Serum 25-Hydroxyvitamin D Concentrations and Incidence of Age-Related Macular Degeneration: The Atherosclerosis Risk in Communities Study

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Purpose. To investigate the association between serum 25-hydroxyvitamin D (25[OH]D) concentrations at visit 2 (1990–1992) and the 18-year incidence of age-related macular degeneration (AMD) between visit 3 (1993–1995) and visit 5 (2011–2013).

Methods. This prospective analysis was conducted in a subset of participants ($n = 1225$) from the Atherosclerosis Risk in Communities Study. We evaluated the incidence of any, early, and late AMD from visit 3 to 5. The 25(OH)D concentrations were assessed in 2012–2013 by using stored serum from visit 2. Retinal fundus photographs taken at both visits were graded side by side to determine the incidence of AMD. Logistic regression was used to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for incident AMD outcomes during 18 years of follow-up (1993–1995 to 2011–2013) by tertile of 25(OH)D adjusted for age, race, and smoking status. *P* for linear trend was estimated by using continuous 25(OH)D concentrations. Sensitivity analyses applied inverse probability weights to account for selection to have eye photographs, death, and loss to follow-up.

Results. There was a decreased odds of any incident AMD ($n = 139$) and large, soft drusen ($n = 80$) in 25(OH)D tertile 3 versus 1, with OR (95% CI) = 0.57 (0.36–0.90), *P* trend = 0.11 and with 0.52 (0.28–0.93), *P* trend = 0.18, respectively. Applying sampling weights attenuated these results to 0.66 (0.38–1.16), *P* trend = 0.32 (any incident AMD) and 0.54 (0.27–1.09), *P* trend = 0.36 (large, soft drusen), respectively, suggesting these associations may be biased by loss to follow-up and sampling for retinal photographs at visit 5. No statistically significant results were observed with pigmentary abnormalities ($n = 46$) or incident late AMD ($n = 26$).

Conclusions. High 25(OH)D concentrations, approximately >70 nM, may be associated with decreased odds of incident early AMD.

Keywords: vitamin D, 25-hydroxyvitamin D, macular degeneration, retinal diseases, epidemiology, cohort studies

For the past decade, vitamin D has been proposed as a novel, modifiable risk factor for age-related macular degeneration (AMD) owing to its immune-modulating and antiangiogenic properties.[1] This area of inquiry has produced many cross-sectional and case-control studies examining the associations between prevalent AMD and the biomarker of 25-hydroxyvitamn D (25[OH]D)[1–12]; however, such study designs cannot determine the temporality of the observed association. Reverse causality could explain the protective associations observed, especially those examining vision-threatening late-stage disease.[8,12] Vision-impaired individuals are more likely to have mobility limitations[13,14] and may be less likely to go outside and be exposed to sunlight for dermal production of vitamin D.

To the best of our knowledge, only two studies of vitamin D status and incident AMD have been published.[15,16] More prospective studies of this association with careful phenotyping of vitamin D and AMD, as well as studies examining the risk of development of early AMD, are needed. Further, only two previous studies on vitamin D and AMD have been conducted in racially diverse samples,[1,15] limiting our understanding of this association in racial groups such as African Americans who have one of the highest burdens of vitamin D deficiency.[17]

We examined the association between 25(OH)D concentrations and the 18-year progression of AMD, using data from the Atherosclerosis Risk in Communities (ARIC) Study, a well-characterized epidemiologic cohort.[18] We hypothesized that

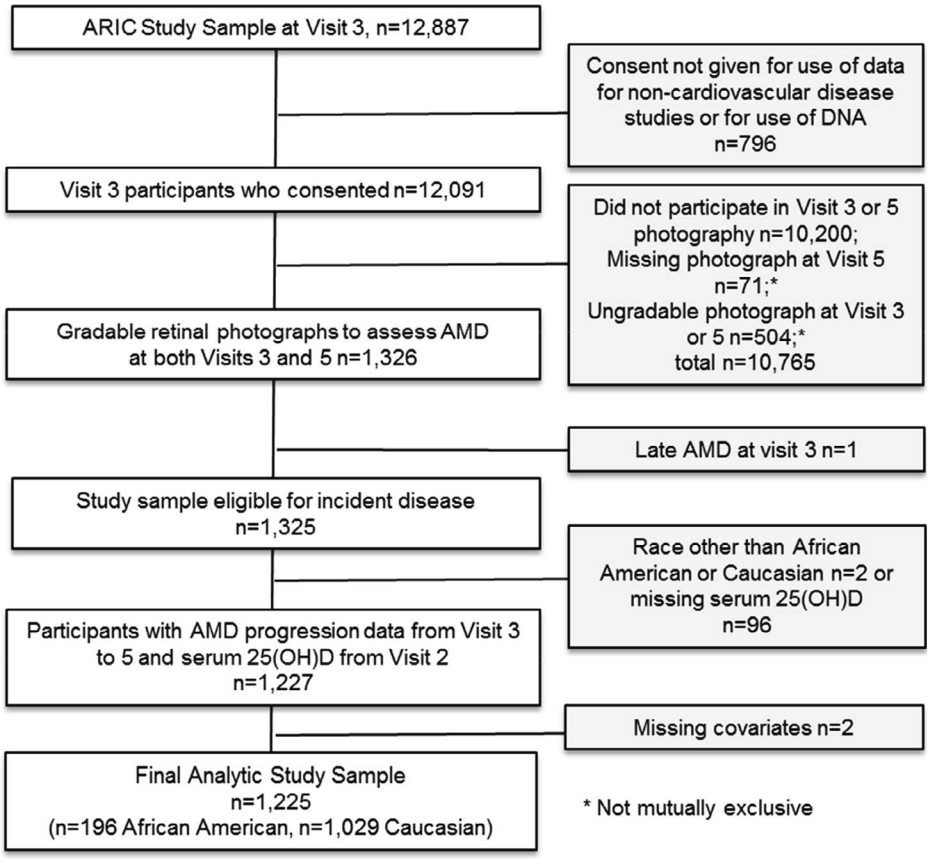

Figure. Study sample selection of ARIC participants with gradable, retinal photographs from visit 3 to visit 5.

ARIC participants with higher 25(OH)D concentrations will have a decreased risk of incident AMD than those with lower 25(OH)D concentrations. We also explored how the observed association varied by age, race, sex, and high-risk AMD genotype.

Methods

Study Design

The ARIC Study is a population-based, prospective study of atherosclerosis with five visits since 1987–1989.[18] Participants were 45 to 65 years of age at visit 1 and recruited from four centers: Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland. For the present analyses, participants needed to have available serum 25(OH)D concentrations from blood drawn at visit 2 (1990–1992) and gradable retinal fundus photographs taken at visits 3 (1993–1995) and 5 (2011–2013) (Fig.). There were 11,863 participants for whom retinal photographs of one randomly chosen eye were taken at visit 3.

Retinal photographs were taken in one or both eyes of a subset (n = 2629) of 6538 participants at visit 5.[19] Of these, approximately a third were recruited from a random sample of participants without any dementia or mild cognitive impairment.[20] Participants with low neurocognitive test scores suggesting dementia or mild cognitive impairment made up the additional sample. Of these, 2124 had gradable images in at least one of two eyes from visit 5, and of these, 1326 also have gradable photographs from a matching eye at visit 3 among the previously noted 11,863 persons with retinal photos at visit 3. Participants were further excluded if they had late AMD at visit 3 (n = 1), were not Caucasian or African American (n = 2), or were missing data on 25(OH)D (n = 96). Two additional participants were excluded owing to missing data on smoking status, leaving an analytic sample of 1225 participants (1029 Caucasians, 196 African Americans). Because of the targeted recruitment of participants with low neurocognitive scores for retinal photographs, our sample included 89 participants with dementia, 440 with mild cognitive impairment, 694 with normal cognitive function, and 2 with unknown cognitive function status.

Participants of this study provided signed informed consent.[21] For those determined to have mild cognitive impairment or dementia, consent to participate was given by a designated proxy and agreement to participate was also obtained from the participant. The study protocol was approved by the institutional review boards at each ARIC study site and complies with the Declaration of Helsinki as revised in 1983.

Data on Participant Characteristics

At all study visits, participants answered questionnaires on their lifestyle habits, medical history,[18] had a physical exam and a blood draw.[18,22] Before the visit, participants were asked to fast for 12 hours and to bring with them any medications or supplements they had taken within the past 2 weeks.[18]

At visits 1 and 3, physical activity was assessed by using a modified version of the previously validated[23,24] Baecke questionnaire.[25] We averaged questionnaire scores obtained at both visits to create a composite physical activity index score ranging from 0 (low overall physical activity) to 6.

Data on the genotypes of two high-risk AMD single nucleotide polymorphisms (SNPs) shown to be associated with increased risk of early AMD[26] were used. *ARMS2* A69S

(rs10490924) was genotyped by using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc., Santa Clara, CA, USA).[27] *CFH* Y402H (rs1061170) was imputed by using the HapMap and 1000 Genomes reference panels (appropriate for race). For *ARMS2* rs10490924, the minor allele frequency and Hardy-Weinberg equilibrium was met. For *CFH* rs1061170, the imputation quality score was >0.8.

Incidence of Early and Late AMD

At visit 3, nonmydriatic retinal film photographs were taken of one randomly chosen eye by using a photograph centered between the disc and fovea. Digital photographs without mydriatics were taken of both eyes (fields 1 [optic nerve] and 2 [macula])[28,29] at visit 5 follow-up. Research shows film and digital grading of AMD to be comparable.[30] Photographs were graded independently by using the Wisconsin Age-Related Maculopathy Grading System,[31] with graders masked to grading from previous visits. Eyes that changed across visits within the three-step ARM severity scale (none, early, late) underwent a side-by-side review to confirm that the change was real and not an error related to image quality/differences or grader (BEKK, RK) error.

Participants were considered to have early AMD if they had any of the following: (1) soft drusen (≥63-µm circle) present with a grid area >500-µm circle or (2) any soft drusen ≥125-µm circle (either distinct of indistinct) present in the grid and any pigmentary abnormality present (increased retinal pigment or depigmentation in the grid) or (3) large (≥125-µm circle) soft indistinct drusen present. Participants were considered to have late AMD if they had any evidence of the following lesions: geographic atrophy, retinal pigment epithelial detachments/retinal detachments, subretinal hemorrhage, subretinal fibrous scar, subretinal new vessels, or history of treatment (laser, photodynamic therapy of intravitreal injections).

Early AMD cases were further subdivided by presence of large, soft drusen (≥1 soft drusen with a diameter ≥125 µm and a grid area >500 µm) or pigmentary abnormalities (presence of increased pigmentation or retinal pigment epithelial depigmentation).

Serum 25-Hydroxyvitamin D

As previously described by Lutsey et al.,[32] serum obtained at visit 2, and stored at −80°C until measurement in 2012–2013, has been assessed for concentrations of 25(OH)D, both D_2 and D_3 forms, with liquid chromatography–tandem-mass spectrometry (Waters Alliance e2795; Waters, Milford, MA, USA) at the University of Minnesota Molecular Epidemiology and Biomarker Research Laboratory (Minneapolis, MN, USA). Quality control measures were applied to minimize laboratory variation across batches of samples sent for analysis. Adjustment of 25(OH)D for season of blood draw was conducted as previously described,[33] and adjusted values were used in all subsequent analyses.

Statistical Analysis

Participants were categorized into tertiles of serum 25(OH)D, and clinically used cut points[34,35] for exploratory analyses; however, these cut points are for bone health and may not apply to ocular health. We compared characteristics of our participants by tertile of 25(OH)D and AMD. Logistic regression was used to relate the log odds of incident AMD by tertile of 25(OH)D with the lowest tertile as the referent category. *P* for linear trend was computed by using continuous 25(OH)D concentrations. We assessed addition of potential confounders (age, race, sex, education, income, health insurance, smoking status, drinking status, ethanol intake, body weight, waist circumference, waist to hip ratio, body mass index [BMI], physical activity, measures of fasting serum total cholesterol, serum high density lipoprotein, serum triglycerides, and use of hormone therapy [in females]) to an age-adjusted model in a stepwise fashion with covariates that changed the OR 10% or more considered for inclusion. A priori we decided to adjust for, at minimum, age, race, and smoking status. Blood pressure, hypertension status, blood glucose concentration, and diabetes status were examined as potential pathway (intermediary) variables. In secondary analyses, we examined the association between 25(OH)D and incident early AMD and incident late AMD, as well as large, soft drusen and pigmentary abnormalities among early incident cases.

In exploratory analyses, we stratified our risk estimates by age, sex, and race. Tertiles 2 and 3 were collapsed for analyses within African Americans as no cases of AMD existed in tertile 3. We examined effect modification of our primary association by high-risk AMD genotype. Genetic analyses were limited to Caucasians owing to an inadequate number of cases of AMD in African Americans. We present *P* values for the interaction terms (e.g., vitamin D * sex) in the logistic regression models, with a *P* value of <0.10 considered statistically significant.

Because of the concern for potential bias resulting from loss to follow-up and selection at visit 5, we compared characteristics of participants who attended visit 3 and were included to those who attended visit 3 and were excluded from these analyses. We also present results with and without the inclusion of inverse probability weights to account for loss to follow-up as previously done,[36] and selection into visit 5, stage 2/3, for retinal photography.[37] We applied weights provided by the ARIC Coordinating Center, specifically S2SAMWT51, from the derived dataset for visit 5,[37] by using PROC SURVEYLOGISTIC.[38] Two participants were missing weights and not included in weighted analyses. The mean weight value was 2.2 with a standard deviation (SD) ± 1.7 in all participants, with 1.9 ± 2.4 (range, 1.0–8.7) in African Americans and 2.3 ± 1.5 (1.1–6.8) in Caucasians. We also compared our primary analysis with and without inclusion of participants with low neurocognitive scores because visit-5 participants with low neurocognitive scores were oversampled for retinal photography. Finally, we conducted a bias analysis where we recategorized some participants considered to not have developed incident AMD to incident outcomes. If a participant's eye was considered to have early or late AMD at visit 5, but not photographed at visit 3, that individual was recategorized as an incident AMD case ($n = 33$). The primary analysis was repeated by considering these individuals as additional cases of incident AMD.

Results

At visit 3, the 1225 participants were on average 58.6 ± 5 (mean ± SD) years old, 58% female, and 16% African American. Follow-up from visits 3 to 5 spanned a mean of 18 years. At visit 3, there were 19 cases of prevalent, early AMD, with 5 developing incident, late AMD. Of participants with no AMD at visit 3, a total of 113 developed early AMD and 21 developed late AMD by visit 5. The incidence of early AMD was 9% ($n = 113$ of 1206 at risk) and 2% for late AMD ($n = 26$ of 1225 at risk). Those who developed incident disease were more likely to be older, Caucasian, current or former smokers, those with greater waist to hip ratios, and those less physically active (Table 1).

Characteristics of individuals with high (tertile 3) compared to low (tertile 1) 25(OH)D were more likely to be older, men, Caucasian, those with health insurance, former smokers, current drinkers, those with smaller waist circumferences and BMIs, those more physically active, those with less

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Table 1. Characteristics* by Incident AMD Status and Tertiles of 25(OH)D Concentrations ($N = 1225$): the ARIC Study

Characteristics	N	AMD Incidence From Visit 3 to Visit 5		Tertiles of 25(OH)D			R (P Value)†
		No (n = 1086)	Yes (n = 139)	Tertile 1 (10.8–53.6)	Tertile 2 (53.7–69.3)	Tertile 3 (69.4–173)	
Season-adjusted serum 25(OH)D, nM, mean (SD)	1225	63 (20.3)	64 (18.7)	**43 (8.7)**	**62 (4.6)**	**85 (14.2)‡**	NA
Prevalent early AMD at visit 3, *n* (%)	19	**14 (1)**	**5 (4)‡**	5 (1)	8 (2)	6 (2)	–
Incident AMD, early or late, *n* (%)	139	**0 (0)**	**139 (100)‡**	47 (12)	48 (12)	44 (11)	–
Demographics							
Age, y, mean (SD)	1225	**55 (5.0)**	**58 (4.9)‡**	**55 (4.9)**	**56 (4.9)**	**56 (5.3)‡**	**0.07 (0.016)**
Sex, *n* (% women)	711	633 (58)	78 (56)	**274 (67)**	**233 (57)**	**204 (50)‡**	–
Race, *n* (% Caucasian)	1029	**896 (83)**	**133 (96)‡**	**272 (67)**	**366 (90)**	**391 (96)‡**	–
Field center, *n* (%)							–
Forsyth County, NC	135	**116 (11)**	**19 (14)‡**	**39 (10)**	**43 (11)**	**53 (13)‡**	
Jackson, MS	185	**179 (17)**	**6 (4)**	**127 (31)**	**41 (10)**	**17 (4)**	
Minneapolis, MN	430	**384 (35)**	**46 (33)**	**117 (29)**	**153 (37)**	**160 (39)**	
Washington County, MD	475	**407 (37)**	**68 (49)**	**125 (30)**	**172 (42)**	**178 (44)**	
Education, visit 1§, *n* (%)							–
Basic or 0 years	144	128 (12)	16 (12)	55 (14)	51 (12)	38 (9)	
Intermediate	545	482 (44)	63 (45)	168 (41)	178 (44)	199 (49)	
Advanced	536	476 (44)	60 (43)	185 (45)	180 (44)	171 (42)	
Health and lifestyle characteristics							
Health insurance, *n* (% yes)	1161	1031 (95)	130 (94)	**377 (92)**	**393 (96)**	**391 (96)‡**	–
Smoking status, *n* (%)							–
Current	178	**160 (15)**	**18 (13)‡**	**67 (16)**	**54 (13)**	**57 (14)‡**	
Former	463	**396 (36)**	**67 (48)**	**123 (30)**	**154 (38)**	**186 (46)**	
Never	584	**530 (49)**	**54 (39)**	**218 (54)**	**201 (49)**	**165 (40)**	
Drinking status, *n* (%)							–
Current	814	716 (66)	98 (71)	**239 (59)**	**280 (68)**	**295 (72)‡**	
Former	178	157 (14)	21 (15)	**65 (16)**	**52 (13)**	**61 (15)**	
Never	233	213 (20)	20 (14)	**104 (25)**	**77 (19)**	**52 (13)**	
Waist circumference, cm, mean (SD)	1225	96 (13.7)	98 (11.8)	**99 (15.4)**	**97 (12.4)**	**93 (12.0)‡**	**−0.14 (<0.001)**
Waist to hip ratio, mean (SD)	1225	**0.91 (0.08)**	**0.93 (0.07)‡**	**0.91 (0.08)**	**0.92 (0.08)**	**0.91 (0.08)‡**	−0.01 (0.713)
BMI category, kg/m^2, *n* (%)							**−0.20 (<0.001)**
Under/normal weight (<25 kg/m^2)	378	341 (31)	37 (27)	**108 (27)**	**108 (26)**	**162 (40)‡**	
Overweight (25–30 kg/m^2)	536	468 (43)	68 (49)	**164 (40)**	**192 (47)**	**180 (44)**	
Obese (≥30 kg/m^2)	311	277 (26)	34 (24)	**136 (33)**	**109 (27)**	**66 (16)**	
Physical activity index visits 1 and 3, mean (SD)	1179	**3.1 (1.3)**	**2.8 (1.4)‡**	**2.8 (1.3)**	**3.0 (1.3)**	**3.3 (1.2)‡**	**0.15 (<0.001)**
Diastolic blood pressure, mm Hg, mean (SD)	1225	72 (9.5)	72 (9.4)	72 (9.3)	72 (9.7)	72 (9.6)	0.004 (0.887)
Systolic blood pressure, mm Hg, mean (SD)	1225	118 (16.1)	118 (14.7)	118 (16.0)	118 (15.0)	118 (16.9)	−0.02 (0.497)
Hypertension, *n* (% yes)‖	305	273 (25)	32 (23)	**118 (29)**	**101 (25)**	**86 (21)‡**	–
Total cholesterol, mg/dL, mean (SD)	1222	208 (37.4)	212 (38.1)	208 (37.5)	212 (40.3)	206 (34.2)	0.01 (0.780)
HDL, mg/dL, mean (SD)	1218	51 (16.6)	50 (14.8)	51 (15.9)	50 (16.1)	51 (17.1)	−0.01 (0.786)
LDL, mg/dL, mean (SD)	1198	131 (33.9)	136 (35.1)	132 (34.7)	134 (36.0)	129 (31.1)	−0.02 (0.511)
Triglycerides, mg/dL, mean (SD)	1222	133 (84.2)	132 (61.0)	**125 (76.5)**	**140 (91.2)**	**132 (76.6)‡**	**0.07 (0.019)**
Glucose, mg/dL, mean (SD)	1225	106 (27.0)	104 (15.5)	**107 (28.4)**	**107 (31.9)**	**102 (13.8)‡**	**−0.12 (<0.001)**
Hormone use, among females, *n* (%)							–
Current estrogen user	132	120 (22)	12 (18)	**47 (20)**	**33 (17)**	**52 (28)‡**	
Current estrogen and progestin user	93	82 (15)	11 (16)	**23 (10)**	**27 (14)**	**43 (24)**	
Never used hormones	363	322 (59)	41 (60)	**161 (67)**	**125 (64)**	**77 (42)**	
Former hormone user	29	25 (4)	4 (6)	**9 (3)**	**9 (5)**	**11 (6)**	

Bolded values represent statistically significant results at a P value of <0.05 or smaller. NA, not applicable.

* Characteristics assessed at visit 2 unless otherwise noted.

† Spearman correlation coefficient and associated P value for the correlation between season-adjusted serum 25(OH)D and the respective continuous variable. Values are not shown (–) for categorical variables.

‡ P value < 0.05 For continuous variables, t-tests or ANOVAs were used to compare means of characteristics between those with and without any incident AMD or across tertiles of 25(OH)D, respectively. For categorical variables, χ^2 tests were used to compare proportions of characteristics between those with and without any incident AMD or across tertiles of 25(OH)D.

§ Education defined as basic or 0 years (≤11 years or less, i.e., high school with no degree or less), intermediate (12–16 years, i.e., high-school graduate or vocational school), or advanced (17–21 years, i.e., college or higher).

‖ Average systolic blood pressure ≥140 mm Hg, or diastolic ≥90 mm Hg, or high blood pressure medication use in the past 2 weeks.

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Table 2. ORs and 95% CIs for AMD Incidence by 25(OH)D Concentrations Defined by Using Both Tertiles and Clinical Outpoints of 25(OH)D (N = 1225): the ARIC Study

	Tertiles of 25(OH)D, nM, Range			
Model	**Tertile 1 (10.8–53.6)**	**Tertile 2 (53.7–69.3)**	**Tertile 3 (69.4–173.0)**	***P* Value for Trend***
No. outcome/total No.	47/408	48/409	44/408	
Age-adjusted	1	0.92 (0.59–1.42)	0.83 (0.53–1.30)	0.96
Weighted†	1	1.11 (0.65–1.90)	0.89 (0.51–1.54)	0.88
Model 1‡	1	0.70 (0.45–1.10)	**0.57 (0.36–0.90)**	0.11
Weighted	1	0.88 (0.53–1.48)	0.66 (0.38–1.16)	0.32
Model 2§	1	0.79 (0.50–1.26)	**0.61 (0.38–0.99)**	0.37
Weighted	1	0.97 (0.57–1.64)	0.73 (0.41–1.31)	0.63
	Clinical Cutpoints Defined by 25(OH)D, nM			
Model	**<50 Deficient/Inadequate**	**50 to <75 Adequate**	**≥75 Adequate**	***P* Value for Trend***
No. outcome/total No.	33/316	69/599	37/310	
Age-adjusted	1	1.02 (0.65–1.60)	1.04 (0.62–1.72)	0.96
Weighted	1	1.52 (0.87–2.66)	1.33 (0.71–2.50)	0.88
Model 1‡	1	0.75 (0.47–1.19)	0.68 (0.40–1.15)	0.11
Weighted	1	1.20 (0.61–2.34)	0.99 (0.48–2.05)	0.32
Model 2§	1	0.87 (0.54–1.42)	0.79 (0.45–1.38)	0.37
Weighted	1	1.38 (0.69–2.77)	1.17 (0.56–2.47)	0.63

Bolded values represent statistically significant results at a P value of < 0.05 or smaller.

* P for trend calculated by using season-adjusted serum 25(OH)D as a continuous variable.

† Inverse probability weights applied; $n = 2$ individuals missing weights.

‡ Model 1: adjusted for age, race, and smoking status.

§ Model 2: adjusted for age, race, and smoking status and composite physical activity index averaging visits 1 and 3 data ($n = 46$ participants were missing this variable).

hypertension, higher triglycerides, lower blood glucose, and those more likely to be current hormone therapy users (Table 1). Individuals excluded, as compared to included, for this analysis had lower 25(OH)D and did not differ by prevalence of any AMD at baseline[33] (Supplementary Table S1). Participants excluded were older, more likely to be men, African American, hypertensive, to have greater waist circumferences, waist to hip ratios, BMI, systolic blood pressure, LDL, and blood glucose. They were less likely to have advanced education, health insurance, and to have never smoked, drank, or taken hormones; and their HDL was lower.

Only adjustment for race and the physical activity index changed the age-adjusted OR for any incident AMD among those in tertile 3 compared to tertile 1 by 10% or more. Therefore, model 1 is adjusted for covariates chosen a priori (age, race, and smoking status). There was a significant decreased odds of any incident AMD (early and late combined) in tertile 3 compared to 1 for 25(OH)D, with a P trend = 0.11 (Table 2). After adjustment for sampling weights, this OR was no longer statistically significant. Further adjustment for physical activity (model 2) attenuated the ORs for tertile 3 compared to 1 and the P trend, but did not remove the statistical significance of the third tertile OR until after weights were applied. Further adjustment for sex and prevalent, early AMD at visit 3, in addition to age, race, and smoking status, also did not substantially change these results (unweighted OR = 0.58 [0.36–0.92], P trend = 0.14 and weighted OR = 0.64 [0.36–1.13], P trend = 0.28). When clinical cut points of 25(OH)D were used, an inverse, but not statistically significant, OR for those with 25(OH)D ≥75 compared to <50 nM was observed in unweighted, but not weighted, analyses.

The inverse association was stronger in those >55 years and women, but no statistically significant age or sex interactions were observed (Table 3). Results in Caucasians paralleled findings in the overall sample. Findings in African Americans showed an inverse association. However, the P trend was only significant in weighted analyses and appeared to be driven by one case with a high weight value. No significant interaction was observed with the *CFH* or *ARMS2* genotypes.

There was a statistically significant decreased odds of incident early AMD and large, soft drusen among incident cases of early AMD in tertile 3 compared to 1, but the significance of these findings was removed after weights were applied (Table 4). No statistically significant associations were observed with pigmentary abnormalities and incident late AMD.

Analyses restricted to those with dementia or cognitive impairment showed similar results to those with normal cognitive functioning. The OR (95% CI), adjusted for age, race, and smoking status, for tertile 3 versus 1 was 0.55 (0.30–1.01), P trend = 0.23 and 0.59 (0.28–1.24), P trend = 0.31, respectively. Our bias analysis showed similar results to our primary findings. When participants' case status was recategorized on the basis of data from both eye photos at visit 5, the OR (95% CI) for any incident AMD in tertile 3 compared to 1 was 0.67 (0.45–1.06) and 0.79 (0.47–1.33) in unweighted and weighted analyses, respectively, with P for trends of 0.31 and 0.32.

Discussion

In this longitudinal study, we observed a statistically significant decreased odds of incident AMD among participants with high compared to low 25(OH)D concentrations during 18 years of follow-up; however, the P for trend for these analyses was not statistically significant. Inverse associations were also observed for incident early AMD and large, soft drusen among early incident cases, but not for pigmentary abnormalities or late AMD. Analyses showed no protective association between 25(OH)D and late AMD or pigmentary abnormalities; however, there were few events for either outcome and thus the risk estimates had poorer precision than examining any early AMD

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Table 3. Adjusted ORs and 95% CIs for Incident AMD From Visit 3 (1993–1995) to Visit 5 (2011–2013) by Tertiles of 25(OH)D Stratified by Race, Age, Sex, Smoking Status, and BMI: ARIC Study Participants With Gradable Eye Photos at Visit 3 and Visit 5 and Available Serum 25(OH)D at Visit 2 (1990–1992) ($N = 1225$)

	Tertiles of 25(OH)D, nM, Range			
Model	**Tertile 1 (10.8–53.6)**	**Tertile 2 (53.7–69.3)**	**Tertile 3 (69.4–173.0)**	***P* Trend***
Age group, y				
≤ Median of 55 years, $n = 642$				
No. with outcome/No. total	10/223	14/199	16/220	
Model 1†	1	1.20 (0.50–2.86)	1.17 (0.50–2.77)	0.85
Weighted‡	1	1.36 (0.52–3.55)	1.31 (0.49–3.47)	0.62
> Median of 55 years, $n = 583$				
No. with outcome/No. total	37/185	34/210	28/188	
Model 1†	1	0.56 (0.33–0.97)	**0.42 (0.24–0.75)**	0.10
Weighted‡	1	0.70 (0.37–1.31)	**0.44 (0.22–0.89)**	0.12
P for interaction§ = 0.74 (weighted‡ = 0.93)				
Sex				
Men, $n = 514$				
No. with outcome/No. total	13/134	22/176	26/204	
Model 1†	1	1.04 (0.49–2.22)	0.90 (0.43–1.91)	0.70
Weighted‡	1	1.16 (0.51–2.66)	0.75 (0.32–1.76)	0.94
Women, $n = 711$				
No. with outcome/No. total	34/274	26/233	18/204	
Model 1†	1	**0.55 (0.31–0.99)**	**0.43 (0.23–0.81)**	**0.03**
Weighted‡	1	0.65 (0.33–1.30)	0.60 (0.28–1.29)	0.18
P for interaction§ = 0.14 (weighted‡ = 0.82)				
Race				
Caucasians, $n = 1029$				
No. outcome/total No.	42/272	47/366	44/391	
Model 1†	1	0.71 (0.45–1.13)	0.57 (0.36–0.92)	0.11
Weighted ‡	1	0.90 (0.53–1.53)	0.66 (0.37–1.19)	0.21
African Americans, $n = 196$‖				
Combining tertiles 2 and 3	**Tertile 1**	**Tertile 2/3**		
No. outcome/total No.	5/136	1/60	–	
Model 1†	1	0.45 (0.05–3.95)	–	0.93
Weighted‡	1	0.53 (0.05–5.31)	–	**0.02**
P for interaction§ = 0.99 (weighted‡ = 0.006)				
CFH genotype, rs1061170				
TT, no high-risk alleles, $n = 348$				
No. with outcome/No. total	12/81	10/129	10/138	
Model 1†	1	0.45 (0.18–1.12)	**0.39 (0.16–0.98)**	0.32
Weighted‡	1	0.53 (0.18–1.54)	0.44 (0.15–1.29)	0.41
CT, 1 high-risk allele, $n = 402$				
No. with outcome/No. total	15/111	19/129	20/162	
Model 1†	1	0.93 (0.43–1.99)	0.71 (0.33–1.52)	0.31
Weighted‡	1	0.95 (0.40–2.30)	0.83 (0.32–2.17)	0.67
CC, 2 high-risk alleles, $n = 127$				
No. with outcome/No. total	9/34	12/51	11/42	
Model 1†	1	0.94 (0.33–2.72)	0.91 (0.31–2.71)	0.70
Weighted‡	1	1.46 (0.45–4.66)	0.96 (0.25–3.74)	0.72
CC/CT, 1–2 high-risk alleles, $n = 529$				
No. with outcome/No. total	24/145	31/180	31/204	
Model 1†	1	0.94 (0.51–1.73)	0.74 (0.40–1.36)	0.24
Weighted‡	1	1.12 (0.55–2.26)	0.82 (0.38–1.78)	0.50
P for interaction§ = 0.61 (weighted‡ = 0.58)				
ARMS2 genotype, rs10490924				
GG, no high-risk alleles, $n = 539$				
No. with outcome/No. total	17/137	20/197	21/205	
Model 1†	1	0.71 (0.35–1.46)	0.65 (0.32–1.33)	0.66
Weighted‡	1	0.86 (0.39–1.91)	0.74 (0.33–1.68)	0.92
TG, 1 high-risk allele, $n = 292$				
No. with outcome/No. total	13/70	18/102	19/120	
Model 1†	1	0.84 (0.37–1.88)	0.69 (0.31–1.53)	0.36
Weighted‡	1	1.05 (0.41–2.73)	0.85 (0.31–2.33)	0.51

Table 3. Continued

Model	Tertiles of 25(OH)D, nM, Range			*P* Trend*
	Tertile 1 (10.8–53.6)	Tertile 2 (53.7–69.3)	Tertile 3 (69.4–173.0)	
TT, 2 high-risk alleles, $n = 46$				
No. with outcome/No. total	6/19	3/10	1/17	
Model 1†	1	0.45 (0.04–4.58)	0.07 (0.004–1.21)	**0.045**
Weighted‡	1	0.46 (0.05–4.39)	**0.03 (0.001–0.92)**	**0.03**
TT/TG, 1–2 high-risk allele, $n = 338$				
No. with outcome/No. total	19/89	21/112	20/137	
Model 1†	1	0.75 (0.36–1.53)	0.52 (0.25–1.07)	0.06
Weighted‡	1	0.86 (0.37–1.99)	0.60 (0.24–01.50)	0.15
P for interaction§ = 0.14 (weighted‡ = 0.14)				

Bolded values represent statistically significant results at a *P* value of < 0.05 or smaller.
* *P* for trend calculated by using season-adjusted serum 25(OH)D as a continuous variable.
† Model 1: adjusted for age, race, and smoking status.
‡ Inverse probability weights applied to adjust for bias due to loss to follow-up from visit 3 to 5; $n = 2$ participants missing weights.
§ Multiplicative interactions were tested by using continuous measures of 25(OH)D, age, and ordinal measures of genotype variables.
|| Results for African Americans are unstable owing to low sample size. Not including one individual with incident AMD and the highest sample weight among incident cases removes the statistically significant *P* trend in the weighted analysis.

as a whole. This article contributes to the literature by providing a longitudinal analysis with assessment of vitamin D status before assessment of AMD incidence. Unlike the previous longitudinal studies[15,16] published, our study had a biomarker for vitamin D status, 25(OH)D, that reflects vitamin D exposure from all sources (diet, supplements, and sunlight) and uses graded retinal photographs to determine disease incidence and progression.

Only two other prospective studies[15,16] have examined the association between vitamin D status and incident AMD. Day et al.[15] have observed no association between vitamin D status, assessed from medical chart claims, and incident AMD determined from medical record codes. These results are consistent by stage of AMD (nonneovascular and neovascular); however, unlike our study, subtype of AMD (soft drusen or pigmentary abnormalities) was not investi-

Table 4. ORs and 95% CIs for Incident AMD (Early, Soft Drusen, Pigmentary Abnormalities) and Incident Late AMD by Tertiles of 25(OH)D ($n = 1225$): the ARIC Study

Model	Tertiles of 25(OH)D, nM, Range			*P* Trend*
	Tertile 1 (10.8–53.6)	Tertile 2 (53.7–69.3)	Tertile 3 (69.4–173.0)	
Incident, early AMD, $n = 1185$				
No. outcome/total No.	39/397	37/392	37/396	
Model 1†	1	0.66 (0.40–1.09)	**0.58 (0.35–0.96)**	0.22
Weighted‡	1	0.84 (0.47–1.48)	0.62 (0.34–1.14)	0.36
Large, soft drusen§, $n = 1165$				
No. outcome/total No.	28/392	28/387	24/386	
Model 1	1	0.69 (0.39–1.20)	**0.52 (0.28–0.93)**	0.18
Weighted	1	0.79 (0.41–1.51)	0.54 (0.27–1.09)	0.39
Large, soft drusen§, $n = 1139$ – further excluding those with both large, soft drusen and pigmentary abnormalities				
No. outcome/total No.	19/383	18/377	17/379	
Model 1	1	0.68 (0.35–1.35)	0.58 (0.29–1.17)	0.48
Weighted	1	0.79 (0.36–1.73)	0.58 (0.25–1.32)	0.63
Pigmentary abnormalities§, $n = 1131$				
No. outcome/total No.	14/378	15/374	17/379	
Model 1	1	0.73 (0.34–1.56)	0.68 (0.32–1.45)	0.49
Weighted	1	1.29 (0.56–2.99)	0.99 (0.40–2.44)	0.72
Incident late AMD, $n = 1225$				
No. outcome/total No.	8/408	11/409	7/408	
Model 1||	1	1.21 (0.47–3.07)	0.72 (0.25–2.05)	0.54
Weighted	1	1.62 (0.57–4.59)	1.27 (0.34–4.84)	0.96

Bolded values represent statistically significant results at a *P* value of < 0.05 or smaller.
* *P* for trend calculated by using season-adjusted serum 25(OH)D as a continuous variable.
† Model 1: adjusted for age, race, and smoking status.
‡ Inverse probability weights applied; $n = 2$ participants missing weights.
§ Large, soft drusen and pigmentary abnormalities were identified among cases of early incident AMD only. In analyses with large, soft drusen, cases of pigmentary abnormalities and no large, soft drusen were excluded. In analyses with pigmentary abnormalities, cases with large, soft drusen and no pigmentary abnormalities were excluded.
|| Model 1 for incident late AMD adjusted only for age and smoking status. The model stability did not hold when adjustment for race was applied.

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gated. Potential exposure and outcome misclassification may explain, in part, these null findings. The second study focuses on the association between vitamin D intake from foods and supplements with the 9.4-year development of advanced AMD among participants with early or intermediate AMD.[16] Although, Merle et al.[16] have found a protective association between dietary vitamin D intake and incident advanced AMD, these findings are not evident with intake from dietary plus supplemental sources of vitamin D, and no data are available on 25(OH)D in blood, allowing for the contribution of vitamin D from sunlight to be considered in the analysis.

Only a few studies[1,2,4,33] have specifically examined associations between vitamin D and measures of soft drusen. We previously have observed no evidence of a cross-sectional association between 25(OH)D and prevalent early AMD or prevalent soft drusen at ARIC visit 3.[33] The definition for soft drusen used in our previous analysis of visit 3 ARIC data differs from the definition used to define large, soft drusen in our current study (at least one soft drusen with a diameter $\geq$125 μm and a grid area >500 μm). The previous grading of visit-3 retinal photographs only identifies drusen larger than $\geq$63 μm and does not differentiate size beyond this threshold. Differently, Parekh et al.[1] have found a statistically significant lower risk of soft drusen with low 25(OH)D in a nationally representative sample, using a similar grading definition for soft drusen (at least one or more drusen >63 μm). Seddon et al.[4] also have found a statistically significant inverse association between dietary intake of vitamin D and drusen size in a study of twins with discordant AMD, and Millen et al.[2] have found an inverse but not statistically significant association with 25(OH)D and soft drusen in a cohort of postmenopausal women. It is possible that vitamin D mitigates development of drusen through its anti-inflammatory properties.

It is also possible that 25(OH)D concentrations are a marker for a healthy lifestyle and that residual confounding exits from such covariates as physical activity. We observed that the protective association of vitamin D on incident AMD remained in unweighted analyses adjusted for physical activity, but was removed after weights were applied. It is also possible that physical activity is a proxy measure for sunlight exposure and its inclusion in the model resulted in overadjustment.[39] We were limited by not having a physical activity measurement at the same point in time as when 25(OH)D was measured. Further, the extent to which physical activity is a known risk factor for AMD is still debated.[40]

Our results were consistent by race when comparing tertiles 3 and 1 for Caucasians and tertiles 3 and 2 combined to tertile 1 for African Americans. The availability of only six cases of AMD among African Americans limited our ability to estimate stable ORs for this subgroup and thus the results should be interpreted with caution.

In exploratory analyses, we did not observe effect modification by either high-risk AMD SNP. Notably, we did not see evidence of a stronger protective association in those with the CC genotype, like previous findings,[41] and the sample size of this study was too small to draw finite conclusions with respect to environment gene interactions. Interactions by *CFH* genotype do vary by single epidemiologic study (e.g., Assel et al.,[42] Ho et al.[43]), and this likely reflects the instability of such interactions in single samples. Larger, pooled studies will be needed to answer questions regarding effect modification by race and genotype adequately.

Limitations of our data included the availability of retinal photographs in only one eye at visit 3, resulting in the potential for misclassified incident disease (false negatives). Our bias analysis suggests that inclusion of possible misclassified cases would have led to slightly attenuated risk estimates. It is possible our findings were also biased by loss to follow-up or death between visit 3 and visit 5, or selection to have retinal photographs taken at visit 5. Even though application of sampling weights attenuated the point estimates in most instances, the attenuation was not extreme. The tradeoff for correcting for bias with sampling weights was the widening of our CIs. At this time, we cannot determine if a larger sample size with greater case number would lead to significant point estimates by increasing our precision and thus tightening our CIs.

Besides sun exposure, supplemental sources of vitamin D are one of the strongest determinants of circulating 25(OH)D concentrations[44]; however, the Age-Related Eye Disease Study (AREDS) supplement formulation does not contain vitamin D.[45] To help fill this current gap in knowledge, the ongoing Vitamin D and Omega-3 Trial (VITAL)[46] has an ancillary study to examine the influence of vitamin D and omega-3 fatty acid supplementation on AMD incidence and progression, which will provide valuable information to this line of inquiry. Supportive findings of a protective effect of vitamin D supplementation on AMD should provide evidence of a causal role of vitamin D deficiency in AMD risk, but null findings could be explained by the duration of the trial (on average 5 years of follow-up), dose of the vitamin D supplement used, or the starting nutrient status of study participants.[47] Blood measures for post hoc stratification analyses are only available in ~65% of the trial participants. Further, VITAL was not originally designed to study AMD and is relying on medical record–confirmed AMD, and also subject to possible false negatives in AMD outcome misclassification.

Additional analyses in other, larger, prospective studies are needed to better understand whether having adequate vitamin D status could be yet another lifestyle factor associated with reduced risk of AMD. Of course, randomized clinical trials will be needed to determine causality. In conclusion, we observed a protective association between 25(OH)D and risk for incident AMD, primarily early AMD. These findings suggest that one's previous 25(OH)D concentrations may influence the early pathology of AMD development but need confirmation in order to better understand the implication of a person's vitamin D status to clinical ophthalmology and public health.

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