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Association of Serum Antioxidant Vitamins and Carotenoids With Incident Alzheimer Disease and All-Cause Dementia Among US Adults

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Abstract

Background. Serum antioxidant vitamins and carotenoids may protect against neurodegeneration with age. We examined associations of these nutritional biomarkers with incident all-cause and AD dementia among U.S. middle-aged and older adults.

Methods. Using data from the third National health and Nutrition Examination Surveys (1988-1994), linked with Centers for Medicare and Medicaid-Medicare follow-up data, we tested associations and interactions of serum vitamins A, C and E, and total and individual serum carotenoids and interactions with incident Alzheimer's Disease (AD) and all-cause dementia. Cox proportional hazards regression models were conducted.

Results. After ≤26y follow-up (mean:16-17y, n=7,283 participants aged 45-90y at baseline), serum lutein+zeaxanthin was associated with reduced risk of all-cause dementia (65+ age group), even in the lifestyle-adjusted model (per SD, HR=0.93, 95%CI: 0.87-0.99, p=0.037), though attenuated in comparison to a socio-economic status (SES)-adjusted model (HR=0.92, 95% CI: 0.86-0.93, p=0.013). An inverse relationship was detected between serum β-cryptoxanthin (per SD increase) and all-cause dementia (45+ and 65+), for age and sex-adjusted models (HR=0.86, 95% CI:0.80-0.93, p<0.001 for 45+; HR=0.86, 95% CI:0.80-0.93, p=0.001 for 65+), a relationship remaining strong in SES-adjusted models (HR=0.89, 95%CI: 0.82-0.96, p=0.006 for

45+; HR=0.88, 95%CI:0.81-0.96, p=0.007 for 65+), but attenuated in subsequent models. Antagonistic interactions indicate putative protective effects of one carotenoid may be observed at lower levels other carotenoids or antioxidant vitamin.

Discussion. Incident all-cause dementia was inversely associated with serum lutein+zeaxanthin and β-cryptoxanthin levels. Further studies with time-dependent exposures and randomized trials are needed to test neuroprotective effects of supplementing the diet with select carotenoids.

Classification of Evidence. This study provides Class II evidence that incident all-cause dementia was inversely associated with serum lutein+zeaxanthin and β-cryptoxanthin levels.

Key Terms: Serum carotenoids; antioxidants; Alzheimer's Disease; Dementia; longitudinal studies; aging

Abbreviations

1995-HEI	Healthy Eating Index, 1995 version
AD	Alzheimer's Disease
AL	Allostatic Load
BMI	Body Mass Index
CI	Confidence Interval
CMS	Centers for Medicare and Medicaid
CPT4	Common Procedural Terminology
DX	Diagnosis
HCPCS	Healthcare Common Procedural Coding System
HHA	Home Health Agency
HMO	Health Maintenance Organization
HOP	Health Options Program
HR	Hazard Ratio
HS	High School
ICD-9	International Classification of Disease-9th revision
ICD-10	International Classification of Disease-10th revision
INCSTAR	Incstar Corp.
MA	Mexican American
MAR	Mean Adequacy Ratio
MEC	Mobile Examination Center
N	Number of participants
NAR	Nutrient Adequacy Ratio
NCHS	National Center for Health Statistics
NDI	National Death Index
NH	Non-Hispanic
NHANES III	Third National Health and Examinations Surveys
PIR	Poverty Income Ratio
PUFA	Polyunsaturated Fatty Acids
RDA	Recommended Dietary Allowance
RDC	Research Data Center
RE	Retinol Equivalent
ROS	Reactive Oxygen Species
SD	Standard Deviation
SE	Standard Error
T1-T3	Tertile
X	Mean

INTRODUCTION

Dementia of all causes and sub-types, including Alzheimer's disease (AD), is a key determinant for disability and long-term institutionalization among older adults [1]. Extending intact cognitive functioning into old age is an increasingly important public health challenge. Such an endeavor would have a sizeable impact on quality of life and costs of care in late life. Thus, a greater focus on and understanding of factors that alter the risk of dementia in general and AD dementia in particular is needed [2].

Oxidative stress has received considerable attention over the past several decades given its possible role in neurodegenerative processes, such as AD, as well as other age-related conditions that affect cardiovascular health and some cancers [3]. Oxidative stress is a form of metabolic stress that emerges due to an imbalance between the production of reactive oxygen species (ROS) and the antioxidant mechanisms that counteract it[2]. The brain comprises a high concentration of lipid and iron content, potentially making neurons especially susceptible to these processes [4]. For example, exposure to ROS can increase brain oxidative processes which may become chronic due to the impaired DNA repair mechanisms that decline with age [5].

Epidemiological studies show that dietary intake of antioxidants (e.g., β-carotene, vitamins A, C and E) may help mitigate oxidative DNA damage through the reduction of ROS [6]. Such antioxidants, consumed via diet or supplements, may protect against neurodegenerative processes including cognitive decline [7]. Studies have also revealed the potential for synergistic effects between some carotenoid and antioxidants [8]. To date, however, no studies have investigated whether carotenoids in general may interact with each other and with vitamins A, C or E in relation to incidence rates of AD or all-cause dementia.

In this report, we use longitudinal data from a large nationally representative sample of middle-aged and older adults, to examine adjusted associations among several serum antioxidants and incidence of AD and all-cause dementia, using a retrospective cohort design. Specifically, we examined relationships of serum vitamins A, C and E with both incident outcomes across levels of serum total carotenoid intake and tested interactions of serum vitamin A, C and E with serum total and individual carotenoids, namely α-carotene, β-carotene, lutein+zeaxanthin, β-cryptoxanthin and lycopene in relation to the two incident outcomes. Finally, interactions between individual carotenoids were also tested in relation to the two incident outcomes of interest.

METHODS

Database

Participants from the Third National Health and Nutrition Examination Survey (NHANES III) comprised a cross-sectional sample representative of the US civilian noninstitutionalized population obtained through a complex multistage probability sample design. Between 1988 and 1994, participants received a household interview and physical examination, including phlebotomy [9]. The NHANES has been linked to several administrative databases, including the Centers for Medicare and Medicaid Service (CMS) and the National Death Index (NDI). Details on these linkage procedures are provided in **eMethods 1**.

Standard Protocol Approvals, Registrations, and Patient Consents

More generally, the procedures followed were in accordance with the ethical standards of the institution or regional committee on human experimentation and approval was obtained from the relevant committee on human subjects at the Center for Disease Control and Prevention,

National Center for Health Statistics. Institutional Review Board (IRB) Approval for the current retrospective analysis of the parent IRB-approved study (i.e. NHANES III linked to CMS-Medicare), was obtained from the National Institutes of Health, Intramural Research Program and the ethics board determined that participant consent was not required or waived.

Study Sample

Details on sample selection criteria are shown in **Figure 1**. We defined our eligible analytic sample to include respondents to the NHANES III who were aged 45-90y (≥45y) at baseline (1988-1994), for whom nutritional biomarkers and linkage to outcome were available, accounting for Health Maintenance Organization (HMO) exclusions. Of 33,199 respondents to the NHANES III aged 1-90y who had complete socio-demographic information, 9,787 were ≥45 years old in their baseline interview. Among these respondents, we further excluded 2,313 respondents for whom nutritional biomarker data were missing or extreme (≥100 μg/dL for α-carotene, n=3 for the 45+ group; ≥300μg/dL for β-carotene, n=6 for the 45+ group), producing an analytic sample of 7,474. Respondents for whom CMS linkage information was missing were assumed to have no event of interest until end of 2013 or censored upon death. Upon further exclusion due to missing covariates of interest and lack of CMS linkage, the sample was reduced to up to 7,283. We included observations that were missing information on some potential confounders and used multiple imputation on these cases. The average rate of missingness on key imputed confounders was<10%. We conducted the same procedure in a further restricted sample to respondents aged ≥65y at baseline for sensitivity analyses (final sample N=3,618 out of an initial sample N=5,252).

Incident all-cause and AD dementia

We used detailed information obtained from the CMS Chronic Condition Data Warehouse to identify cases of AD and all-cause dementia as well as onset time. Diagnostic categories contained 21 chronic conditions with varying reference time periods, numbers and types of claims to qualify, exclusions and a set of ICD-9/CPT4/HCPCS codes. Details are provided in e**Methods 1**. We used age on study (in years to the nearest month) as the underlying time scale, with baseline age defined as the earliest examination date obtained from the Medical Examination Center (MEC). The follow-up period was 1999-2013 for the pre-estimated earliest occurrence date. Follow-up time was truncated to January 1, 2014. We used the same algorithm to estimate AD/dementia's earliest diagnosis date during 1991-1998. [10] Thus, for most participants, the follow-up time could go up to 26y, with a mean of ~16-17 y, depending on the outcome.

Serum carotenoid and antioxidant exposures

Serum levels of vitamin A (retinol), vitamin E (α-tocopherol), retinyl esters and carotenoids were measured by isocratic high performance liquid chromatography with detection at wavelengths of 300, 325, and 450 nm. Quantitation was accomplished by comparison of peak heights with a standard solution.[11] Serum concentrations of vitamin C were measured using a total vitamin C, fully reduced method using high-performance liquid chromatography with electrochemical detection analysis.

Covariates

Socio-demographic and SES covariates. Covariates added in multivariable models were previously shown to be related either to the outcomes or the exposures, or both. Those included

age at baseline (in years), sex, race (NH white (ref), NH black, Mexican American, others), urban-rural residence, household size, marital status (Never married, married, divorced, widowed, other), poverty income ratio and completed years of education.

Lifestyle and health-related covariates. We accounted for lifestyle and health-related covariates which included smoking, alcohol use, diet, physical activity, and social support. Smoking was defined by the number of cigarettes smoked per day as well as the person-years of smoking (i.e., number of years that a respondent smoked cigarettes). A single 24- hour dietary recall was elicited from NHANES III participants by trained interviewers in a private room in the MEC. Data were collected on personal computers using the Dietary Data Collection system, an automated, interactive data collection and coding system. Interviewers were fluent in Spanish and English and had a set of measuring guides to help respondents estimate portion sizes. Data were collected for all days of the week. NHANES III data were coded with the seven-digit food codes from the US Department of Agriculture survey nutrient database [12]. Nutrient intakes were calculated with a database provided for NHANES III [13]. Alcohol was assessed as part of a single 24-hr dietary recall from which nutrient and food group intakes were derived. Alcohol use in this study was measured in g/day. Diet quality was assessed using the 1995-Healthy Eating Index (1995-HEI) and the mean adequacy ratio score (MAR) (**eMethods 2**). We classified physical activity using three survey items that assessed (1) the respondent's relative change in activity over the past month to the past year (0=less, 1=same, 2=more), (2) self-reported activity levels among respondents relative to men/women their age (0=less, 1=same, 2=more), and (2) self-reported activity levels among respondents relative to their levels of activity 10 years ago (0=less, 1=same, 2=more). Five survey items were used to define social support, which included (1) "In a typical week, how many times do you talk on the telephone with family, friends, or

neighbors?", (2) "How often do you get together with friends or relatives; I mean things like going out together or visiting in each other's homes? (per year)", (3) "About how often do you visit with any of your other neighbors, either in their homes or in your own? (per year)", (4) "How often do you attend church or religious services? (per year)", (5)"Altogether, how often do you attend meetings of the clubs or organizations (per year)".

We defined a health construct using measures on four health assessments, including self-reated health (excellent, very good, good fair, poor), comorbidity index (arthritis, congestive heart failure, stroke, asthma, chronic bronchitis, emphysema, hay fever, cataracts, goiter, thyroid disease, lupus, gout, skin cancer, other cancer), body mass index (BMI), and allostatic load (AL) which was defined using 9 biochemical and anthropometric indices detailed in **eMethods 2**. AL was defined such that higher scores reflected poorer health. [14]

Other nutritional biomarkers. The INCSTAR 25(OH)D assay consists of a two-step procedure. The first step involves rapid extraction of 25(OH)D and other hydroxylated metabolites from serum or plasma with acetonitrile. The second step involves assaying the treated sample using an equilibrium RIA procedure. [11] In the NHANES III, serum folate, which is required in cellular metabolism and hematopoiesis, is measured by using the Bio-Rad Laboratories "Quantaphase Folate" radioassay kit. [11]

Statistical analysis

Analyses were completed with Stata release 16 [15]. All Covariates aside from carotenoids, antioxidants and other nutritional biomarkers were multiple imputed (5 imputations, 10 iterations), assuming missingness at random. Description of key variable distributions were presented for the total sample and stratified by tertiles (T) of total carotenoids, for the total

eligible sample (45+y at baseline). Means of continuous variables across tertiles were compared using linear regression models, first to examine trends across tertiles, and then to contrast T_2 vs. T_1 and T_3 vs. T_1. Multiple linear, logistic, and multinomial logit models were used to test those differences across carotenoid tertiles, while adjusting for age, sex, race, and poverty income ratio. The analyses testing the main hypotheses consisted of several Cox proportional hazards regression models that were stratified by total carotenoid intake tertiles [16]. In each model, and for each stratum, outcomes included one of 2 incident outcomes (all-cause or AD dementia) with up to 26y of follow-up, and predictors were each of 5 individual carotenoids, total carotenoids, vitamins A, C and E measured at baseline. All models accounted for number of years elapsed between age at entry ≥45y (delayed entry) and age at outcome of interest or censoring by end of follow-up or age of death. All participants were dementia-free at baseline, by design, and models included potentially confounding baseline covariates. These covariates (listed under the *Covariates* section) included other antioxidants and total carotenoids, socio-demographic, lifestyle and health-related factors. Modeling was done in six steps. In model 1, minimal adjustment was made on the other two antioxidants, total carotenoids and age. Model 2 further adjusted for sex, race, marital status, urban-rural area of residence, and household size. Model 3 further adjusted for poverty income ratio and years of education. In model 4, further adjustment was made lifestyle and social support variables. Model 5 was Model 4 further adjusted for health-related factors, as well as additional nutritional biomarkers (i.e. serum folate and 25-hydroxyvitamin D). The model was conducted overall and stratified by serum total carotenoid tertiles. Two-way interaction terms were added to test heterogeneity of antioxidant effects on outcomes across tertiles of total serum carotenoids, in the overall unstratified model. Most of the main analyses were also conducted in the 65+ age group, as a sub-analysis. Dose-response

relationships were tested by including tertiles of total carotenoids as an ordinal variable. Individual carotenoids and antioxidants were examined as standardized z-scored exposures, with a per 1 SD increase interpretation. Finally, to test synergism and antagonism, two-way interaction terms were added alternately between each individual carotenoid and each antioxidant vitamin (45+y), while adjusting for the remaining factors and nutritional biomarkers (i.e. the full model), and including their main effects. The 2-way interaction was interpreted as synergism if negative, and antagonism if positive, given the expected protective effects on each outcome. A similar approach was applied to interactions among individual carotenoids, presenting only the final model among those aged 45+y, and adjusting for the remaining carotenoids in all models.

Type I errors for each main effect and interaction term was set at 0.05 and 0.10, respectively,[17] prior to multiple testing correction. A familywise Bonferroni approach was applied for this adjustment, accounting only outcome multiplicity. We thus assumed that each outcome was a distinctive substantive hypothesis [18]. Thus, significance levels for main effects were adjusted to $p < 0.025$ (0.05/2); 0.10/2=0.05 for the two-way interaction terms [19].

RESULTS

Characteristics of Study Participants by Total Carotenoid Tertiles

Study sample characteristics are presented in **Table 1** and **eTable 1** across baseline serum total carotenoid tertiles. There was a linear increase in all nutritional biomarkers between the lowest and the uppermost tertiles of serum total carotenoids ($p<0.001$), that was independent of age, sex, race and PIR. Similarly, the proportion of males (39.4% vs. 52.1%) was significantly lower in the uppermost tertile vs. the lowest tertile of total carotenoids, as was the proportion of

NH whites (80.2% vs. 84.5%), the percentage living in rural areas (48.1% vs. 60.0%), number of cigarettes and years smoked cigarettes ($p<0.001$), alcohol consumption ($p=0.003$), percentage with fair/poor self-rated health ($p<0.001$), mean co-morbidity index ($p=0.045$), mean allostatic load ($p<0.001$), and mean BMI ($p<0.001$). In contrast, the uppermost tertile of total carotenoids vs. the lowest was more likely to report being more active than age peers or self 10 years ago ($p<0.05$), and more frequently attended church or meetings in clubs, independently of age, sex, race or poverty income ratio. Other results are summarized in e**Results 1**.

All-cause and AD dementia vs. individual/total carotenoids and other antioxidants: Cox proportional hazards models

Table 2 shows results from Cox PH models examining associations of total and individual carotenoids with incidence of AD and all-cause dementia. In the 45+ baseline age group, age and sex-adjusted models indicated an inverse relationship between total carotenoids and both outcomes of interest (per SD of total carotenoids, HR=0.92, 95%CI: 0.86-0.98, p=0.012 for all-cause dementia; HR=0.89, 95%CI:0.80-0.98, p=0.029 for AD). However, these associations were attenuated upon adjustment for other socio-demographic and SES factors, including education and poverty income ratio ($p<0.10$), and became null upon further adjustment for diet quality and other lifestyle factors (Model 3). Nevertheless, when examining individual carotenoids, lutein+zeaxanthin plasma concentration was associated with reduced risk of all-cause dementia in the 65+ baseline age group, even upon adjustment for lifestyle factors such as diet quality (HR=0.93, 95%CI: 0.87-0.99, p=0.037), though a marked attenuation compared to Model 2 (HR=0.92, 95% CI: 0.86-0.93, p=0.013). The relationship became non-significant when health-related factors such as the allostatic load were introduced into the model (HR=0.92, 95% CI: 0.84-1.00, P=0.062). A strong inverse relationship was also detected between serum β-

cryptoxanthin and all-cause dementia in both age groups for the age and sex-adjusted models (HR=0.86, 95% CI:0.80-0.93, p<0.001 for 45+; HR=0.86, 95% CI:0.80-0.93, p=0.001 for 65+). This relationship remained strong in models adjusted for other socio-demographic and SES factors (HR=0.89, 95%CI: 0.82-0.96, p=0.006 for 45+; HR=0.88, 95%CI:0.81-0.96, p=0.007 for 65+). Nevertheless, it was attenuated upon further adjustment for diet quality and other lifestyle factors, suggesting mediation through healthy dietary patterns. The inverse relationship between β-cryptoxanthin and incident AD was detected in the 45+ group, retaining statistical significance in Model 2. Unlike lutein+zeaxanthin and β-cryptoxanthin, the initial inverse relationship between lycopene and all-cause dementia was highly confounded by SES factors (Model 2 vs. Model 1). No association was found between α-carotene or β-carotene and any of the outcomes within both age groups of interest. Upon correction for multiple testing, only inverse associations in Models 1 and 2 of lutein+zeaxanthin (45+ and 65+) and β-cryptoxanthin (45+) with all-cause dementia (and AD for β-cryptoxanthin, 45+) remained statistically significant (p<0.025).

All-cause and AD dementia vs. vitamin antioxidants, overall and across total carotenoid tertiles: Cox proportional hazards models

eTable 2 displays findings from a series of Cox proportional hazards models in the 45+ and 65+ age groups and show findings of associations for antioxidant vitamins A, C and E with all-cause and AD dementia at increasing level of covariate adjustment, in the total population and across tertiles of serum total carotenoids. Overall, serum vitamin C was inversely associated with incident all-cause dementia only in the age and sex-adjusted model (i.e. Model 1), with a stronger effect shown in the 45+ age group. The association remained statistically significant in the model adjusting for other socio-demographic and SES factors, though it was attenuated in both age groups. In model 3, which added diet quality and other lifestyle factors among adjusted

covariates, the association between vitamin C and all-cause dementia was no longer detected, as was the case for model 4. When examining interaction with total carotenoids, only one passed the threshold of statistical significance, in model 3 (which adjusted for diet and other lifestyle factors), indicating that at higher levels of carotenoids, vitamin A may potentially increase the risk for all-cause dementia in the older group (65+ at baseline), an association that differed significantly between the top and bottom tertiles of serum carotenoids.

All-cause and AD dementia vs. interaction between individual carotenoids and other antioxidants: Cox proportional hazards models

Table 3 presents key findings from Cox proportional hazards models for all-cause and AD dementia incidence among participants aged 45+ at baseline, in the full models, with 2-way interactions added between each individual carotenoid and each antioxidant vitamin. In those fully adjusted models, antagonistic interactions were observed between serum vitamin A and α-carotene vs. all-cause dementia (β±SEE:+0.039±0.016, p=0.017); vitamin A and α-carotene vs. AD dementia (β±SEE: +0.080±0.016, p<0.001); vitamin A and β-carotene vs. AD incidence (β±SEE: +0.088±0.021, P<0.001); vitamin E and lycopene vs. AD incidence (β±SEE: +0.078±0.022, P=0.001).

All-cause and AD dementia vs. interactions among individual carotenoids: Cox proportional hazards models

Table 4 shows findings from full models with interactions added between individual carotenoids in relation to incidence all-cause and AD dementia, within the 45+ baseline age group. Only one interaction was deemed statistically significant, namely a potential antagonistic interaction between lycopene and β-carotene vs. incident AD (C1×C2: β±SE: +0.057±0.028,

p=0.046), indicating that putative protective effects on incident AD of lycopene are reduced at higher levels of β-carotene. Other relevant results showing findings from Table 2, Model 4 for covariates included in the model are presented in **eResults 1**. This study provides Class II evidence that incident all-cause dementia was inversely associated with serum lutein+zeaxanthin and β-cryptoxanthin levels.

DISCUSSION

Main findings

In this study, we evaluated whether carotenoids and other antioxidants act synergistically in their association with AD- and all-cause dementia using a nationally representative prospective cohort of US adults with administrative linkage. Inverse associations of total and individual carotenoid plasma concentrations with both outcomes were detected, with lutein+zeaxanthin and β-cryptoxanthin meeting statistical significance upon multiple testing adjustment. Specifically, lutein+zeaxanthin was associated with reduced risk of all-cause dementia (65+ age group), even in the lifestyle-adjusted model (per SD, HR=0.93, 95%CI: 0.87-0.99, p=0.037), though attenuated in comparison to socio-demographic and socio-economic status (SES) factors-adjusted model (HR=0.92, 95% CI: 0.86-0.93, p=0.013). A strong inverse relationship was detected between serum β-cryptoxanthin (per SD increase) and all-cause dementia (45+ and 65+), for age and sex-adjusted models (HR=0.86, 95% CI:0.80-0.93, p<0.001 for 45+; HR=0.86, 95% CI:0.80-0.93, p=0.001 for 65+), a relationship remaining strong in socio-demographic and SES factor-adjusted models (HR=0.89, 95%CI: 0.82-0.96, p=0.006 for 45+; HR=0.88, 95%CI:0.81-0.96, p=0.007 for 65+), but attenuated in subsequent models. In fully adjusted models, antagonistic interactions were observed between serum vitamin A and α-carotene vs. all-

cause dementia; vitamin A and α-carotene; vitamin E and lycopene; vitamin A and β-carotene; lycopene and β-carotene vs. AD incidence.

Previous studies

To date, studies examining the link between dietary antioxidant intake and the risk of dementia have produced mixed findings. For example, Laurin and colleagues [20] reported no association between midlife dietary intake of vitamins E and C and incident dementia, a finding that was consistent with five other cohort studies with respect to these two dietary antioxidants [21-24]. A study by Jama et al., however, found that carotenoids, particularly β-carotene intake, may have beneficial effects on various cognitive outcomes, [22] whereas associations between cognitive outcomes and other carotenoids were not detected in other studies[23-25].

Among carotenoids, lutein or lutein+zeaxanthin were found to have beneficial cognitive effects in older men and women as indicated by a recent randomized controlled trial [26] and a large cohort study[27] as did two recent meta-analyses of randomized controlled trials and cohort studies, which came to the conclusion that carotenoids in general, and lutein in particular, may have cognitive benefits[28, 29]. In the first study[26], the cognitive benefit of Docosahexaenoic acid (DHA), an essential omega-3 fatty acid, and lutein in unimpaired elder women were explored in a 4-month, double-blind, intervention trial supplementing DHA and lutein for eye health. Most notably, the study's results indicated that memory scores and rate of learning improved significantly in the combined treatment group vs. placebo ($P < 0.03$) [26]. The second study indicated that higher total carotenoid intake was indeed linked to substantially lower hazard of AD after controlling for age, sex, education, participation in cognitively stimulating activities, ApoE4 status and physical activity level[27]. Comparing the uppermost to the lowest quintile

(median intake: 24.8 compared with 6.7 mg/d) of total carotenoids, the multivariate HR (95% CI) was 0.52 (0.33, 0.81), P-trend < 0.01. A similar association was observed for lutein+zeaxanthin, with a weaker inverse relationship observed for β-carotene, and a marginally significant inverse association found for β-cryptoxanthin. In the deceased group, higher total carotenoids consumption (Uppermost vs. lowest tertile, 18.2 compared with 8.2 mg/d) had less global AD pathology (b: -0.10; SE = 0.04; P-trend = 0.01). For individual carotenoids, lutein+zeaxanthin and lycopene were inversely related to brain global pathology, whereas lutein+zeaxanthin exhibited an additional inverse association with AD diagnostic score, neuritic plaque severity, and neurofibrillary tangle density and severity[26]. Our study had comparable findings for lutein+zeaxanthin in serum in relation to AD and all-cause dementia in the older group (65+y of age at baseline), with some additional evidence for a protective effect of β-cryptoxanthin. Nevertheless, a recent randomized controlled trial of over 3000 participants with age-related macular degeneration (AREDS2 study) showed that supplementation with omega-3 fatty acids and lutein/zeaxanthin had no significant effect on cognitive function[30].

Serum concentrations of antioxidant vitamins may be a better biomarker for oxidative stress status whether derived from dietary intake or supplementation. Several recent cohort studies [31-33] reported an inverse relationship between serum vitamin E levels and cognitive impairment and disorders. In one of these studies, researchers observed a U-shaped association between blood tocopherol sub-types and cognitive impairment [32]. Moreover, numerous other studies reported protective associations between serum carotenoids and cognitive impairment [34-42], including a recent study conducted in our same cohort that detected similar potentially protective associations between plasma lutein+zeaxanthin, lycopene and AD mortality[42]. Taken together, the previous literature indicates that both carotenoids and serum antioxidant vitamins tended to be protective

against various adverse cognitive outcomes, including incident AD and all-cause dementia. However, only one recent study has examined interactions between those bioactive micronutrients and cognitive performance or decline in mid-life [43]. The findings indicated that among others, there was a synergistic interaction between vitamin E and total carotenoids, particularly lycopene, whereby vitamin E was directly associated with baseline performance on a test of verbal memory at higher carotenoid levels, with antagonistic interactions detected between vitamin A and some carotenoids in relation to visual memory decline[43]. Our current study did not detect any synergistic interactions or a potential protective effect of vitamin E against incidence of all-cause or AD dementia. In contrast, vitamin E and lycopene exhibited an antagonistic interaction in our present study in relation to AD incidence, suggesting that interactions between carotenoids and antioxidant vitamins are patterned differently across time. A study conducted on brain tissues acquired from frontal and temporal cortices of 47 centenarians from the Georgia Centenarian Study, indicated that brain nutrient pattern explained mainly by carotenoid concentrations is correlated with cognitive function among subjects who had no dementia, re-enforcing the biological plausibility of our detected associations[44]. Other related biological mechanisms are summarized in **eDiscussion 1**.

Strengths and limitations

Our study has notable strengths. First, we used a nationally representative study that sufficiently powered our analyses to detect interactions between various nutritional biomarkers of antioxidant status in relation to two key cognitive impairment outcomes, namely all-cause and AD dementia. We used a nationally representative sample together with administrative linkages which allowed us to combine detailed demographic and behavioral health information with

medical records. In prior work, studies have typically relied solely on medical claims information which do not necessarily contain demographic and behavioral health information [45] Second, advanced statistical techniques such as multiple Cox proportional hazards models were used with multiple imputed covariates, thus reducing selection bias and preserving statistical power within the eligible sample with complete exposure and outcome data. Third, it is among few studies to examine serum nutritional biomarkers of antioxidant status, rather than dietary intakes, the latter being known for reflecting only short-term exposure and having considerable measurement error. Fourth, our analyses were carried out among middle-aged and older adults, with a sub-analysis carried out among older adults (aged 65+) to determine the influence of age at exposure on the outcome.

Our study also has limitations. First, in terms of outcome, those diagnosed earlier may be at worse overall health and/or have better access to health care than those who were diagnosed later. In addition, baseline exclusion of dementia or cognitive impairment cases was based on a household screener[46] rather than a formal set of cognitive performance tests. Nevertheless, the vast majority of incident dementia cases were diagnosed after at least 10 years of follow-up thus reducing the possibility of reverse causality. Second, although nutritional biomarkers are an improvement over dietary intakes, their association with the key outcomes may be confounded by other biomarkers. In addition, despite some genetic effect, dietary influence on these nutritional biomarkers is often pre-dominant. In addition, the serum antioxidant levels reflect current intakes and may not accurately reflect the person's lifetime habitual intakes. Another class of antioxidants, the flavonoids, have been shown to be protective against oxidative DNA damage [47] but were not accounted for in this study because of the lack of a flavonoid database. While some drugs like aspirin and L-dopa preparations can affect antioxidant systems [48], this

study did not control for these drugs. Moreover, the levels of serum antioxidants needed to beneficially modify the aging of the brain are unknown resulting in the need for further exploration of the association between serum antioxidant levels and dementia. Finally, two other limitations of the study are the unavailability of vitamin E isoforms in the data and the possibility of regression dilution due to elongated follow-up periods.

Conclusions

In sum, incident all-cause dementia was inversely associated with serum lutein+zeaxanthin and β-cryptoxanthin levels. Antagonistic interactions indicate putative protective effects of one carotenoid may be observed at a lower level of another carotenoid or antioxidant vitamin. Further studies with time-dependent exposures and randomized trials are needed to test neuroprotective effects of supplementing the diet with select carotenoids.

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Table 1. Study sample characteristics by tertile of serum total carotenoids for sub-sample with complete and valid plasma nutritional biomarker data, NHANES III 1988-1994 [a]

	Overall (45+y at baseline)	**By plasma total carotenoids tertiles (µg/dL)** [a]			
		T_1	**T_2**	**T_3**	***p*** [b]
	(X ± SEM), %	(X ± SEM), %	(X ± SEM), %	(X ± SEM), %	
	(N=7,283)	**(N = 2,469)**	**(N =2,404)**	**(N =2,410)**	
Serum carotenoids and other antioxidants					
Serum total carotenoids, µg/dL	84.23±0.94	45.45±0.34	76.56±0.26	128.20±1.15	<0.001[d]
α-carotene, µg/dL	5.45±0.14	2.47±0.06	4.64±0.1	9.04±0.28	<0.001[d]
β-carotene, µg/dL	24.09±0.5	10.25±0.14	19.25±0.27	41.88±1.03	<0.001[d]
Lutein+zeaxanthin, µg/dL	24.27±0.35	14.83±0.20	23.04±0.25	34.35±0.71	<0.001[d]
β-cryptoxanthin, µg/dL	9.64±0.22	5.05±0.08	8.61±0.14	14.96±0.39	<0.001[d]
Lycopene, µg/dL	20.79±0.31	12.85±0.27	21.03±0.32	27.97±0.47	<0.001[d]
Serum vitamin A, µg/dL	62.72±0.46	59.36±0.81	62.84±0.56	65.75±0.58	<0.001[d]
Serum vitamin C, mg/dL	0.82±0.02	0.62±0.02	0.82±0.02	0.99±0.02	<0.001[d]
Serum vitamin E, µg/dL	1354.99±12.60	1137.12±15	1358.24±23.96	1555.73±21.81	<0.001[d]
Baseline socio-demographic, lifestyle and health-related variables					
Sex, % male	46.03	52.11	46.96	39.43	<0.001[d]
Age at v_1, yrs.	61.41±0.35	61.42±0.36	60.92±0.44	61.89±0.56	0.4294
Race/ethnicity, %					0.0253[d]
NH white	82.67	84.49	83.41	80.24	
NH black	8.23	8.71	7.59	8.41	
Mexican-American	3.00	2.98	3.03	3.00	
Other	6.09	3.82	5.97	8.35	
Urban/rural area of residence					0.0008[d]
Urban	46.18	40.05	46.19	51.88	
Rural	53.82	59.95	53.81	48.12	
Household size	2.43±0.37	2.42±0.04	2.52±0.05	2.36±0.06	0.3271[d]

Marital status					0.6129[d]
Never married	4.27	4.53	3.85	4.45	
Married	66.39	64.21	69.05	65.79	
Divorced	9.32	10.6	7.52	9.93	
Widowed	16.2	16.31	15.82	16.48	
Other	3.81	4.35	3.76	3.35	
Poverty income ratio	3.27±0.07	2.92±0.09	3.31±0.08	3.55±0.09	<0.001[d]
Education, yrs. Completed	11.69±0.11	11.14±0.13	11.67±0.13	12.23±0.15	<0.001[d]
Cumulative incidence of AD and all-cause dementia, %					
AD dementia	10.77	10.52	10.63	11.13	0.6548
All-cause dementia	21.16	20.24	21.24	21.95	0.3153

Abbreviations: 1995-HEI=Healthy Eating Index 1995; AD=Alzheimer's Disease; AL=Allostatic Load; BMI=Body Mass Index;. NHANES III=Third National Health and Nutrition Examination Survey.

[a]Values are means (X) ±SEM for continuous variables and % for categorical variables. The sample selected has complete data on nutritional biomarkers, including carotenoids and vitamins A, C and E. The same visit and approach were applied to other dietary factors, including other antioxidants. Tertiles of total carotenoids were determined using the final analytic sample. Standard deviation (SD) and coefficient of variation (CV) values for total carotenoids and for each individual carotenoid were as follows (based on the final 45+ sample): total carotenoids, SD=43.6, CV=51.8% ; α-carotene: SD=4.87, CV=89%; β-carotene: SD=22.77, CV=93.8%; lutein+zeaxanthin: SD=15.44, CV=63.6%; β-cryptoxanthin: SD=8.59, CV=89.5%; Lycopene: SD=11.14, CV=53.4%; serum vitamin A: SD=18.0, CV=28.7%; serum vitamin C: SD=0.49, CV=59.7% ; serum vitamin E: SD=578.5, CV=42.7% . Note that CV was computed using observed SD and weighted means ($SD_{obs}/Mean_{weighted}$).

[b] *p*-value from OLS linear regression models with carotenoid tertile as the only covariate for continuous variables, and multinomial logit model with carotenoid tertile as the only covariate for categorical variables, with carotenoid tertile as an ordinal variable.

[c] See Methods section for definitions for AL and the co-morbidity index.

[d] p<0.05 upon further adjustment for age, sex, race, and poverty income ratio in multiple linear, logistic and multinomial logit models with carotenoid tertile entered as an ordinal variable.

*$p < 0.05$** $p < 0.01$; *** $p < 0.001$, *t*-test for null hypothesis of no between-tertile differences, taking T_1 as the referent.

Table 2. Associations of serum total and individual carotenoids (z-scores) with incident all-cause and AD dementia (45+ and 65+ age at baseline): Cox proportional hazards models , NHANES III, 1988-1994[a,b,c]

	Exposure=Serum Carotenoids, z-scores					
	Total carotenoids		**α-carotene**		**β-carotene**	
	45+	65+	45+	65+	45+	65+
	β±SE[b]	β±SE	β±SE	β±SE	β±SE	β±SE
All-cause dementia	(N=7,257)	(N=3,593)	(N=7,257)	(N=3,593)	(N=7,257)	(N=3,593)
Model 1	-0.083±0.033, p=0.012	-0.064±0.034, p=0.070	-0.042±0.033, p=0.21	-0.033±0.029, p=0.25	-0.015±0.030, p=0.61	-0.000±0.027, p=0.87
Model 2	-0.057±0.032, p=0.079	-0.046±0.036, p=0.21	-0.003±0.033, p=0.94	-0.002±0.028, p=0.94	-0.000±0.027, p=0.99	+0.016±0.032, p=0.62
Model 3	-0.020±0.032, p=0.54	-0.009±0.036, p=0.80	+0.039±0.031, p=0.23	+0.040±0.027, p=0.15	+0.028±0.027, p=0.31	+0.044±0.032, p=0.18
Model 4	-0.026±0.033, p=0.43	-0.026±0.037, p=0.49	+0.064±0.034, p=0.068	+0.044±0.029, p=0.14	+0.028±0.030, p=0.34	+0.049±0.034, p=0.15
AD	(N=7,283)	(N=3,618)	(N=7,283)	(N=3,618)	(N=7,283)	(N=3,618)
Model 1	-0.120±0.053, p=0.029	-0.097±0.056, p=0.091	-0.061±0.043, p=0.16	-0.065±0.045, p=0.16	-0.040±0.045, p=0.37	-0.026±0.046, p=0.57
Model 2	-0.096±0.049, p=0.054	-0.077±0.056, p=0.18	-0.025±0.039, p=0.53	-0.028±0.042, p=0.51	-0.024±0.040, p=0.54	-0.017±0.045, p=0.72
Model 3	-0.042±0.050, p=0.41	-0.031±0.059, p=0.61	+0.032±0.032, p=0.38	+0.024±0.042, p=0.57	+0.015±0.041, p=0.72	+0.017±0.050, p=0.73
Model 4	-0.059±0.053, p=0.27	-0.057±0.064,	+0.069±0.042, p=0.10	+0.036±0.047,	+0.007±0.048, p=0.88	+0.001±0.061,

		p=0.38		p=0.45		p=0.98
	Lutein+zeaxanthin		**β-cryptoxanthin**		**Lycopene**	
	45+	65+	45+	65+	45+	65+
	β±SE	β±SE	β±SE	β±SE	β±SE	β±SE
All-cause dementia	(N=7,257)	(N=3,593)	(N=7,257)	(N=3,593)	(N=7,257)	(N=3,593)
Model 1	-0.082±0.036, p=0.028	-0.092±0.035, p=0.012	-0.149±0.038, p<0.001	-0.148±0.041, p=0.001	-0.101±0.038, p=0.011	-0.085±0.043, p=0.052
Model 2	-0.084±0.033, p=0.016	-0.088±0.034, p=0.013	-0.115±0.040, p=0.006	-0.126±0.045, p=0.007	-0.069±0.037, p=0.070	-0.065±0.042, p=0.13
Model 3	-0.061±0.032, p=0.063	-0.071±0.033, p=0.037	-0.070±0.041, p=0.092	-0.076±0.047, p=0.11	-0.052±0.038, p=0.18	-0.046±0.041, p=0.28
Model 4	-0.061±0.041, p=0.14	-0.083±0.044, p=0.062	-0.072±0.047, p=0.13	-0.074±0.054, p=0.18	-0.041±0.040, p=0.31	-0.029±0.044, p=0.51
AD	(N=7,283)	(N=3,618)	(N=7,283)	(N=3,618)	(N=7,283)	(N=3,618)
Model 1	-0.140±0.071, p=0.055	-0.132±0.073, p=0.076	-0.188±0.063, p=0.004	-0.138±0.063, p=0.033	-0.094±0.052, p=0.079	-0.073±0.061, p=0.23
Model 2	-0.138±0.066, p=0.044	-0.121±0.070, p=0.090	-0.162±0.063. p=0.013	-0.119±0.067, p=0.079	-0.074±0.054, p=0.18	-0.061±0.066, p=0.36
Model 3	-0.100±0.063, p=0.12	-0.100±0.070, p=0.17	-0.089±0.065, p=0.18	-0.047±0.069, p=0.50	-0.052±0.052, p=0.32	-0.038±0.063, p=0.55
Model 4	-0.100±0.066, p=0.15	-0.109±0.080, p=0.17	-0.080±0.073, p=0.28	-0.018±0.067, p=0.79	-0.035±0.053, p=0.51	-0.014±0.064, p=0.83

Abbreviations: AD=Alzheimer's Disease; NHANES III=Third National Health and Nutrition Examination Survey.

[a] Values are β=Log_e(HR) with their associated Standard Errors (SE) for main effect of each carotenoid on the two main outcomes: all-cause and AD dementia. Analyses are conducted on the total eligible sample (45+) and a sub-analysis is conducted among older adults (65+).

[b] Standard deviation (SD) values for total carotenoids and for each individual carotenoid were as follows (based on the final 45+ sample): total carotenoids, SD=43.6 ; α-carotene: SD=4.87; β-carotene: SD=22.77; lutein+zeaxanthin: SD=15.44; β-cryptoxanthin: SD=8.59; Lycopene: SD=11.14. Units are μg/dL.

[c] Model 1: age- and sex-adjusted; Model 2: Model 2 + other demographic factors, education and income; Model 3: Model 2+ lifestyle-related factors, including diet quality indices; Model 4: Model 3+health-related factors and other nutritional biomarkers (serum folate and 25-hydroxyvitamin D, antioxidant vitamins and the remaining carotenoids, if applicable).

*P<0.05 **P<0.01 ***P<0.001 for null hypothesis of $Log_e(HR)=0$.

Table 3. Interactions between individual carotenoids and other antioxidants (vitamins A, E and C) in relation to incident all-cause and AD dementia (45+ group) [a,b]

	Carotenoid, z-scores				
	Alpha-Carotene	Beta-Carotene	Lutein+zeaxanthin	Beta-Cryptoxanthin	Lycopene
Vitamin A, z-scores					
All-cause dementia	(N=7,257)	(N=7,257)	(N=7,257)	(N=7,257)	(N=7,257)
Vitamin A	+0.013±0.043	+0.010±0.045	+0.020±0.044	+0.020±0.043	+0.020±0.044
Carotenoid	+0.059±0.034	+0.012±0.033	-0.037±0.044	-0.080±0.050	-0.048±0.038
Carotenoid×Vitamin A	+0.039±0.016*	+0.033±0.020	-0.051±0.028	+0.030±0.050	+0.027±0.038
AD dementia	(N=7,283)	(N=7,283)	(N=7,283)	(N=7,283)	(N=7,283)
Vitamin A	-0.090±0.053	-0.101±0.058	-0.078±0.055	-0.077±0.056	-0.078±0.056
Carotenoid	+0.058±0.043	-0.034±0.049	-0.091±0.071	-0.082±0.074	-0.048±0.050
Carotenoid×Vitamin A	+0.080±0.016***	+0.088±0.021***	-0.020±0.050	+0.011±0.084	+0.075±0.042
Vitamin C, z-scores					
All-cause dementia	(N=7,257)	(N=7,257)	(N=7,257)	(N=7,257)	(N=7,257)
Vitamin C	-0.047±0.046	-0.047±0.046	-0.048±0.044	-0.058±0.046	-0.035±0.046
Carotenoid	+0.34±0.042	+0.004±0.036	-0.053±0.042	-0.029±0.054	-0.056±0.037
Carotenoid×Vitamin C	+0.047±0.035	+0.029±0.024	-0.045±0.035	-0.074±0.047	+0.051±0.042
AD dementia	(N=7,283)	(N=7,283)	(N=7,283)	(N=7,283)	(N=7,283)
Vitamin C	-0.059±0.070	-0.059±0.070	-0.059±0.066	-0.047±0.070	-0.048±0.070
Carotenoid	+0.029±0.057	-0.027±0.054	-0.087±0.067	-0.090±0.088	-0.058±0.051

Carotenoid×Vitamin C	+0.052±0.039	+0.042±0.031	-0.057±0.077	+0.017±0.067	+0.065±0.041
Vitamin E, z-scores					
All-cause dementia	(N=7,257)	(N=7,257)	(N=7,257)	(N=7,257)	(N=7,257)
Vitamin E	+0.026±0.038	+0.027±0.038	+0.030±0.038	+0.028±0.037	+0.024±0.037
Carotenoid	+0.058±0.035	+0.022±0.035	-0.054±0.043	-0.065±0.046	-0.052±0.038
Carotenoid×Vitamin E	+0.017±0.031	+0.006±0.022	-0.014±0.037	-0.014±0.051	+0.039±0.020
AD dementia	(N=7,283)	(N=7,283)	(N=7,283)	(N=7,283)	(N=7,283)
Vitamin E	+0.094±0.053	+0.094±0.051	+0.092±0.051	+0.093±0.050	+0.090±0.050
Carotenoid	+0.050±0.049	-0.003±0.055	-0.108±0.068	-0.109±0.079	-0.060±0.050
Carotenoid×Vitamin E	+0.041±0.042	+0.010±0.037	+0.021±0.041	+0.045±0.045	**+0.078±0.022*** **

Abbreviations: AD=Alzheimer's Disease; NHANES III=Third National Health and Nutrition Examination Survey.

[a] Models were adjusted for age, sex, other demographic factors, education and income, lifestyle-related and health-related factors, other nutritional biomarkers including other antioxidant vitamins, other carotenoids, serum folate and 25-hydroxyvitamin D.

[b] Values are the main effects and the 2-way interaction terms between each serum carotenoid exposure and the antioxidant vitamin exposure, each expressed as z-score. See Table 1 for SD estimates for serum antioxidant vitamins and carotenoids for the 45+ group.

*P<0.05 **P<0.01 ***P<0.001 for null hypothesis of Log_e(HR)=0.

Table 4. Interactions among individual carotenoids in relation to incident all-cause and AD dementia (45+ group) [a,b]

	Carotenoid 1 (C1), z-score				
	Alpha-Carotene	Beta-Carotene	Lutein+zeaxanthin	Beta-Cryptoxanthin	Lycopene
Carotenoid 2 (C2), z-score					
Alpha-Carotene					
All-cause dementia	__	(N=7,257)	(N=7,257)	(N=7,257)	(N=7,257)
C1	…	+0.035±0.033	-0.053±0.040	-0.058±0.050	-0.052±0.038
C2	…	+0.083±0.044	+0.073±0.036	+0.070±0.034	+0.045±0.030
C1×C2	…	-0.008±0.009	-0.020±0.024	-0.012±0.016	+0.037±0.020
AD dementia	__	(N=7,283)	(N=7,283)	(N=7,283)	(N=7,283)
C1	…	+0.014±0.051	-0.096±0.068	-0.071±0.079	-0.049±0.051
C2	…	+0.085±0.058	+0.069±0.043	+0.070±0.041	+0.043±0.044
C1×C2	…	-0.006±0.015	-0.002±0.025	-0.004±0.014	+0.043±0.022
Beta-Carotene					
All-cause dementia	__	__	(N=7,257)	(N=7,257)	(N=7,257)
C1	…	…	-0.040±0.041	-0.040±0.052	-0.056±0.038
C2	…	…	+0.035±0.030	+0.040±0.031	+0.031±0.030
C1×C2	…	…	-0.019±0.016	-0.039±0.036	+0.048±0.030
AD dementia	__	__	(N=7,283)	(N=7,283)	(N=7,283)
C1	…	…	-0.093±0.072	-0.088±0.088	-0.055±0.052

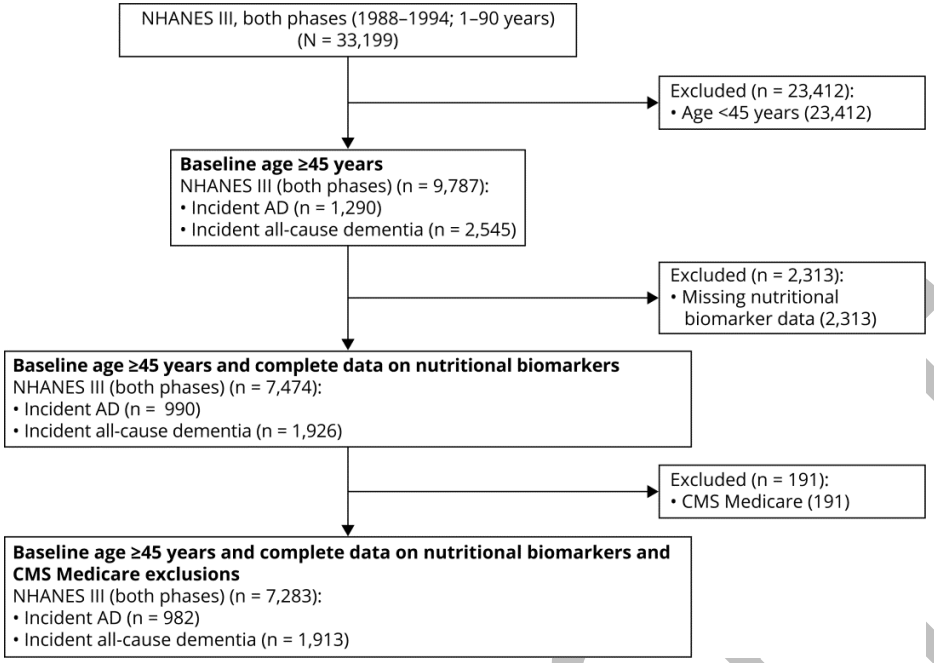
C2	…	…	+0.005±0.050	+0.018±0.051	+0.014±0.050
C1×C2	…	…	-0.004±0.022	+0.010±0.037	+0.057±0.028*
Lutein+zeaxanthn					
All-cause dementia	__	__	__	(N=7,257)	(N=7,257)
C1	…	…	…	-0.082±0.049	-0.041±0.041
C2	…	…	…	-0.064±0.042	-0.059±0.041
C1×C2	…	…	…	+0.017±0.023	+0.003±0.026
AD dementia	__	__	__	(N=7,283)	(N=7,283)
C1	…	…	…	-0.090±0.078	-0.031±0.053
C2	…	…	…	-0.095±0.066	-0.089±0.065
C1×C2	…	…	…	+0.024±0.046	+0.002±0.044
Beta-Cryptoxanthin					
All-cause dementia	__	__	__	__	(N=7,257)
C1	…	…	…	…	-0.041±0.040
C2	…	…	…	…	-0.075±0.047
C1×C2	…	…	…	…	+0.023±0.042
AD dementia	__	__	__	__	(N=7,283)
C1	…	…	…	…	-0.033±0.052
C2	…	…	…	…	-0.085±0.071
C1×C2	…	…	…	…	+0.045±0.069

Abbreviations: AD=Alzheimer's Disease; C1=Carotenoid 1; C2=Carotenoid 2; NHANES III=Third National Health and Nutrition Examination Survey.

[a] Model adjusted for age, sex, other demographic factors, education, income, lifestyle-related factors, health-related factors and other nutritional biomarkers including other antioxidant vitamins, other carotenoids, serum folate and 25-hydroxyvitamin D.

[b] Values are main effects of each carotenoid and their 2-way interaction, expressed as z-scores. See Table 1 for SD estimates for serum antioxidant vitamins and carotenoids for the 45+ group.

*P<0.05 **P<0.01 ***P<0.001 for null hypothesis of $Log_e(HR)=0$.



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