Association Between Vitamin D and Age-Related Macular Degeneration in the Third National Health and Nutrition Examination Survey, 1988 Through 1994

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Objective: To evaluate the associations between levels of vitamin D (25-hydroxyvitamin D) in serum and prevalent age-related macular degeneration (AMD).

Methods and Design: Cross-sectional associations of serum vitamin D and early and advanced AMD, assessed from nonmydriatic fundus photographs, were evaluated in the third National Health and Nutrition Examination Survey, a multistage nationally representative probability sample of noninstitutionalized individuals (N=7752; 11% with AMD).

Results: Levels of serum vitamin D were inversely associated with early AMD but not advanced AMD. The odds ratio (OR) and 95% confidence interval (CI) for early AMD among participants in the highest vs lowest quintile of serum vitamin D was 0.64 (95% CI, 0.5-0.8; *P* trend <.001). Exploratory analyses were conducted to evaluate associations with important food and supplemental sources of vitamin D. Milk intake was inversely associated with early AMD (OR, 0.75; 95% CI, 0.6-0.9). Fish intake was inversely associated with advanced AMD (OR, 0.41; 95% CI, 0.2-0.9). Consistent use vs nonuse of vitamin D from supplements was inversely associated with early AMD only in individuals who did not consume milk daily (early AMD: OR, 0.67; 95% CI, 0.5-0.9).

Conclusion: This study provides evidence that vitamin D may protect against AMD. Additional studies are needed to confirm these findings.

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AGE-RELATED MACULAR DEgeneration (AMD), a progressive degenerative condition of the retina, is the leading cause of legal blindness among older Americans. In the United States, 7 million individuals older than 40 years are diagnosed with early AMD and 1.75 million have advanced stages.[1] With increasing longevity, and with the projected doubling of the population aged 65 years and older by 2020, almost 3 million people will have AMD[1] unless risk for the condition is lowered with changes in diet, lifestyle, and medical treatments. High-dose antioxidants have been shown to slow progression from intermediate to late AMD,[2] but long-term benefits and risks are unknown. Slowing the onset in earlier stages may further alleviate the economic burden of health care costs.

Several potential risk factors for the development and progression of AMD have been identified. The most consistent risk factors include age,[3] cigarette smoking,[4] hypertension,[3] and family history of the disease.[5] Other potential risk factors associated less consistently in previous studies include cardiovascular disease,[6] sunlight exposure,[3,7] and diets low in lutein and zeaxanthin[8,9] or other dietary antioxidants[9,10] or diets high in fats.[11-14] Recently, inflammation has received attention as a potential risk factor for this disease.[15-19] Immune components, including immunoglobulins, complement factors, and fibrinogen, have been observed to be entrapped within drusen.[15,20] A major proportion of AMD cases have been identified in several independent cohorts to specific polymorphisms in the complement regulatory gene *CFH* (recently reviewed[21,22]) and implicate local inflammation and activation of the complement cascade, a mechanism in host immunologic defense in the development of drusen.[23] The inflammatory na-

ture of AMD pathogenesis is also supported by subsequently reported associations of AMD with other gene loci involved with the alternative complement pathway (Factor B) or in regions of genes suspected to be involved in cellular immunity (LOC 387715 and PLEKHA1) (as reviewed[22]) and by enhanced AMD risk among persons with markers of chronic or acute inflammation and a history of smoking, which enhances inflammation.[24] A number of studies suggest an anti-inflammatory role for vitamin D in vitro and in vivo.[25-28] There is also evidence that it reduces the proliferation of cells of the immune system.[25-28] Evidence suggests that an inverse relationship exists between vitamin D and several chronic conditions associated with inflammation.[29-32] Since histological studies confirm immune involvement in drusen biogenesis,[18,33] it is possible that vitamin D may protect against AMD by virtue of its anti-inflammatory properties.

The primary purpose of this research was to examine the relationship between serum vitamin D level and prevalent AMD using the third National Health and Nutrition Examination Survey (NHANES III), 1988 through 1994. We hypothesized that participants in the highest quintile compared with the lowest quintile of serum vitamin D level would have decreased prevalent AMD. We also explored the relationships between the consumption of specific food and supplemental sources of vitamin D and the prevalence of AMD to ascertain whether they were consistent with the associations with serum vitamin D level.

METHODS

STUDY POPULATION

The NHANES III, conducted by the Centers for Disease Control and Prevention, is a nationally representative stratified probability sample of the noninstitutionalized civilian population in the United States. Data were collected in 2 phases over a 6-year period between 1988 and 1994. Oversampling of non-Hispanic black individuals, Mexican American individuals, and adults aged 60 years and older was done to allow more accurate estimates for these individual subgroups. Details of sampling strategy have been described elsewhere.[34]

Of the targeted 14 464 participants aged 40 years and older who were eligible for the survey, 11 448 persons (79%) were interviewed. Participants with ungradeable or missing fundus photographs or missing AMD data (n=3240); missing serum vitamin D data (n=271); and missing serum cotinine levels (n=185), a biomarker for smoking status; were excluded for statistical analyses. The final analyses included 7752 individuals (53% of targeted sample) and consisted of non-Hispanic white (n=3889), non-Hispanic black (n=1820) and Mexican American individuals (n=1742) and people of other races and ethnicities (all Hispanics who were not Mexican American and all non-Hispanic individuals from racial groups other than white or black) (n=301).

DEMOGRAPHIC, DIETARY, SUPPLEMENT USE, AND OTHER COVARIATE DATA

A medical examination that included blood and urine collection and fundus photography was performed.[34] In the same visit, in-person interviews were conducted to obtain demographic, socioeconomic, health, supplement use, and dietary history data via both food frequency questionnaires and 24-hour dietary recalls. Race and ethnicity were self-reported by the participants.

Intake of vitamin D and other micronutrients was estimated from the 24-hour diet recall interview using food composition data from the National Coordinating Center database. Milk and fish intake was computed from responses to the food frequency questionnaire. The nonquantitative 60-item food frequency questionnaire queried intake of foods in the past 1-month period prior to the interview.[35] Collection of other covariate data such as smoking and alcohol consumption were determined from the interviews and medical examinations.

COMPARISON OF PARTICIPANTS INCLUDED IN VS EXCLUDED FROM THE ANALYSES

On comparing characteristics of the participants who were included in (n=7752) with those excluded from (n=3696) these analyses, it was observed that those included differed from those excluded. Included participants were younger (aged 56 vs 65 years; $P<.001$), had a higher intake of dietary zinc (11 vs 10 mg/d; $P<.001$) and vitamin E (9 vs 8 mg/d; $P<.001$), had a lower prevalence of hypertension (47% vs 52%; $P<.001$), and smoked less (19% vs 23%; $P<.001$) compared with excluded participants.

SERUM DATA

Approximately 100 mL of whole blood was collected in evacuated containers during the medical examination held in mobile examination centers. Serum specimens were immediately frozen at −70°C and were subsequently used to estimate serum vitamin D levels within 2 weeks of collection as previously detailed.[36] The NHANES III estimated serum 25-hydroxyvitamin D, the predominant form of circulating vitamin D in humans,[37] using the Incstar 25(OH)D assay (now DiaSorin Inc, Stillwater, Minn) based on a radioimmunoassay method. The mean value obtained for serum vitamin D using this assay was 23.04 ng/mL (57.5 nmol/L) with a 2 SD range of 9.01 to 37.66 ng/mL (22.5-94 nmol/L).[38] Serum cotinine levels were analyzed using the competitive enzyme immunoassay method. Serum C-reactive protein was quantified by latex enhanced nephelometry. Details of these procedures are described in the NHANES III manual of laboratory protocols.[39]

FUNDUS PHOTOGRAPHY AND GRADING

Nonmydriatic fundus photographs were taken in one eye during study visits as previously described.[40] Characteristics of early AMD, large drusen and pigmentary abnormalities, and advanced AMD were identified by the University of Wisconsin Age-Related Maculopathy Grading Center.[41,42] Soft drusen were defined as the presence of 1 or more drusen larger than 63 µm in diameter. Pigmentary abnormalities were defined as retinal pigment epithelial depigmentation or the presence of increased retinal pigment (presence of gray or black pigment clumps in or beneath the retina) and were graded as present or absent. Overall early AMD was defined as either the presence of soft drusen (grid area of >375 µm) or any type of drusen with pigmentary abnormalities in the absence of advanced AMD. Advanced AMD was defined as the presence of exudative macular degeneration (detachment of the neurosensory retina and/or retinal pigment epithelium, subretinal hemorrhage, retinal scarring) or geographic atrophy visualized as distinct areas with retinal pigment epithelial cells absent and areas with choroidal vessels more visible than in surrounding areas of the retina, after exclusion of mimicking retinal disorders.

STATISTICAL ANALYSES

Logistic regression analyses were performed and odds ratios (ORs) for AMD (early and advanced) were computed to ex-

amine the associations between prevalent AMD and quintile of serum vitamin D level. Odds ratios for AMD and 95% confidence intervals, adjusted for age only, were generated for overall early AMD, drusen, pigmentary abnormalities, and advanced AMD in quintiles 2 through 5 compared with quintile 1, the lowest level of serum vitamin D.

The distribution of possible risk factors for AMD were investigated by quintile of serum vitamin D level. Potential confounders tested in the model were age (continuous in years); body mass index (continuous; calculated as weight in kilograms divided by height in meters squared); cardiovascular disease (dichotomous; reported as personal history of stroke, heart attack, or angina); hypertension (dichotomous; defined as blood pressure >140/90 mm Hg or current antihypertensive medications); diabetes mellitus (dichotomous; excluding gestational diabetes); serum cotinine level (continuous in ng/mL); alcohol consumption (continuous in g/d); C-reactive protein level (continuous in mg/dL); fibrinogen level (continuous in g/L); and levels of dietary lutein and zeaxanthin, zinc, and vitamin E (continuous in mg/d). Confounders were defined as variables that changed the crude, age-adjusted ORs for AMD and serum vitamin D level by 10% or more when entered singly into the logistic regression model. The identified confounders of the relationship of AMD and serum vitamin D level were added to the final logistic regression models.

Blood vitamin D levels vary by race due to differing capacities to produce vitamin D.[43] For this reason and because this sample was enriched with non-Hispanic black and Mexican American individuals who differ from non-Hispanic white individuals in age and response rates, we explored the associations of serum vitamin D level and early AMD by the 3 major race groups represented by this sample: non-Hispanic white, non-Hispanic black, and Mexican American. The NHANES III sample weights were applied in all the analyses to account for individual selection probabilities, nonresponse, and poststratification that resulted from the complex survey design. We used the jackknife replication method to obtain appropriate variance estimates in regression analyses to account for clustering, which resulted from the complex survey design in the NHANES III. All analyses were done using SAS version 9 (SAS Institute Inc, Cary, NC).

EXPLORATORY ANALYSES OF FOOD AND SUPPLEMENT SOURCES OF VITAMIN D

In separate logistic regression models, we explored the association between prevalent AMD and dietary intake of 2 concentrated food sources of vitamin D: milk and fish. Monthly servings of milk were categorized into logical consumption categories that were approximate tertiles (less than weekly, weekly to less than daily, and daily or more) and monthly servings of fish were categorized as less than bimonthly, bimonthly to weekly, and more than weekly. Individuals with missing milk and fish data were excluded from these analyses. Age-adjusted ORs were computed by frequency of intake of these foods. Correlations of milk and fish intake with serum vitamin D level were computed using Pearson correlation.

We next examined the relationship between use of vitamin D–containing supplements and AMD among consistent supplement users vs nonusers in the overall sample (n=7752; 16% consistent supplement users) as well as among people with less than daily milk intake (n=4531, 14% consistent supplement users). Age-adjusted ORs for AMD were computed for individuals with consistent vitamin D supplement use, defined as the consumption of greater than 200 IU (international units) per week from either vitamin D single supplements or multivitamins for at least 1 year, vs nonusers of vitamin D–containing supplements.

RESULTS

PARTICIPANT CHARACTERISTICS

Participant characteristics in the NHANES III were examined by quintile of serum vitamin D level (**Table 1**). Non-Hispanic black participants had lower serum vitamin D levels than the other racial subgroups. Individuals in the highest quintile of serum vitamin D level had higher intakes of dietary vitamin D, ω-3 fatty acids, zinc, vitamin E, and milk and lower intake of lutein and zeaxanthin. Individuals in the highest quintile of serum vitamin D level were less likely to have hypertension and diabetes. Prevalence of drusen was significantly lower among people in the highest quintile of serum vitamin D level.

SERUM VITAMIN D

As summarized in **Table 2**, serum vitamin D level was inversely associated with early AMD after adjusting for age and serum cotinine level in the overall population and in non-Hispanic white participants. There was a significant decrease in odds of early AMD with increasing quintile medians for serum 25-hydroxyvitamin D level before and after adjusting for age and serum cotinine level in the overall population (P trend $<.001$) and among non-Hispanic white participants (P trend $=.003$). Relationships between serum 25-hydroxyvitamin D level and prevalent early AMD were in the same direction among non-Hispanic black and Mexican American individuals but were not statistically significant. In the crude and adjusted models for the overall population, there was also a statistically significant trend for decreasing odds of drusen with increasing quintile medians for serum 25-hydroxyvitamin D level. Relationships between serum 25-hydroxyvitamin D level and drusen were also in the same direction among specific ethnic groups but not statistically significant (data not shown). There were no associations observed between serum vitamin D level and risk for pigmentary abnormalities or advanced AMD. Further adjustment for sex and other covariates did not influence the ORs. Interactions for race, sex, and age were not significant (data not shown).

MILK AND FISH CONSUMPTION

We explored the relationship between food sources rich in vitamin D and risk of AMD. Milk consumption was positively correlated with serum vitamin D level (Pearson correlation coefficient, 0.2; $P<.001$). As seen in **Table 3**, reported intake of weekly to daily consumption of milk per month compared with less frequent consumption of milk was inversely associated with early AMD and drusen but not pigmentary abnormalities before and after adjusting for age and race. Odds ratios for early AMD associated with daily or greater consumption of milk were also less than 1 and statistically significant. Associations were in the same direction for advanced AMD but were not statistically significant.

Inverse associations were observed for drusen in individuals who consumed fish bimonthly to weekly

Table 1. Weighted and Age-Adjusted Rates and Least Squared Means by Quintile of Serum Vitamin D Level in NHANES III Participants, 1988-1994 (n = 7752)*†

Variable	Quintile (Serum Vitamin D Level, nmol/L) 1 (<42)	2 (42-54)	3 (54-68)	4 (68-84)	5 (>85)	*P* Trend‡
Demographic						
Non-Hispanic white, %	59	73	84	89	93	<.001
Non-Hispanic black, %	26	12	7	4	2	
Mexican American, %	5	5	3	2	2	
Other races/ethnicities, %	9	10	6	5	3	
Women, %	71	64	54	49	44	<.001
Age, median, y	57	57	57	56	56	.003
Serum						
Median serum vitamin D level, nmol/L§‖						
Whole population	32	48	61	76	104	<.001
Non-Hispanic white participants	40	57	70	84	112	<.001
Non-Hispanic black participants	26	37	46	58	84	<.001
Mexican American participants	32	46	56	70	95	<.001
C-reactive protein, mg/dL	0.6	0.5	0.4	0.4	0.4	<.001
Serum fibrinogen, g/L	3.1	3.1	3.1	3.0	3.1	<.001
Cotinine, ng/mL	86	67	63	58	66	<.001
Diet						
Vitamin D, µg per 1000 kcal	2.0	2.6	2.6	2.9	2.9	<.001
Total dietary fat, % kcal	34	34	33	34	33	.30
ω-3 Fatty acids, mg/d	86	141	112	121	149	.004
Lutein and zeaxanthin, µg/d	2190	2017	1881	1631	1585	<.001
Zinc, mg/d	9	10	11	12	12	<.001
Vitamin E, mg/d	8	9	9	10	10	<.001
Alcohol intake, g/d	7	6	7	8	8	.06
Milk, servings/mo	15	19	23	26	27	<.001
Fish, servings/mo	4.5	4.6	4.8	5	4.7	.05
Medical Factors, %						
Hypertension	47	46	45	40	41	<.001
Cardiovascular disease	13	14	14	12	12	.18
Diabetes	12	11	8	8	5	<.001
AMD Outcomes, No. (%)§						
Early AMD	164 (11)	191 (12)	152 (10)	162 (11)	154 (10)	.05
Soft drusen	160 (10)	190 (12)	157 (10)	157 (109)	160 (10)	.02
Pigmentary abnormalities	25 (2)	37 (2)	37 (2)	44 (3)	42 (3)	.45
Advanced AMD	10 (0.6)	10 (0.6)	7 (0.4)	12 (0.8)	15 (1)	.50

Abbreviations: AMD, age-related macular degeneration; NHANES III, third National Health and Nutrition Examination Survey.
SI conversion factors: To convert serum 25-hydroxyvitamin D to ng/mL, divide by 2.496.
*The NHANES III sample weights were applied in all the analyses to account for individual selection probabilities, nonresponse, and poststratification that resulted from the complex survey design.
†Rates, expressed as percentages, were directly standardized to the NHANES III age groups (40-49, 50-59, 60-69, 70-79, and 80+ years).
‡*P* values for general association were generated by using multiple regression analyses.
§Unweighted values for the actual sample were used (N = 7752) in these analyses.
‖Quintiles were reassigned for each racial subgroup for all racial subgroup analyses.

compared with those who consumed fish less frequently. Odds ratios were in a similar direction for drusen in individuals who consumed fish weekly or more but were marginally significant. Fish consumption was not related to pigmentary abnormalities and overall early AMD. Weekly or greater consumption of fish was inversely associated with advanced AMD. However, reported fish intake was not significantly correlated with serum vitamin D level in this population (r=0.02; P=.10). The frequency of consumption of this rich source of vitamin D was low. Approximately half of the population consumed fish weekly or more, often with the median intake of 4-monthly servings among these individuals.

SUPPLEMENT USE

Consistent use of vitamin D–containing supplements was not associated with early AMD in the overall population (data not shown). However, consistent users of vitamin D–containing supplements in a subgroup of people consuming less than one serving of milk daily had decreased prevalent early AMD (**Table 4**). Associations were similar for drusen and pigmentary abnormalities but were not statistically significant.

SUNLIGHT

There are no estimates of sunlight exposure in the NHANES III to explore associations with this source of

Table 2. Adjusted Odds Ratios and 95% Confidence Intervals for AMD in the NHANES III (1988-1994) by Quintiles of Serum Vitamin D Level in the Overall Population and by Race*†

Characteristic	No. at Risk	No. With Outcome	Quintile (Serum Vitamin D Level, nmol/L) 1 (<42)	2 (42-54)	3 (54-68)	4 (68-84)	5 (>84)	*P* Trend‡
Early AMD§								
Whole population	7692	823						
Crude OR (95% CI)			1.0	0.97 (0.7-1.3)	0.75 (0.6-1.0)	0.70 (0.5-0.9)	0.64 (0.5-0.8)	<.001
Adjusted OR (95% CI)			1.0	0.98 (0.7-1.3)	0.75 (0.6-0.9)	0.69 (0.5-0.9)	0.64 (0.5-0.8)	<.001
Non-Hispanic white individuals	3843	478						
Crude OR (95% CI)			1.0	0.81 (0.6-1.1)	0.65 (0.5-0.9)	0.52 (0.5-0.8)	0.65 (0.4-0.9)	.003
Adjusted OR (95% CI)			1.0	0.81 (0.6-1.1)	0.65 (0.5-0.9)	0.52 (0.5-0.8)	0.64 (0.4-0.9)	.003
Non-Hispanic black individuals	1816	147						
Crude OR (95% CI)			1.0	0.78 (0.5-1.5)	0.88 (0.5-1.5)	0.84 (0.5-1.5)	0.80 (0.5-1.5)	.50
Adjusted OR (95% CI)			1.0	0.75 (0.5-1.3)	0.87 (0.5-1.4)	0.85 (0.5-1.4)	0.80 (0.5-1.4)	.60
Mexican American individuals	1739	162						
Crude OR (95% CI)			1.0	1.40 (0.8-2.5)	1.55 (0.9-2.6)	1.21 (0.7-2.2)	0.73 (0.4-1.4)	.20
Adjusted OR (95% CI)			1.0	1.39 (0.8-2.5)	1.54 (0.9-2.7)	1.21 (0.7-2.2)	0.73 (0.4-1.4)	.20
Soft Drusen								
Whole population	7750	824						
Crude OR (95% CI)			1.0	0.94 (0.7-1.2)	0.94 (0.7-1.2)	0.71 (0.5-0.9)	0.76 (0.6-1.0)	.007
Adjusted OR (95% CI)			1.0	0.94 (0.7-1.2)	0.94 (0.7-1.2)	0.71 (0.5-0.9)	0.76 (0.6-0.96)	.006
Pigmentary Abnormalities								
Whole population	7752	185						
Crude OR (95% CI)			1.0	1.38 (0.8-2.7)	1.25 (0.7-2.1)	1.01 (0.6-1.8)	1.01 (0.6-1.7)	.40
Adjusted OR (95% CI)			1.0	1.41 (0.8-2.5)	1.28 (0.7-2.2)	1.04 (0.6-1.8)	1.02 (0.6-1.8)	.40
Advanced AMD								
Whole population	7698	54						
Crude OR (95% CI)			1.0	0.73 (0.3-2.5)	0.36 (0.1-1.3)	0.65 (0.2-2.0)	1.20 (0.5-3.1)	.30
Adjusted OR (95% CI)			1.0	0.74 (0.3-2.2)	0.37 (0.1-1.3)	0.66 (0.2-2.0)	1.16 (0.5-3.1)	.30

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; NHANES III, third National Health and Nutrition Examination Survey; OR, odds ratio.

SI conversion factors: To convert serum 25-hydroxyvitamin D to ng/mL, divide by 2.496.

*Adjusted for age and serum cotinine.

†The NHANES III sample weights were applied in all the analyses to account for individual selection probabilities, nonresponse, and poststratification that resulted from the complex survey design. The jackknife replication method was used to obtain appropriate variance estimates in regression analyses.

‡*P* for trend was calculated using quintile medians.

§Individuals with drusen area smaller than 375 µm or having advanced AMD were not included in the early AMD end point per the NHANES III grading protocol.

vitamin D alone. However, we explored relationships of serum vitamin D level to AMD after excluding persons who reported consuming milk at least daily and people who reported to consistently use vitamin D in supplements. This left a sample of people for whom endogenous vitamin D would represent the predominant source. In this sample, odds for early AMD were significantly lower among people in the highest vs lowest quintile for serum vitamin D level (**Table 5**). Associations were similar for soft drusen. There were too few cases of advanced AMD or pigmentary abnormalities to conduct exploratory analyses with these outcomes.

COMMENT

We observed evidence of an inverse association between vitamin D status and the prevalence of early AMD. Higher serum vitamin D levels were inversely associated with prevalent early AMD and with soft drusen specifically in the American population aged 40 years and older. These associations were consistent across all 3 major ethnic groups, although not statistically significant in non-Hispanic black and Mexican American individuals for whom sample sizes were considerably smaller. We observed no associations of serum vitamin D level with pigmentary abnormalities or with advanced AMD. This might reflect less reliable ORs for these less common and more advanced end points or might indicate that vitamin D level is more specifically related to the formation of drusen.

We speculate that vitamin D may reduce the risk of AMD by its anti-inflammatory properties. Several putative mechanisms support the anti-inflammatory role of vitamin D. Studies have reported that vitamin D decreases proliferation of T helper cells,[44] T cytotoxic cells, and natural killer cells[45] and enhances T suppressor cell activity.[30] Vitamin D also decreases the production of proinflammatory agents such as IL-2,[25,28] IL-6,[46] IL-8,[26] and IL-12.[27] In addition, a recent study has shown that vitamin D intake reduces C-reactive protein, a marker of systemic inflammation.[47]

There is laboratory and epidemiologic evidence of inflammation underlying AMD pathology. A common polymorphism in complement factor H, a key regulator of the alternate complement pathway identified in a region of a gene responsible for binding heparin and C-reactive pro-

Table 3. Odds Ratios and 95% Confidence Intervals for Early AMD and Advanced AMD Among Participants Aged 40 Years and Older in High vs Low Milk and Fish Intake Groups in the NHANES III, 1988-1994*†

	Milk Intake			Fish Intake		
Type of AMD	Less Than Weekly‡	Weekly to Daily§	Daily or More‖	Less Than Bimonthly¶	Bimonthly to Weekly#	Weekly or More**
Early AMD††						
Cases per total, No.	211/2080	207/2412	402/3186	267/2158	151/1565	404/3968
Crude OR (95% CI)	1.0	0.66 (0.5-0.8)	0.74 (0.6-0.9)	1.0	0.80 (0.6-1.0)	0.91 (0.7-1.1)
Adjusted OR (95% CI)	1.0	0.67 (0.5-0.8)	0.75 (0.6-0.9)	1.0	0.81 (0.6-1.0)	0.91 (0.7-1.1)
Soft drusen						
Cases per total, No.	211/2087	218/2423	393/3220	272/2179	149/1577	402/3987
Crude OR (95% CI)	1.0	0.76 (0.6-0.9)	0.77 (0.7-0.9)	1.0	0.72 (0.6-0.9)	0.80 (0.7-0.96)
Adjusted OR (95% CI)	1.0	0.78 (0.6-0.9)	0.80 (0.7-0.98)	1.0	0.73 (0.6-0.9)	0.80 (0.7-0.97)
Pigmentary abnormalities						
Cases per total, No.	35/2088	39/2423	110/3221	48/2180	46/1577	91/3988
Crude OR (95% CI)	1.0	0.90 (0.6-1.4)	1.18 (0.8-1.8)	1.0	1.51 (1.0-2.3)	0.95 (0.6-1.4)
Adjusted OR (95% CI)	1.0	0.88 (0.5-1.4)	1.15 (0.8-1.7)	1.0	1.50 (1.0-2.3)	0.95 (0.6-1.4)
Advanced AMD						
Cases per total, No.	8/2088	11/2423	35/3221	22/2180	12/1577	20/3988
Crude OR (95% CI)	1.0	0.70 (0.2-1.9)	0.76 (0.3-1.8)	1.0	0.73 (0.3-1.7)	0.41 (0.2-0.9)
Adjusted OR (95% CI)	1.0	0.64 (0.2-1.8)	0.71 (0.3-1.7)	1.0	0.72 (0.3-1.7)	0.41 (0.2-0.9)

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; NHANES III, third National Health and Nutrition Examination Survey; OR, odds ratio.

*Adjusted for race and age.

†The NHANES III sample weights were applied in all the analyses to account for individual selection probabilities, nonresponse, and poststratification that resulted from the complex survey design. The jackknife replication method was used to obtain appropriate variance estimates in regression analyses.

‡Less than 4 servings of milk per month.

§Four to 30 servings of milk per month.

‖More than 30 servings of milk per month.

¶Less than 2 servings of fish per month.

#Two to 4 servings of fish per month.

**More than 4 servings of fish per month.

††Individuals with drusen area smaller than 375 μm or having advanced AMD were not included in the early AMD per the NHANES III grading protocol.

Table 4. Odds Ratios and 95% Confidence Intervals for Early AMD and Advanced AMD by Consistent Supplement Use Among People Consuming Milk Less Than Daily*†

Type of AMD	No. at Risk	No. With Outcome	Nonusers of Vitamin D Supplements (n = 3895)	Consistent Users of Vitamin D Supplements (n = 636)‡
Early AMD§	4512	421		
Crude OR (95% CI)			1.0	0.65 (0.5-0.9)
Adjusted OR (95% CI)			1.0	0.67 (0.5-0.9)
Soft drusen	4530	431		
Crude OR (95% CI)			1.0	0.81 (0.6-1.1)
Adjusted OR (95% CI)			1.0	0.84 (0.6-1.1)
Pigmentary abnormalities	4531	75		
Crude OR (95% CI)			1.0	0.61 (0.3-1.2)
Adjusted OR (95% CI)			1.0	0.60 (0.3-1.2)
Advanced AMD	4531	18		
Crude OR (95% CI)			1.0	2.53 (0.9-7.1)
Adjusted OR (95% CI)			1.0	2.40 (0.9-6.9)

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; NHANES III, third National Health and Nutrition Examination Survey; OR, odds ratio.

*Adjusted for race and age.

†The NHANES III sample weights were applied in all the analyses to account for individual selection probabilities, nonresponse, and poststratification that resulted from the complex survey design. The jackknife replication method was used to obtain appropriate variance estimates in regression analyses.

‡Consistent supplement use was defined as consumption of at least 200 IU of vitamin D per week from multivitamins or single supplements for 1 or more years.

§Individuals with drusen area smaller than 375 μm or having advanced AMD were not included in the early AMD per the NHANES III grading protocol.

tein, was associated with higher risk for AMD in several previous studies.[21,48-51] Using histological methods, Anderson et al[20] identified immuno-proteins entrapped within drusen, implying local inflammation, and Hageman et al[33] proposed a mechanism by which local inflammation may contribute to drusen development. Associations between

Table 5. Odds Ratios and 95% Confidence Intervals for Early AMD and Drusen in NHANES III (1988-1994) in People Who Neither Reported Daily Milk Drinking Nor Consistently Used Vitamin D–Containing Supplements (n = 3895) by Quintile of Serum Vitamin D Level*†

Type of AMD	No. at Risk	No. With Outcome	Serum Vitamin D Level, nmol/L					*P* Trend‡
			<37	37-48	48-60	61-78	>78	
Early AMD	3895	365						
Crude OR (95% CI)			1.0	1.2 (0.8-1.8)	1.0 (0.7-1.4)	0.8 (0.5-1.2)	0.6 (0.4-0.9)	<.001
Adjusted OR (95% CI)			1.0	1.3 (0.8-1.9)	1.0 (0.7-1.5)	0.9 (0.6-1.3)	0.6 (0.4-0.9)	.001
Soft drusen	3895	374						
Crude OR (95% CI)			1.0	1.0 (0.7-1.6)	1.0 (0.7-1.5)	0.8 (0.5-1.2)	0.7 (0.4-1.0)	.003
Adjusted OR (95% CI)			1.0	1.1 (0.7-1.7)	1.1 (0.7-1.6)	0.9 (0.6-1.3)	0.7 (0.5-1.0)	.02

Abbreviations: AMD, age-related macular degeneration; CI, confidence interval; NHANES III, third National Health and Nutrition Examination Survey; OR, odds ratio.

SI conversion factors: To convert serum 25-hydroxyvitamin D to ng/mL, divide by 2.496.

*Adjusted for age, serum cotinine, and race.

†The NHANES III sample weights were applied in all the analyses to account for individual selection probabilities, nonresponse, and poststratification that resulted from the complex survey design. The jackknife replication method was used to obtain appropriate variance estimates in regression analyses.

‡*P* for trend was calculated using quintile medians.

markers of inflammation (such as C-reactive protein) and AMD have been observed in some[52,53] but not all[54] previous epidemiological studies. Anti-inflammatory drug use was significantly related to AMD in one study[55] but not other previous studies.[56,57] Recently results of the Beaver Dam Eye Study[19] indicated an association between histories of gout and emphysema, two diseases associated with inflammation, and intermediate and late stages of AMD.[19]

Alternatively, vitamin D might protect against AMD by virtue of its antiangiogenic properties. There is recent evidence of vitamin D being a potent inhibitor of angiogenesis by its effects on endothelial cells[58-60] and by interrupting signaling pathways that are key to angiogenesis, specifically in tumorigenesis. We speculate that by virtue of its antiangiogenic role, vitamin D may protect against "wet" advanced AMD, which involves growth of new blood vessels in the retina. Since we had few cases of wet advanced AMD, we were not able to examine the associations of serum vitamin D level and advanced AMD. However, further research on advanced AMD and disease progression is warranted due to the biological plausibility of this association.

Vitamin D is provided in some foods and is made endogenously on exposure to sunlight. The serum levels of 25-hydroxyvitamin D assessed in this study reflect vitamin D from all sources combined. Observations in this study are consistent with the idea of lower risk for early AMD only among people who are exposed to 3 main sources of vitamin D: milk, supplements, and sunlight. Milk consumption was associated with lower odds for AMD. Consistent supplement use was inversely associated with AMD among people who did not consume at least 1 daily serving of milk. However, supplement use was not associated with AMD in people who consume milk daily. Milk is a rich source of vitamin D since all fluid milk in the United States is fortified with vitamin D with 400 IU added to a quart or 946 mL of milk.[61] We can speculate that supplement use may not be necessary to lower risk for AMD if vitamin D is obtained through diet. Finally, after excluding people whose usual daily intake of vitamin D is likely to be below 100 IU (Table 5), because of not drinking milk or taking supplements containing vitamin D regularly, serum vitamin D level in the highest vs lowest quintile was associated with 40% lower risk for early AMD. This observation is consistent with the idea that higher serum vitamin D derived from sunlight could be associated with lower risk for AMD.

Fish can be a rich source of vitamin D and may be protective against AMD. In this study population, fish intake was not correlated with serum vitamin D level, possibly due to a low frequency of fish consumption. Another reason we did not have observed associations with high fish intake and early AMD may be because we were not able to distinguish between fatty fish, a rich source of vitamin D, and other fish, which may contain lower levels of vitamin D. We report modest inverse associations of drusen with consumption of fish intake at least once a week. Consistent with the previous NHANES III[62] and findings of 4 previous epidemiological studies,[12-14,63] we observed inverse associations of advanced AMD with higher fish consumption. The protective effects of fish on AMD may be explained in part by its high concentration of ω-3 fatty acids, which may modulate the release of proinflammatory cytokines.[64]

Our results support the idea that lower serum vitamin D levels may lead to progression of chronic diseases, specifically those associated with inflammation.[29-32,65] This may be important to the health of older Americans. Studies have reported a strikingly high incidence of insufficient vitamin D intake in the US population and recognize inadequate vitamin D status as a public health problem.[66,67] Looker et al[68] reported that in summer months, about 21% to 49% of NHANES III participants living at higher altitudes were vitamin D insufficient and had serum vitamin D levels of less than 25 ng/mL (62.5 nmol/L) with 1% to 3% adults being deficient. Consequently, because poor dietary choices and sun avoidance behavior persist, attention to the health effects of vitamin D insufficiency is warranted. Furthermore, vitamin D availability and metabolism declines with age,[69] which enhances the concern in older adults.

Several potential limitations of the present investigation must be considered in drawing conclusions from the results. In particular, AMD was ascertained only in one

eye, resulting in a possible underestimation of AMD cases; however, studies have shown that AMD development is typically symmetric.[40] Further, AMD was identified using nonmydriatic fundus photography without dilating the pupils, which may have lead to potential misclassification of cases. In estimating milk and fish intake, the food frequency questionnaire used in this study was not validated and the measurement error was unknown. The serum 25-hydroxyvitamin D values would reflect sun exposure and food intake over recent weeks, rather than years, which would have enhanced random measurement error. Therefore, associations reported are likely to be biased toward the null. Next, the results of this study may be influenced by unknown or unmeasured risk or protective factors for AMD that are more common among persons with high compared with low serum levels of vitamin D. For example, family history of AMD, which was not ascertained in the current study, is known to influence AMD prevalence.[5,70] Also, high blood vitamin D levels may be related to other unknown and unmeasured healthy lifestyles that protect against AMD. Finally, the cross-sectional study design limited the ability to assess whether vitamin D was antecedent to the development of AMD. The results of our study provide strong support for further investigation of this hypothesis.

The strategy of blood collection in the NHANES III may be a potential source of bias and may affect the interpretation of serum 25-hydroxyvitamin D levels. Blood sampling in the NHANES III was carried out in a mobile examination center to control examination conditions nationwide. Blood was collected in the northern states during summer and in the southern states during winter. However, we found that median serum vitamin D levels of the 2 seasonal subpopulations were not statistically different (data not shown). Additionally, there is no existing evidence that AMD prevalence varies with latitude. Due to the blood collection strategy in the NHANES III, we were unable to examine vitamin D level in relation to AMD in individuals who live in the northern United States, a group that is likely have the lowest vitamin D levels. Moreover, because the NHANES III consisted of individuals who were free living, we were unable to evaluate the relationship of vitamin D level and AMD in the institutionalized population, who may be at a high risk for AMD due to the presence of comorbidities such as atherosclerosis or hypertension, 2 postulated risk factors of AMD, in addition to the possibility of low sunlight exposure.

In conclusion, the present study conducted in a large, representative sample of the US population provides evidence for inverse associations between AMD and higher serum vitamin D levels and higher intake of milk. We also observed reduced prevalence of AMD among consistent vitamin D–supplement users who consumed milk less than daily. However, at this time there is insufficient epidemiologic evidence of the relationship between vitamin D level and AMD to make recommendations regarding optimum serum vitamin D levels or milk and fish intake to protect against AMD or its progression. The results of the present research warrant further investigation for confirmation of the vitamin D–AMD association in other population studies.

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