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

Amy E. Millen, PhD; Rick Voland, PhD; Sherie A. Sondel, MS; Niyati Parekh, PhD; Ronald L. Horst, PhD; Robert B. Wallace, MD; Gregory S. Hageman, PhD; Rick Chappell, PhD; Barbara A. Blodi, MD; Michael L. Klein, MD; Karen M. Gehrs, MD; Gloria E. Sarto, MD, PhD; Julie A. Mares, PhD; for the CAREDS Study Group

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 in participants of the Carotenoids in Age-Related Eye Disease Study.

Methods: Stereoscopic fundus photographs, taken from 2001 to 2004, assessed AMD status. Baseline (1994-1998) serum samples were available for 25(OH)D assays in 1313 women with complete ocular and risk factor data. Odds ratios (ORs) and 95% confidence intervals (CIs) for early AMD (n=241) of 1287 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=.47). A significant age interaction (*P*=.002) sug- gested selective mortality bias in women aged 75 years and older: serum 25(OH)D was associated with decreased odds of early AMD in women younger than 75 years (n=968) and increased odds in women aged 75 years or older (n=319) (OR for quintile 5 vs 1, 0.52; 95% CI, 0.29-0.91; *P* for trend=.02 and OR, 1.76; 95% CI, 0.77-4.13; *P* for trend=.05, respectively). Further adjustment for body mass index and recreational physical activity, predictors of 25(OH)D, attenuated the observed association in women younger than 75 years. Additionally, among women younger than 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 younger than 75 years.

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AGE-RELATED MACULAR DE-generation (AMD), a chronic, late-onset disease that results in degeneration of the macula, is the leading cause of adult irreversible vision loss in developed countries.[1] Age-related macular degeneration affects approximately 9% (8.5 million) of Americans aged 40 years and older.[2] Earlier stages of AMD, which increase the odds of developing advanced disease,[3] are the most common, affecting 8% of persons aged 43 to 54 years and 30% of those older than 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 fac- tors 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 the variation in risk of AMD. Recently, a strong protective association between vitamin D status, as reflected by serum concentrations 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 exist in 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]

Author Affiliations are listed at the end of this article.

The purpose of this 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), in which 25(OH)D concentrations were assessed 6 years prior to AMD status. The CAREDS study 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 an average of 6 years later at the 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.

METHODS

THE CAREDS STUDY SAMPLE

The CAREDS population consists of women (ages, 50-79 years) who were enrolled in the observational study of the Women's Health Initiative at 3 of 40 sites: the University of Wisconsin, Madison, the University of Iowa, Iowa City, and the Kaiser Center for Health Research, Portland, Oregon. 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 3143 women who fulfilled these criteria, 96 died or were lost to follow-up between the selection year (2000) and enrollment in CAREDS (2001-2004). Those who remained were mailed letters inviting them to participate.

A total of 1042 women declined participation, and 2005 were enrolled (64%). Of those enrolled, 1894 participated in study visits. Gradable fundus photographs were obtained for 1853 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 1787 participants, 474 women had insufficient serum for assays, leaving a sample size for the analysis of 1313. 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 fast of 10 or more hours and stored at −80°C.[19] From 2004 to 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. The 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 to 3 hours for serum 25(OH)D concentration (nmol/L) using the LIAISON (DiaSorin, Stillwater, Minnesota) chemiluminescence method. Previous research shows that blood 25(OH)D concentrations 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) on separate days from the 25(OH)D assessment. C-reactive protein has been shown to remain stable for 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 concentrations, 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, North Carolina),[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 from 2001 through 2004. Of the 1857 participants with ocular data, 5% (n=95) self-reported a diagnosis of AMD at the 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] Presence of AMD was classified as any, early, or advanced AMD (at least 1 eye). There were 241 cases of early AMD in 1287 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 ≥650 µm when soft indistinct drusen are absent]) or pigmentary abnormalities (increased or decreased pigmentation accompanied by at least 1 drusen ≥63 µm). Of this sample, 26 women were classified as having advanced AMD (the presence of geographic atrophy in the center subfield or neovascular or exudative macular degeneration). Owing to the minimal number of advanced AMD outcomes, these analyses focus on early AMD.

SOURCES OF VITAMIN D (DIETARY, SUPPLEMENT, AND SUNLIGHT DATA)

At Women's Health Initiative baseline, vitamin D intake from foods was estimated from a self-administered food frequency questionnaire,[26] to assess usual dietary intake during the previous 3 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 food frequency questionnaire 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 the CAREDS baseline visit, participants were asked to report their sunlight exposure for each city/town in which they resided from 18 years of age 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 or more 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 in the last 20 years was estimated.[30]

STATISTICAL ANALYSIS

Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (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 of early AMD was investigated: study site, age, race/ethnicity, smoking pack years, recreational physical activity, body mass index (BMI; calculated as weight in kilograms divided by height in meters squared), 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,[31] they are also significant determinants of serum 25(OH)D status.[32] Addition of BMI and physical activity to the model could potentially overadjust 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,[33] and overall healthy diet, as indicated by the HEI 2005 score.[34]

Next, it was investigated whether consistent relationships were observed between early AMD and 2 sources of vitamin D: sunlight exposure and oral intake. The odds of early AMD were estimated among women self-reporting more than 3 and 1 to 3 compared with less than 1 h/d in direct sunlight at WHIOS baseline, and among women in high compared with low quintiles for baseline intake of vitamin D from foods, supplements, and foods and supplements combined.

The 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. $P < .10$ was considered statistically significant. Analyses were conducted stratified by identified effect modifiers.

All analyses were conducted using SAS version 9.2.

RESULTS

PARTICIPANT CHARACTERISTICS

Participants with high compared with low vitamin D status after adjustment for month of blood draw were more likely to be 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 concentrations of CRP (**Table 1**). Participants with high vitamin D status were also more likely to have higher calorie consumption, lower

Table 1. Characteristics of Participants in Low and High Quintiles for 25(OH)D, Assessed at Baseline, Adjusted for Month of Blood Draw[a]

Characteristic (n=1313)	Patients, %		P Value[b]
	Quintile 1	Quintile 5	
25(OH)D concentration, nmol/L, median (range)	30 (7-38)	85 (75-165)	
Demographic			
Age at eye photography, mean (SE), y	69 (0.4)	69 (0.4)	.17
Ethnicity (white)	95	98	.03
Income, ≥75,000/y	11	22	.005
Study site			.23
Iowa	32	35	
Oregon	37	27	
Wisconsin	32	38	
Lifestyle			
Smoking, pack-years			.22
Never	55	63	
0-7	21	21	
>7	24	16	
Alcohol, g/wk			.03
Nondrinker	46	36	
0.4 to <4.0	33	29	
≥4 to <127	22	35	
Recreational physical activity, MET, h/wk			<.001
0-3	37	17	
3-10	26	21	
10-21	20	27	
≥21	17	35	
Average ocular visible sun exposure in the last 20 y, Maryland sun-years, mean (SE)	0.77 (0.03)	0.91 (0.03)	<.001
Ocular and medical factors			
Iris color blue	44	42	.90
Family history of macular degeneration	13	18	.16
Body mass index[c]			<.001
<22.5	10	30	
≤22.5 to <25	12	24	
≤25 to <30	36	31	
≤30 to <35	22	13	
≤35	21	2	
Hypertension	35	28	.01
Cardiovascular disease	27	24	.67
Diabetes	3.1	1.2	.28
Hormone replacement therapy			.39
Never	37	27	
Past	15	13	
Current	49	60	
CRP concentration, mg/L, mean (SE)	5.2 (0.3)	4.2 (0.3)	.03

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; MET, metabolic equivalent; SE, standard error.

[a] 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).

[b] P values are for general associations. For categorical variables, the Cochran-Mantel-Haenszel statistic for a general association is used. For continuous variables, an analysis of variance to compare least square means by level of categorical predictor (quintile of serum vitamin D) is used. A P value for the analysis of variance 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).

[c] Calculated as weight in kilograms divided by height in meters squared.

Table 2. Energy, Nutrient Intake, and Serum Nutrient Concentrations, Assessed at Baseline in Participants in the Low and High Quintiles of 25(OH)D, Adjusted for Month of Blood Draw[a]

| Variable (n=1313) | Mean (SD) | | P Value[b] | Spearman Correlation |
	Quintile 1	Quintile 5		
25(OH)D concentration, nmol/L, median (range)	30 (7-38)	85 (75-165)		
Total energy, kcal	1572 (40)	1706 (39)	.12	0.06
Total fat, kcal, %	33.9 (0.5)	30.0 (0.5)	<.001	−0.15
Polyunsaturated, kcal, %	6.9 (0.1)	6.0 (0.1)	<.001	−0.13
Dietary fiber, g/d	17.0 (0.6)	20.2 (0.6)	.004	0.11
Micronutrients, mg/d				
Lutein and zeaxanthin from food[c]	1.6 (0.07)	1.8 (0.07)	.27	0.06
Vitamin C from foods and supplements	344 (35)	515 (34)	<.001	0.16
Vitamin D from foods and supplements, µg/d	7.9 (0.4)	15.1 (0.4)	<.001	0.33
Vitamin E from foods and supplements	141 (22)	237 (22)	<.001	0.16
Zinc from foods and supplements	16.3 (0.8)	23.9 (0.8)	<.001	0.22
Fruit intake, servings/d	1.9 (0.1)	2.4 (0.1)	<.001	0.12
Vegetable intake, servings/d	2.2 (0.1)	2.6 (0.1)	.10	0.07
Milk intake, servings/d	0.46 (0.03)	0.73 (0.03)	<.001	0.21
Fortified cereal intake, servings/d	0.04 (0.01)	0.05 (0.01)	.07	0.03
Margarine intake, g/d	6.2 (0.5)	5.5 (0.5)	.31	−0.01
Fish intake, servings/d	0.19 (0.01)	0.20 (0.01)	.49	0.03
Healthy Eating Index 2005 score	63 (0.4)	65 (0.4)	<.001	0.16
Supplement user (yes), %[d]	60	87	<.001	. . .

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; ellipses, no spearman correlation computed for the relationship between supplement user (yes/no) and serum 25(OH)D concentrations, as supplement users was a categorical variable; SE, standard error.

[a]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).

[b]P values are for general associations. For categorical variables, the Cochran-Mantel-Haenszel statistic for a general association is used. For continuous variables, an analysis of variance to compare least square means by level of categorical predictor (quintile of serum vitamin D) is used. A P value for the analysis of variance 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 ($\leq$69; 70-74; $\geq$75).

[c]Data on lutein and zeaxanthin intake from diet plus supplements is not presented, as lutein supplements were not recorded at baseline of the Women's Health Initiative.

[d]Supplement user defined as a user of any of the single or combination nutrient supplements (missing values are considered as nonuser for a given supplement).

intake of fat, greater fiber intake, and greater intake of antioxidant nutrients ($P \leq .20$). They consumed a greater amount of fruit, milk, and fortified cereal servings, had higher scores on the HEI 2005, and were more likely to use supplements compared with individuals with low vitamin D status (**Table 2**).

SERUM 25(OH)D STATUS AND AMD

Table 3 shows the odds of having AMD among participants in quintiles 2 through 5 compared with 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 concentration was modified by age (P for interaction=.002). The ORs for early AMD among participants younger than 75 years were in the opposite direction of ORs for early AMD among women aged 75 or older, suggesting selective mortality bias in the older age group. Subsequently, further analyses were conducted using the sample of individuals younger than 75 years without advanced disease.

In the multivariate model, participants younger than 75 years had 48% decreased odds of early AMD (OR for quintile 5 vs 1, 0.52; 95% CI, 0.29-0.91; P for trend=.02)

(Table 3). In women younger than 75 years, there were 57% decreased odds of pigmentary abnormalities (OR for quintile 5 vs 1, 0.43; 95% CI, 0.18-0.96; P for trend=.02) and the OR for quintile 5 compared with 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. Further adjustment of the multivariate model for CRP strengthened relationships.

The inverse association between early AMD and 25(OH)D in women younger than 75 years was not explained by dietary intake of lutein plus zeaxanthin or polyunsaturated fat (**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 less than 1.0. There was no statistically significant ($P < .10$) effect modification of the relationship between 25(OH)D status and early AMD in women younger than 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 for early AMD among women in tertile 3 vs 1 for serum 25(OH)D: low intake, 0.94; 95% CI, 0.50, 1.76; high intake, 0.46; 95% CI, 0.22, 0.93; P for interaction=.04).

Table 3. Odds Ratios for AMD, Assessed From 2001-2004 Among Participants in Quintiles 2 Through 5 Compared With 1 for 25(OH)D, Assessed in 1993-1998

| Variable | OR (95% CI) by Quintile | | | | | P Value for Trend[a] |
	1	2	3	4	5	
Serum 25(OH)D concentration, nmol/L, median (range)	30 (7-38)	44 (>38-50)	56 (>50-61)	67 (>61-75)	85 (>75-165)	
Early AMD[b]						
All ages (n=1287)						
Patients with AMD/patients in quintile, No.	57/251	42/260	49/259	48/257	45/260	
Age-adjusted model[c]	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)	.28
Multivariate model[d]	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)	.47
Multivariate model + BMI + PA[e]	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)	.71
Multivariate model + CRP[f]	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)	.37
<75 y (n=968)						
Patients with AMD/patients in quintile, No.	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)	.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)	.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)	.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)	.01
≥75 y (n=319)						
Patients with AMD/patients in quintile, No.	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)	.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)	.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)	.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)	.06
Advanced AMD[g]						
All ages (n=1313)						
Patients with AMD/patients at risk, No.	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)	.95

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AMD, age-related macular degeneration; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CI, confidence interval; CRP, concentration of C-reactive protein; PA, physical activity; OR, odds ratio.
[a] A P value was obtained for the linear trend by replacing the categorical predictor with the continuous variable (serum vitamin D).
[b] Analyses for early AMD do not include women with advanced AMD.
[c] Worse eye, adjusted for age at photography.
[d] 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).
[e] Multivariate model further adjusted for BMI and PA.
[f] Multivariate model further adjusted for CRP.
[g] There were insufficient cases of AMD to run stable risk estimates for other multivariate models of advanced AMD.

Table 4. Multivariate[a] Model Odds Ratios for Early AMD, Assessed From 2001-2004, of Participants Younger Than 75 Years in Quintiles 2-5 Compared With 1 for Serum 25(OH)D, Assessed in 1993-1998, Further Adjusted for Potential Dietary Confounders

| Variable | OR (95% CI) by Quintile | | | | | P Value for Trend[b] |
	1	2	3	4	5	
25(OH)D, nmol/L, median (range)	30 (7-38)	44 (>38-50)	56 (>50-61)	67 (>61-75)	85 (>75-165)	
Patients with AMD/patients in quintile, No.	42/196	23/190	28/199	24/184	22/199	
Multivariate model	1.0 [Reference]	0.50 (0.28-0.87)	0.66 (0.38-1.12)	0.58 (0.33-1.01)	0.52 (0.29-0.91)	.02
+ Lutein and zeaxanthin intake from foods	1.0 [Reference]	0.52 (0.29-0.90)	0.67 (0.39-1.14)	0.59 (0.33-1.03)	0.53 (0.30-0.94)	.02
+ Polyunsaturated fatty acid intake from foods	1.0 [Reference]	0.51 (0.28-0.88)	0.67 (0.39-1.15)	0.61 (0.34-1.06)	0.55 (0.31-0.97)	.04
+ Healthy eating index 2005	1.0 [Reference]	0.53 (0.30-0.93)	0.74 (0.42-1.27)	0.66 (0.36-1.16)	0.57 (0.32-1.01)	.06

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.
[a] Model worse eye, adjusted for age at photography, month of blood draw, and risk factors for AMD (smoking pack years, iris pigmentation, family history of AMD, cardiovascular disease, diabetes, and hormone use status).
[b] A P value was obtained for the linear trend by replacing the categorical predictor with the continuous variable (serum vitamin D).

SOURCES OF VITAMIN D AND EARLY AMD IN WOMEN YOUNGER THAN 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 concentrations (<10%), a 59% reduced odds of early AMD in quintile 5 compared with 1 for vitamin D from food and supplements combined (associated P for trend = .15) was observed. Decreased, but not statistically significant, odds of early AMD in high compared

Table 5. Multivariate Model Odds Ratios for Early AMD, Assessed From 2001-2004, of Participants Younger Than 75 Years Who Reported High vs Low Levels of Sunlight Exposure and High vs Low Intake of Vitamin D From Foods and Supplements, Assessed in 1993-1998

Variable	OR (95% CI) by Measures					P Value for Trend[a]
Time Spent in Sunlight						
	h/d					
	<1	**1-3**	**>3**			
Patients with AMD/patients in sunlight, No.	49/384	75/483	15/101			
Multivariate model[b]	1.0 [Reference]	1.15 (0.78-1.72)	1.15 (0.59-2.15)			.46
Vitamin D From Supplements						
	µg/d					
	0	**>0 to <10**	**10**	**>10**		
Patients with AMD/patients in supplement use category, No.	64/407	17/120	44/301	14/140		
Multivariate model	1.00 [Reference]	0.85 (0.46-1.50)	0.89 (0.58-1.36)	0.59 (0.30-1.09)		.60
Vitamin D From Food						
Quintile						
	1	**2**	**3**	**4**	**5**	
Intake, µg/d, 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)	
Patients with AMD/patients in quintile, No.	30/206	34/198	35/201	21/189	19/174	
Multivariate model	1.00 [Reference]	1.20 (0.69-2.08)	1.24 (0.72-2.16)	0.75 (0.41-1.37)	0.74 (0.39-1.38)	.04
Vitamin D From Food and Supplements						
Quintile						
	1	**2**	**3**	**4**	**5**	
Intake, µg/d, 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)	
Patients with AMD/patients in quintile, No.	36/211	24/200	37/196	28/177	14/184	
Multivariate model	1.00 [Reference]	0.67 (0.38-1.18)	1.09 (0.65-1.84)	0.90 (0.51-1.56)	0.41 (0.20-0.78)	.15

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; OR, odds ratio.
[a] A P value was obtained for the linear trend by replacing the categorical predictor with the continuous variable.
[b] Worse eye, adjusted for age at photography, and risk factors for AMD (smoking pack years, iris pigmentation, family history of AMD, cardiovascular disease, diabetes, and hormone use status).

with low intake of vitamin D from foods was observed, with a significant P for trend of .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).

COMMENT

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 younger than 75 years, having 25(OH)D concentrations higher than 38 nmol/L was significantly associated with a 48% decreased odds of early AMD. This association was consistent across subtypes 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, polyunsaturated fat, 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 owing to multicolinearity between serum 25(OH)D and HEI 2005 score levels. A marginally statistically significant (P for trend=.05) direct association between 25 (OH)D and early AMD was observed in women aged 75 years or older.

The observed significant age interaction is consistent with previous observations in CAREDS. Exposures (macular pigment density,[35] 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, suggesting selective mortality bias.[36] We propose that, as people age, a greater proportion of those susceptible to early AMD than unsusceptible individuals with low 25(OH)D concentrations die of other chronic diseases prior to developing early AMD.[36] 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 50% decreased odds of early AMD in quintile 2 compared with 1 for 25(OH)D. The odds of early AMD did not further decrease after 25(OH)D concentrations rose higher than 38 nmol/L. This is higher than what is considered severely deficient, less than 25 nmol/L,[37] but not high enough to be considered sufficient by some investigators who suggest that concentrations lower than 50 nmol/L[16] or even 75 to 80 nmol/L are deficient.[38] A previous cross-sectional analysis[14] observed at least a 25% significant decreased odds of early AMD among persons with 25(OH)D concentrations of 54 nmol/L or higher. It is possible that measured serum 25(OH)D concentrations in this article slightly underestimated the true 25(OH)D concentrations due to degradation in storage, although this has been shown to minimally occur.[22] If degradation occurred, it seems likely that 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 most circulating vitamin D in most individuals is derived from 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 of developing 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, and (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 the 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. Significantly decreased odds of developing early AMD of similar magnitude to that observed with serum 25(OH)D was observed among persons in quintile 5 (estimated intake of 18 µg/d [720 IU/d]) compared with 1 for total vitamin D intake. This is greater than the Dietary Reference Intakes, which recommend 600 IU/d.[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 retinal pigment epithelium–choroid interface in early stages of AMD.[8] The vitamin D receptor is expressed on cells of the human immune system and 1,25-dihydroxyvitamin D has been shown to suppress proinflammatory cytokines in vitro, perhaps in part by altering T-cell function toward a T-helper 2 (anti-inflammatory) rather than a T-helper 1 (proinflammatory) response.[15,55]

A possible role of vitamin D in ocular functioning is supported by evidence that the vitamin D receptor 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 AMD; however, we could not assess this in CAREDS. Vitamin D has been shown to inhibit angiogenesis in cultured endothelial cells[59] and within the retinas 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 white 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 of 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, 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 participants who were eligible (n = 3143) for CAREDS declined to participate. Those persons younger than 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 the Women's Health Initiative year 3 follow-up. For this reason, we suspect that nonparticipation 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 nongenetic 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 developing 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 of early AMD.

Author Affiliations: Department of Social and Preventive Medicine, School of Public Health and Health Professions, University at Buffalo, Buffalo, New York (Dr Millen); Department of Ophthalmology and Visual Sciences (Drs Voland and Mares and Ms Sondel), Depart-

ment of Biostatistics and Medical Informatics, School of Medicine and Public Health, Clinical Science Center (Dr Chappell), and Department of Obstetrics and Gynecology, School of Medicine and Public Health (Dr Sarto), University of Wisconsin, Madison; Department of Nutrition, Food Studies and Public Health, New York University, New York (Dr Parekh); Department of Animal Science, Iowa State University, Ames (Dr Horst); Department of Epidemiology, General Hospital Office, University of Iowa, Iowa City (Dr Wallace); Translational Research Institute, John A. Moran Eye Center, University of Utah, Salt Lake City (Dr Hageman); Fundus Photograph Reading Center, Madison, Wisconsin (Dr Blodi); Department of Ophthalmology, Oregon Health and Science University/Casey Eye Institute, Portland (Dr Klein); and the Center for Retina and Macular Disease, Winter Haven, Florida (Dr Gehrs).

Correspondence: Amy E. Millen, PhD, Department of Social and Preventive Medicine, University at Buffalo, Ferber Hall 240, Buffalo, NY 14214 (aemillen@buffal.edu).

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