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Vitamin D Status and Early Age-Related Macular Degeneration in Postmenopausal Women

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Abstract

Objective—The relationship between serum 25-hydroxyvitamin D (25(OH)D) concentrations (nmol/L) and the prevalence of early age-related macular degeneration (AMD) was investigated among participants of the Carotenoids in Age-Related Eye Disease Study.

Methods—Stereoscopic fundus photographs, taken from 2001–2004, assessed AMD status. Baseline (1994–1998) serum samples were available for 25(OH)D assays in 1,313 women with complete ocular and risk factor data. Odds ratios (ORs) and 95% confidence intervals (CIs) for early AMD (n=241), among 1,287 without advanced disease, were estimated with logistic regression and adjusted for age, smoking, iris pigmentation, family history of AMD, cardiovascular disease, diabetes, and hormone therapy use.

Results—In multivariate models, no significant relationship was observed between early AMD and 25(OH)D (OR for quintile 5 vs. 1=0.79, 95% CI=0.50–1.24; p for trend=0.47). A significant age interaction (p=0.0025) suggested selective mortality bias in women ≥75 years: serum 25(OH)D was associated with decreased odds of early AMD in women <75 years (n=968) and increased odds in women ≥75 years (n=319) (OR for quintile 5 vs. 1=0.52, 95% CI=0.29–0.91; p for trend=0.02 and 1.76, 95% CI=0.77–4.13; p for trend=0.05, respectively). Further adjustment for body mass index and recreational physical activity, predictors of 25(OH)D, attenuated the observed association in women <75 years. Additionally, among women <75 years, intake of vitamin D from foods and supplements was related to decreased odds of early AMD in multivariate models; no relationship was observed with self-reported time spent in direct sunlight.

Conclusions—High serum 25(OH)D concentrations may protect against early AMD in women <75 years.

Keywords

vitamin D; 25-hydroxyvitamin D; sunlight; diet; macular degeneration; cohort studies; epidemiology

INTRODUCTION

Age-related macular degeneration (AMD), a chronic, late-onset disease resulting in degeneration of the macula, is the leading cause of adult irreversible vision loss in developed countries (1). AMD affects approximately 9% (8.5 million) of Americans 40 years and older (2). Earlier stages of AMD, which increase the odds for developing advanced disease (3), are the most common, reported to affect 8% of persons 43 to 54 years and 30% among those over 75 years (4). There is no cure for this condition (5). Limited treatment is available to slow its progression, and no established means of prevention exits (5). Therefore, it is important to identify modifiable risk factors that may reduce disease occurrence or prevent progression to advanced stages.

The pathogenesis of AMD is likely to involve a complex interaction of multiple factors, including light damage (6), oxidative stress (7), inflammation (8), possible disturbance in the choroidal blood vessels (9), and genetic predisposition (10). Nonmodifiable genetic risk factors (11,12), especially those associated with inflammatory response, and the modifiable risk factor of smoking (12,13), appear to explain a large percentage of variation in risk for AMD. Recently, a strong protective association between vitamin D status, as reflected by serum levels of 25-hydroxyvitamin D (25(OH)D), and the prevalence of early AMD was

reported in a nationally representative, cross-sectional study (14). Research suggests that vitamin D affects immune modulation and perhaps the prevention of diseases with inflammatory etiologies (15). Currently there is evidence that vitamin D deficiency and insufficiency exists among individuals worldwide; and that the risk of developing many chronic diseases of aging have been shown to be inversely associated with vitamin D status (16).

The purpose of this current study was to investigate whether the previously observed protective association of vitamin D status and AMD could be confirmed in a second study, the Carotenoids in Age-related Eye Disease Study (CAREDS), where 25(OH)D status was assessed six years prior to AMD status. CAREDS is an ancillary study within the Women's Health Initiative Observational Study (WHIOS) which was initiated to investigate relationships of carotenoids in the diet, serum, and retina to AMD (17) and cataract (18). Using CAREDS data, the relationship between individually measured serum 25(OH)D concentrations at WHIOS baseline (1993–1998) and the prevalence of early AMD, assessed on average six years later at CAREDS baseline (2001–2004), was investigated. Additionally, analyses sought to determine whether associations between all sources of vitamin D (sunlight, food, and supplements) and AMD supported associations observed between serum 25(OH)D and AMD.

MATERIALS AND METHODS

The CAREDS Study Sample

The CAREDS population consists of women (50–79 years) who were enrolled in the observational study of the WHI at 3 of 40 sites: the University of Wisconsin (Madison, WI), the University of Iowa (Iowa City, IA), and the Kaiser Center for Health Research (Portland, OR). Participants with baseline WHIOS (1993–1998) intakes of lutein plus zeaxanthin above the 78th and below the 28th percentiles, as assessed at WHIOS baseline (1993–1998), were recruited. Of the 3,143 women who fulfilled these criteria, 96 died or were lost to follow-up between selection year (2000) and enrollment in CAREDS (2001–2004). Those remaining were mailed letters inviting them to participate.

A total of 1,042 women declined participation and 2,005 were enrolled (64%). Of those enrolled, 1,894 participated in study visits. Gradable fundus photographs were obtained for 1,853 participants; an additional 4 participants were included who did not have AMD photographs but had a doctor's confirmation of AMD. One participant was excluded because her lutein data were determined to be unreliable. Sixty-nine participants were further excluded because of missing important AMD risk factor data. Of the remaining 1,787 participants, 474 women had insufficient serum for assays, leaving a sample size for the analysis of 1,313. All procedures conformed to the Declaration of Helsinki and were approved by the Institutional Review Board at each University.

Serum Assays

Serum 25(OH)D is the preferred biomarker for vitamin D status as it reflects vitamin D exposure from both oral sources and sunlight (16). Serum samples were drawn at WHIOS baseline after a ≥10 hour fast and stored at −80°C (19). From 2004–2005, serum lutein and zeaxanthin concentrations were determined at Tufts University, Boston Massachusetts (17), where samples were stored at −70°C and thawed at room temperature. Remaining serum was refrozen at −70°C and remained frozen until the day of vitamin D assay (in fall 2008) at which time they were thawed at room temperature and assayed within 2–3 hours for serum 25(OH)D (nmol/L) using the Diasorin LIAISON® chemiluminescence method. . Previous research shows that blood serum 25(OH)D levels are minimally affected by multiple freeze-

thaw cycles (20) or extended years in storage (21,22). C-reactive protein (CRP) (mg/L) concentrations were assessed using the high sensitivity CRP assay kit (DiaSorin, Stillwater MN) on separate days from the 25(OH)D assessment. CRP has been shown to be stable up to 5 freeze/thaw cycles (23). Both 25(OH)D and CRP assays were conducted by Heartland Assays, Inc. (Ames, Iowa). The coefficient of variation determined using blind duplicates was 8.9% for 25(OH)D and 18.8% for CRP.

As sun exposure and thus 25(OH)D levels vary by season at Northern climates, 25(OH)D concentrations were adjusted for month of blood acquisition. Residuals from local regression of 25(OH)D on month of blood draw, with application of the local regression (Loess) procedure (PROC LOESS in SAS v.9.2, SAS Institute, Cary, NC) (24), were added to the overall population mean (57.31 nmol/L). The Loess method applies a nonparametric curve to smooth the means between adjacent months using weighted polynomial regression. Means for each month determined from the smooth curve are used for the adjustment.

AMD Classification

Prevalent AMD was determined from stereoscopic retinal fundus photographs taken in 2001–2004. Of the 1,857 participants with ocular data, 5% (n=95) self-reported a diagnosis of AMD at WHIOS year 3 follow-up (prior to fundus photography), 94% self-reported no AMD, and 1% (n=24) had missing data. Photographs were graded by the University of Wisconsin Fundus Reading Center using the Age-Related Eye Disease Study protocol for grading maculopathy (25). AMD was classified as the following: any, early, or advanced AMD (at least one eye). There were 241 cases of early AMD among 1,287 women without advanced AMD. Early AMD was further classified as large drusen (≥ 1 large drusen (≥125 μm) or extensive intermediate drusen (area ≥360 μm when soft indistinct drusen are present or an area of ≥650 μm when soft indistinct drusen are absent)) or pigmentary abnormalities (increased or decreased pigmentation accompanied by at least 1 drusen ≥63 μm). Among this sample, 26 women were classified with advanced AMD (the presence of geographic atrophy in the center subfield, or neovascular or exudative macular degeneration). Due to the minimal number of advanced AMD outcomes, these analyses focus on early AMD.

Sources of Vitamin D (Dietary, Supplement, and Sunlight Data)

At WHI baseline, vitamin D intake from foods was estimated from a self-administered food frequency questionnaire (FFQ) (26), to assess usual dietary intake over the previous three months. An interviewer-administered form was used to collect information on the dose, frequency, and duration of current supplement use at WHIOS baseline (27,28). Total vitamin D intake was calculated by summing vitamin D intake from foods and supplements. Using FFQ data, dietary pattern scores were estimated for the 2005 Health Eating Index (HEI 2005), without inclusion of the oil subscore, as previously described (29).

At CAREDS baseline, participants were asked to report their sunlight exposure for each city/town in which they resided from age 18 to their age at CAREDS. Specifically, for each residence they were asked to report the number of daytime hours (<1, 1–3, >3) spent in direct sunlight between 10 am to 4 pm, in the months of April through September, during weekdays and leisure time. They also reported daytime activity on the water for ≥3 hours and whether they used protective gear (hats, sunglasses, and protective lenses). From these data, participants' estimation of reported time spent in direct sunlight at WHIOS baseline, corresponding in time to 25(OH)D assessment, was ascertained, and chronic ocular exposure to visible light over the last 20 years was estimated (30).

STATISTICAL ANALYSES

Logistic regression was used to estimate ORs and 95% CIs for AMD by quintile of serum 25(OH)D adjusting for age. Additional adjustment of the age adjusted model for the following potential confounders or explanatory variables of early AMD was investigated: study site, age, race/ethnicity, smoking pack years, recreational physical activity, body mass index (BMI), and family history of AMD. Only BMI and physical activity changed the ORs by 10% or more. Although both measures of adiposity and physical activity have been reported as risk factors for AMD in the literature (reviewed in (31)), they are also significant determinants of serum 25(OH)D status (32). Addition of BMI and physical activity to the model could potentially over adjust and explain the relationship of vitamin D status to early AMD. For this reason, the ORs were first investigated adjusted for early AMD risk factors identified *a priori* that were not strong determinants of serum vitamin D status: smoking pack years, iris pigmentation, self-reported family history of AMD, cardiovascular disease, diabetes, and hormone therapy use. In a second step, this multivariate model was further adjusted for BMI and physical activity. Next, we adjusted the multivariate model for CRP, a marker for systemic inflammation, to explore whether this association was potentially acting through an inflammatory pathway. As an exploratory analysis, we adjusted the multivariate model for other dietary factors highly correlated with 25(OH)D concentrations and associated with AMD in previous CAREDS analyses: dietary intake of lutein and zeaxanthin (17), dietary intake of polyunsaturated fat (PUFAs) (33), and overall healthy diet, as indicated by the HEI 2005 score (Julie Mares, University of Wisconsin-Madison, unpublished manuscript).

Next, it was investigated whether consistent relationships were observed between early AMD and sources of vitamin D: sunlight exposure and oral intake. The odds of early AMD were estimated among women self-reporting >3 and 1 to 3 compared to <1 hours/day in direct sunlight at WHIOS baseline, and among women in high compared to low quintiles for baseline intake of vitamin D from foods, supplements, and foods and supplements combined.

Effect modification of the associations between serum 25(OH)D status and early AMD by age, BMI, physical activity, lutein plus zeaxanthin intake, a healthy dietary pattern (HEI 2005 score), hormone therapy use, and self-reported family history of AMD was investigated. Effect modification of the association between total vitamin D intake and AMD by sun exposure was also investigated. A p-value <0.10 was considered statistically significant. Analyses were conducted stratified by identified effect modifiers.

All analyses were conducted using SAS® version 9.2; SAS Institute Inc., Cary, NC.

RESULTS

Participant characteristics

Participants with high compared to low vitamin D status, after adjustment for month of blood draw, were more likely to be Non-Hispanic White, have a higher income, consume more alcohol, engage in a higher level of recreational physical activity, report greater ocular visible sun exposure, have a family history of AMD, have a lower BMI, be less hypertensive, and have lower levels of CRP ($p \leq 0.20$) (Table 1). Participants with high vitamin D status were also more likely to have higher calorie consumption, lower intake of fat, greater fiber intake, and greater intake of antioxidant nutrients ($p \leq 0.20$). They consumed a greater number of fruit, milk, and fortified cereal servings, had higher scores on the HEI 2005, and were more likely to use supplements compared to individuals with low vitamin D status (Table 2).

Serum 25(OH)D status and AMD

Table 3 shows the odds of AMD among participants in quintiles 2–5 compare to 1. In models adjusted for age and further adjusted for *a priori* early AMD risk factors (multivariate model), there was no significant relationship between vitamin D status and early or advanced AMD. The same was observed for drusen and pigmentary abnormalities (data not shown). However, the association between early AMD and 25(OH)D level was modified by age (p for interaction=0.0025). ORs for early AMD among participants <75 years were in the opposite direction of ORs for early AMD among women ≥75, suggesting selective mortality bias in the older age group. Subsequently, further analyses were conducted using the sample of individuals <75 years without advanced disease.

In the multivariate model, participants <75 years had a 48% decreased odds of early AMD (OR [95% CI] for quintile 5 vs. 1=0.52 [0.29–0.91]; pfor trend=0.02) (Table 3). In women <75 years, there was a 57% decreased odds of pigmentary abnormalities (OR [95% CI] for quintile 5 vs. 1=0.43 [0.18–0.96]; p for trend=0.02) and the OR for quintile 5 compared to 1 for large drusen was also less than 1.0 but not statistically significant. Further adjustment of these relationships for BMI and physical activity, determinants of 25(OH)D status as well as potential confounders, attenuated these relationships.. Differently, further adjustment of the multivariate model for CRP strengthened relationships.

The inverse association between early AMD and 25(OH)D in women <75 years was not explained by dietary intake of lutein plus zeaxanthin or polyunsaturated fat (PUFAs) (Table 4). After adjustment for HEI 2005 score, the statistically significant relationship between 25(OH)D and AMD was attenuated, although the OR was still <1.0. There was no statistically significant (p<0.10) effect modification of the relationship between 25(OH)D status and early AMD in women <75 years by BMI, physical activity, HEI 2005 score, hormone therapy, or self-reported family history of AMD. However, the relationship between early AMD and 25(OH)D was stronger among women with higher than lower intakes of lutein plus zeaxanthin (adjusted OR (95% CI) for early AMD among women in tertile 3 vs. 1 for serum 25(OH)D: low intake 0.94 (0.50, 1.76), high intake 0.46 (0.22, 0.93); p for interaction=0.04).

Sources of vitamin D (sunlight, diet, and supplements) and early AMD in women <75 years

There was no observed protective effect of reported hours spent in direct sunlight at WHIOS baseline on early AMD, as hypothesized (Table 5). Although oral sources of vitamin D accounted for only a small variation in serum 25(OH)D levels (<10%), a 59% reduced odds of early AMD in quintile 5 compared to 1 for vitamin D from food and supplements combined (associated p for trend=0.15) was observed. A decreased, but not statistically significant, odds of early AMD in high compared to low intake of vitamin D from foods was observed, with a significant p for trend of 0.04. The top food sources of vitamin D in this sample included milk, fish, fortified margarine and fortified cereal. Exploratory analyses revealed no statistically significant effect modification of the relationship between early AMD and total vitamin D intake by sunlight exposure (data not shown).

DISCUSSION

Analyses in the present sample of postmenopausal women confirm a protective association of vitamin D status to the prevalence of AMD, similar to that previously observed in the American population (14). In women <75 years, having 25(OH)D concentrations above 38 nmol/L was significantly associated with a 48% decreased odds of early AMD. This association was consistent across sub-types of early AMD. Attenuation of the multivariate model after adjustment for BMI and physical activity is most likely explained by the strong

correlation between these factors (predictors of vitamin D status) and 25(OH)D concentrations. Adjustment of the multivariate model for intake of lutein plus zeaxanthin, PUFAs, or CRP, a marker of systemic inflammation, did not explain the observed association, but the relationship was attenuated after adjustment for dietary pattern score. Some of the association between vitamin D status and early AMD may be explained by dietary patterns, but may also have resulted in overadjustment of the multivariate model due to multicollinearity between serum 25(OH)D and HEI 2005 score levels. Differently, a marginally statistically significant (p for trend=0.05) direct association between 25(OH)D and early AMD was observed in women ≥75 years.

The observed, significant age interaction is consistent with previous observations in CAREDS. Exposures (macular pigment density (34), lutein and zeaxanthin intake (17), and fat intake (33)) associated with decreased odds of early AMD in younger women were associated with increased odds in older women, suggestive of selective mortality bias (35). We propose, that as people age, a greater proportion of early AMD susceptible, compared to unsusceptible, individuals with low 25(OH)D concentrations die from other chronic diseases prior to developing early AMD (35). Subsequently, a direct association between 25(OH)D and early AMD was observed in the oldest women. One way to avoid the influence of this bias is to examine associations in the youngest age group, as we have done in this investigation.

We observed a possible threshold effect with a 50% decreased odds of early AMD in quintile two compared to 1 for 25(OH)D. The odds of early AMD did not further decrease after 25(OH)D concentrations rose above 38 nmol/L. This is above what is considered severely deficient, <25 nmol/L (36), but not high enough to be considered sufficient by some investigators who suggest levels below 50 nmol/L (16) or even 75–80 nmol/L are deficient (37). A previous cross-sectional analysis (14) observed at least a 25% significant decreased odds of early AMD among persons with 25(OH)D levels >54 nmol/L. It is possible that measured serum vitamin D levels in this paper slightly underestimated the true 25(OH)D concentrations due to degradation in storage, although this has been shown to minimally occur (38). If degradation occurred, it seems likely it would have been fairly uniform across samples and not greatly affected the risk estimate.

We did not observe an association between early AMD and reported time spent outside in direct sunlight, although the majority of circulating vitamin D in most individuals is derived from ultraviolet B (UVB)-induced dermal production of vitamin D (39). Previous relationships between sunlight exposure and AMD have been investigated because chronic sunlight exposure is hypothesized to increase risk (40). Of the observational studies (41–53) investigating this relationship, only a few found direct associations between sunlight and AMD (43,45,50,53). Detrimental effects may be limited to blue light exposure (6,43), which is not measured in all studies. Two studies found that sunlight exposure was related to lower odds for AMD (46,48). In another cohort, AMD was directly associated with leisure time outdoors in summer (45,50,53), not associated with ambient UVB exposure, but (in some instances) inversely associated with UVB exposure after accounting for use of sunglasses and hats with brims (45,50,53). Perhaps some amount of UVB exposure, necessary for dermal vitamin D synthesis, may be protective for AMD when the eyes are also protected from blue light exposure. In CAREDS, measurement error in assessment of sun exposure may have biased the results toward the null; or sunlight's protective effect via vitamin D synthesis and its detrimental effect from blue light ocular damage, negate any findings between AMD and ambient sun exposure.

The inverse association between early AMD and 25(OH)D was supported by analyses of vitamin D intake. A significant decreased odds for early AMD, of a similar magnitude to

that observed with serum 25(OH)D, was observed among persons in quintile 5 (estimated intake of 18 μg/day (720 IU/day)) compared to 1 for total vitamin D intake. This is greater than the Dietary Reference Intakes (DRIs) which recommend 400 IU/day for adults 50–70 years and 600 IU/day for adults >70 years (54).

Inflammation is thought to be involved in the pathogenesis of AMD. Vitamin D, because of its anti-inflammatory, immune modulating properties (55) may suppress the cascade of destructive inflammation that occurs at the level of the RPE-choroid interface in early stages of AMD (8). VDR is expressed on cells of the human immune system and $1,25(OH)_2D$ has been shown to suppress pro-inflammatory cytokines *in vitro* perhaps in part by altering T-cell function toward T-helper 2 (anti-inflammatory) rather than a T-helper 1(pro-inflammatory) response (reviewed in (15,55)). A possible role of vitamin D in ocular functioning is supported by evidence that the vitamin D receptor (VDR) is located in vertebrate retinal tissue (56,57) and is expressed in human cultured retinal endothelial cells (58). Additionally, vitamin D may play a role in preventing AMD progression from early to neovascular, however, we could not assess this in CAREDS. Vitamin D has been shown to inhibit angiogenesis in cultured endothelial cells (59) and within the retina's of animal models of retinoblastoma (60) and oxygen-induced ischemic retinopathy (61).

Although we saw an inverse association between prevalent early AMD and 25(OH)D, assessed 6 years earlier, we cannot firmly establish causality with this study design. Conclusions from this analysis can only be extrapolated to US postmenopausal women and Caucasian women, as CAREDS had limited minority representation and was not a nationally representative sample. Additionally, CAREDS is limited by a lack of measures for genetic risk factors which strongly predict risk for AMD (11).

As previously described (17) selection bias is a potential concern in this study. As eligibility of participation in CAREDS was based on lutein plus zeaxanthin intake in order to maximize dietary diversity, we investigated the associations between AMD and serum 25(OH)D stratified by lutein plus zeaxanthin intake (low and high). Regardless of intake, the relationship between AMD and vitamin D status was inverse, although stronger in those with higher lutein and zeaxanthin intake, suggesting that serum vitamin D status and lutein plus zeaxanthin intake may synergistically be important to eye health.

Thirty-six percent of eligible participants (n=3,143) for CAREDS declined to participate. Those persons <75 years who participated were healthier with respect to dietary and lifestyle factors, and had slightly greater self-reported AMD (3.4 vs. 2.0%) at WHI year 3 follow-up. For this reason, we suspect that non-participation would have biased our results toward the null. We cannot completely dismiss the possibility of selection bias in this study. This association needs to be observed in other longitudinal studies.

This is the second study to present an association between AMD status and 25(OH)D, and our data support the previous observation that vitamin D status may potentially protect against development of AMD. In CAREDS we were able to adjust for the major non-genetic risk factors for AMD, as well as explore relationships between other surrogate measures for vitamin D status, such as oral sources of vitamin D and sun exposure. In conclusion, vitamin D status may significantly affect a woman's odds of early AMD. More studies are needed to verify this association prospectively; as well as to better understand the potential interaction between vitamin D status and genetic and lifestyle factors with respect to risk for early AMD.

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Table 1

Sociodemographic, lifestyle, and health-related characteristics among participants in low and high quintiles for serum 25-hydroxyvitamin D (25(OH)D) (nmol/L), assessed at the Women's Health Initiative Observational Study baseline (1993–1998), adjusted for month of blood draw [†]: the Carotenoids and Age-Related Eye Disease Study (n=1,313).

25(OH)D, nmol/L (median (range))	**Quintile 1** **30 (7, 38)**	**Quintile 5** **85 (75, 165)**	**p-value***
Demographic			
Age at eye photography, years (mean (SE [‡]))	69 (0.4)	69 (0.4)	0.17
Ethnicity (% Non-Hispanic White)	95	98	0.03
Income, ≥ $75,000/year (%)	11	22	<0.01
Study site (%)			0.23
Iowa	32	35	
Oregon	37	27	
Wisconsin	32	38	
Lifestyle			
Smoking pack-years (%)			0.22
Never	55	63	
0–7	21	21	
>7	24	16	
Alcohol, g/week (%)			0.03
Non-drinker	46	36	
0.4 to < 4.0	33	29	
≥4 to < 127	22	35	
Recreational physical activity, MET hrs/wk (%)			<0.01
None – 3	37	17	
3 – 10	26	21	
10 – 21	20	27	
≥21	17	35	
Average ocular visible sun exposure in the last 20 years, Maryland sun-years (mean (SE))	0.77 (0.03)	0.91 (0.03)	<0.01
Ocular and medical factors			
Iris color (% blue)	44	42	0.90
Family history of macular degeneration (% yes)	13	18	0.16
Body mass index, kg/m^2 (%)			<0.01
< 22.5	10	30	
22.5 ≤ to < 25	12	24	
25 ≤ to < 30	36	31	
30 ≤ to < 35	22	13	
35 ≤	21	2	
Hypertension (% yes)	35	28	0.01
Cardiovascular disease (% yes)	27	24	0.67

25(OH)D, nmol/L (median (range))	**Quintile 1** **30 (7, 38)**	**Quintile 5** **85 (75, 165)**	**p-value***
Diabetes (% yes)	3.1	1.2	0.28
Hormone replacement therapy (%)			0.39
Never	37	27	
Past	15	13	
Current	49	60	
C-reactive protein, mg/L (mean (SE))	5.2 (0.3)	4.2 (0.3)	0.03

*P-values are for general associations. For categorical variables, the Cochran-Mantel-Haenszel statistic for a general association is used. For continuous variables, an ANOVA to compare least square means by level of categorical predictor (quintile of serum vitamin D) is used. A p-value for the ANOVA was obtained for the linear trend by replacing the categorical predictor with the continuous variable (serum vitamin D). P-values do not necessarily represent a linear trend for either type of variable. Continuous variables are adjusted for age as a continuous variable and categorical variables are adjusted for age using a variable with 3 categories (≤69; 70–74; ≥75).

†Serum vitamin D values were adjusted for month of blood draw by adding the residuals from a Loess fit to the overall population mean (57.31 nmol/L).

‡SE =Standard Error

Table 2

Energy, nutrient intake and serum nutrient concentrations, assessed at the Women's Health Initiative Observational Study (WHIOS) baseline (1993–1998) among participants in low and high quintiles for serum 25-hydroxyvitamin D (25(OH)D) (nmol/L), assessed at WHIOS baseline, adjusted for month of blood draw [†]: the Carotenoids and Age-Related Eye Disease Study (n=1,313).

25(OH)D, nmol/L (median (range))	**Quintile 1 30 (7, 38)**	**Quintile 5 85 (75, 165)**	**p-value***	**Spearman Correlation**
Total energy, kcals (mean (SE[‡]))	1572 (40)	1706 (39)	0.12	0.06
Total fat, % kcals (mean (SE))	33.9 (0.5)	30.0 (0.5)	<0.01	−0.15
Polyunsaturated, % kcals	6.9 (0.1)	6.0 (0.1)	<0.01	−0.13
Dietary fiber, g/day (mean (SE))	17.0 (0.6)	20.2 (0.6)	<0.01	0.11
Micronutrients (mean (SE))				
Lutein and zeaxanthin from foods [¶]*, mg/day*	1.6 (0.07)	1.8 (0.07)	0.27	0.06
Vitamin C from foods and supplements, mg/day	344 (35)	515 (34)	<0.01	0.16
Vitamin D from foods and supplements, mcg/day	7.9 (0.4)	15.1 (0.4)	<0.01	0.33
Vitamin E from foods and supplements, mg/day	141 (22)	237 (22)	<0.01	0.16
Zinc from foods and supplements, mg/day	16.3 (0.8)	23.9 (0.8)	<0.01	0.22
Fruit intake, servings/day (mean (SE))	1.9 (0.1)	2.4 (0.1)	<0.01	0.12
Vegetable intake, servings/day (mean (SE))	2.2 (0.1)	2.6 (0.1)	0.10	0.07
Milk intake, servings/day (mean (SE))	0.46 (0.03)	0.73 (0.03)	<0.01	0.21
Fortified cereal intake, servings/day (mean (SE))	0.04 (0.01)	0.05 (0.01)	0.07	0.03
Margarine intake, g/day (mean (SE))	6.2 (0.5)	5.5 (0.5)	0.31	−0.01
Fish intake, servings/day (mean (SE))	0.19 (0.01)	0.20 (0.01)	0.49	0.03
Healthy Eating Index 2005 (mean (SE))	63 (0.4)	65 (0.4)	<0.01	0.16
Supplement user (% yes) [§]	60	87	<0.01	

*P-values are for general associations. For categorical variables, the Cochran-Mantel-Haenszel statistic for a general association is used. For continuous variables, an ANOVA to compare least square means by level of categorical predictor (quintile of serum vitamin D) is used. A p-value for the ANOVA was obtained for the linear trend by replacing the categorical predictor with the continuous variable (serum vitamin D). P-values do not necessarily represent a linear trend for either type of variable. Continuous variables are adjusted for age as a continuous variable and categorical variables are adjusted for age using a variable with 3 categories (≤69; 70–74; ≥75).

†Serum vitamin D values were adjusted for month of blood draw by adding the residuals from a loess fit to the overall population mean (57.31 nmol/L).

‡SE =Standard Error

¶Data on lutein and zeaxanthin intake from diet plus supplements is not presented as lutein supplements were not recorded at WHI-baseline.

§Supplement user defined as a user of any of the single or combination nutrient supplements (missing values are considered as non-user for a given supplement).

Table 3

Odds ratios and 95% confidence intervals for age-related macular degeneration (AMD), assessed from 2001–2004, among participants in quintiles 2–5 compared to one for serum 25-hydroxyvitamin D (nmol/L), assessed in 1993–1998: the Carotenoids and Age-Related Eye Disease Study (n=1,313).

	Serum 25-Hydroxyvitamin D (25(OH)D)					
Quintiles of serum 25(OH)D, nmol/L	**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	
Median (range)	**30 (7, 38)**	**44 (>38, 50)**	**56 (>50, 61)**	**67 (>61, 75)**	**85 (>75, 165)**	
Early AMD †						***P* for trend** *
All ages (n=1,287)						
# with AMD/# in quintile	57/251	42/260	49/259	48/257	45/260	
Age-adjusted model ‡	1.0	0.61 (0.38–0.95)	0.79 (0.51–1.23)	0.74 (0.48–1.15)	0.72 (0.46–1.13)	0.28
Multivariate model ¶	1.0	0.63 (0.39–0.99)	0.83 (0.53–1.30)	0.78 (0.50–1.23)	0.79 (0.50–1.24)	0.47
Multivariate model + BMI + PA§	1.0	0.65 (0.40–1.04)	0.89 (0.55–1.41)	0.82 (0.51–1.31)	0.85 (0.52–1.38)	0.71
Multivariate model + CRP ††	1.0	0.61 (0.38–0.97)	0.78 (0.49–1.24)	0.73 (0.46–1.16)	0.74 (0.46–1.19)	0.37
<75 yrs (n=968)						
# with AMD/# in quintile	42/196	23/190	28/199	24/184	22/199	
Age-adjusted model	1.0	0.49 (0.28–0.85)	0.62 (0.36–1.04)	0.57 (0.32–0.98)	0.48 (0.27–0.83)	0.01
Multivariate model	1.0	0.50 (0.28–0.87)	0.66 (0.38–1.12)	0.58 (0.33–1.01)	0.52 (0.29–0.91)	0.02
Multivariate model + BMI + PA	1.0	0.55 (0.30–0.97)	0.76 (0.43–1.33)	0.74 (0.40–1.32)	0.68 (0.37–1.24)	0.19
Multivariate model + CRP	1.0	0.49 (0.27–0.86)	0.58 (0.33–1.01)	0.51 (0.28–0.90)	0.49 (0.27–0.88)	0.01
≥75 yrs (n=319)						
# with AMD/# in quintile	15/55	19/70	21/60	24/73	23/61	
Age-adjusted model	1.0	1.00 (0.45–2.22)	1.43 (0.65–3.21)	1.30 (0.61–2.85)	1.62 (0.74–3.61)	0.08
Multivariate model	1.0	1.10 (0.48–2.57)	1.52 (0.66–3.60)	1.55 (0.69–3.58)	1.76 (0.77–4.13)	0.05
Multivariate model + BMI + PA	1.0	0.84 (0.35–2.04)	1.26 (0.52–3.10)	1.10 (0.47–2.64)	1.28 (0.52–3.17)	0.18
Multivariate model + CRP	1.0	1.05 (0.44–2.54)	1.64 (0.69–4.02)	1.58 (0.69–3.72)	1.62 (0.69–3.95)	0.06
Advanced AMD ‡‡						
All ages (n=1,313)						
# with AMD/# at risk	5/256	5/265	6/265	7/264	3/263	
Age-adjusted model	1.0	0.89 (0.24–3.26)	1.15 (0.34–4.07)	1.25 (0.39–4.32)	0.59 (0.12–2.46)	0.95

* A *P*-value was obtained for the linear trend by replacing the categorical predictor with the continuous variable (serum vitamin D).

[†] Analyses for early AMD do not include women with advanced AMD.

[‡] Worse eye, adjusted for age at photography.

[¶] Multivariate model: Worse eye, adjusted for age at photography, and risk factors for age-related macular degeneration (smoking pack years, iris pigmentation, family history of AMD, cardiovascular disease, diabetes, and hormone use status).

[§] Multivariate model further adjusted for body mass index (BMI) and physical activity (PA).

[††] Multivariate model further adjusted for C-reactive protein (CRP).

[‡‡] There were insufficient cases of AMD to run stable risk estimates for other multivariate models of advanced AMD.

Table 4

Multivariate† model odds ratios and 95% confidence intervals for Early Age-Related Macular Degeneration (AMD), assessed from 2001–2004, among participants (<75 years) in quintiles 2–5 compared to one for serum 25-hydroxyvitamin D, assessed in 1993–1998, further adjusted for potential dietary confounders: the Carotenoids and Age-Related Eye Disease Study (n=968)

	Serum 25-Hydroxyvitamin D (25(OH)D)					*P* for trend *
Quintiles of serum 25(OH)D, nmol/L	**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	
Median (range)	**30 (7, 38)**	**44 (>38, 50)**	**56 (>50, 61)**	**67 (>61, 75)**	**85 (>75, 165)**	
Early AMD						
# with AMD/# in quintile	42/196	23/190	28/199	24/184	22/199	
Multivariate model	1.0	0.50 (0.28–0.87)	0.66 (0.38–1.12)	0.58 (0.33–1.01)	0.52 (0.29–0.91)	0.02
+ *lutein and zeaxanthin intake from foods*	1.0	0.52 (0.29–0.90)	0.67 (0.39–1.14)	0.59 (0.33–1.03)	0.53 (0.30–0.94)	0.02
+ *polyunsaturated fatty acid intake from foods*	1.0	0.51 (0.28–0.88)	0.67 (0.39–1.15)	0.61 (0.34–1.06)	0.55 (0.31–0.97)	0.04
+ *healthy eating index 2005*	1.0	0.53 (0.30–0.93)	0.74 (0.42–1.27)	0.66 (0.36–1.16)	0.57 (0.32–1.01)	0.06

* A *P*-value was obtained for the linear trend by replacing the categorical predictor with the continuous variable (serum vitamin D).

† Model Worse eye, adjusted for age at photography, month of blood draw, and risk factors for age-related macular degeneration (AMD) (smoking pack years, iris pigmentation, family history of AMD, cardiovascular disease, diabetes, and hormone use status).

Table 5

Multivariate † model odds ratios and 95% confidence intervals for early age-related macular degeneration (AMD), assessed from 2001–2004, among participants (<75 years) reporting high compared to low levels of sunlight exposure and consuming high compared to low intake of vitamin D from foods and supplements, assessed in1993–1998: Carotenoids in Age-Related Eye Disease Study CAREDS (n=968).

Time spent in sunlight (hours/day)			**< 1 hours**	**1 to 3 hours**	**>3 hours**	***P* for trend***
# with AMD/# in sunlight category			49/384	75/483	15/101	
Multivariate model ‡			1.0	1.15 (0.78–1.72)	1.15 (0.59–2.15)	0.46
Total vitamin D intake from foods and supplements combined	**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	***P* for trend***
Quintiles, µg/day (median (range))	2.8 (0.4–4.5)	6.5 (>4.5–9.5)	12.2 (>9.5–14)	15.8 (>14–18)	21.4 (>18–61)	
# with AMD/# in quintile	36/211	24/200	37/196	28/177	14/184	
Multivariate model	1.00	0.67 (0.38–1.18)	1.09 (0.65–1.84)	0.90 (0.51–1.56)	0.41 (0.20–0.78)	0.15
Vitamin D intake from supplements, µg/day		**None**	**>0 to <10 µg**	**10 µg**	**>10 µg**	***P* for trend***
# with AMD/# in supplement use category		64/407	17/120	44/301	14/140	
Multivariate model		1.00	0.85 (0.46–1.50)	0.89 (0.58–1.36)	0.59 (0.30–1.09)	0.60
Vitamin D intake from foods	**Quintile 1**	**Quintile 2**	**Quintile 3**	**Quintile 4**	**Quintile 5**	***P* for trend***
Quintiles, µg/day (median (range))	2.0 (0.4–2.7)	3.5 (>2.7–4.2)	5.0 (>4.2–5.8)	7.0 (>5.8–8.6)	10.6 (>8.6–30.4)	
# AMD/# in quintile	30/206	34/198	35/201	21/189	19/174	
Multivariate model	1.00	1.20 (0.69–2.08)	1.24 (0.72–2.16)	0.75 (0.41–1.37)	0.74 (0.39–1.38)	0.04

*A P-value was obtained for the linear trend by replacing the categorical predictor with the continuous variable.

†Worse eye, adjusted for age at photography, and risk factors for age-related macular degeneration (smoking pack years, iris pigmentation, family history of AMD, cardiovascular disease, diabetes, and hormone use status).