

Dietary Antioxidants and the Long-term Incidence of Age-Related Macular Degeneration

The Blue Mountains Eye Study

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Purpose: To assess the relationship between baseline dietary and supplement intakes of antioxidants and the long-term risk of incident age-related macular degeneration (AMD).

Design: Australian population-based cohort study.

Participants: Of 3654 baseline (1992–1994) participants initially 49 years of older, 2454 were reexamined after 5 years, 10 years, or both.

Methods: Stereoscopic retinal photographs were graded using the Wisconsin Grading System. Data on potential risk factors were collected. Energy-adjusted intakes of α -carotene; β -carotene; β -cryptoxanthin; lutein and zeaxanthin; lycopene; vitamins A, C, and E; and iron and zinc were the study factors. Discrete logistic models assessed AMD risk. Risk ratios (RRs) and 95% confidence intervals (CIs) were calculated after adjusting for age, gender, smoking, and other risk factors.

Main Outcome Measures: Incident early, late, and any AMD.

Results: For dietary lutein and zeaxanthin, participants in the top tertile of intake had a reduced risk of incident neovascular AMD (RR, 0.35; 95% CI, 0.13–0.92), and those with above median intakes had a reduced risk of indistinct soft or reticular drusen (RR, 0.66; 95% CI, 0.48–0.92). For total zinc intake the RR comparing the top decile intake with the remaining population was 0.56 (95% CI, 0.32–0.97) for any AMD and 0.54 (95% CI, 0.30–0.97) for early AMD. The highest compared with the lowest tertile of total β -carotene intake predicted incident neovascular AMD (RR, 2.68; 95% CI, 1.03–6.96; $P = 0.029$, for trend). Similarly, β -carotene intake from diet alone predicted neovascular AMD (RR comparing tertile 3 with tertile 1, 2.40; 95% CI, 0.98–5.91; $P = 0.027$, for trend). This association was evident in both ever and never smokers. Higher intakes of total vitamin E predicted late AMD (RR compared with the lowest tertile, 2.83; 95% CI, 1.28–6.23; and RR, 2.55; 95% CI, 1.14–5.70 for the middle and highest tertiles, respectively; $P = 0.22$, for trend).

Conclusions: In this population-based cohort study, higher dietary lutein and zeaxanthin intake reduced the risk of long-term incident AMD. This study confirmed the Age-Related Eye Disease Study finding of protective influences from zinc against AMD. Higher β -carotene intake was associated with an increased risk of AMD. *Ophthalmology* 2008;115:334–341 © 2008 by the American Academy of Ophthalmology.

Age-related macular degeneration (AMD) is a leading cause of blindness with limited treatment options.¹ Although the exact etiology of AMD is largely unknown, considerable recent

work has identified key genetic risk factors.² Oxidative stress is hypothesized to be one of the pathogenetic mechanisms in AMD. The retina is thought to be highly susceptible to oxidative stress given its high oxygen consumption, high concentration of polyunsaturated fatty acids and photosensitizers, and exposure to light. Phagocytosis by the retinal pigment epithelium also leads to oxidative stress.³

The Age-Related Eye Disease Study (AREDS), a large, multicenter, randomized, placebo-controlled clinical trial, provided evidence that high dose zinc and antioxidant vitamin supplementation may slow AMD progression in relatively advanced early AMD cases.⁴ Trial participants who received a daily high-dose supplement of vitamins C (500 mg) and E (400 IU), β -carotene (15 mg), and zinc (80 mg) had an approximately 25% reduced risk of progression from relatively advanced early AMD stages to late AMD.⁴ Recently, the Rotterdam Study reported that above-median dietary intake of all 4 of these nutrients was associated with

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a statistically significant 35% reduction in incident AMD risk, greater than that observed in the AREDS from supplement use.⁵ Other studies have not provided consistent evidence to support a protective effect of antioxidants on AMD.⁴⁻¹¹ In this report, the authors aimed to investigate the association between dietary antioxidants from daily foods and supplements and the 10-year incidence of AMD in an older Australian cohort, particularly high intakes of combined β -carotene, zinc, and vitamins C and E.

Patients and Methods

The Blue Mountains Eye Study is a population-based cohort study of vision, common eye diseases, and other health outcomes in an urban, predominantly white, Australian population located west of Sydney. Study methods and procedures have been reported.¹² Baseline (1992–1994) eye examinations of 3654 residents aged 49 years or older were conducted (82.4% participation). Of the baseline participants, 2335 (75.1% of survivors) returned for the 5-year follow-up examinations (1997–1999), and 1952 participants (53.4% of the original cohort, or 76.6% of survivors) returned for the 10-year follow-up examinations (2002–2004). Of those seen at either 5- or 10-year follow-up, 2454 (67.2% of the original cohort) underwent stereoscopic retinal photography. The mean follow-up period was 5.1 years and 10.5 years for the 5- and 10-year follow-up examinations, respectively. This study followed the recommendations of Declaration of Helsinki and was approved by the Western Sydney Area Human Research Ethics Committee. Written, informed consent was obtained from all participants.

At each examination, participants underwent a comprehensive eye examination after pupil dilation and 30° stereoscopic retinal photographs were obtained using a Zeiss FF3 fundus camera (Carl Zeiss, Oberkochen, Germany). At the baseline, 5-, and 10-year examinations, photographs of both eyes were obtained in 98%, 98%, and 85% of participants, respectively, and photographs of at least 1 eye were obtained in 99%, 99%, and 87% of participants. Details of the photographic grading for AMD lesions performed in the Blue Mountains Eye Study were reported¹² and closely followed the Wisconsin Age-related Maculopathy Grading System.¹³ When any lesions were identified at follow-up, side-by-side grading of the baseline and follow-up photographs was performed. Assessments of intergrader and intragrader reliability showed good agreement for identifying AMD lesions.¹²

Dietary Assessment

Participants completed a 145-item, semiquantitative food frequency questionnaire (FFQ) modified from an early FFQ by Willett et al¹⁴ for Australian diet and vernacular. This included questions on dietary supplements including strength and frequency of supplement uses. The FFQ was attempted and returned by 3267 participants at baseline (89.4%), with 2900 (88.8% of those who attempted the FFQ, 79.4% of those who attended examination) usable FFQs after excluding those considered unreliable. Characteristics of the FFQ respondents and exclusion criteria have been reported.^{15,16} Briefly, subjects were excluded if more than 12 FFQ questions or an entire page remained blank, or daily energy intakes were less than 2500 kJ or more than 18 000 kJ. The FFQ was found to be reliable in the population and to have reasonable concurrent validity compared with weighed food records collected over 1 year (energy-adjusted Spearman correlations of more than 0.5 for most nutrients).¹⁶

The electronic version of the Australian Tables of Food Composition 1990¹⁷ was used to calculate the intake of most nutrients. β -Carotene intake was estimated using the Australian Tables of

Food Composition 1990. This database represented the most up-to-date nutrient composition information based on laboratory analyses of Australian food products available at that time. However, only 1 carotenoid, β -carotene, was included in this database. The United States Department of Agriculture Carotenoid Food Composition database¹⁸ was used to estimate the intakes of other carotenoids, including α -carotene, β -cryptoxanthin, combined lutein and zeaxanthin, and lycopene.

Assessment of Confounders

Information on potential confounders was collected at baseline. A standardized questionnaire was used to ascertain demographic information; family history; medications taken; self-reported diagnoses of diabetes, acute myocardial infarction, angina, or stroke; and smoking history. Participants were asked what their main job had been in their working life. Occupational prestige was assessed from the principal job using the Daniel Occupational Prestige Scale,¹⁹ where participants were assigned scores ranging from 1.4 to 6.9 (highest to lowest prestige, respectively). Those with scores of less than 5 were classified as having a high-prestige job, and those with scores of 5 or more were classified as having a low-prestige job. Fasting blood specimens were drawn for clinical biochemistry assessment. Diabetes was diagnosed either from medical history or fasting blood glucose of 7.0 mmol/l or more. Age was defined as age at the baseline examination.

Definitions of Age-Related Macular Degeneration

Late AMD was defined to include neovascular AMD and geographic atrophy (GA), as described in the international classification.²⁰ All detected late AMD cases were adjudicated and confirmed by a retinal specialist (PM). Incident late AMD was defined by the appearance at follow-up of neovascular AMD or GA in either eye of persons without late AMD lesions in either eye at baseline. Incident neovascular AMD was defined after excluding baseline neovascular AMD cases. Cases with baseline GA were not excluded, however, because the authors considered that neovascular AMD may still develop in eyes with GA. Persons with both GA and neovascular AMD at baseline or follow-up were not considered to have incident GA.

Early AMD was defined as the presence at the macula¹³ of large ($>125 \mu\text{m}$ in diameter) indistinct soft or reticular drusen, or combined large distinct soft drusen and retinal pigmentary abnormalities,²⁰ without late AMD signs. Incident early AMD was defined in persons without AMD at baseline by the appearance at follow-up of these lesions in either eye of persons without late AMD at follow-up. Incidence of the principal early AMD lesions, soft indistinct or reticular drusen, or retinal pigmentary abnormalities was defined by the appearance of these lesions at follow-up in either eye of persons without late or early AMD at baseline or follow-up, and without corresponding lesions in either eye at baseline. Any AMD was defined in participants without any AMD lesions at baseline by the appearance at follow-up of early or late AMD.

Statistical Methods

Statistical Analysis System software version 9 (SAS Institute, Cary, NC) was used for all analyses. The authors examined the association of baseline dietary antioxidant intake and the 10-year incidence of early, late, or any AMD and each specific lesion. The study factors included α -carotene; β -carotene; β -cryptoxanthin; combined lutein and zeaxanthin; lycopene; vitamins A (retinol equivalents), C, and E; and iron and zinc as cofactors for antioxidant enzymes. Dietary intakes were energy adjusted using the residual method described by Willett and Stampfer.²¹ Total intake

Table 1. Comparison of Baseline Characteristics for Those Who Attended Follow-up between Participants Who Completed or Did Not Complete a Baseline Food Frequency Questionnaire in the Blue Mountains Eye Study*

Baseline Characteristic	Food Frequency Questionnaire Not Completed (n = 371)	Food Frequency Questionnaire Completed (n = 2083)	P Value [†]
Mean age (yrs)	66.3	64.0	<0.0001
Female gender (%)	62.0	56.8	0.064
Smoking status (%)			
Never	55.3	50.9	
Past	28.8	36.7	0.0036
Current	15.9	12.4	0.064
High job prestige (%)	54.7	63.8	0.0008
History of diabetes (%)	7.5	6.1	0.31
History of cardiovascular disease (%)	19.1	16.0	0.14
Family history of age-related macular degeneration (%)	2.2	2.8	0.46
Total cholesterol (mmol/L)	6.1	6.0	0.89
HDL cholesterol (mmol/L)	1.4	1.4	0.93
Total-to-HDL cholesterol ratio	4.5	4.6	0.63
White cell count (10 ⁹ cells/l)	6.4	6.4	0.83

HDL = high-density lipoprotein.

*For participants who attended either the 5- or 10-yr follow-up examinations and from whom retinal photographs were obtained.

[†]P values obtained by comparing those who completed a food frequency questionnaire with those who did not. The chi-square test was used to compute P values for discrete variables; the t test was used for continuous variables (Satterthwaite for unequal variances, otherwise pooled t test).

(from diet and supplements) was calculated for β -carotene, retinol equivalents, iron, zinc, and vitamins C and E by adding the energy-adjusted dietary intake and crude supplement intake.

Relative risks (RRs) and 95% confidence intervals (CIs) were calculated using discrete linear logistic models, initially adjusted for age, gender, and smoking (current versus never or past). Final multivariate models also adjusted for other factors significantly associated with AMD in the cohort, including white cell count (10⁹ cells/l), AMD family history (present, absent), and job prestige (high vs. low). The authors additionally adjusted for intake of total fat in the analysis of the fat-soluble vitamin E because of reported associations between total fat and AMD.²²

Antioxidant intakes were analyzed as continuous variables (per standard deviation [SD]) and as tertiles. The lowest tertile was the referent group. The authors calculated the trend in RR across tertiles by modeling an ordinal variable corresponding to the median value for each antioxidant tertile. These analyses tested for continuous trends between antioxidant intake and AMD risk. The authors also examined possible threshold effects of antioxidant intake by modeling dummy variables to compare the top 50%, tertile, 20%, and 10% of intake with the rest of the population for each nutrient. Each cut-point was assessed using the chi-square test statistic. Similar methods were used to analyze associations between selected foods and AMD, where those foods were known to contribute a high proportion to the nutrients of interest.

The authors studied the combination of nutrients administered at high dose in the AREDS⁴ using the same method as van Leeuwen et al.⁵ The median intake of total β -carotene, zinc, and vitamins C and E, based on the total sample, was used as the cut-off value. The high-intake group was defined as participants with an above-median intake of each of the 4 nutrients, and the low-intake group had a below-median intake of each of the nutrients. Participants in between were considered the referent group.

Results

Table 1 presents the baseline characteristics for participants with and without a baseline FFQ from among those who attended at

least 1 follow-up examination and underwent retinal photography. Of the 2454 participants who were followed up, 2083 (84.9%) had completed an FFQ at baseline. Those who completed the FFQ were more likely to be slightly younger, to be noncurrent smokers, and to have higher prestige occupations. Table 2 presents the baseline mean energy-adjusted antioxidant intakes for participants followed up and not followed up. Those lost to follow-up were more likely than those followed up to have lower intakes of vitamin E and iron from diet alone.

In threshold analyses, the authors found that those in the top decile of total zinc intake (≥ 15.8 mg/day) were significantly less likely to develop early AMD (RR, 0.54; 95% CI, 0.30–0.97; $P = 0.041$) or any AMD (RR, 0.56; 95% CI, 0.32–0.97; $P = 0.040$) compared with the remaining population. Corresponding results for dietary zinc alone were not significant (RR, 0.86; 95% CI, 0.52–1.45, for early AMD; RR, 0.91; 95% CI, 0.56–1.48, for any AMD). The authors also found that for dietary lutein and zeaxanthin intake, participants within the top tertile (≥ 942 μ g/day), compared with the remaining population, were significantly less likely to develop neovascular AMD (RR, 0.35; 95% CI, 0.13–0.92; $P = 0.033$), and those above the median (743 μ g) also were less likely to develop indistinct soft or reticular drusen (RR, 0.66; 95% CI, 0.48–0.92; $P = 0.013$).

Table 3 shows the significant or near significant associations between late AMD and nutrient intakes. Increasing β -carotene intake from diet alone predicted incident neovascular AMD (RR, 1.36; 95% CI, 1.02–1.81 per 1-SD increase; $P = 0.039$), with a significant trend across increasing tertiles of dietary β -carotene intake (Table 3). After stratifying by smoking status (ever vs. never), a borderline significant increased risk of neovascular AMD for each SD increase of dietary β -carotene intake was found for both ever smokers (RR, 1.48; 95% CI, 0.99–2.23) and never smokers (RR, 1.38; 95% CI, 1.00–1.91). However, trends across tertiles were nonsignificant for both smoking subgroups (results not shown). Similar associations were observed between total β -carotene intake and neovascular AMD (Table 3), with a significant trend across tertiles for ever smokers (results not shown).

Each SD increase in dietary α -carotene predicted incident neovascular AMD (RR, 1.37; 95% CI, 1.03–1.81; $P = 0.029$). Each

Table 2. Comparison of Baseline Daily Energy-Adjusted Antioxidant Intakes between Participants Followed up and Not Followed up over 10 Years in the Blue Mountains Eye Study*

Nutrient	Mean Intakes (Standard Deviation)		P Value [†]
	Followed up	Not Followed up or No Retinal Photographs Obtained	
α -carotene (μ g)	2607 (1765)	2586 (1967)	0.86
β -carotene (μ g; diet)	7177 (4090)	7007 (4356)	0.49
β -carotene (μ g; diet+supplements)	7494 (4593)	7108 (4535)	0.16
β -cryptoxanthin (μ g)	269 (296)	257 (282)	0.50
Lutein and zeaxanthin (μ g)	826 (482)	842 (507)	0.58
Lycopene (μ g)	3781 (3099)	3926 (3307)	0.43
Vitamin A (μ g; retinol equivalents, diet)	2001 (1161)	2066 (1188)	0.35
Vitamin A (μ g; retinol equivalents, diet+supplements)	2217 (1537)	2326 (1494)	0.23
Vitamin C (mg; diet)	185 (88)	183 (91)	0.65
Vitamin C (mg; diet+supplements)	344 (384)	355 (410)	0.64
Vitamin E (mg; diet)	9.8 (7.6)	8.9 (6.1)	0.024
Vitamin E (mg; diet+supplements)	39.7 (109.9)	42.7 (122.7)	0.68
Iron (mg; diet)	12.8 (2.6)	12.5 (2.6)	0.043
Iron (mg; diet+supplements)	13.8 (7.1)	14.2 (13.3)	0.59
Zinc (mg; diet)	11.8 (2.3)	11.7 (2.5)	0.26
Zinc (mg; diet+supplements)	13.0 (7.9)	12.6 (5.1)	0.18

*For participants who completed a food frequency questionnaire at baseline.

[†]P values obtained by comparing those who were not followed up or had not undergone retinal photography with those followed up, excluding those who died. The *t* test was used to compute P values (Satterthwaite for unequal variances, otherwise pooled *t* test).

SD increase in total vitamin E intake (RR, 1.15; 95% CI, 1.03–1.29; *P* = 0.015) predicted the incidence of indistinct soft or reticular drusen. The RR of incident late AMD for participants in the middle and highest tertile of total vitamin E intake was 2.83 (95% CI, 1.28–6.23; *P* = 0.0099) and 2.55 (95% CI, 1.14–5.70; *P* = 0.022), respectively, compared with the lowest tertile (Table 3). The test of trend across tertiles for each of these associations, however, was not significant (results not shown for indistinct soft drusen). Further controlling for total fat intake did not alter the results for vitamin E.

For each of the above findings, the authors created multinutrient models that simultaneously evaluated all of the nutrients that were found significantly associated with each AMD subtype. However, α -carotene and β -carotene were not included in the same models because they were highly correlated (correlation, 0.84). All of the findings reported above were similar in the multinutrient models.

The median total intakes for total β -carotene, vitamin C, vitamin E, and zinc were 6836 μ g, 206 mg, 8.2 mg, and 12 mg, respectively. After dividing the cohort into those with above-median and below-median intakes of all 4 of these nutrients, with reference to those with intakes between these, no significant associations were found between either the higher or lower intake groups and incident AMD (data not shown). These results remained similar after analyzing the combined intake of these 4 nutrients from diet alone.

Associations between AMD and major food groups known to contribute high amounts of zinc, lutein, or zeaxanthin in this population also were examined, including total meat, red meat, vegetables, fruits, dairy, and breads and cereals. The authors found that those in the top tertile of intake of vegetables, compared with the remaining population, had a significantly reduced risk of developing any AMD (RR, 0.64; 95% CI, 0.41–0.98; *P* = 0.040) and a borderline nonsignificant reduced risk of incident early AMD (RR, 0.65; 95% CI, 0.42–1.02) and GA (RR, 0.13; 95% CI, 0.02–1.10).

To adjust for the multiple tests, the authors further applied the

Bonferroni correction (*k* = 10). Most of the results, however, were nonsignificant after applying this correction. Each SD increase in total β -carotene still predicted neovascular AMD (RR, 1.47; 95% CI, 1.17–1.85; *P* = 0.009). After stratifying by smoking status, each SD increase in total β -carotene significantly predicted neovascular AMD only among ever smokers (RR, 1.62; 95% CI, 1.24–2.11; *P* = 0.004).

Discussion

In this population-based longitudinal study, those in the highest decile of total zinc intake (≥ 15.8 mg/day) were found to be significantly less likely to develop early or any AMD compared with the remaining population. Similarly, for dietary lutein and zeaxanthin intake, those in the top tertile had a reduced risk of incident neovascular AMD, and those with above-median intakes had a reduced risk of incident indistinct soft or reticular drusen. This is further supported by the finding that those in the highest tertile of vegetable intake, compared with the remaining population, were less likely to develop any AMD. The authors found that increasing β -carotene intake, either from diet alone or from diet and supplements combined (total intake), was associated with a higher risk of neovascular AMD. These findings also suggested an association between higher intakes of dietary α -carotene and risk of neovascular AMD, or high total vitamin E intake and risk of late AMD or indistinct soft or reticular drusen. The authors were unable to confirm the protective effect shown by the AREDS⁴ and the Rotterdam Study⁵ of a combination of high doses of zinc, β -carotene, and vitamins C and E on AMD.

These results suggest a possible threshold effect of total zinc intake on risk of early or any AMD. A protective effect

Table 3. Association between Baseline Dietary Antioxidant Intake and 10-Year Incident Late Age-Related Macular Degeneration in the Blue Mountains Eye Study

Nutrient (Tertile)	Late Age-Related Macular Degeneration			Neovascular Age-Related Macular Degeneration		
	No. of Outcomes/No. at Risk	Adjusted Relative Risk (95% Confidence Interval)*	Adjusted Relative Risk (95% Confidence Interval)†	No. of Outcomes/No. at Risk	Adjusted Relative Risk (95% Confidence Interval)*	Adjusted Relative Risk (95% Confidence Interval)†
β-carotene (diet)						
1	14/672	1.00	1.00	7/675	1.00	1.00
2	17/683	1.11 (0.52–2.36)	0.86 (0.39–1.93)	10/684	1.37 (0.50–3.74)	0.94 (0.30–2.88)
3	28/680	1.68 (0.85–3.34)	1.65 (0.83–3.28)	21/681	2.32 (0.95–5.65)	2.41 (0.98–5.91)
P value for trend		0.11	0.10		0.048	0.027
β-carotene (diet+supplements)						
1	12/674	1.00	1.00	6/676	1.00	1.00
2	20/684	1.55 (0.73–3.30)	1.28 (0.58–2.82)	12/686	1.90 (0.69–5.24)	1.47 (0.49–4.38)
3	27/677	1.83 (0.89–3.76)	1.86 (0.90–3.84)	20/678	2.49 (0.97–6.40)	2.68 (1.03–6.96)
P value for trend		0.11	0.080		0.059	0.029
Lutein and zeaxanthin						
1	19/673	1.00	1.00	13/675	1.00	1.00
2	23/682	1.18 (0.62–2.25)	1.11 (0.58–2.13)	16/684	1.19 (0.56–2.54)	1.12 (0.52–2.41)
3	17/680	0.76 (0.38–1.56)	0.72 (0.34–1.50)	9/681	0.46 (0.18–1.19)	0.37 (0.13–1.05)
P value for trend		0.41	0.36		0.097	0.061
Vitamin A, retinol equivalents (diet)						
1	14/679	1.00	1.00	7/682	1.00	1.00
2	15/680	0.99 (0.46–2.13)	0.97 (0.44–2.12)	10/682	1.39 (0.52–3.73)	1.42 (0.51–3.91)
3	30/676	1.78 (0.91–3.49)	1.66 (0.84–3.30)	21/676	2.26 (0.93–5.52)	2.12 (0.85–5.28)
P value for trend		0.065	0.11		0.057	0.093
Vitamin A, retinol equivalents (diet+supplements)						
1	13/677	1.00	1.00	7/681	1.00	1.00
2	18/681	1.23 (0.58–2.59)	1.17 (0.55–2.49)	11/682	1.50 (0.57–3.94)	1.41 (0.52–3.81)
3	28/677	1.71 (0.85–3.44)	1.60 (0.79–3.25)	20/677	2.10 (0.86–5.16)	1.94 (0.77–4.87)
P value for trend		0.11	0.17		0.098	0.15
Vitamin E (diet + supplements)						
1	11/677	1.00	1.00	8/680	1.00	1.00
2	28/677	2.71 (1.27–5.75)	2.83 (1.28–6.23)	18/678	1.97 (0.81–4.78)	2.03 (0.79–5.24)
3	20/681	2.21 (1.02–4.80)	2.55 (1.14–5.70)	12/682	1.57 (0.62–3.96)	1.96 (0.74–5.15)
P value for trend		0.4	0.22		0.82	0.44

*Adjusted for age, gender, and smoking.

†Adjusted for age, gender, smoking, white cell count, family history of age-related macular degeneration, and job prestige.

from high intakes of zinc is biologically plausible. Zinc is concentrated in the retina and is a cofactor for many enzymes, including the antioxidant enzymes superoxide dismutase and catalase that are present in human retinal pigment epithelium.^{23,24} Zinc is also a cofactor for vitamin A metabolism and is essential for the synthesis of retinol binding protein.²⁴ Animal models also support a protective role for zinc in AMD.²⁴ Epidemiologic studies on the association between zinc intake and AMD have been inconsistent. Some studies show no significant association,^{23,25} including the baseline and 5-year follow-up Blue Mountains Eye Study reports.^{6,15} However, other studies have reported a protective effect from zinc on AMD.^{8,11,26} Importantly, the AREDS reported that zinc supplementation significantly slowed AMD progression in relatively advanced early AMD cases, and the protective effect shown with a combined zinc and antioxidant supplement seemed driven largely by zinc.⁴ The recently reported findings from the Rotterdam Study also seemed to be driven by zinc, and perhaps vitamin E.⁵

These results also suggest a possible threshold protective effect of dietary lutein and zeaxanthin intake on the risk of incident neovascular AMD or indistinct soft drusen. Lutein and zeaxanthin are the only carotenoids that concentrate in the macula, where they are the main components of macular pigment.²⁷ They are obtained naturally only through diet, particularly leafy dark-green vegetables.²⁸ Proposed mechanisms by which lutein and zeaxanthin may protect against AMD include effects on the ability to absorb blue light, quench free radicals, or increase membrane stability.²⁷ A relative decrease of these carotenoids has been shown in eyes of patients with AMD,²⁹ and a higher intake of lutein and zeaxanthin from foods or supplements increases serum levels of these carotenoids and macular pigment density in humans.^{30–32} A role for dietary intake of lutein and zeaxanthin in AMD prevention is supported further by animal models.^{33,34} However, studies on the association between dietary, or serum levels, of lutein and zeaxanthin and AMD have been inconsistent. A 12-month randomized trial of lutein and antioxidant supplementation in people with GA

demonstrated that daily 10-mg lutein supplements, with or without additional nutrients, improved visual function.³⁵ Other studies also have shown a protective association between dietary intake, or serum levels, of these carotenoids and AMD,¹¹ particularly case-control^{10,36} or cross-sectional studies.^{27,37–39} However, several studies have found no significant association.^{6,8,15,40–42} The role of lutein and zeaxanthin supplementation on AMD progression is being tested in the current AREDS extension trial (AREDS II).⁴³

There is a strong known association between current smoking and incident AMD. Further, there is evidence that β -carotene may have potentially harmful effects among smokers, with a reported increased risk of lung cancer among smokers taking daily β -carotene supplements (30 mg).⁴⁴ However, the authors found that high β -carotene intake was associated with high risk of neovascular AMD even after adjusting for smoking status, and also in both subgroups of ever and never smokers. This suggests that the observed association between β -carotene and neovascular AMD is not fully explained by smoking. The findings of an association between higher intake of β -carotene, and possibly dietary α -carotene, and an increased risk of AMD are inconsistent with other reports. In addition to the previously mentioned findings of AREDS⁴ and the Rotterdam Study,⁵ the Eye Disease Case-Control Study Group also reported a reduced risk for AMD with higher dietary intakes of carotenoids, particularly β -carotene and lutein and zeaxanthin.¹⁰ The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group randomly assigned participants to α -tocopherol, β -carotene, or placebo for 5 to 8 years and found no significant difference in AMD prevalence between treatment groups at the final examinations.⁴⁵ Other studies also have shown no statistically significant effect of dietary β -carotene on AMD risk.^{6,8,11,15} The authors are not aware of other studies reporting a higher risk of AMD with increasing dietary intake of β -carotene, and they have no biological explanation for this finding. Thus, these findings need to be taken with caution. The AREDS II also is examining the effect of eliminating β -carotene from the current AREDS formulation in patients with moderate to high AMD risk.⁴³ This may provide further insights into the association between β -carotene and AMD.

The current findings of a possible association between increasing total vitamin E and AMD risk also are inconsistent with other studies that have reported a protective effect on AMD progression⁴ or development,^{5,11} or no effect.^{6,8–10,15,46} There have been reports linking high-dose vitamin E supplements (≥ 400 IU) with an increased risk of heart failure among people with vascular disease or diabetes⁴⁷ or all-cause mortality.⁴⁸ Proposed mechanisms are that high doses of vitamin E may have prooxidant effects and could displace other fat-soluble vitamins.⁴⁸ Animals studies suggest that vitamin E also may affect the conversion of β -carotene to vitamin A, but the relevance of this in humans has not been established.⁴⁸ The authors are not aware of other studies reporting a harmful effect of dietary vitamin E on AMD risk. Similarly, although the authors reported increasing total vitamin C intake predicted early AMD after the 5-year follow-up of this population,⁶ they could not confirm this association in the current study, and other studies have

reported no significant association between vitamin C and AMD.^{7–11}

This study population differs from the AREDS study sample, which considered only participants with AMD.⁴ Although this may help to explain the difference in findings between the present study and the AREDS, it does not explain the difference between these study findings and those from the Rotterdam study.⁵ Although findings on the dietary antioxidant and AMD association are inconsistent, it is important to emphasize that a healthy diet with a variety of fresh fruit and vegetables will have many benefits and is unlikely to be harmful.⁴⁹

Strengths of this study include prospective observation from a population-based sample, reasonable follow-up, and use of the gold standard method to assess AMD incidence, including photographic grading by the same personnel at all examinations and a detailed side-by-side comparison of the baseline and follow-up photographs to ensure negligible misclassification of AMD diagnosis. Although there were moderate losses to follow-up, only the findings for dietary vitamin E and iron were likely to be affected by these losses because of the significant difference in levels of these nutrients between those followed up and not followed up.

These results must be interpreted with caution, particularly because the authors have examined many associations, and several of their findings were of borderline significance. For example, most of the results were nonsignificant after applying the Bonferroni method to adjust for multiple tests. However, it is well known that the Bonferroni method is extremely conservative and may mask findings with relatively lower levels of significance. Additionally, some of the results were based on relatively small outcome numbers. The possibility of chance findings therefore cannot be ruled out, and replication of these findings in other studies is needed. It also is difficult to analyze individual nutrient intakes in the absence of other antioxidants, although the authors have tried to do this by creating multinutrient models and by analyzing the combination of nutrients administered in the AREDS. Although bias from healthy behaviors among participants with high lutein and zeaxanthin intakes after early AMD was diagnosed also cannot be ruled out, the dietary measures were made at a time before widespread publicity about AMD or its risk factors was common knowledge. Patients also may overestimate or have a greater recall of their dietary intake of certain food groups if they have knowledge of the implications to eye disease. The likelihood of recall bias operating in a longitudinal study also is limited. This is particularly so, given that those with early AMD at baseline in whom incident late AMD developed over the follow-up were initially asymptomatic.

In conclusion, in this population-based cohort study, the authors found that higher dietary intake of lutein and zeaxanthin may confer protection against long-term incident neovascular AMD or indistinct soft or reticular drusen and that zinc intake from diet and supplements combined may protect against early or any AMD. A high intake of vegetables also was protective for any AMD. The authors could not confirm the protective effect of the combined high intakes of β -carotene, zinc, and vitamins C and E, reported by the AREDS and the Rotterdam Study. Given the incon-

sistency of dietary findings to date, and the potentially important therapeutic implications, further longitudinal studies and randomized clinical trials, such as AREDS II, are needed to clarify these associations.

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