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Vitamin D status and prevalent early age-related macular degeneration in African Americans and Caucasians: the Atherosclerosis Risk in Communities (ARIC) Study

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Abstract

Objective—Vitamin D status has been hypothesized to protect against development of early age-related macular degeneration (AMD) via its anti-inflammatory properties and its possible

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Conflict(s) of interest

Other co-authors had no conflicts of interest to disclose.

Access to Data Statement(s), Data Analysis, Methods, and Contribution of Authors

Dr. Amy Millen had full access to all of the data in the study and takes responsibility for the integrity for the data and the accuracy of the data analysis.

Contribution of authors: AEM, MJL, PLL, JAM, BEKK, KJM, CAA, RK obtained funding and designed the study. AEM directed analyses with MWS and JN conducting the analyses and aiding in data interpretation. AEM and MWS wrote the primary manuscript, with all co-authors aiding in the interpretation of the data analysis and drafting of the manuscript.

ETHICAL STANDARDS

All participants provided signed informed consent and the study protocol was approved by the institutional review boards at each ARIC study site and complies with the Helsinki Declaration as revised in 1983.

Reference to prior publication of the study in abstract form:

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beneficial influence on blood pressure control. We investigated the association between vitamin D status and prevalent early AMD in a community-based cohort.

Design—This was a cross-sectional study.

Setting—This was a secondary data analysis of already existing data from the Atherosclerosis Risk in Communities Study (ARIC) cohort study collected from 1990 to 1995.

Participants—There were 9,734 (7,779 Caucasians, 1,955 African American) ARIC participants (aged 46 to 70 at visit 2 [1990-1992]) with 25(OH)D data available at visit 2, AMD assessment at visit 3 (1993-1995), and complete covariate data.

Measurements—Vitamin D status was assessed with serum 25-hydroxyvitamin D (25(OH)D) concentrations from bloods drawn at visit 2. Prevalent, early AMD (n=511) was assessed at visit 3 (1993-95) with nonmydriatic retinal photographs of one randomly chosen eye. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for early AMD by categories of 25(OH)D in nmol/L (deficient <30, inadequate 30-<50, and two categories of adequate status: 50-<75 and ≥75). Linear trend was estimated using continuous 25(OH)D concentrations. ORs were adjusted for age, race, and smoking status. We further adjusted for hypertension status to examine if vitamin D status influenced early AMD via its effects on blood pressure. Exploratory analyses of effect modification by age, gender, race and high risk genotypes [Y402H complement factor H (*CFH*) rs1061170 and the A69S age-related maculopathy susceptibility 2 (*ARMS2*) rs10490924 polymorphisms] were conducted.

Results—The prevalence of early AMD was 5%, and 5% of participants were vitamin D deficient. The adjusted OR (95% CIs) for early AMD among those with adequate (≥75 nmol/L) compared to deficient (<30 nmol/L) vitamin D status was 0.94 (0.59-1.50), p-trend=0.86. Further adjustment for hypertension status did not influence results (OR [95% CI]=0.95 [0.59-1.52], p-trend=0.84). Results did not vary significantly by age, race, gender, early AMD subtype (soft drusen or retinal pigment epithelium depigmentation), *ARMS2* genotype, or *CFH* genotype in African Americans. Although the p for multiplicative interaction between 25(OH)D and *CFH* genotype was 0.06 in Caucasians, OR [95% CIs] for AMD in participants with 25(OH)D ≥50 compared to <50 nmol/L were similar in each *CFH* genotype and not statistically significant.

Conclusions—Vitamin D status was not associated with early AMD in this cohort sample.

Keywords

vitamin D; 25-hydroxyvitamin D; macular degeneration; retinal diseases; epidemiology; cohort studies

INTRODUCTION

Clinical trial data has shown that nutrients with antioxidant and anti-inflammatory properties (vitamins C, E, beta-carotene and zinc) protect against progression of age-related macular degeneration (AMD) from early to late disease (1). More remains to be learned about the influence of other nutrients, such as vitamin D, which has been hypothesized to reduce risk of AMD (2) because of its immune modulating, anti-inflammatory (3–5), and anti-

angiogenic properties (6); however it is relatively understudied with respect to AMD in cohort studies, particularly those with racial diversity.

Existing literature supports a protective association between prevalent AMD, either early or late, and vitamin D status, assessed with the blood biomarker 25-hydroxyvitamin D [25(OH)D] reflecting sunlight exposure as well as intake from foods and supplements (2, 7–10). However, not all studies support this association (11–14). Only three previous studies were conducted in racially/ethnically diverse samples, inclusive of non-Hispanic whites (2, 12), non-Hispanic blacks (2, 12), Mexican Americans (2), and Koreans (9), limiting our understanding of this association in racial/ethnic groups such as African Americans, who have one of the highest burdens of vitamin D deficiency (15). The 2011 Institute of Medicine's (IOM) report on the Dietary Reference Intakes (16) of vitamin D and calcium only supports a role for vitamin D in bone health stating not enough scientific evidence exists to make conclusions on vitamin D status in relation to other health outcomes. A better understanding of the role of vitamin D status in diseases with anti-inflammatory etiologies, such as AMD (17–19), is needed.

We examined the association between serum 25(OH)D concentrations and prevalent, early AMD assessed three-years later with non-mydriatic retinal fundus photographs among African American (n=1,955) and Caucasian (n=7,779) participants of the Atherosclerosis Risk in Communities (ARIC) Study, a well-characterized population-based cohort (20). We also explored the extent to which this potential association was modified by variants in high risk AMD genes. Only one previous study (21), conducted in post-menopausal women, the majority of whom were Caucasian, has investigated effect modification of the vitamin D and AMD association by high risk AMD genotype. We hypothesized that individuals with higher 25(OH)D concentrations would have lower odds of early AMD than participants with lower concentrations of 25(OH)D and that this association would be strongest in those with high genetic risk.

METHODS

Study Sample

The ARIC Study is a population-based, prospective study designed to investigate the causes and natural history of atherosclerotic diseases and variation in risk factors for heart disease (20). Participants (45 to 65 years at visit 1 [1987-1989]) were recruited from four centers: Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland. The present analyses use data collected at visits 1 (1987-1989), 2 (1990-1992) and 3 (1993-1995). The study sample for the proposed analyses consists of African American and Caucasian participants with gradable retinal fundus photographs taken at visit 3 and serum 25(OH)D measures assessed at visit 2.

There were 15,792 participants enrolled at visit 1, a total of 14,348 attended visit 2, of which 12,887 attended visit 3. We excluded 796 participants who did not consent for use of their data to study outcomes other than cardiovascular disease and an additional 1,255 participants who were missing data on AMD status (228 missing retinal photos and 1,027 with ungradable photos). Additional participants were excluded if they had prevalent late

AMD (n=14), were missing serum 25(OH)D values (n=1,018), were neither African American nor Caucasian (n=29), or were missing data on pertinent covariates (smoking, waist circumference or hypertension status) (n=221), leaving an analytic sample of 9,734. These exclusions were not mutually exclusive. Analyses of effect modification by high risk AMD genes had varying sample sizes (n<9,734) due to missing genetic data.

We compared characteristics of individuals included and excluded from these analyses. We compared those included (n=9,734) to those excluded but who attended visit 1 (n=5,192), and to those excluded but who attended visit 3 (n=2,257). In general excluded individuals had lower serum 25(OH)D concentrations, were older, more likely to be African American, less educated, and more likely to have risk factors for AMD. Although there was no statistically significant difference in prevalent AMD in the participants excluded with gradable fundus photographs but missing other relevant covariates, participants excluded were more likely to have self-reported having AMD.

At each visit, trained study personnel collected information on participants' demographic factors, personal and family health history, smoking behavior, medication use and other potential risk factors for cardiovascular disease (20). Participants had a clinic exam and blood draw (20). Prior to the visit, participants were asked to fast for twelve hours and to bring with them any medications or supplements taken within the past two weeks (20). For these analyses, all data, with the exception of information on education, income, and physical activity, were assessed at visit 2, corresponding in time to the assessment of serum 25(OH)D concentrations. Physical activity was assessed at visit 1 and 3 using a modified version (22) of the previously validated (23, 24) Baecke questionnaire from which we created a composite physical activity index score ranging from 0 (low overall physical activity) to 6.

Retinal Photography

At visit 3, prevalent early AMD was determined from a 45-degree nonmydriatic retinal photograph, taken with a Canon CR-45UAF nonmydriatic film camera (Canon USA, Itasca, IL) with nonpharmocological pupil dilution of one randomly selected eye (). The photograph was centered to include the optic disc and the macula () and graded for the presence of AMD at the University of Wisconsin Fundus Photograph Reading Center using a standard grading system for participants, the modified Arlie House classification scheme (). Early AMD was defined as presence of either soft drusen ≥63 μm in diameter or retinal pigment epithelium depigmentation, in the absence of advanced AMD (presence of geographic atrophy or choroidal neovascularization).

Assessment of 25(OH)D and other biomarkers

Fasting blood collected at visit 2 was used to assess serum 25(OH)D concentrations (sum of $25[OH]D_2$ and $25[OH]D_3$) using liquid chromatography in tandem with high-sensitivity mass spectrometry (Waters Alliance e2795; Waters, Milford, MA, USA) at the University of Minnesota Molecular Epidemiology and Biomarker Research Laboratory (Minneapolis, MN) (). Bloods drawn at visit 2 were stored at −80°C until analysis in 2012-2013 (). Differences in 25(OH)D concentrations due to season were accounted for using local

regression (). 25(OH)D was regressed on day of blood draw separately for African Americans and Caucasians. Residuals were added back to the sample mean (47.7 and 65.4 nmol/L for African Americans and Caucasians, respectively). These season-adjusted values were used in all analyses. Blood collected at visit 2 was also used to measure total plasma cholesterol, plasma triglyceride, low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol concentrations ().

Genetic data

Genetic data was available on two common, single nucleotide polymorphisms (SNPs) shown to be associated with increased risk of early AMD (): complement factor H (*CFH)* Y402H [rs1061170] and age-related maculopathy susceptibility gene 2 (*ARMS2)* A69S [rs10490924]. Genotyping of SNPs in ARIC was completed using the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Inc., Santa Clara, CA) (). *ARMS2* rs10490924 was directly genotyped from the Affymetrix array. *CFH* rs1061170 was imputed using the HapMap and 1000 Genomes reference panels as appropriate for Caucasians and African-Americans. For *ARMS2* rs10490924, the minor allele frequency and Hardy-Weinberg equilibrium was met. The imputation quality score was >0.8 for *CFH* rs1061170 in both the Caucasian and African-American datasets.

Statistical Analysis

Guided by the IOM (), vitamin D status was defined using 25(OH)D concentrations as deficient (<30 nmol/L) and inadequate (>30 to ≤50 nmol/L). We divided participants with adequate concentrations into two categories (>50 to <75 and ≥75 nmol/L) to better understand associations between vitamin D status and AMD at higher 25(OH)D concentrations (1 nmol/L=2.5 ng/mL). Participant characteristics and risk factors for AMD were examined by vitamin D status as well as by presence of early AMD (any early AMD versus none). Differences in characteristics by vitamin D or AMD status were tested using t-tests, ANOVAs or chi-square tests, as appropriate.

Logistic regression models were used to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs) for any prevalent early AMD by vitamin D status with the referent category of deficient status (<30 nmol/L). We explored associations between vitamin D status and early AMD. We estimated a p-for trend for early AMD using continuous 25(OH)D concentrations.

We examined different factors as possible confounders of the age-adjusted association between vitamin D status and AMD association. These included sex, race, health insurance status, smoking status, drinking status, ethanol intake, physical activity, body mass index (BMI), waist circumference, and blood lipid concentrations. Using a stepwise process, we identified confounders as covariates which changed the ORs ≥10%. A decision was made *a priori* to examine the multivariable model with and without adjustment for hypertension status() to examine whether vitamin D status influenced AMD through a potential influence on hypertension (),().

These associations were explored in strata defined by age group, sex and race. We also explored the association between vitamin D status and AMD by genotype of *ARMS2*

rs10490924 and *CFH* rs1061170, separately. Vitamin D status was dichotomized into two groups: deficient/inadequate [25(OH)D < 50 nmol/L] and adequate (25(OH)D ≥ 50 nmol/L]). The OR (95% CI) for AMD in participants with adequate compared to deficient/inadequate vitamin D status and the p-for trend for the odds of AMD across continuous concentrations of 25(OH)D were estimated among participants with each genotype. Multiplicative interactions between vitamin D status and age, race, sex, and genotype were tested by adding interaction terms to our logistic regression models. A p-value <0.10 for the interaction term was considered statistically significant.

Results

Five and twenty-five percent of the sample had deficient and inadequate vitamin D status, respectively (Table 1). Participants with adequate status were older, on average, than those with deficient or inadequate status. Participants with adequate status were also more likely to be men, Caucasian, and have less advanced education, but more intermediate education.

There were no differences in the prevalence of early AMD by vitamin D status (Table 2). Participants with adequate status compared to inadequate or deficient status were more likely to be former smokers, current drinkers, not obese, physically active, and not hypertensive. They were more likely to have higher mean HDL and triglyceride concentrations, lower LDL and glucose concentrations, and more likely to be current hormone users (if women).

There was no statistically significant association observed between vitamin D status and prevalent early AMD in the age adjusted model (Table 3). There were no potential confounders that, after adjustment with age, changed the OR 10% or more, however race and waist circumference change the OR the greatest percent (8 and 5%, respectively). Based on previous knowledge of AMD risk factors, we chose to adjust the model for age, race, smoking status, and waist circumference, after which, there was still no statistically significant association between vitamin D status and AMD. We chose to show the multivariable model with and without adjustment for waist circumference as this is also a strong determinant of circulating 25(OH)D concentrations and could lead to over-adjustment. Further adjustment for the proposed mediator of hypertension status also had no influence on the model. Exploratory analyses of vitamin D status and AMD were conducted using quintiles of serum 25(OH)D rather than the clinically utilized cut points. Results were consistent with the results presented in Table 3 (data not shown).

There was no association with 25(OH)D when we analyzed the data by AMD subtype (soft drusen or RPE depigmentation) (Table 3). There were no statistically significant interactions by age group, sex (data not shown), or race (Table 3). Similarly, there was no effect modification of the vitamin D and AMD association by *ARMS2* genotype (rs10490924) or by *CFH* genotype (rs1061170) in African Americans (Table 4). We did observe a statistically significant interaction between vitamin D status and *CFH* in Caucasians, but the ORs in each genotype strata were close to 1.0 and not statistically significant. Removal of one influential participant with a 25(OH)D serum value of 185 nmol/L removed the statistically significant interaction.

DISCUSSION

We observed no association between 25(OH)D concentrations and prevalent, early AMD assessed 3 years later in this community-based cohort of African Americans and Caucasians. These null associations were present regardless of age, sex, and race. Despite biological plausibility for a protective association between vitamin D status and AMD, associations between serum 25(OH)D and AMD across different study populations have been inconsistent. The body of literature, to date, consists of primarily cross sectional studies (,, , , ,), with one retrospective cohort study of incident AMD cases.

Data from three large cross-sectional studies (,,) have observed protective associations between 25(OH)D and AMD in the overall sample or in a subgroup, but taken together do not suggest that this potential association is limited to a particular subset of individuals. Data from a survey of US adults showed a protective association between 25(OH)D and early, but not late, AMD (n=7692) (). Similar results were observed in each racial-ethnic subgroup (non-Hispanic white, non-Hispanic black and Mexican American), however, the association was only statistically significant among non-Hispanic whites. Data from a nationally representative survey of Korean adults (n=17,045) () found no overall statistically significant association between 25(OH)D and any, early, or late AMD, but did find a statistically significant association with late AMD in men. A cohort study of postmenopausal women () (primarily Caucasian) observed a protective association between early AMD and 25(OH)D in a subset of women less than 75 years of age (n=913). In this study there was an interaction between 25(OH)D concentrations and *CFH* rs1061170 genotype (). Our current analyses in ARIC suggests no variation in the association between vitamin D status and genotype of certain high risk AMD genes.

Some studies suggest no association between vitamin D status and risk of AMD. A retrospective cohort of Medicare beneficiaries (n=13,932) () observed no association between Medicare claims for vitamin D deficiency and incident AMD diagnosis, even after stratification by race (Caucasian or African American) and presence of neovascular disease. Similar null results were observed in a cross-sectional analysis of members of a health maintenance organization (n=9,169) (). Both studies were likely subject to measurement error when classifying vitamin D and AMD status, perhaps biasing study findings toward the null. Medical diagnosis codes were used to identify AMD in both studies and vitamin D status was identified by either Medicare 5% claim files () or vitamin D tests conducted at clinical exams (). Although some studies have found null results, the data trend towards a protective association. Morrison et al. observed lower mean 25(OH)D concentrations in 50 individuals with neovascular AMD in at least one eye compared to a sibling without AMD (). No statistically significantly differences between groups were observed. Adjustment for factors other than month of blood draw were not conducted. Data from an elderly cohort of French individuals (n=697) also observed inverse, but not statistically significant, associations between 25(OH)D concentrations and any, early and late AMD (). Current data still remains inconclusive regarding associations between vitamin D and AMD.

A limitation of our current analysis includes the availability of a photograph of only one randomly chosen eye per participant, leaving the potential for outcome misclassification

(false negative). Although our grading of retinal photographs involved the use of reliable, standardized protocols (), it is also possible that our grading scale was not sufficiently refined, leading to measurement error in early AMD diagnosis. Graders identified drusen ≥63 μm in size, but differentiation of drusen sizes ≥125 μm was not conducted, making it impossible to currently differentiate between what is now considered the Age-Related Eye Disease Study (AREDS) category 2 (early) versus 3 (intermediate) (), with category 3 having a greater probability of progression than category 2 (). As we would not expect differential misclassification of AMD status by vitamin D status, the results would likely be biased towards the null. Our participants had early, not late AMD, and were relatively young (66% <60 years of age). Therefore, it is possible that vitamin D does not influence the development of early AMD, but rather later staged disease which we were unable to robustly assess.

The prevalence of vitamin D deficiency (25(OH)D <30 nmol/L) in ARIC was ~5%, comparable to the ~5% observed in a survey of US adults in the same time period (1988-94) and in which a protective association between 25(OH)D and early AMD was observed (), therefore our null findings are likely not explained by minimal between person variation in 25(OH)D concentrations. We suspect that our study had limited error in serum 25(OH)D assessment as we used quality control measures to ensure minimal laboratory variation across batches of samples sent for analysis.

Attrition from visit 2 to visit 3 may have attenuated our study findings as the comparison of individuals with and without retinal photographs showed that individuals who did not have retinal photographs were more likely to have characteristics predictive of AMD and lower vitamin D status (,) likely attenuating our results toward the null.

There is a lack of data about associations between vitamin D status and advanced AMD in robust samples. Although studies to date have examined associations between 25(OH)D and specifically late or neovascular AMD (,, , ,), findings have been inconsistent, and cases minimal (,, ,) or not specified (). A recent manuscript compared mean serum 25(OH)D between patients with neovascular AMD (n=146), non-neovascular AMD (n=216), and a control group of patients without AMD (n=100), found lower mean concentrations of 25(OH)D in neovascular AMD patients (). This previous study is the largest sample size to date comparing vitamin D status in people with neovascular AMD, but no multivariable analyses were conducted. Further, analyses presenting data stratified by subtype of AMD (drusen versus pigmentary abnormalities) also present mixed findings, leaving more research needed to better understand associations between 25(OH)D by AMD stage or subtype.

It is possible that no association exists between vitamin D status and AMD and that other studies that have observed protective associations are influenced by reverse causality, since individuals with AMD are less mobile, spending less time outside exposed to sunlight. However, our 25(OH)D measures were assessed 3 years prior to measures of AMD and 25(OH)D concentrations are thought to be relatively stable over time (). It is possible that vitamin D is a marker for a healthy lifestyle, and that prior significant findings occurred as a consequence of residual confounding. Challenges exist when controlling for some factors that determine vitamin D levels but may also be risk factors for AMD (body fatness and

physical activity) (). We did not have incident disease, but fewer individuals likely had AMD at visit 2, when 25(OH)D was assessed, than at 3 when retinal photographs were taken. Prospectively designed studies can help address the possibility of reverse causality, and if the evidence for a vitamin D and AMD association continued to grow, randomized clinical trials of vitamin D and eye health will allow for better control of confounding.

In conclusion, there were no associations between vitamin D status and prevalent AMD three years later in this community-based ARIC cohort which includes African Americans and Caucasians. These findings add to the inconsistent relationships of vitamin D status to AMD observed in the body of epidemiological evidence to date. Prospectively designed studies over longer periods of time and including a larger number of advanced AMD cases are needed to better assess the potential protective influence of adequate vitamin D status on AMD.

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Kristin Meyers' affiliation was with the University of Wisconsin during her efforts on this manuscript. As of February 2015, she has been an employee of Eli Lilly and Company and her efforts on this manuscript have been limited to critical review.

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Table 1

Demographic characteristics (n [%] or mean [standard deviation])[*] by vitamin D status of ARIC Study participants with gradable eye photo at visit 3 (1993-1995) and serum 25(OH)D concentrations at Visit 2 (1990-1992) (N=9,734)

	No. (%) of Participants by Vitamin D Status determined by 25(OH)D				p-value[†]
	Deficient (<30 nmol/L) (n=465)	Inadequate (30 to <50 nmol/L) (n=2,430)	Adequate (50 to <75 nmol/L) (n=4,437)	Adequate (≥75 nmol/L) (n=2,402)	
Age (years) [‡]	55 (5.5)	56 (5.6)	57 (5.7)	57 (5.7)	<0.001
Sex (% women)	358 (77%)	1,604 (66%)	2,314 (52%)	1,184 (49%)	<0.001
Race (% Caucasian)	211 (45%)	1,507 (62%)	3,781 (85%)	2,280 (95%)	<0.001
Education[§] **(%)**					<0.001
Basic or 0 years	80 (17%)	526 (22%)	812 (18%)	415 (17%)	
Intermediate	186 (40%)	1,007 (42%)	1,890 (43%)	1,071 (45%)	
Advanced	199 (43%)	891 (37%)	1,732 (39%)	911 (38%)	
Field center (%)					<0.001
Forsyth County, NC	103 (22%)	513 (21%)	1,199 (27%)	786 (33%)	
Jackson, MS	206 (44%)	795 (33%)	575 (13%)	110 (5%)	
Minneapolis, MN	77 (17%)	567 (23%)	1,389 (31%)	789 (33%)	
Washington County, MD	79 (17%)	555 (23%)	1,274 (29%)	717 (30%)	

[*] Characteristics assessed at Visit 2 unless otherwise noted.

[†] P-value for associated ANOVA or chi-square test for differences in characteristics by vitamin D status.

[‡] Spearman correlation coefficient for the correlation between season-adjusted serum 25(OH)D and age was 0.08 with a p-value of <0.001.

[§] Education defined at visit 1 as basic or 0 years (high school with no degree or less), intermediate (high school graduate or vocational school), or advanced (college or higher).

Table 2

Health and lifestyle **c**haracteristics (n [%] or mean [standard deviation])[*] by vitamin D status of ARIC Study participants with gradable eye photo at visit 3 (1993-1995) and serum 25(OH)D concentrations at Visit 2 (1990-1992) (N=9,734)

	No. (%) of Participants by Vitamin D Status determined by 25(OH)D				p-value	r[‡]
	Deficient (<30 nmol/L) (n=465)	Inadequate (30 to <50 nmol/L) (n=2,430)	Adequate (50 to < 75 nmol/L) (n=4,437)	Adequate (≥75 nmol/L) (n=2,402)		
Early AMD at Visit 3 (%)	23 (5%)	112 (5%)	237 (5%)	139 (6%)	0.313	§
Smoking status (%)					<0.001	§
Current	127 (27%)	577 (24%)	842 (19%)	431 (18%)		
Former	128 (28%)	830 (34%)	1,724 (39%)	1,066 (44%)		
Never	210 (45%)	1,023 (42%)	1,871 (42%)	905 (38%)		
Drinking status (%)					<0.001	§
Current	230 (50%)	1,262 (52%)	2,701 (61%)	1,541 (64%)		
Former	101 (22%)	532 (22%)	827 (19%)	414 (17%)		
Never	134 (29%)	634 (26%)	908 (21%)	447 (19%)		
Waist circumference (cm)	101.4 (17.4)	100.8 (15.6)	97.9 (13.5)	93.9 (12.3)	<0.001	−0.17‖
Waist to hip ratio	0.918 (0.083)	0.926 (0.080)	0.927 (0.081)	0.915 (0.084)	<0.001	−0.03¶
BMI category (%)					<0.001	−0.22‖
Under/normal weight (<25 kg/m^2)	115 (25%)	569 (24%)	1,346 (30%)	998 (42%)		
Overweight (25-30 kg/m^2)	151 (33%)	900 (37%)	1,855 (42%)	1,015 (42%)		
Obese (≥30 kg/m^2)	198 (43%)	957 (39%)	1,233 (28%)	388 (16%)		
Average physical activity index of visit 1 and visit 3##	2.4 (1.2)	2.6 (1.3)	2.9 (1.3)	3.2 (1.3)	<0.001	0.18‖
Diastolic blood pressure[**] **(mm Hg)**	74 (10.7)	73 (10.2)	72 (10.1)	71 (9.8)	<0.001	−0.06‖
Systolic blood pressure[**] **(mm Hg)**	123 (19.4)	122 (18.7)	120 (17.8)	119 (17.3)	<0.001	−0.08‖
Hypertension[**] **(% yes)**	198 (43%)	961 (40%)	1,446 (33%)	663 (28%)	<0.001	§
Total cholesterol (mg/dL) ##	208 (41.2)	209 (39.3)	210 (38.1)	209 (38.2)	0.654	0.002
HDL (mg/dL) ##	51 (16.9)	50 (16.3)	49 (16.1)	52 (17.7)	<0.001	0.02
LDL (mg/dL) ##	132 (38.7)	134 (36.9)	134 (35.5)	131 (35.7)	0.022	−0.02

	No. (%) of Participants by Vitamin D Status determined by 25(OH)D				p-value	r‡
	Deficient (<30 nmol/L) (n=465)	Inadequate (30 to <50 nmol/L) (n=2,430)	Adequate (50 to < 75 nmol/L) (n=4,437)	Adequate (≥75 nmol/L) (n=2,402)		
Triglycerides (mg/dL) ##	127 (97.7)	132 (76.2)	139 (88.2)	133 (76.2)	<0.001	0.02††
Glucose (mg/dL)	121 (58.8)	118 (46.4)	112 (38.5)	106 (25.6)	<0.001	−0.12‖
Hormone use (in women) (%)					<0.001	§
Current estrogen user	54 (18%)	223 (17%)	393 (20%)	309 (30%)		
Current estrogen and progestin user	24 (8%)	94 (7%)	213 (11%)	181 (17%)		
Never used hormones	211 (71%)	946 (71%)	1,235 (64%)	486 (47%)		
Former hormone user	10 (3%)	68 (5%)	104 (5%)	64 (6%)		
Health insurance (%yes)	391 (84%)	2,163 (89%)	4,168 (94%)	2,310 (96%)	<0.001	§
Prevalent coronary heart disease (%yes) §§	15 (3%)	129 (5%)	235 (5%)	137 (6%)	0.180	§
Prevalent stroke (%yes) §§	10 (2%)	40 (2%)	75 (2%)	26 (1%)	0.153	§

*Characteristics assessed at Visit 2 unless otherwise noted.

†P-value for associated ANOVA or chi-square test for differences in characteristics by vitamin D status.

‡Spearman correlation coefficient for the correlation between season-adjusted serum 25(OH)D and the respective continuous variable.

§Correlation coefficient not presented because characteristic was not a continuous variable.

‖Associated p-value for Spearman correlation coefficient <0.001.

¶Associated p-value for Spearman correlation coefficient <0.01.

**Diastolic and systolic blood pressure measures in mm Hg are an average of the second and third repeated blood pressure measures out of three. Hypertension is defined as the average systolic blood pressure ≥ 140 mm Hg, or diastolic ≥ 90 mm Hg, or high blood pressure medication use in the past 2 weeks.

††Associated p-value for Spearman correlation coefficient <0.05.

##Denotes a variable with missing data. The n's are provided for categorical data within the table above. For the following continuous variables the numbers of missing were as follows: 389 for average physical activity index, 18 for total cholesterol, 48 for HDL, 184 for LDL, 20 for triglycerides.

§§Prevalent coronary heart disease and stroke are defined in the ARIC Exam 2 Derived Variable Dictionary (42).

Table 3

ORs and 95% CIs for early AMD (any, soft drusen or RPE depigmentation) by vitamin D status among ARIC Study participants with gradable eye photos at visit 3 (1993-1995) and available serum 25(OH)D concentrations at Visit 2 (1990-1992) (N=9,734)

	Vitamin D status defined by serum 25(OH)D concentrations				
	Deficient <30 nmol/L	**Inadequate30 to <50 nmol/L**	**Adequate 50 to <75 nmol/L**	**Adequate ≥75 nmol/L**	**P for trend***
Season-adjusted 25(OH)D, mean (SD) (nmol/L)	24 (5.1)	42 (5.5)	62 (7.0)	89 (14.1)	
Early AMD at Visit 3 (%)	23 (5%)	112 (5%)	237 (5%)	139 (6%)	
Any early AMD					
Whole Sample					
# with AMD/# in category	23/465	112/2,430	237/4,437	139/2,402	
Age-adjusted model	1	0.87 (0.55-1.38)	0.97 (0.63-1.51)	1.04 (0.66-1.64)	0.42
Model 1†	1	0.84 (0.53-1.34)	0.90 (0.58-1.42)	0.94 (0.59-1.50)	0.86
Model 1 + waist circumference	1	0.85 (0.53-1.35)	0.93 (0.59-1.46)	0.99 (0.62-1.59)	0.58
Model 1 + hypertension status‡	1	0.84 (0.53-1.34)	0.91 (0.58-1.42)	0.95 (0.59-1.52)	0.84
Caucasians					
# with AMD/# in category	14/211	73/1507	210/3781	133/2280	
Age-adjusted model	1	0.71 (0.39-1.29)	0.80 (0.46-1.41)	0.83 (0.47-1.48)	0.84
Model 1	1	0.72 (0.39-1.30)	0.80 (0.46-1.41)	0.83 (0.47-1.47)	0.89
African Americans					
# with AMD/# in category	9/254	39/923	27/656	6/122	
Age-adjusted model	1	1.13 (0.54-2.37)	1.07 (0.49-2.31)	1.26 (0.44-3.64)	0.81
Model 1	1	1.12 (0.53-2.34)	1.06 (0.49-2.29)	1.22 (0.42-3.55)	0.84
Soft Drusen ‡					
Whole Sample					
# with AMD/# in category	22/464	100/2418	190/4390	122/2385	
Age-adjusted model	1	0.81 (0.51-1.30)	0.82 (0.52-1.29)	0.95 (0.60-1.52)	0.58
Model 1	1	0.80 (0.50-1.29)	0.79 (0.50-1.26)	0.91 (0.56-1.48)	0.77
RPE Depigmentation ‡					
Whole Sample					

	Vitamin D status defined by serum 25(OH)D concentrations				
	Deficient <30 nmol/L	**Inadequate30 to <50 nmol/L**	**Adequate 50 to <75 nmol/L**	**Adequate ≥75 nmol/L**	**P for trend***
# with AMD/# in category	24/2784§		63/4263	27/2290	
Age-adjusted model	1		1.64 (1.02-2.63)	1.28 (0.73-2.22)	0.64
Model 1	1		1.27 (0.79-2.06)	0.90 (0.51-1.59)	0.37

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

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

‡Hypertension status is defined as in table 2.

‡The total n for this analysis does not equal that for the whole sample. For the soft drusen analyses, individuals with early AMD and no soft drusen were excluded. For the RPE depigmentation analysis, individuals with AMD and no RPE depigmentation were excluded.

§Due to the small number of outcomes of individuals with RPE among those with 25(OH)D <30 nmol/L, this category was combined with the ≥ 30 to <50 nmol/L category to create a referent group.

Table 4

Adjusted[*] ORs and 95% CIs for early AMD by vitamin D status and genotype[†] among ARIC Study participants with gradable eye photo at visit 3 (1993-95), and available serum 25(OH)D concentrations Visit 2 (1990-92) (N=8,201[‡])

	Total n	AMD cases (%)	Deficient/Inadequate (25[OH]D <50 nmol/L)	Adequate (25[OH]D ≥50 nmol/L)	P for trend[§]	p-value for interaction[‖]
***CFH* (rs1061170)**						
Caucasians (n=6,563)						
TT (noncarrier)	2,733	107 (4%)	1.0	0.95 (0.59-1.53)	0.57	0.15[¶]
CT	2,978	158 (5%)	1.0	1.15 (0.77-1.72)	0.49	
CC	852	79 (9%)	1.0	1.04 (0.58-1.87)	0.12[¶]	
African Americans (n=1,470)						
TT (noncarrier)	542	22 (4%)	1.0	0.59 (0.24-1.50)	0.31	0.26
CT	755	28 (4%)	1.0	1.37 (0.64-2.93)	0.17	
CC	173	13 (8%)	1.0	1.04 (0.33-3.30)	0.77	
***ARMS2* (rs10490924)**						
Caucasians (n=6,563)						
GG (noncarrier)	4,048	196 (5%)	1.0	1.20 (0.83-1.73)	0.39	0.30
TG	2,223	114 (5%)	1.0	0.96 (0.61-1.52)	0.53	
TT	292	34 (12%)	1.0	0.83 (0.36-1.92)	0.97	
African Americans (n=1,638)						
GG (noncarrier)	965	40 (4%)	1.0	0.85 (0.44-1.64)	0.71	0.78
TG	582	21 (4%)	1.0	1.01 (0.42-2.45)	0.74	
TT	91	7 (8%)	1.0	1.23 (0.25-6.07)	0.86	

[*] Odds ratios adjusted for age and smoking status.

[†] Presented as heterozygous/homozygous carriers or noncarriers for the high risk alleles.

[‡] The sample size is less than 9,734 due to missing genetic data.

[§] P for trend calculated using serum 25(OH)D as a continuous variable.

[‖] P for interaction calculated using serum 25(OH)D as a continuous variable and genotype as an ordinal variable.

¶This p for trend in the CC genotype category and the p for interaction were estimated after removal of one influential participant with a serum 25(OH)D concentration of 185 nmol/L. All data presented in the table, with the exclusion of the p for trend in the CC genotype strata and the p for interaction includes this individual. The p for trend in the CC category is 0.03 and the p for interaction is 0.06 when this participants is included.