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Intakes of Lutein, Zeaxanthin, and Other Carotenoids and Age-Related Macular Degeneration During 2 Decades of Prospective Follow-up

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Interviews

Abstract

Importance Despite strong biological plausibility, evidence from epidemiologic studies and clinical trials on the relations between intakes of lutein and zeaxanthin and age-related macular degeneration (AMD) has been inconsistent. The roles of other carotenoids are less thoroughly investigated.

Objective To investigate the associations between intakes of carotenoids and AMD.

Design, Setting, and Participants Prospective cohort study, with cohorts from the Nurses' Health Study and the Health Professionals Follow-up Study in the United States. A total of 63 443 women and 38 603 men were followed up, from 1984 until May 31, 2010, in the Nurses' Health Study and from 1986 until January 31, 2010, in the Health Professionals Follow-up Study. All participants were aged 50 years or older and were free of diagnosed AMD, diabetes mellitus, cardiovascular disease, and cancer at baseline.

Main Outcomes and Measures Predicted plasma carotenoid scores were computed directly from food intake, assessed by repeated food frequency questionnaires at baseline and follow-up, using validated regression models to account for bioavailability and reporting validity of different foods, and associations between predicted plasma carotenoid scores and AMD were determined.

Results We confirmed 1361 incident intermediate and 1118 advanced AMD cases (primarily neovascular AMD) with a visual acuity of 20/30 or worse by medical record review. Comparing extreme quintiles of predicted plasma lutein/zeaxanthin score, we found a risk reduction for advanced AMD of about 40% in both women and men (pooled relative risk comparing extreme quintiles = 0.59; 95% CI, 0.48-0.73; *P* for trend < .001). Predicted plasma carotenoid scores for other carotenoids, including β-cryptoxanthin, α-carotene, and β-carotene, were associated with a 25% to 35% lower risk of advanced AMD when comparing extreme quintiles. The relative risk comparing extreme quintiles for the predicted plasma total carotenoid index was 0.65 (95% CI, 0.53-0.80; *P* for trend < .001). We did not identify any associations of carotenoids, either as predicted plasma score or calculated intake, with intermediate AMD.

Conclusions and Relevance Higher intake of bioavailable lutein/zeaxanthin is associated with a long-term reduced risk of advanced AMD. Given that some other carotenoids are also associated with a lower risk, a public health strategy aimed at increasing dietary consumption of a wide variety of fruits and vegetables rich in carotenoids may reduce the incidence of advanced AMD.

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world,[1,2] affecting 6.5% of persons aged 40 years and older in the United States, with 0.8% having advanced AMD.[3] The prevalence of AMD

is projected to increase by 50% in the next couple of decades[4-6] as a consequence of exponential population aging and the lack of a cure or any effective means of primary prevention other than smoking cessation.[7]
Carotenoids are fat-soluble plant pigments found in red, yellow, orange, and dark green fruits and vegetables. Of more than 600 carotenoids, 6 are commonly found in the human diet and serum: lutein, zeaxanthin, α-carotene, β-carotene, lycopene, and β-cryptoxanthin.[8] Lutein and zeaxanthin are selectively concentrated in the macula,[9,10] where they are hypothesized to protect against AMD by absorbing blue light, quenching free radicals, and stabilizing cell membranes.[11] However, despite compelling biological plausibility, epidemiologic studies have not yielded consistent findings[12-15] and long-term, well-powered prospective cohort studies are lacking. The recently concluded Age-Related Eye Disease Study 2 (AREDS2) trial was unable to confidently demonstrate protective effects of lutein/zeaxanthin,[16,17] and whether lutein/zeaxanthin may protect against early AMD also remains unknown. Some other carotenoids such as α-carotene, β-carotene, and lycopene found in the retinal pigment epithelium (RPE) and choroid[18] have been inconsistently linked to a lower risk of AMD.[12-15,19,20]
We previously reported a suggestive inverse association of lutein/zeaxanthin with advanced AMD[21] and some associations for other carotenoids.[22] With an additional decade of follow-up and the occurrence of a large number of additional incident AMD cases, we aimed to provide more detailed insights into the roles of carotenoids in the development of AMD.

At a Glance

- Antioxidant carotenoids are hypothesized to lower the risk of age-related macular degeneration (AMD); however, results from prior epidemiologic studies have been inconsistent.
- Comparing extreme quintiles, intake of bioavailable lutein and zeaxanthin was associated with a pooled relative risk of advanced AMD of 0.59 (95% CI, 0.48-0.73).
- An association of any of these carotenoids with development of intermediate AMD was not identified.
- Although not yet supported by randomized clinical trials, this study suggests that carotenoids may slow worsening of AMD once it occurs.

Methods

Study Population

The Nurses' Health Study (NHS) is an ongoing prospective cohort initiated in 1976 that includes 121 700 US female registered nurses aged 30 to 55 years at baseline. The Health Professionals Follow-up Study (HPFS) was initiated in 1986 and includes 51 529 US male health professionals aged 40 to 75 years at baseline. Both cohorts are predominantly white (NHS, >98%; HPFS, >91%) and have high rates of long-term follow-up (>95%). Both cohort studies have approval by the human subjects committees at the Brigham and Women's Hospital and the Harvard T. H. Chan School of Public Health. Cohort participants or family members provided written informed consent.
We restricted the study population to participants aged 50 years or older and censored participants at age 90 years to alleviate concerns of low reporting validity (NHS, n = 0; HPFS, n = 526). At baseline, we excluded participants who did not return the initial food frequency questionnaire (FFQ), left the entire fruit and vegetable sections blank or had more than 70 food items blank, reported implausible dietary intake (<500 or >3500 kcal/d for NHS and <800 or >4200 kcal/d for HPFS) (dietary exclusions: NHS, n = 46 142; HPFS, n = 1647), or had prevalent AMD, cancer (except nonmelanoma skin cancer), diabetes mellitus, or cardiovascular disease (disease exclusions: NHS, n = 8536; HPFS, n = 5709). To minimize detection bias, we also excluded participants who never reported an eye examination during follow-up (NHS, n = 3362; HPFS, n = 4763) and excluded from analysis the person-time during any 2-year interval in which a participant did not report an eye examination. In sensitivity analyses including intervals lacking an eye examination, results did not materially change. Participants contributed person-time to the analysis from return of the

baseline questionnaire or reaching age 50 years to the confirmed diagnosis of AMD, death, loss to follow-up, or the end of follow-up (May 31, 2010, for the NHS and January 31, 2010, for the HPFS), whichever occurred first. By 2010, a total of 63 443 women and 38 603 men contributed to the analysis.

AMD Ascertainment

Our case definition has been previously validated.[23] When a participant reported a diagnosis of AMD on a biennial questionnaire, we requested written informed consent and then contacted the participant's eye care professional to confirm the diagnosis by review of medical records. We excluded cases with only small hard drusen (<63 μm in diameter). We defined intermediate AMD as having at least 1 of the following signs: intermediate drusen (≥63 and <125 μm), pigment abnormalities, large drusen (≥125 μm), or any noncentral geographic atrophy (GA). We defined a subgroup of intermediate AMD as having at least 1 large druse or any noncentral GA, the most likely to progress to advanced AMD.[24] We defined neovascular AMD as having any of the following: RPE detachment, subretinal neovascular membrane, disciform scar, or history of treatment with laser, photodynamic, or anti–vascular endothelial growth factor therapy for AMD. Central GA was defined as having a central GA lesion involving the center of the macula. Advanced AMD included neovascular AMD and central GA. Additionally, our case definition included a visual acuity of 20/30 or worse due primarily to AMD. The person was used as the unit of analysis, and the worse eye was used for classification.

Dietary Assessment of Carotenoids

We began follow-up in 1984 for the NHS and 1986 for the HPFS, when the first comprehensive FFQ with an expanded section on fruit and vegetable intake was administered, and updated dietary intake every 4 years. On the FFQs, commonly used units or portion sizes (eg, 1 orange or half cup of broccoli) are specified for each item. The FFQs contained at least 15 questions for fruit and juice intake and 30 questions for vegetable intake. Participants were asked to report how often, on average during the past year, they had consumed each food item (responses ranging from ≤1 time/mo to ≥6 times/d). Use and dosage of beta carotene and multivitamin supplements were assessed by biennial questionnaires with additional information on brands for multivitamins, whereas lycopene supplements were only inquired about from 2002 onward. We calculated nutrient intakes by multiplying the consumption frequency of each food by the nutrient content of the specified food portion and summing across all foods. Nutrient values were energy adjusted using the residual method.[25] The FFQ has been validated in both cohorts and had good reproducibility and validity in measuring a wide range of foods and nutrients.[26-30]
Because of variation in assessment validity and bioavailability across carotenoid-containing foods, calculated intakes of carotenoids from FFQs may not adequately represent the more biologically relevant internal dosage. We thus used a previously validated empirical prediction model among 4180 nonsmoking women in the NHS that related carotenoid-containing foods directly to the measured plasma carotenoid level using linear regression.[31] The regression coefficient of each food in the model reflected a weighted contribution to the bioavailable level. We then derived predicted plasma carotenoid scores for all participants by multiplying the consumption frequency of each food by its regression coefficient and summing across all foods. We created a total carotenoid index by first categorizing each predicted plasma score into quintiles and then summing the quintile scores across all carotenoids, yielding a final score ranging from 5 to 25. The empirical prediction model demonstrated improved assessment of carotenoid intakes compared with the conventional food composition–based method.[31]

Statistical Analysis

We calculated the cumulative average for predicted plasma carotenoid scores by averaging scores from all available FFQs up to the start of each 2-year risk interval. We used time-varying multivariate Cox proportional hazards model to estimate the hazard ratios and 95% confidence intervals controlling for known and suspected risk factors. We assessed the linear trend across categories by modeling the median level of each category as a continuous variable. We examined the possible nonlinear relations between carotenoids and AMD nonparametrically by the likelihood ratio test, comparing a model with only the linear term vs a model with the linear and restricted cubic splines with 4 knots.[32]
To assess whether the associations between carotenoids and AMD would vary by prespecified risk factors including age, body mass index (calculated as weight in kilograms divided by height in meters squared), smoking status, and postmenopausal hormone use, we created interaction terms between carotenoids and these variables and tested their significance using likelihood ratio tests. In exploratory analysis, we investigated the independent association of each carotenoid adjusting for all other carotenoids as a composite variable (sum of the quintile score of each carotenoid).

We performed the analyses separately in each cohort and pooled the results with an inverse variance-weighted meta-analysis using the fixed-effects model. We used an α level of .05 without adjustment for multiple comparisons. We used SAS version 9.3 statistical software (SAS Institute, Inc) to perform the analyses.

Results

During 26 years of follow-up in the NHS and 24 years in the HPFS, we confirmed 1361 incident intermediate and 1118 advanced AMD cases (>96% neovascular AMD). The median age at AMD onset was 73 years in women and 76 years in men.

In 1996 (the middle of follow-up), participants at the highest cumulative average predicted plasma score of lutein/zeaxanthin were likely to be more physically active, smoke less, consume more fruits and vegetables, and score higher in an alternative healthy eating index. They also had higher calculated intakes of lutein/zeaxanthin and other carotenoids (**Table 1**).

Predicted plasma carotenoid scores were strongly correlated with their respective calculated intakes (Spearman correlation, $r = 0.67$-0.90) and with each other (eg, $r = 0.64$ between lutein/zeaxanthin and food-sourced β-carotene; $r = 0.67$ between α-carotene and food-sourced β-carotene). Lycopene had the weakest correlations with all other carotenoids ($r \leq 0.18$).

Comparing extreme quintiles, we identified an inverse association with advanced AMD for predicted plasma carotenoid scores of lutein/zeaxanthin (relative risk [RR] = 0.59; 95% CI, 0.48-0.73; *P* for trend < .001), β-cryptoxanthin (RR = 0.73; 95% CI, 0.60-0.89; *P* for trend = .002), α-carotene (RR = 0.69; 95% CI, 0.56-0.84; *P* for trend < .001), food-sourced β-carotene (RR = 0.64; 95% CI, 0.52-0.79; *P* for trend < .001), total carotene from food (RR = 0.64; 95% CI, 0.51-0.79; *P* for trend < .001), and total carotenoid index (RR = 0.65; 95% CI, 0.53-0.80; *P* for trend < .001) (**Table 2**). Predicted plasma lutein/zeaxanthin score and total carotenoid index had a linear relationship with advanced AMD within the range of dietary intake (**Figure 1**). Carotenoids other than lycopene had a similar linear relation (all *P* for linearity < .05; all *P* for nonlinearity > .10; graphs not shown). For the outcome of intermediate AMD, we did not observe any association for any predicted plasma carotenoid scores (**Table 2**). The results did not materially change when restricted to a subgroup of intermediate AMD with large drusen or noncentral GA (n = 283 in the NHS and n = 80 in the HPFS; data not shown).

Because AREDS2 raised the concern for the competitive absorption between lutein/zeaxanthin and β-carotene, in a sensitivity analysis we excluded β-carotene supplement users (4.5% of the person-years in the NHS and 10% in the HPFS) from the analysis of predicted plasma lutein/zeaxanthin score. However, neither the RR for advanced AMD (RR = 0.58; 95% CI, 0.47-0.72; *P* for trend < .001) nor the RR for intermediate AMD (RR = 0.90; 95% CI, 0.75-1.09; *P* for trend = .26) was essentially altered.

In secondary analyses using calculated intakes of carotenoids, lutein/zeaxanthin (*P* for trend = .003), β-cryptoxanthin (*P* for trend = .009), α-carotene (*P* for trend < .001), and food-sourced β-carotene (*P* for trend < .001) were also inversely related to advanced AMD in the NHS (eTable 1 in the **Supplement**). However, none were associated with advanced AMD in the HPFS (eTable 2 in the **Supplement**).

The primary food sources for measured plasma carotenoid levels in the NHS31 were cooked and raw spinach for lutein/zeaxanthin, oranges and orange juice for β-cryptoxanthin, cooked and raw carrots for both α- and β-carotene, and tomato sauce for lycopene (**Figure 2**), consistent with the National Health and Nutrition Examination Survey.33 These foods were generally inversely related to advanced AMD, although with variation for specific forms of these foods (**Figure 2**). The inverse association between tomato sauce and advanced AMD was primarily attributed to that in the HPFS (comparing ≥2 servings/wk vs almost never, RR = 0.60; 95% CI, 0.39-0.93; *P* for trend = .01). Cooked spinach had an inverse association with intermediate AMD (comparing ≥1 serving/wk vs almost never, RR = 0.71; 95% CI, 0.56-0.90; *P* for trend = .02), as did orange juice (comparing ≥1 small glass/d vs almost never, RR = 0.77; 95% CI, 0.66-0.91; *P* for trend = .003) (**Figure 2**). Although not the primary source of α-carotene, consumption of bananas, which predicted plasma α-carotene level in our sample, was inversely relate to intermediate AMD (comparing ≥5 pieces/wk vs almost never, RR = 0.83; 95% CI, 0.70-1.00; *P* for trend = .003).

In an exploratory analysis adjusted for all other carotenoids as a composite score, only lutein/zeaxanthin and α-carotene persisted with an inverse association with advanced AMD (**Figure 3**). Collectively, β-cryptoxanthin, β-carotene, and lycopene did not have an inverse association after accounting for lutein/zeaxanthin and α-carotene, nor did β-cryptoxanthin and β-carotene combined after accounting for all other carotenoids. In the sensitivity analysis in which we entered each individual carotenoid in the same model, the inverse association for lutein/zeaxanthin and α-carotene persisted (lutein/zeaxanthin: RR = 0.66; 95% CI, 0.50-0.87; α-carotene: RR = 0.75; 95% CI, 0.59-0.96).

Plasma carotenoid scores except lycopene seemed to be associated with a lower risk of total AMD among postmenopausal women currently using exogenous hormones compared with those not currently using them (eFigure 1 in the **Supplement**). We also found a suggestive stronger inverse association for all predicted plasma carotenoid scores except lycopene with advanced AMD in those aged 75 years and older, and this was most pronounced for lutein/zeaxanthin (*P* for interaction = .04) (eFigure 2A in the **Supplement**). We found similar RRs in never smokers compared with ever smokers (all *P* for interaction > .25) (eFigure 2B in the **Supplement**).

Discussion

Our findings from 2 large, long-running prospective cohorts with repeated dietary assessments suggest that a higher intake of bioavailable lutein/zeaxanthin is associated with a 40% lower risk of advanced AMD. Higher intakes of other bioavailable carotenoids also contribute to a reduced risk of advanced AMD. In contrast, intakes of carotenoids were not associated with intermediate AMD, suggesting an effect on AMD progression rather than initiation.

Although the inverse association between lutein/zeaxanthin and advanced AMD was consistent with a number of previous studies,12,13,19-21,34 the observational nature of our study precludes the level of causal inference that could be derived from a randomized trial. Unfortunately, the primary analyses of the AREDS2 trial failed to prove a protective effect of lutein/zeaxanthin.16 However, when restricted to participants at the bottom 20% of dietary intake of lutein/zeaxanthin, there was a 26% risk reduction.17 The subgroup result is consistent with the hypothesis that supplements may be more effective when the background dietary intake is below a biologically sufficient threshold. Given the unlikely occurrence of another well-designed large-scale randomized trial, long-running large prospective cohort studies like ours provide the best available evidence to further strengthen the evidence base for a protective role of lutein/zeaxanthin.

Lutein and zeaxanthin form macular pigments that may protect against AMD by reducing oxidative stress, absorbing blue light, and stabilizing cell membranes.11 Cross-sectional studies (reviewed by Beatty et al35) and experimental studies36-38 have shown a significant correlation between serum lutein and zeaxanthin and macular pigment optical density. Increasing evidence also suggests that genetic variants related to lutein and zeaxanthin metabolism are associated with macular pigment optical density or AMD.39-42 Therefore, multiple independent lines of evidence point to a protective role of lutein and zeaxanthin in the development of advanced AMD.

Several mechanisms could explain the protective roles of other carotenoids including α-carotene, β-carotene, and β-cryptoxanthin, which are nonmacular pigment carotenoids. All carotenoids are potent antioxidants, which could reduce systemic oxidative stress that indirectly influences the macula. The original AREDS formula containing β-carotene, antioxidant vitamins, and minerals but not lutein and zeaxanthin reduced the risk of AMD progression by a quarter.43 Carotenoids including α-carotene, β-carotene, and lycopene have been found in human RPE and choroid18 and could protect this tissue against light-induced oxidative damage and locally produced free radicals.

The integrity of the RPE and choroid could further affect the retina's uptake of lutein and zeaxanthin from the circulating blood. We also speculate that other carotenoids may directly protect lutein and zeaxanthin from oxidative damage in both blood and the RPE/choroid. Among a subsample of women in the NHS, we found that measured plasma lutein/zeaxanthin could be significantly predicted by every other plasma carotenoid apart from its own food sources (all $P < .001$), in accordance with a separate study.44

We did not find an association between carotenoids and intermediate AMD. While one previous case-control study13 and one cross-sectional study45 reported an inverse association, only one34 of 3 prospective cohort studies15,34,46 reported a significant inverse association between intake of lutein/zeaxanthin and intermediate AMD. One nested case-control study based on 41 cases found an inverse association for serum lutein/zeaxanthin but not for other carotenoids,47 whereas 2 other case-control studies14,20 found an inverse association only for serum lycopene.

Our study has some limitations. Although our results did not appreciably change after adjusting for many known and suspected risk factors including an alternative healthy eating index, an indicator of a healthy dietary pattern,48 residual confounding from unaccounted or imprecise measurement cannot be excluded. However, similar associations among ever and never smokers assured us that results were unlikely to be confounded by smoking, the strongest modifiable risk factor for advanced AMD.49 Because our nutrient and blood database assessed lutein and zeaxanthin together, we were unable to estimate the individual effect of each nutrient to inform the optimal ratio for supplementation. Although the relationship between lutein/zeaxanthin and advanced AMD was linear within the range consumed in our cohorts (0.8-10.7 mg/d), we could not evaluate the effect of the higher dosage (10 mg of lutein plus 2 mg of zeaxanthin) used in the AREDS2 formula. Some patients with intermediate AMD in the later follow-up may have been using the AREDS formula, which was not ascertained by our FFQs; this may have resulted in underestimation of the true associations between carotenoids and advanced AMD because dietary effects of carotenoids could be masked under intake of pharmacological doses of antioxidant vitamins and minerals.

Strengths of this study included a prospective cohort design with high follow-up that minimized recall and selection biases. Another strength lies in our creation of predicted plasma carotenoid scores to better estimate the true variation of carotenoid exposures accounting for variations in bioavailability across different foods,50,51 preparation methods,52 accuracy of responses to various FFQ items, and food composition databases. Analyses using the predicted plasma scores strengthened the association between lutein/zeaxanthin and advanced AMD and modestly improved associations with other carotenoids. Differences in the impact of substituting estimated bioavailable nutrient levels among specific carotenoids might be attributable to variation in the accuracy of FFQ data for specific food items. The predicted plasma score for lutein/zeaxanthin (median, 16.9 μg/dL; eTable 1 and eTable 2 in the **Supplement**) in our cohorts was comparable with the baseline serum level in AREDS2 participants (mean, 17.9 μg/dL) and the general population participants older than 60 years sampled from the 2005-2006 National Health and Nutrition Examination Survey (mean, 15.0 μg/dL).16

Conclusions

Higher intakes of bioavailable carotenoids, particularly lutein/zeaxanthin and α-carotene, are associated with reduced risk of advanced AMD. This study lends further support to the causal role of lutein/zeaxanthin in protecting against the development of advanced AMD. Because other carotenoids may also have a protective role, a public health strategy of increasing the consumption of a wide variety of fruits and vegetables rich in carotenoids could be most beneficial and is compatible with current dietary guidelines.

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