthin; and spinach, broccoli, and peas for lutein/zeaxanthin. Foods providing the greatest contribution to vitamin A intake were romaine lettuce and carrots; to retinol intake, milk; to vitamin C intake, orange juice; to vitamin E intake, cold cereal and olive oil; and to folate intake, cold cereal.

The validity and reproducibility of the SFFQ has been described previously (21, 22). In validation studies of the SFFQ compared with detailed 1-wk diet records, the correlation coefficients were 0.79 for total vitamin A from food only, 0.59 for retinol from food only (20), 0.49 for total vitamin A (including contributions from food and supplements), 0.75 for total vitamin C, and 0.77 for total folate (21). The correlation between the questionnaire and measured serum folate was 0.63 (23). For dietary information that was collected before 1998, data on the folate content of foods reflect values before mandatory fortification of the food supply.

Ascertainment of outcome

On the 2009 questionnaire, participants were asked, “Do you have a hearing problem?” (response options: no, mild, moderate, severe) and, if so, “At what age did you first notice a change in your hearing?” (<30, 30–39, 40–44, 45–49, 50–54, 55–59, 60+ y). The primary outcome, self-reported hearing loss, was determined based on the response to this question, and a case was defined as a hearing problem first noticed after 1991. Although hearing loss can be subtle in onset, incident cases were
defined as hearing loss at the age it was first noticed by the participant. We did not have information on severity of hearing loss at time of onset, and thus we could not perform prospective analyses that considered severity of hearing loss as the outcome. Hearing loss measured by pure-tone audiometry (PTA) is considered the gold standard for hearing loss evaluation; however, questionnaires have been used in large populations to assess hearing loss and have been found to be reasonably reliable in previous studies (24, 25).

In addition, a recent study in NHANES that examined the accuracy of subjective assessment of hearing (self-report based on a single question that asked respondents to report their level of hearing without the use of hearing aids as excellent, good, a little trouble, a lot of trouble, or deaf) compared with objective assessment of hearing impairment, defined as PTA (0.5,1,2,4 kHz) >25 dB in the better-hearing ear, found that 77.4% of women aged 50–59 y classified their hearing status correctly (26). The wording used on our questionnaire has not been validated against other surveys and may not be sensitive to mild or slight hearing loss, which could lead to misclassification of the outcome and bias the results toward the null.

Our participants were asked whether they had a hearing problem. Although not all hearing problems may be hearing loss per se, hearing loss is highly prevalent. Although we were not able to separate out hearing problems such as difficulty understanding speech in noise or hyperacusis, hearing loss is the most prevalent hearing problem, and the finding in our study population of 19.5% of the women reporting a hearing problem is consistent with data from NHANES 1999–2004 that show the prevalence of bilateral hearing loss, defined as PTA (0.5,1,2,4 kHz) ≥25 dB in both ears, was 7.5%; unilateral hearing loss, defined as PTA (0.5,1,2,4 kHz) ≥25 dB in 1 ear only, was 12%; and high-frequency hearing loss, defined as PTA (3,4,6 kHz) ≥25 dB in either ear, was 34% in white women aged 50–59 y (3). We herein use the term hearing loss based on the assumption that hearing loss describes the hearing problem reported by most participants.

Ascertaining of covariates

Potential confounders of the relation between carotenoids and vitamin intake and the risk of hearing loss that were considered covariates in multivariable analyses included age (3); race (3); socioeconomic status (3); smoking (27); BMI (in kg/m²) (28); waist circumference (28); physical activity (28); intake of alcohol (27), vitamin B-12, magnesium (29), potassium (30), and long-chain omega-3 PUFAs (31); history of hypertension (32); history of diabetes (33); acetaminophen use (34); ibuprofen use (34); and tinnitus (35). Covariate information was obtained from the biennial questionnaires or the SFFQs and updated throughout the analysis whenever new information became available.

Statistical analysis

Person-time of follow-up was calculated from the date of return of the 1991 SFFQ until the date of self-reported hearing loss or end of follow-up in 2009. Participants who reported cancers other than nonmelanoma skin cancer were excluded at baseline or when cancer was reported during follow-up. Date of reported hearing loss was considered the midpoint of the category for reported age of first noticing a change in hearing. During follow-up, participants with missing dietary data were skipped for the associated time period.

Total intakes of carotenoids, retinol, total vitamin A, and vitamin E were categorized into quintiles based on the distribution of the entire analytic cohort. Total vitamin A was measured in retinol activity equivalents, a measure of vitamin A activity based on the capacity to convert provitamin carotenoids containing at least one unsubstituted ionone ring to retinaldehyde (1 μg retinol activity equivalents = 1 mg retinol = 12 mg β-carotene = 24 mg other vitamin A precursor carotenoids) (36). We chose to use prespecified cutoffs to categorize both vitamin C and folate intake—from less than the US Recommended Dietary Allowance of 75 mg/d (referent) up to ≥1000 mg/d for vitamin C and <200 μg/d up to ≥1000 μg/d for folate—because of the very skewed distribution of the intakes of these vitamins, to examine commonly used high doses, and to be consistent with previous literature (37, 38). For β-carotene; vitamins A, C, and E; and folate, we also analyzed the association between hearing loss and intake from foods and supplements separately. In our final multivariable models, the nutrient covariates that were not confounders but were highly correlated with the exposures of interest (magnesium, potassium, trans fat, and vitamin B-12) were removed from the multivariable model to reduce the influence of collinearity.

In addition to analyzing the association for each specific carotenoid, we also analyzed total carotenoid intake by summing the intake of the specific carotenoids to create a total carotenoid intake variable. We also calculated a total carotenoid score by summing the quintile score for each carotenoid (scores ranged from 5 to 25) to reflect the difference in intake amounts across the specific carotenoids (39).

Descriptive analyses for baseline characteristics in 1991 were examined for the entire cohort and by categories of nutrient intake. We used Cox proportional hazards regression models with age and questionnaire period as the time scale to estimate RRs and 95% CIs by using the lowest category of intake of each nutrient as the referent group for the carotenoids and vitamins A, C, and E. For folate, relatively few women had intake <200 μg/d; therefore, we used intake 200–399 μg/d as the referent category. We used the Anderson-Gill (40) data structure, with a new data record created for each biennial questionnaire cycle in which the participant was at risk with covariates set to represent the value from the latest returned questionnaire to handle time-varying covariates efficiently. For all RRs, we calculated 95% CIs.

All analyses were prospective, using information on dietary intake that was collected before the date when a change in hearing was first noticed. The temporal relation between these micronutrients and risk of hearing loss is unknown; therefore, dietary intake was examined in 3 ways: baseline intake (1991 SFFQ), most recently reported intake before the change in hearing, and cumulative average intake. Cumulative average intake was calculated by assigning the 1991 intake to the 1991–1995 follow-up period; the average of the 1991 and 1995 intake to the 1995–1999 follow-up period; the average of the 1991, 1995, and 1999 intake to the 1999–2003 follow-up period; and so forth. We present the result for the cumulative average models because this method captures long-term dietary intake and reduces measurement error due to within-person variation over time, minimizing misclassification (41).
Tests for linear trend for the exposures of interest were performed by assigning the median value of each category to all participants in that group. We conducted analyses stratified by age <50 and ≥50 y, smoking status (never, past, frequency of current smoking), and amount of magnesium intake to examine potential interactions between antioxidant vitamin intake because these factors may increase oxidative stress or have been implicated as modifying factors (42). In addition, the relation between folate intake and incident hearing loss was analyzed stratified by amount of alcohol intake, categorized as low (0–5 g/d), medium (5.1–19.9 g/d), and high (≥20 g/d). All P values are 2-tailed. Statistical tests were performed with SAS statistical software, version 9.3 (SAS Institute Inc.).

RESULTS

Baseline characteristics are presented in Table 1. The mean ± SD age of the cohort at baseline was 36.3 ± 4.6 y, the participants were predominantly white women, and the mean ± SD BMI was 24.5 ± 5.2. Two-thirds of the women had never been smokers, 44% took multivitamins, 20.1% took a vitamin C supplement, and 6.7% took supplemental vitamin E. Baseline characteristics according to category of specific nutrients are presented in Supplemental Tables 1-5. Overall, women with higher intake of carotenoids, vitamins A, C, and E; and folate tended to be more physically active, were less likely to be current smokers, were more likely to take multivitamins and vitamin C or E supplements, and had higher intakes of other vitamins and minerals.

After 1,084,598 person-years of follow-up, 12,789 cases of hearing loss were reported to have occurred. Higher intakes of carotenoids, specifically of β-carotene and β-cryptoxanthin, were statistically significantly associated with a lower risk of hearing loss (Table 2). For example, in comparison with women in the lowest quintile of β-carotene intake, the multivariable-adjusted RR of hearing loss in women in the highest quintile was 0.88 (95% CI: 0.81, 0.94; P-trend < 0.001). In comparison with women in the lowest quintile of β-cryptoxanthin intake, the multivariable-adjusted RR of hearing loss in women in the highest quintile was 0.90 (95% CI: 0.84, 0.96; P-trend < 0.001). A higher total carotenoid score was also significantly associated with lower risk (RR: 0.84; 95% CI: 0.79, 0.90; P-trend < 0.001). No significant associations were observed for intakes of α-carotene, lycopene, or lutein/zeaxanthin.

A higher intake of vitamin C was associated with a higher risk of hearing loss (Table 3). In comparison with women whose intake of vitamin C was <75 mg/d, the multivariable-adjusted RR of hearing loss in women was 1.13 (95% CI: 1.02, 1.26) for vitamin C intake 75–249 mg/d, 1.18 (95% CI: 1.05, 1.32) for intake 250–499 mg/d, 1.19 (95% CI: 1.06, 1.35) for intake 500–999 mg/d, and 1.22 (95% CI: 1.06, 1.42; P-trend = 0.02) for intake ≥1000 mg/d. More than half of the women in the higher intake categories of total vitamin C took vitamin C supplements. Although the difference in the point estimates in the age-adjusted and multivariable-adjusted models indicates the presence of negative confounding, no individual covariate predominantly influenced the results. A higher intake of vitamin E was modestly associated with higher risk; however, there was no significant trend between intake of vitamin E and risk of hearing loss (P-trend = 0.27).

A higher intake of folate tended to be associated with a lower risk of hearing loss (P-trend = 0.04). In comparison with women whose intake of folate was 200–399 μg/d, the multivariable-adjusted RR of hearing loss in women with lower folate intake (<200 μg/d) was 1.19 (95% CI: 1.01, 1.41). In comparison with women whose intake of folate was 200–399 μg/d, higher folate intake (≥600 μg/d) was marginally associated with lower risk; the multivariable-adjusted RR of hearing loss in women was 0.90 (95% CI: 0.84, 0.96) with folate intake 600–799 μg/d, 0.92 (95% CI: 0.84, 1.00) with folate intake 800–999 μg/d, and 0.88 (95% CI: 0.78, 1.00) with folate intake ≥1000 μg/d.

The relations between vitamin intake and hearing loss did not vary by age, smoking status, or amount of magnesium intake (P-interaction ≥ 0.09 for age; P-interaction ≥ 0.3 for smoking; P-interaction ≥ 0.2 for magnesium intake). In addition, stratifying by amount of alcohol intake did not influence the relation between folate intake and hearing loss (P-interaction = 0.3). We had limited power to examine this relation in women with higher alcohol intake (≥20 g/d). When dietary intake was also examined by using baseline intake (1991 SFFQ) or simple updating (most recently reported intake before the change in

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**TABLE 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>36.3 ± 4.6&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>24.5 ± 5.2</td>
</tr>
<tr>
<td>Waist circumference (1993), cm</td>
<td>77.9 ± 12.5</td>
</tr>
<tr>
<td>Physical activity, METs</td>
<td>12.6 (5.2–26.5)&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Smoking status, %</td>
<td>66.6</td>
</tr>
<tr>
<td>Never</td>
<td>22.3</td>
</tr>
<tr>
<td>Past</td>
<td>10.9</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>6.1</td>
</tr>
<tr>
<td>History of diabetes, %</td>
<td>0.8</td>
</tr>
<tr>
<td>History of tinnitus, %</td>
<td>3.7</td>
</tr>
<tr>
<td>White race, %</td>
<td>95.1</td>
</tr>
<tr>
<td>Ibuprofen use, %</td>
<td>9.2</td>
</tr>
<tr>
<td>Acetaminophen use, %</td>
<td>7.4</td>
</tr>
<tr>
<td>Alcohol intake, g/d</td>
<td>0.9 (0.0–3.5)</td>
</tr>
<tr>
<td>Vitamin B-12 intake, µg/d</td>
<td>7.0 (5.0–11.0)</td>
</tr>
<tr>
<td>Potassium intake, mg/d</td>
<td>2941 (532)</td>
</tr>
<tr>
<td>Magnesium intake, g/d</td>
<td>316 (74)</td>
</tr>
<tr>
<td>Long-chain ω-3 PUFA intake, g/d</td>
<td>0.2 (0.2)</td>
</tr>
<tr>
<td>Multivitamin use, %</td>
<td>44.0</td>
</tr>
<tr>
<td>Vitamin C supplement use, %</td>
<td>20.1</td>
</tr>
<tr>
<td>Vitamin E supplement use, %</td>
<td>6.7</td>
</tr>
</tbody>
</table>

<sup>1</sup>Values are standardized to the age and distribution of the study population. Values of polytomous variables may not sum to 100% because of rounding. METs, metabolic equivalent tasks from recreational and leisure-time activities.

<sup>2</sup>Mean ± SD (all such values).

<sup>3</sup>Median; IQR in parentheses (not age standardized) (all such values).

<sup>4</sup>Two days per week or more.

<sup>5</sup>Nutrient intakes are adjusted for total energy intake.
TABLE 2
Age- and multivariable-adjusted relative risks (95% CIs) for hearing loss according to specific carotenoid intake in the Nurses’ Health Study II, 1991–2009 (n = 65,521)$^1$

<table>
<thead>
<tr>
<th>Carotenoid</th>
<th>Quintile of carotenoid intake</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>(P)-linear trend$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)-Carotene</td>
<td>Median intake, (\mu g/)d</td>
<td>155</td>
<td>379</td>
<td>573</td>
<td>829</td>
<td>1470</td>
<td></td>
</tr>
<tr>
<td>Cases, (n)</td>
<td>2462</td>
<td>2706</td>
<td>2559</td>
<td>2659</td>
<td>2403</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.99 (0.94, 1.05)</td>
<td>0.92 (0.87, 0.97)</td>
<td>0.93 (0.88, 0.98)</td>
<td>0.86 (0.81, 0.91)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>1.03 (0.97, 1.09)</td>
<td>0.99 (0.93, 1.06)</td>
<td>1.03 (0.96, 1.11)</td>
<td>1.01 (0.93, 1.10)</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td>(\beta)-Carotene$^3$</td>
<td>Median intake, (\mu g/)d</td>
<td>1478</td>
<td>2469</td>
<td>3373</td>
<td>4541</td>
<td>7028</td>
<td></td>
</tr>
<tr>
<td>Cases, (n)</td>
<td>2480</td>
<td>2678</td>
<td>2600</td>
<td>2656</td>
<td>2375</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.95 (0.90, 1.00)</td>
<td>0.89 (0.84, 0.94)</td>
<td>0.88 (0.83, 0.93)</td>
<td>0.79 (0.75, 0.84)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.97 (0.91, 1.03)</td>
<td>0.94 (0.88, 1.00)</td>
<td>0.95 (0.89, 1.01)</td>
<td>0.88 (0.81, 0.94)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>(\beta)-Cryptoxanthin$^3$</td>
<td>Median intake, (\mu g/)d</td>
<td>46</td>
<td>79</td>
<td>115</td>
<td>164</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>Cases, (n)</td>
<td>2585</td>
<td>2777</td>
<td>2646</td>
<td>2498</td>
<td>2283</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.99 (0.94, 1.05)</td>
<td>0.93 (0.88, 0.98)</td>
<td>0.87 (0.82, 0.92)</td>
<td>0.81 (0.76, 0.85)</td>
<td>&lt;0.001</td>
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<tr>
<td>Multivariable-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>1.02 (0.96, 1.08)</td>
<td>0.96 (0.91, 1.02)</td>
<td>0.94 (0.88, 1.00)</td>
<td>0.90 (0.84, 0.96)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Lycopene</td>
<td>Median intake, (\mu g/)d</td>
<td>3293</td>
<td>4741</td>
<td>6070</td>
<td>8144</td>
<td>12,487</td>
<td></td>
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<tr>
<td>Cases, (n)</td>
<td>2442</td>
<td>2521</td>
<td>2650</td>
<td>2723</td>
<td>2453</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.98 (0.92, 1.04)</td>
<td>0.98 (0.92, 1.04)</td>
<td>1.01 (0.95, 1.07)</td>
<td>0.96 (0.91, 1.02)</td>
<td>0.30</td>
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<tr>
<td>Multivariable-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.98 (0.92, 1.04)</td>
<td>1.01 (0.92, 1.04)</td>
<td>1.04 (0.98, 1.11)</td>
<td>0.99 (0.93, 1.05)</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Lutein + zeaxanthin</td>
<td>Median intake, (\mu g/)d</td>
<td>952</td>
<td>1618</td>
<td>2290</td>
<td>3116</td>
<td>4863</td>
<td></td>
</tr>
<tr>
<td>Cases, (n)</td>
<td>2516</td>
<td>2703</td>
<td>2612</td>
<td>2655</td>
<td>2303</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.97 (0.91, 1.02)</td>
<td>0.91 (0.87, 0.97)</td>
<td>0.90 (0.85, 0.95)</td>
<td>0.79 (0.75, 0.84)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>1.02 (0.96, 1.09)</td>
<td>1.01 (0.94, 1.09)</td>
<td>1.02 (0.95, 1.11)</td>
<td>0.95 (0.87, 1.04)</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Total carotenoid score$^4$</td>
<td>Median score</td>
<td>7</td>
<td>12</td>
<td>15</td>
<td>18</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Cases, (n)</td>
<td>2675</td>
<td>2696</td>
<td>2751</td>
<td>2224</td>
<td>2443</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.96 (0.91, 1.01)</td>
<td>0.91 (0.86, 0.95)</td>
<td>0.89 (0.84, 0.94)</td>
<td>0.78 (0.74, 0.83)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Multivariable-adjusted RR (95% CI)</td>
<td>1.00 (referent)</td>
<td>0.97 (0.92, 1.03)</td>
<td>0.94 (0.89, 1.00)</td>
<td>0.94 (0.88, 1.00)</td>
<td>0.84 (0.79, 0.90)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Cumulative average intake of energy-adjusted nutrients. Cox proportional hazards regression was used to estimate RRs (95% CIs). Multivariable models were adjusted for age, race, socioeconomic status, BMI, waist circumference, physical activity, alcohol intake, long-chain \(\omega\)-3 fatty acid intake, smoking, hypertension, diabetes, tinnitus, ibuprofen use, acetaminophen use, retinol intake, vitamin C intake, vitamin E intake, and intake of the other carotenoids, unless otherwise indicated.

$^2$A linear trend was calculated by using the Wald test statistic.

$^3$The multivariable model was adjusted for all of the above except for intakes of retinol, \(\alpha\)-carotene, lycopene, and lutein/zeaxanthin.

$^4$The total carotenoid score was derived from summing the quintile scores for total \(\beta\)-carotene intake and dietary intakes of \(\alpha\)-carotene, \(\beta\)-cryptoxanthin, lycopene, and lutein/zeaxanthin.

DISCUSSION
In this large prospective study of vitamin intake and risk of hearing loss in 65,521 US women, higher intakes of \(\beta\)-carotene and \(\beta\)-cryptoxanthin were independently associated with lower risk of hearing loss, whereas higher vitamin C intake (from supplements) was associated with higher risk. Higher folate intake tended to be associated with lower risk. No significant associations were observed between intake of vitamin A, other carotenoids, or vitamin E and risk of hearing loss in these women.

Acquired hearing loss in adults is multifactorial and results from the cumulative influence of a number of intrinsic and extrinsic factors over the course of a lifetime. Factors that contribute to hearing loss include insufficient cochlear blood supply that leads to hypoxia and ischemic damage, oxidative stress, mitochondrial dysfunction and cell injury, and peripheral and central auditory neurodegeneration (43). There are several potential mechanisms by which carotenoids and vitamins may...
influence auditory function, including providing protection against oxidative damage and mediating vascular and membrane function (4, 44, 45). Some animal evidence suggests that antioxidant nutrients may attenuate cell damage after exposure to noise or ototoxins (46, 47); however, results have been inconsistent, and their role in age-related hearing loss is unclear (48, 49). Although results from animal studies of antioxidant treatment are promising, data in humans are limited and not conclusive (50, 51).

Carotenoids may reduce oxidative stress and DNA damage by scavenging free radicals. β-Carotene is efficient at quenching singlet oxygen and inhibits oxidative modification of LDL cholesterol. Both β-carotene and β-cryptoxanthin may increase intracellular glutathione concentrations, modulate cytokines, and alter lipid metabolism (52). Our finding of an inverse association between higher β-carotene intake and hearing loss is consistent with those from some human cross-sectional studies (42, 53); however, it differs from findings from 2 previous longitudinal studies, one in older US men, the Health Professionals Follow-Up Study (HPFS) (n = 26,273), and one in older Australian adults, the Blue Mountains Hearing Study (BMHS) (n = 798), in which no prospective associations were observed (10, 13). Possibly, the larger size and longer follow-up in our study afforded better ability to detect an association. However, differences in the study populations and the use of self-report in our study compared with hearing threshold measurements in the BMHS to assess the outcome may account for the differing results. To our knowledge, no other prospective study has examined the independent association between intake of β-cryptoxanthin and risk of hearing loss in women.

Vitamins A, C, and E have been shown to be scavengers of singlet oxygen, reduce peroxyl radicals, and inhibit lipid peroxidation (54–56). Although some cross-sectional studies observed an association between higher vitamin A intake and better hearing thresholds (10, 53), others have reported worse auditory function or no association (11, 12). We found no prospective association between vitamin A intake and risk of incident hearing loss, consistent with previous prospective studies (10, 13).

Vitamin C can have antioxidant activity and may influence vascular remodeling, endothelial function (57), and atherosclerosis (58). In some cross-sectional studies, individuals with higher intake of vitamin C tended to have better hearing...
 tween dietary and total intake for those nutrients most com-
cancer, and mortality found no effect in healthy populations
the effects of dietary supplementation with vitamins A, C, and
and cardiovascular disease and cancer. However, trials examining
with the hope they will prevent chronic diseases, including
daily oral folic acid supplementation slowed a 3-y decline in
a randomized controlled trial in the Netherlands that found that
of hearing loss. This is consistent with our prospective findings
higher folate intake tended to be inversely associated with risk
its beneficial effects on endothelial function (62). In our study,
more association with lower plasma concentrations of uric acid (38),
relation between low plasma uric acid and hearing loss is
unclear. Uric acid production may be elevated in human peri-
conditions of oxidative stress (60) and perhaps may
play a role in mediating oxidative damage. This relation merits
further exploration.

In addition to acting as a scavenger of free radicals, vitamin E
may play a role in lipid peroxidation, inhibition of foam cell
formation, and platelet function (61). An association between
higher vitamin E intake and better prevalent hearing thresholds
was observed in some cross-sectional studies (10, 12, 42) but not
in another (11). Prospective findings from both the HPFS and
BMHS suggested that higher vitamin E intake was not longitudi-
inally associated with the risk of incident hearing loss (10, 13).
Folate may favorably influence cochlear blood flow through
its beneficial effects on endothelial function (62). In our study,
higher folate intake tended to be inversely associated with risk
of hearing loss. This is consistent with our prospective findings
from the HPFS (13). This is also consistent with findings from
a randomized controlled trial in the Netherlands that found that
daily oral folic acid supplementation slowed a 3-y decline in
hearing thresholds (16).

In the United States, dietary supplements are commonly taken
with the hope they will prevent chronic diseases, including
vascular disease and cancer. However, trials examining
the effects of dietary supplementation with vitamins A, C, and
E or folate on the primary prevention of cardiovascular disease,
cancer, and mortality found no effect in healthy populations
(63). In addition, findings from a recent large cohort study did
not support the use of supplemental intake of vitamin A, C, or
E to increase longevity (64). We explored the relations be-
tween dietary and total intake for those nutrients most com-
monly consumed as individual supplements or in multivitamins.
For β-carotene, vitamin E, and folate, we did not observe any
material differences in the results for diet or total intake. For
women with very low dietary intake of folate (<200 μg/d),
supplemental folate intake may help lower the elevated risk.
Because it is difficult to achieve the highest amounts of vitamin
C intake without supplements, we did not have enough women
who derived these higher amounts from diet alone, so we were
unable to draw conclusions.

It has been suggested that the relation between antioxidant
nutrient intake and hearing loss may be modified by the
amount of magnesium intake. In a cross-sectional study,
higher intakes of β-carotene, vitamin C, and magnesium were
associated with better prevalent pure-tone thresholds, and higher
intakes of β-carotene or vitamin C combined with high
magnesium compared with low intakes of both nutrients were
associated with better prevalent high frequency pure-tone
thresholds (42). Examined prospectively, we found that the
association between intake of any of the vitamins and risk of
incident hearing loss did not vary by amount of magnesium intake.

Our study has limitations. Dietary information was self-
reported, and thus nondifferential misclassification of our
exposures may have resulted in an underestimation of the
associations of interest. Although this could possibly explain null
findings for some of the antioxidant nutrients examined, we
averaged multiple dietary assessments, which helps reduce ran-
dom measurement error (20). We have previously detected im-
portant diet and hearing loss relations for other self-reported
nutrients in this cohort (31). Although the correlations for some
of the nutrients may be moderate, these measures can still be used
to detect significant associations. Nevertheless, misclassification
is a possibility. Notably, the correlation coefficients we report
from the validation study do not take into account the improve-
ment in accuracy when cumulative averaging is used, as we did
in this study. With cumulative average updated dietary data, we
were able to enhance the precision of dietary assessments and
account for changes in nutrient intakes over time and reduce
misclassification of intake. The lack of an association observed
for certain nutrients examined in this study might reflect non-
differential measurement error of intake, which results in bias
toward the null. Assessment of hearing loss was based on self-
report. Hearing decline is often subtle in onset, and thus there is
imprecision in the assessment of date of onset. Standard PTA
is the gold-standard measure for evaluation of hearing loss;
however, assessment of hearing loss based on self-report has
been found to be reasonably reliable (24, 65). Assessment of
hearing loss was based on participant report in 2009 regarding
date of onset, yet all information on exposures and covariates
was collected before the reported date of hearing loss onset;
therefore, the relations were examined prospectively. We did
not have information on hearing loss severity at the time of
onset, and thus severity of hearing loss could not be considered.
Participants reported whether they had a hearing problem yet
may have had auditory problems other than hearing loss per se.
Hearing loss is highly prevalent (3), and we used the term
hearing loss based on the assumption that this describes the
hearing problem reported by most participants. This is an ob-
servational study, and therefore residual confounding could have
influenced the results; nevertheless, we carefully adjusted for
many potentially confounding variables in our analyses. After
fortification of the US food supply in 1998, total folate intake
<200 μg/d became relatively uncommon, and thus we were
limited in our ability to explore the relation between very low
intake and risk of hearing loss. Our study was limited to pre-
dominantly non-Hispanic white women, and thus further re-
search in additional populations is warranted.

These findings from a large prospective study of carotenoid
and vitamin intake and risk of hearing loss among US women
suggest that higher intake of β-carotene, β-cryptoxanthin, and
folate, whether total or from diet, and avoidance of vitamin C
supplements could play a role in the prevention of acquired
hearing loss.

The authors’ responsibilities were as follows—SGC, KMS, RDE, MW,
MJS, and GCC: contributed to the study concept and design and critically
revised the manuscript for important intellectual content; SGC, RDE, MW,
MJS, and GCC: contributed to the analysis, acquisition, or interpretation
of the data; SGC: drafted the manuscript; MW and GCC: provided statistical
expertise; GCC: provided study supervision and had primary responsibility
for the final content; and all authors: read and approved the final manuscript.
None of the authors reported a conflict of interest.
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