Antioxidant vitamins and magnesium and the risk of hearing loss in the US general population[1–4]

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ABSTRACT

Background: The protective effects of antioxidant vitamins on hearing loss are well established in animal studies but in few human studies. Recent animal studies suggest that magnesium intake along with antioxidants may act in synergy to prevent hearing loss.

Objective: We examined associations between intake of antioxidant vitamins (daily β-carotene and vitamins C and E) and magnesium and hearing thresholds and explored their joint effects in US adults.

Design: We analyzed cross-sectional data from 2592 participants aged 20–69 y from NHANES 2001–2004. Hearing thresholds as pure tone averages (PTAs) at speech (0.5, 1, 2, and 4 kHz) and high frequencies (3, 4, and 6 kHz) were computed.

Results: When examined individually, modeled as quartiles, and after adjustment for potential confounders, higher intakes of β-carotene, vitamin C, and magnesium were associated with lower (better) PTAs at both speech and high frequencies. High intakes of β-carotene or vitamin C combined with high magnesium compared with low intakes of both nutrients were significantly associated with lower (better) PTAs at high frequencies (−14.82%; 95% CI: −20.50% to −8.74% for β-carotene + magnesium and −10.72%; 95% CI: −16.57% to −4.45% for vitamin C + magnesium). The estimated joint effects were borderline significantly larger than the sums of the individual effects [high β-carotene/low magnesium (−4.98%) and low β-carotene/high magnesium (−0.80%), *P*-interaction = 0.08; high vitamin C/low magnesium (−1.33%) and low vitamin C/high magnesium (2.13%), *P*-interaction = 0.09].

Conclusion: Dietary intakes of antioxidants and magnesium are associated with lower risks of hearing loss. *Am J Clin Nutr* 2014;99:148–55.

INTRODUCTION

Hearing loss is a major public health concern affecting more than 36 million people (17% of the adult population) in the United States (1). The prevalence of hearing loss is rising rapidly because of an aging population, our increasingly noisy environment, and the growing use of listening devices (2). Hearing loss affects communication, compromising quality of life, and is therefore associated with social costs as well as the economic costs of medical treatment or hearing aids (3). Recently, hearing impairment has been associated with increased cognitive dysfunction in the elderly (4). Identifying protective factors and avoiding risk factors are important to lessen the burden of hearing loss.

The discovery that the formation of free radicals in the inner ear is a key factor in hearing loss (5–10) suggests that antioxidants may play a preventive or therapeutic role. Indeed, animal experiments have shown a therapeutic effect of antioxidant β-carotene (metabolized to vitamin A in vivo) and vitamins C and E on hearing loss, with reduced free radical formation (5, 8, 11–17). Despite biological plausibility, only a few epidemiologic studies have examined the relations between antioxidant nutrients and hearing loss, and the results are not consistent (18–23). Possible reasons for null findings include *1*) that dietary assessment was misclassified or did not account for long-term intake (23) or *2*) imprecise outcome assessments, based on self-reports, were used (21). Furthermore, antioxidants may be most effective when working together with other nutrients. Recently, several animal studies have suggested that antioxidants act synergistically with magnesium intake to prevent hearing loss (7, 10). Magnesium is known to reduce noise-induced vasoconstriction that occurs as a result of free radical formation (7, 24–26). Few epidemiologic studies have shown a protective effect of magnesium intake on hearing loss (20, 27), and we know of no epidemiologic studies that have examined the potential joint effects of antioxidants and magnesium.

The demonstration that increased consumption of dietary antioxidants, with or without magnesium, is associated with a reduction in age-related hearing loss could have profound potential effects on the quality of life of many millions and a related reduction in associated health care costs. Thus, the primary aim of this study was to examine the association between intake of antioxidants (β-carotene and vitamins C and E) and

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magnesium, estimated from both food and dietary supplements, and the risk of hearing loss and their joint effects in a well-defined general US population by using data from NHANES 2001–2004.

SUBJECTS AND METHODS

Study population

NHANES is an ongoing series of cross-sectional surveys conducted by the National Center for Health Statistics (NCHS[5]; CDC) that are designed to assess health and nutritional information from a representative sample of the US civilian, noninstitutionalized US population. The survey includes an initial extensive household interview and a subsequent physical examination, with additional interviews, at a specially equipped Mobile Examination Center. The participants are selected at random, based on a broad demographic distribution (28, 29).

Data for this study included NHANES 2001–2004 (30, 31). We did not use NHANES 1999–2000 because its carotenoid data were insufficiently detailed. In each survey cycle, half of the subjects aged 20–69 y were randomly assigned to participate in the Audiometry Examination Component. Participants who wore hearing aids that could not be removed for testing or who had ear pain and could not tolerate test headphones were excluded (32). We combined 2 survey cycles to analyze 4 y of data, per NCHS recommendations (28). The initial sample size eligible for the audiometric examination was 3935 participants; 2046 in 2001–2002 and 1889 in 2003–2004. Additional participants were excluded for nonresponse ($n = 324$) or unreliable response ($n = 3$) at the audiometric examination as described below, yielding 3608 observations. We further excluded participants with unilateral hearing loss, defined as a >10-dB difference between the pure-tone averages (PTAs) of left and right ears ($n = 297$), those with missing information on dietary or supplement intake ($n = 111$), and those with missing data on occupational, recreational, or firearm noise exposure ($n = 199$) or other key demographic or clinical risk variables ($n = 409$). Therefore, 2592 participants were eligible for data analyses. Compared with those excluded, included participants were more likely to have lower PTAs at high frequencies, be less exposed to recreational noise, and be non-Hispanic white (*see* Supplemental Table 1 under "Supplemental data" in the online issue). To better interpret regression results and to avoid adding a constant before log transformation of hearing thresholds, we additionally excluded 49 participants at speech frequencies and 57 participants at high frequencies, with hearing thresholds ≤ 0 dB from linear regression models, leaving a total of 2543 and 2535 participants at speech and high frequencies, respectively. NHANES is a publicly available data set, and all participants provided written informed consent, consistent with approval by the NCHS Institutional Review Board.

Audiometric measurement

Audiometry examination was performed in a sound-isolated room by health technicians trained by an audiologist certified by the National Institute for Occupational Safety and Health. Instrumentation for the Audiometry Component included an audiometer (Interacoustics model AD226) with standard headphones (TDH-39) and insert earphones (Etymotic EarTone 3A) (32).

Pure-tone air conduction hearing thresholds were obtained for each ear at frequencies from 0.5 to 8 kHz over an intensity range of -10 to 120 dB. We computed hearing thresholds (dB) as a PTA at speech frequencies (0.5, 1, 2 and 4 kHz; speech-PTA) and at high frequencies (3, 4, and 6 kHz; high-PTA) (3). Hearing loss was defined as a speech-PTA >25 dB in either ear (33). Audiograms for participants who did not respond to at least one frequency were coded as nonresponses. To measure reliability of the participant's responses, the 1-kHz frequency was tested twice in each ear, and audiograms with a $\geq$10-dB difference between 2 tests were classified as unreliable responses (32).

Intake of antioxidants and magnesium

Dietary β-carotene, vitamin C, vitamin E, and magnesium intakes in NHANES were estimated by 24-h dietary recall (DR) interview. DR interviews request a list of all foods and beverages consumed by the respondent, except plain drinking water, during the 24-h period before the interview, amounts of the reported foods, and detailed food descriptions (30, 31, 34). Food consumption data were converted to USDA standard reference codes, and food intake was linked to the USDA survey nutrient composition database. Daily nutrient intakes from selected food items were determined by multiplying the content of each nutrient in each food item by its daily consumption. Data on participants' daily dietary intake were estimated as the sum of those from all food sources. Each nutrient intake reported was adjusted for total energy intake by using the residual method (35). The information on supplement use during the 30 d before the interview was gathered. Individual nutrient intakes from the supplements were calculated by using the supplement product ingredient information, and participants' daily intake was computed as the sum of all supplement sources intake. Estimated total nutrient intake was the sum of food and supplement intakes (36). Further details are available elsewhere (*see* "Supplemental data" in the online issue).

We created 2 composite scores of antioxidant intake using a dietary composite scoring system based on percentile ranks of each nutrient's intake developed by Chiu et al (37): *1*) antioxidant composite 1 (AC1) = β-carotene plus vitamin C, indicating water-soluble antioxidant vitamins; and *2*) antioxidant composite 2 (AC2) = β-carotene plus vitamin C plus vitamin E, indicating both water- and fat-soluble antioxidant vitamins. We first ranked and clustered the 2592 participants into percentiles by ordering intake from the lowest to the highest for each nutrient. We then defined the antioxidant composite scores of *1*) AC1 ($0 \leq$ scale ≤ 200) by summing the 2 rank scores of β-carotene and vitamin C intake and *2*) AC2 ($0 \leq$ scale ≤ 300) by summing the 3 rank scores of β-carotene, vitamin C, and vitamin E intake. Note that magnesium was not included as part of these composite scores.

Demographic/clinical risk factors

BMI was calculated as weight (in kg)/height (in m)^2. Use of ototoxic medication was defined as self-reported use of aminoglycoside, loop diuretics, antineoplastic drugs, or nonsteroidal

[5]Abbreviations used: AC1, antioxidant composite 1; AC2, antioxidant composite 2; DR, dietary recall; DRI, Dietary Reference Intake; NCHS, National Center for Health Statistics; O*NET, Occupational Network; PTA, pure-tone average.

antiinflammatory drugs. Smoking pack-years were computed, and participants were grouped into nonsmokers, smokers <20 pack-years, or smokers with ≥20 pack-years. Hypertension was defined as a self-reported physician diagnosis, current use of antihypertensive medication, systolic blood pressure ≥140 mm Hg, or diastolic blood pressure ≥90 mm Hg at the time of examination. Type 2 diabetes was defined as a self-reported physician diagnosis or current use of antihyperglycemic medication (38).

Occupational noise exposures were defined based on the job title held for the longest time in the participant's working life and the Occupational Network (O*NET) database (39). Exposures to nonoccupational firearm noise and recreational noise were defined as exposed by questionnaire if a respondent indicated exposure outside of work to the noise of a firearm and if he or she indicated exposure outside of work to loud noise (eg, power tools or loud music) at least once a month for 1 y.

Statistical analysis

All statistical analyses were performed by using the SAS survey procedures (version 9.2; SAS Institute Inc) to account for the complex survey design and sample weights of NHANES 2001–2004 (40). We computed 4-y sample weights per NCHS recommendations and adjusted for oversampling and nonresponse of subjects such as ethnic minorities, elderly persons, and low income (28). The statistical significance level was set as $P < 0.05$.

Hearing thresholds in PTA were highly skewed and, thus, log transformed to normalize distributions. Individual nutrients and the antioxidant composite scores were each categorized into quartiles. All regression models were adjusted for age, age^2 to capture nonlinear effects, sex, race-ethnicity [non-Hispanic white (reference), non-Hispanic black, Mexican American, or other], BMI, ototoxic medication use (yes or no), pack-years of cigarette smoking [never smoker (reference), <20 pack-years, or ≥20 pack-years], hypertension (yes or no), type 2 diabetes (yes or no), and occupational (O*NET score, continuous), recreational (yes or no), and firearm noise exposures (yes or no). We estimated the percentage change in hearing threshold by comparing each of the upper 3 quartiles with the lowest quartile. *P* values for linear trend were evaluated by fitting the nutrient quartile as an ordinal variable. Logistic regression was used to estimate the ORs for hearing loss.

To evaluate joint effects of antioxidants (β-carotene, vitamin C, vitamin E, AC1, or AC2) and magnesium on log-transformed PTA, we made a combined categorical intake variable classified as low antioxidant and low magnesium (reference), low antioxidant and high magnesium, high antioxidant and low magnesium, or high antioxidant and high magnesium. Low and high categories were based on the Estimated Average Requirements of Dietary Reference Intake (DRI) (41). We used the median for β-carotene, AC1, and AC2, because no DRI values are available. Their joint effects were also examined in subgroups stratified by age (20–39, 40–59, or ≥60 y), BMI (<30 or ≥30), and occupational noise exposure (<2.84 or ≥2.84 O*NET score).

Sensitivity analysis

We computed speech-PTAs and high-PTAs with different sets of frequencies (averages of 0.5, 1, and 2 kHz for speech-PTA and 3, 4, 6, and 8 kHz for high-PTA) according to the method used by Shargorodsky et al (21) and Bainbridge et al (38) and conducted sensitivity analyses.

RESULTS

Characteristics of our study population are shown in **Table 1**. After sampling weights, clusters, and strata of the NHANES

TABLE 1
General characteristics of the study participants (n = 2592)[1]

Characteristic	Distribution
β-Carotene (μg/d)	
Total, diet plus supplement	1931 ± 99.4[2]
Diet only	1931 ± 99.4
Vitamin C (mg/d)	
Total, diet plus supplement	212 ± 11.1
Diet only	90.9 ± 3.4
Vitamin E (mg/d)	
Total, diet plus supplement	49.2 ± 4.23
Diet only	3.69 ± 0.17
AC1 (nutritional score)[3]	
Total, diet plus supplement	98.6 ± 1.51
Diet only	96.6 ± 1.62
AC2 (nutritional score)[4]	
Total, diet plus supplement	150 ± 2.23
Diet only	145 ± 2.28
Magnesium (mg/d)	
Total, diet plus supplement	331 ± 4.85
Diet only	286 ± 3.87
Speech-frequency PTA (dB)[5]	12.7 ± 0.35
High-frequency PTA (dB)[6]	18.8 ± 0.59
Hearing loss (%)	11.7[7]
Age (y)	42.1 ± 0.33
BMI (kg/m^2)	28.0 ± 0.14
Noise exposures	
Occupational noise[8] (O*NET score)	3.1 ± 0.02
Recreational noise (exposed %)	27.5
Firearm noise (exposed %)	7.8
Sex (male %)	47.8
Race ethnicity (%)	
Non-Hispanic white	73.0
Non-Hispanic black	11.2
Mexican American	6.8
Other	9.0
Ototoxic medication (current use %)	14.3
Cumulative cigarette pack-years (%)	
Never	54.4
<20	33.2
≥20	12.4
Current hypertension (%)	23.5
Current diabetes mellitus (%)	4.5

[1] Participants included individuals with all variables of interest: hearing thresholds, nutrient intake, age, BMI, sex, race-ethnicity, ototoxic medication use, cumulative cigarette pack-years, hypertension, diabetes, and occupational, recreational, and firearm noise. AC1, antioxidant composite 1; AC2, antioxidant composite 2; O*NET, Occupational Network; PTA, pure-tone average.
[2] Four-year weighted mean ± SE (all such values).
[3] β-Carotene + vitamin C (0 < nutritional score < 200).
[4] β-Carotene + vitamin C + vitamin E (0 < nutritional score < 300).
[5] At 0.5, 1, 2, and 4 kHz.
[6] At 3, 4, and 6 kHz.
[7] Weighted percentages (all such values).
[8] 1 < O*NET noise scale < 5.

TABLE 2
Means and 95% CIs of nutrient intake by participants' characteristics (n = 2592)[1]

Characteristic	Participants[2]	β-Carotene	Vitamin C	Vitamin E	Magnesium
	n (%)	*μg/d*	*mg/d*	*mg/d*	*mg/d*
Total	2592	1931 (1728, 2134)	212 (189, 235)	49.2 (40.6, 57.9)	331 (321, 341)
Age					
20–39 y	1145 (44.1)	1679 (1455, 1904)*	159 (139, 180)*	17.3 (13.3, 21.2)*	294 (282, 305)*
40–59 y	988 (44.6)	1969 (1652, 2287)	245 (207, 283)	63.9 (46.6, 81.2)	355 (337, 373)
60–69 y	459 (11.3)	2765 (2243, 3286)	288 (248, 328)	115.9 (95.6, 136.3)	381 (345, 417)
Sex					
Male	1202 (47.8)	1781 (1508, 2053)*	191 (163, 219)*	42.0 (33.6, 50.3)†	325 (310, 341)
Female	1390 (52.2)	2069 (1868, 2270)	231 (207, 256)	55.9 (42.8, 68.9)	336 (327, 345)
BMI					
<30 kg/m^2	1726 (68.3)	2080 (1831, 2329)*	224 (199, 250)*	50.0 (39.5, 60.5)*	339 (326, 352)*
≥30 kg/m^2	866 (31.7)	1612 (1369, 1855)	185 (150, 221)	47.6 (37.8, 57.4)	313 (303, 323)
Race ethnicity					
Non-Hispanic white	1315 (73.0)	1949 (1682, 2217)	229 (197, 261)*	57.1 (47.3, 66.9)*	345 (331, 360)*
Non-Hispanic black	565 (11.2)	2099 (1723, 2475)	149 (130, 167)	22.2 (16.0, 28.4)	264 (252, 276)
Mexican American	516 (6.8)	1780 (1445, 2114)	159 (130, 188)	18.7 (12.3, 25.2)	317 (305, 329)
Other	196 (9.0)	1692 (1274, 2110)	192 (139, 245)	41.6 (10.5, 72.7)	308 (291, 324)
Occupational noise exposure[3]					
Low, <2.84	1311 (53.6)	2265 (1998, 2533)*	248 (223, 273)*	60.6 (47.6, 73.5)*	349 (337, 361)*
High, ≥2.84	1281 (46.4)	1546 (1360, 1732)	171 (141, 201)	36.1 (26.7, 45.6)	310 (294, 325)
Firearm noise exposure					
No	2422 (92.2)	1925 (1737, 2113)	214 (190, 238)	50.3 (40.7, 59.9)	330 (322, 338)
Yes	170 (7.8)	2010 (1230, 2790)	191 (124, 258)	36.9 (23.8, 49.9)	341 (288, 395)
Recreational noise exposure					
No	1952 (72.5)	1935 (1720, 2150)	216 (190, 242)	49.9 (38.9, 60.9)	333 (322, 343)
Yes	640 (27.5)	1921 (1527, 2315)	202 (171, 234)	47.4 (37.4, 57.4)	326 (310, 342)

[1] *$P < 0.05$ and †$P < 0.1$ (survey t test for binominal groups and the Wald F test for categorical groups). O*NET, Occupational Network.
[2] Weighted percentages from survey frequency.
[3] 1 < O*NET noise scale < 5.

complex design were accounted for, the mean (±SE) age was 42.1 ± 0.33 y, and means of speech-PTA and high-PTA were 12.7 ± 0.35 and 18.8 ± 0.59 dB, respectively. Overall, 309 participants (11.7%) had hearing loss (speech-PTA > 25 dB) in one or both ears. Weighted means of daily β-carotene, vitamin C, vitamin E, and magnesium intakes were 1931 ± 99.4 μg, 212 ± 11.1 mg, 49.2 ± 4.23 mg, and 331 ± 4.85 mg, and those of AC1 and AC2 scores were 98.6 ± 1.51 and 150 ± 2.23, respectively. Weighted means of individual nutrients, by participant's characteristics, are shown in **Table 2**. Each of the nutrient intake was likely to be higher in older individuals (compared with younger) and in participants with low BMI (compared with high BMI) and low occupational noise exposure (compared with high exposure).

After adjustment for potential confounders, all individual nutrients and antioxidant composite scores across each quartile showed dose-dependent trends with lower (better) speech-PTA, and all factors except vitamin E showed trends toward lower high-PTA (**Table 3**). Compared with participants in the lowest AC1 quartile, there was significantly lower speech-PTA in the highest quartile (quartile 4: −14.95%; 95% CI: −20.82 to −8.65; P-trend < 0.001). Participants in the highest quartile of AC2 had significantly lower speech-PTA (quartile 4: −14.81%; 95% CI, −20.80 to −8.37; P-trend < 0.001) than did those in the lowest quartile. Magnesium intake was also associated with significantly lower speech-PTA (quartile 4: −13.82%; 95% CI: −21.07 to −5.90; P -trend = 0.008). When modeled as diet-only variables, similar but less significant dose-dependent relations were found with β-carotene, vitamins C and E, magnesium, and AC1 (*see* Supplemental Table 2 under "Supplemental data" in the online issue). We found consistent results in sensitivity analyses by using different definitions of speech-PTA and high-PTA (*see* Supplemental Table 3 under "Supplemental data" in the online issue). By using logistic regression hearing loss, we found significant dose-dependent reductions in the odds of hearing loss across quartiles of vitamins C and E and AC2 (**Table 4**).

In fully adjusted models, we evaluated the joint effect of antioxidant intake combined with magnesium intake on reduced hearing thresholds (**Table 5**). Low compared with high cutoff points for vitamins C (75 mg/d for men; 60 mg/d for women) and E (12 mg/d) and magnesium (350 mg/d for men; 265 mg/d for women) were defined as Estimated Average Requirements of DRI, whereas those for β-carotene, AC1, and AC2 were at the medians. Participants with both high β-carotene and magnesium intakes (compared with both low) had significantly lower (better) high-PTA (−14.82%; 95% CI: −20.50%, −8.74%), and this was almost 3 times the additive effects of high β-carotene only (−4.98%; 95% CI: −11.54%, 2.06%) or of high magnesium only (−0.80%; 95% CI: −8.86%, 7.99%), with a marginally significant multiplicative joint effect (P-interaction = 0.082). For vitamins C and magnesium intakes, participants with both high intakes had a significant reduction (−10.72%; 95% CI: −16.57, −4.45%) in high-PTA that was marginally significantly larger than the additive effects of high vitamin C only (−1.33%; 95% CI: −9.37%, 7.42%) and high magnesium only (2.13%; 95% CI: −8.49%, 13.97%) (P-interaction = 0.090). Similar but less

TABLE 3
Multivariate-adjusted percentage changes (95% CIs) of hearing thresholds by total nutrient intake quartiles[1]

	Q1	Q2	Q3	Q4	*P*-trend
Speech-frequency PTA (n = 2543)[2]					
β-Carotene[3]	0 (Reference)	−8.90 (−16.66, −0.41)	−12.90 (−19.81, −5.39)	−14.31 (−21.03, −7.03)	<0.001
Vitamin C[4]	0 (Reference)	−6.96 (−15.39, 2.30)	−7.98 (−15.63, 0.36)	−14.17 (−22.04, −5.50)	0.004
Vitamin E[5]	0 (Reference)	3.06 (−8.78, 16.43)	−1.92 (−10.25, 7.18)	−9.52 (−17.29, −1.02)	0.005
Magnesium[6]	0 (Reference)	−15.69 (−22.92, −7.77)	−13.43 (−20.65, −5.55)	−13.82 (−21.07, −5.90)	0.008
AC1[7]	0 (Reference)	−7.35 (−13.58, −0.68)	−16.59 (−22.30, −10.45)	−14.95 (−20.82, −8.65)	<0.001
AC2[8]	0 (Reference)	−5.32 (−12.74, 2.72)	−12.07 (−18.75, −4.86)	−14.81 (−20.80, −8.37)	<0.001
High-frequency PTA (n = 2535)[9]					
β-Carotene[3]	0 (Reference)	−2.93 (−11.49, 6.45)	−9.65 (−16.18, −2.62)	−14.46 (−20.83, −7.59)	<0.001
Vitamin C[4]	0 (Reference)	−3.18 (−11.36, 5.74)	−7.64 (−15.16, 0.54)	−12.31 (−19.69, −4.26)	0.002
Vitamin E[5]	0 (Reference)	7.65 (−5.10, 22.11)	1.80 (−6.71, 11.10)	−6.78 (−15.60, 2.97)	0.061
Magnesium[6]	0 (Reference)	−13.55 (−24.33, −1.23)	−10.58 (−17.73, −2.81)	−11.93 (−20.02, −3.02)	0.024
AC1[7]	0 (Reference)	−1.10 (−10.32, 9.08)	−11.07 (−19.13, −2.20)	−13.75 (−19.48, −7.62)	<0.001
AC2[8]	0 (Reference)	−2.32 (−11.33, 7.61)	−9.97 (−17.68, −1.54)	−13.72 (−20.15, −6.77)	<0.001

[1] Values were adjusted for age, age^2, sex, race-ethnicity, BMI, ototoxic medication use, pack-years of cigarette smoke, hypertension, diabetes, occupational noise, recreational noise, and firearm noise. Q1 is the reference. AC1, antioxidant composite 1; AC2, antioxidant composite 2; PTA, pure-tone average; Q, quartile (~25th percentile, ~median, ~75th percentile, ~100th percentile).

[2] At 0.5, 1, 2, and 4 kHz.

[3] β-Carotene quartile cutoff points: 375.63, 771, and 2034 μg/d.

[4] Vitamin C quartile cutoff points: 44.623, 107, and 213.5 mg/d.

[5] Vitamin E quartile cutoff points: 0.944, 6.444, and 22.2 mg/d.

[6] Magnesium quartile cutoff points: 233.49, 294.75, and 374.45 mg/d.

[7] β-carotene + vitamin C (0 < nutritional score < 200); quartile cutoff points: 66.3, 101, and 137.2.

[8] β-carotene + vitamin C + vitamin E (0 < nutritional score < 300); quartile cutoff points: 101.2, 150.6, and 200.3.

[9] At 3, 4, and 6 kHz.

significant joint effects were found in speech-PTA. No significant joint effects were found in combination with AC1 or AC2 (data not shown). We did not observe significant effect modification by age, BMI, and occupational noise groups (data not shown).

DISCUSSION

In a representative sample of US adults, the current study suggests apparent protective dose-response relations of hearing thresholds with intakes of antioxidant vitamins and magnesium. We found significant dose-dependent reductions in hearing thresholds with antioxidants (β-carotene and vitamins C and E) and magnesium intakes, at levels currently observed in the general US population. These trends were observed in models after adjustment for socioeconomic factors, noise exposures, and other potential risk factors.

Free radical formation in the inner ear is a key mechanism for hearing loss (7, 42), causing cell death and consequence vasoconstriction and a rebound of cochlear blood flow, which, such as stroke, further contributes to free radical formation. Animal experiments have shown that pretreatment with β-carotene and/or vitamins C and E reduced noise-induced formation of free

TABLE 4
Multivariate-adjusted ORs (95% CIs) of hearing loss by total nutrient intake quartile (n = 2592)[1]

ORs of hearing loss >25 dB[2]	Q1	Q2	Q3	Q4	*P*-trend
β-Carotene[3]	1 (Reference)	0.87 (0.54, 1.39)	0.90 (0.56, 1.54)	0.74 (0.48, 1.14)	0.232
Vitamin C[4]	1 (Reference)	1.02 (0.63, 1.64)	0.98 (0.62, 1.57)	0.62 (0.40, 0.96)	0.030
Vitamin E[5]	1 (Reference)	1.13 (0.62, 2.04)	1.25 (0.75, 2.11)	0.67 (0.44, 1.04)	0.019
Magnesium[6]	1 (Reference)	0.60 (0.33, 1.11)	0.84 (0.44, 1.58)	0.68 (0.39, 1.19)	0.434
AC1[7]	1 (Reference)	1.07 (0.70, 1.63)	0.78 (0.49, 1.22)	0.72 (0.47, 1.09)	0.053
AC2[8]	1 (Reference)	0.83 (0.46, 1.49)	0.97 (0.62, 1.51)	0.54 (0.34, 0.84)	0.008

[1] Values were adjusted for age, age^2, sex, race-ethnicity, BMI, ototoxic medication use, pack-years of cigarette smoke, hypertension, diabetes, and occupational, recreational, and firearm noise. Q1 is the reference. AC1, antioxidant composite 1; AC2, antioxidant composite 2; PTA, pure-tone average; Q, quartile (~25th percentile, ~median, ~75th percentile, ~100th percentile).

[2] Hearing loss was defined as speech-PTA (0.5, 1, 2, and 4 kHz) >25 dB in either ear.

[3] β-Carotene quartile cutoff points: 375.63, 771, and 2034 μg/d.

[4] Vitamin C quartile cutoff points: 44.623, 107, and 213.5 mg/d.

[5] Vitamin E quartile cutoff points: 0.944, 6.444, and 22.2 mg/d.

[6] Magnesium quartile cutoff points: 233.49, 294.75, and 374.45 mg/d.

[7] Antioxidant composite 1: β-carotene + vitamin C (0 < nutritional score < 200); quartile cutoff points: 66.3, 101, and 137.2.

[8] Antioxidant composite 2: β-carotene + vitamin C + vitamin E (0 < nutritional score < 300); quartile cutoff points: 101.2, 150.6, and 200.3.

TABLE 5
Percentage changes (95% CIs) in hearing thresholds (dB) by joint effect between antioxidant and magnesium intakes[1]

	Change (95% CI)		
	Low magnesium	High magnesium	*P*-interaction
	%		
Speech-frequency PTA (n = 2543)[2]			
Low β-carotene	0 (Reference)	−7.87 (−14.94, −0.21)*	0.670
High β-carotene	−9.32 (−16.74, −1.23)*	−14.21 (−20.26, −7.71)‡	
Low vitamin C	0 (Reference)	−1.68 (−12.26, 10.17)	0.329
High vitamin C	−3.39 (−11.42, 5.38)	−11.87 (−18.60, −4.59)†	
Low vitamin E	0 (Reference)	−3.23 (−12.46, 6.97)	0.425
High vitamin E	−5.75 (−13.42, 2.60)	−13.74 (−18.02, −9.23)‡	
High-frequency PTA (n = 2535)[3]			
Low β-carotene	0 (Reference)	−0.80 (−8.86, 7.99)	0.082
High β-carotene	−4.98 (−11.54, 2.06)	−14.82 (−20.50, −8.74)‡	
Low vitamin C	0 (Reference)	2.13 (−8.49, 13.97)	0.090
High vitamin C	−1.33 (−9.37, 7.42)	−10.72 (−16.57, −4.45)†	
Low vitamin E	0 (Reference)	−4.68 (−12.73, 4.11)	0.934
High vitamin E	−6.89 (−15.90, 3.08)	−11.71 (−16.78, −6.32)‡	

[1] Low compared with high cutoff points for vitamins C (75 mg/d for men; 60 mg/d for women) and E (12 mg/d) and magnesium (350 mg/d for men; 265 mg/d for women) were defined as the Estimated Average Requirements of Dietary Reference Intakes. We used the median for β-carotene (771 μg/d) because no Estimated Average Requirements for β-carotene were available. All models were adjusted for age, age^2, sex, race-ethnicity, BMI, ototoxic medication use, pack-years of cigarette smoke, hypertension, diabetes, occupational noise, recreational noise, and firearm noise. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. PTA, pure-tone average.
[2] At 0.5, 1, 2, and 4 kHz.
[3] At 3, 4, and 6 kHz.

radicals, hearing loss, and sensory cell pathology in the inner ear consistent with a preventive or therapeutic role in hearing loss (5, 8, 10–17). Despite extensive basic research evidence, previous epidemiologic studies have failed to support a protective association between antioxidant vitamin intake and hearing loss. A previous study of elderly adults in the Blue Mountains reported a significant association of dietary antioxidant intake with prevalence, but not with 5-y incidence, of hearing loss, possibly because of the short follow-up time (18). A study of noise-induced hearing loss in the work place observed no significant difference in blood concentrations of vitamins A (β-carotene potentially converts to vitamin A) or E between patients and a control group (23). A follow-up study of 26,273 US men between 1986 and 2004 reported that a higher intake of β-carotene and vitamins C and E did not reduce the risk of hearing loss (21). Measurement errors in antioxidant intake based on biomarkers with short half-lives (23) and in outcome assessment based on self-reports (21) may explain why the previous studies did not find associations. Dietary intake that excludes intake from supplements may lead to misclassification of exposure. In fact, we found less significant associations when only food-based antioxidant intake was used. Dietary assessments based on both food and supplements would seem to ensure a more valid measure of antioxidant intake and may enable us to find stronger associations between antioxidants and hearing loss.

The current study provides suggestive evidence of a synergistic effect on hearing loss by the combined intake of antioxidants (β-carotene and vitamins C) and magnesium, as was suggested by Le Prell's animal experiment (10), with marginal significance on the multiplicative interaction. Le Prell et al suggest that the most effective strategy to prevent hearing loss is to inhibit the "excessive free radical formation" that is triggered by aging, noise, and other environmentally mediated trauma. Their studies show that free radical scavengers, such as vitamins A, C, and E, act in synergy with magnesium to reduce changes in hearing thresholds more reliably than treatment with any single agent (10). Other animal studies have shown that magnesium reduces noise-induced vasoconstriction (7, 10, 24–26) and alone may reduce noise-induced hearing loss (27). These studies provide the scientific rationale to support our epidemiologic findings.

Such protective effects could roughly compensate for risk factors of diabetes, cigarette smoke (heavy compared with nonsmoker), race ethnicity (non-Hispanic white compared with black), aging of 4 y (when age is fit linearly), an 18-kg/m^2 increase of BMI, and one-unit increase of O*NET occupation noise scores (corresponding to the noise exposure difference between occupation categories of "Fabricators, assemblers, inspectors, and samplers" and "Executive, administrators, and managers"), which indicates that beneficial effects may be greater than adverse effects from traditional risk factors (*see* Supplemental Table 4 under "Supplemental data" in the online issue).

The chief strengths of this study are *1*) its use of a representative sample of the US general population that justifies generalization of our findings and *2*) adjustment for potential confounders of the association between nutrients and hearing loss, especially exposure to occupational noise through the newly developed assessment tool by using O*NET (39), exposure to nonoccupational noise, and use of ototoxic medication.

This study had several limitations to be considered. Although NHANES data reflect a well-defined and representative sample of the US population, NHANES is cross-sectional in design; thus, causal inferences cannot be drawn between nutrients and hearing loss. Reverse causality cannot be excluded: subjects with severe hearing loss may have had diet rich in antioxidants and/or

magnesium. One might argue that the observed associations could differ depending on personal characteristics. In the current study, nutrient intake was likely to be higher in older adults and in people with low occupational noise and BMI (Table 2); however, we confirmed that the nutritional influence on hearing thresholds does not depend on those factors. We also cannot rule out recall bias from dietary assessment based on a 24-h DR. This method is known to be inadequate for capturing day-to-day variability in diet patterns; however, such error would be random and influence results toward the null.

In conclusion, higher intakes of antioxidants and magnesium, individually and in combination, appear to be associated with a lower risk of hearing loss that may be greater than adverse effects on hearing from traditional risk factors. Although the cross-sectional finding does not allow for the conclusion that intake of antioxidants and magnesium has a causal protective effect on hearing loss, our study has important public health implications. Given inevitable risk factors, our findings suggest that a diet rich in antioxidant vitamins (β-carotene and vitamin C) combined with magnesium may contribute to a reduced risk of hearing loss. In addition to the supplements available over the counter, dietary β-carotene is found in many yellow and orange vegetables; vitamin C is plentiful in citrus fruit; and magnesium is commonly found in green leafy vegetables, legumes, nuts, and whole grains (43). Identification of protective factors coupled with avoidance of risk factors is important, especially for chronic conditions experienced by older adults.

The authors' responsibilities were as follows—Y-HC and SKP: designed and conceived the study and analyzed and interpreted the data; Y-HC: acquired the data, had full access to all of the data in the study, performed the statistical analysis, had final responsibility for the decision to submit for publication, and drafted the manuscript; JMM, KLT, HH, and SKP: critically revised the manuscript for important intellectual content; SKP: obtained funding; and HH and SKP: supervised the study. JMM is co-inventor of a University of Michigan patent for the use of β-carotene, vitamins C and E, plus magnesium to prevent noise-induced hearing loss and is a minority stock holder in a company licensed to commercialize this treatment. A Conflict Management Plan is in place. None of the other authors reported a conflict of interest.

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