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Prospective Study of Plasma Homocysteine Level and Risk of Age-related Macular Degeneration in Women

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Abstract

Purpose—Prospective data to examine the association of homocysteine and age-related macular degeneration (AMD) are limited. We examined the prospective relation of plasma homocysteine level and AMD in a large cohort of apparently healthy women.

Methods—We evaluated the relationship between baseline levels of plasma homocysteine and incident AMD among 27,479 female health professionals aged 40 years or older. Main outcome measures were total AMD, defined as a self-report documented by medical record evidence of an initial diagnosis after randomization, and visually significant AMD, defined as confirmed incident AMD with visual acuity of 20/30 or worse attributable to this condition.

Results—During an average of 10 years of follow-up, a total of 452 cases of AMD, including 182 cases of visually-significant AMD, were documented. Women in the highest versus lowest quartile of plasma homocysteine had modestly, but statistically non-significant, increased risks of total (hazard ratio [HR], 1.24; 95% confidence interval [CI], 0.95–1.63; p for trend, 0.07) and visually-significant AMD (HR, 1.41; 95% CI, 0.92–2.17; p for trend, 0.052) in age- and treatment-adjusted analyses.

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Dr. Christen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analyses.

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Conclusions—These prospective data from a large cohort of apparently-healthy women do not support a strong role for homocysteine in AMD occurrence.

Keywords

age-related macular degeneration; homocysteine; prospective cohort; risk factor; Women's Health Study

Introduction

Age-related macular degeneration (AMD) is a degenerative condition of the central retina responsible for high resolution vision. An estimated 9 million U.S. adults exhibit signs of AMD.¹ This includes 1.75 million individuals with advanced AMD (ie, geographic atrophy or neovascular AMD), which accounts for most cases of severe vision loss.¹ An additional 7.3 million persons have early AMD,¹ which is usually associated with little or no vision loss,² but increases the risk of developing advanced AMD.³ In spite of its common occurrence, however, information on risk factors is limited and its pathogenesis remains unclear.

AMD and cardiovascular disease (CVD) are postulated to share similar underlying mechanisms and risk factors.⁴ One possible risk factor is elevated plasma homocysteine which has been linked to increased vascular inflammation and endothelial dysfunction in basic research studies,⁵ and has been associated with increased risks of CVD in numerous observational studies.⁶ Similarly, results of most observational studies, primarily cross-sectional and case-control, suggest a positive association between elevated plasma homocysteine and risk of AMD, particularly advanced AMD.^{7–19} However, the temporal relationship between exposure and disease can be difficult to determine in cross-sectional and case-control studies, and thus it is unclear whether elevated homocysteine in these studies is a potential cause of AMD, or simply a consequence of AMD and its underlying mechanisms. Prospective data can provide more reliable evidence to evaluate a possible causal link between homocysteine and AMD, but are limited to a single report from Australia.¹⁰

Herein, we examine the prospective relationship of plasma homocysteine and AMD among 27,479 participants in the Women's Health Study (WHS) who were free of a reported diagnosis of AMD at baseline.

Methods

Study design

Study participants were drawn from the WHS, a completed randomized trial of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer among 39,876 apparently healthy female health professionals aged 45 years and older, who did not have a history of cardiovascular disease, cancer (except nonmelanoma skin cancer), or other major illnesses at study entry.^{20–22} Blood samples were collected from 28,345 (71%) women at baseline. All women completed baseline questionnaires providing information on height, weight, cigarette smoking, history of alcohol use, blood pressure level, cholesterol level,

history of diabetes, history of multivitamin use, post-menopausal hormone use, and history of an eye exam in the last two years. Women also provided information on a personal history of AMD. A total of 27,479 women who provided a baseline blood sample and were free of a reported diagnosis of AMD at baseline are included in these analyses. Informed consent was obtained from all participants. This research was performed according to the guidelines of the Declaration of Helsinki, and the research protocol was reviewed and approved by the institutional review board at Brigham and Women's Hospital in Boston. This study is registered at clinicaltrials.gov (NCT00000161).

Ascertainment and definition of endpoints

Women who reported a prior diagnosis of AMD at baseline were excluded. On annual questionnaires, women were asked about new diagnoses in the past year including "macular degeneration right eye" and "macular degeneration left eye". Women who responded affirmatively were asked to provide the month and year of the diagnosis and to complete a consent form granting permission to examine medical records pertaining to the diagnosis. Ophthalmologists and optometrists were contacted by mail and asked to complete an AMD questionnaire that requested information on the date of initial diagnosis, the best-corrected visual acuity at the time of diagnosis, and the date when best-corrected visual acuity reached 20/30 or worse (if different from the date of initial diagnosis). The questionnaire also asked about signs of AMD observed (drusen, retinal pigment epithelium [RPE] hyperpigmentation/hypopigmentation, geographic atrophy, RPE detachment, subretinal neovascular membrane, or disciform scar) when visual acuity was first noted to be 20/30 or worse, and the date when exudative neovascular AMD, if present, was first noted (defined by presence of RPE detachment, subretinal neovascular membrane, or disciform scar). If ocular abnormalities were noted that could explain or contribute to vision loss, the ophthalmologist or optometrist was asked to indicate whether the AMD, by itself, was significant enough to cause the best-corrected visual acuity to be reduced to 20/30 or worse. Ophthalmologists/optometrists could also provide the requested information by supplying photocopies of the relevant medical records. Medical record data were obtained for 85.2% of participants reporting AMD.

Two endpoints were defined: 1) total AMD, defined as a self-report confirmed by medical record evidence of an initial diagnosis after randomization but before March 31, 2004, the scheduled end of the trial, and 2) visually-significant AMD, defined as above, but with best-corrected visual acuity loss to 20/30 or worse attributable to AMD.

Homocysteine measurement

Blood samples were obtained at baseline and stored in vapor phase liquid nitrogen (-170°C) until analysis. The concentration of homocysteine was determined using an enzymatic assay on the Hitachi 917 analyzer (Roche Diagnostics) using reagents and calibrators from Catch, Inc. Blood samples were blinded and randomly analyzed in triplicate to minimize systematic bias and interassay variation. The sensitivity of the homocysteine assay is $0.24\text{ }\mu\text{mol/L}$, and its coefficient of variation is 1.8% at $15.6\text{ }\mu\text{mol/L}$.

Data analysis

Participants were followed up beginning at the time of randomization and continuing until the initial diagnosis of AMD, death, or March 31, 2004, whichever came first. Participants were categorized into quartiles of plasma homocysteine concentration. We examined the distribution of baseline characteristics that were possible risk factors for AMD according to homocysteine quartiles. P-values for statistical comparisons across quartiles were obtained from Cochran-Mantel Haenszel test for trend for categorical variables and linear regression for continuous variables expressed as medians. Cumulative probabilities of total and visually-significant AMD were calculated according to homocysteine quartiles. Cox proportional hazards regression models were used to calculate hazard ratios (HR) and 95% confidence intervals (95% CIs) according to homocysteine quartiles. We used the median value for each quartile to perform tests for trends across quartiles. Initial models adjusted for age (continuous) and randomized treatment assignment (aspirin, vitamin E, beta carotene). We then adjusted the models for smoking, alcohol use, body mass index (BMI), menopausal status and use of hormone therapy (HT), history of hypertension, history of hyperlipidemia, current multivitamin use, and history of eye exam in the last 2 years. We also examined whether any association of homocysteine and AMD differed among those who did and did not report current multivitamin use at baseline. P for interaction was calculated by including a product term for log-transformed homocysteine and multivitamin use at baseline in Cox models. Finally, we analyzed two cut-points used in prior studies to mark the hyperhomocysteinemic range ($\geq 15 \mu\text{mol/L}$, 95th percentile).²³

Individuals, rather than eyes, were the unit of analysis because eyes were not examined independently, and participants were classified according to the status of the worse eye as defined by disease severity.²⁴ When the worse eye was excluded because of visual acuity loss attributed to other ocular abnormalities, the fellow eye was considered for classification.

Results

In this cohort of apparently healthy women, median (interquartile range [IQR]) plasma homocysteine level was 10.5 (8.7, 12.9) $\mu\text{mol/L}$. Table 1 shows the distribution of baseline characteristics according to quartiles of plasma homocysteine. Women with higher homocysteine levels were older, smoked more and were more likely to use alcohol. They also weighed more, exercised less, and were more likely to report high blood cholesterol and hypertension, but less likely to report hormone use and use of multivitamins. These women also were less likely to report having had an eye exam in the past two years.

During a mean follow-up of 10 years, a total of 452 cases of AMD, including 182 cases of visually-significant AMD were documented. Most AMD cases were characterized by some combination of drusen and RPE changes indicating an early stage of disease development. Cumulative incidence rates of AMD according to year of follow-up are shown in Figures 1 and 2. For both total (Figure 1) and visually-significant AMD (Figure 2), cumulative incidence varied significantly among the four homocysteine groups ($p < 0.001$ for each). In age- and treatment-adjusted analyses, the risk of total AMD for women in the lowest to highest quartiles of homocysteine at baseline were 1.00 (referent), 1.02, 1.03, and 1.24 (p for trend = 0.07) (Table 2). Adjustment for other AMD risk factors slightly attenuated the HRs.

For visually-significant AMD, the age- and treatment-adjusted HRs for increasing quartiles of homocysteine level were 1.00 (reference), 1.04, 0.90, and 1.41 (p for trend = 0.052) (Table 3). HRs were attenuated after adjustment for other AMD risk factors.

The association of homocysteine and AMD appeared to vary according to reported multivitamin use at baseline. Among current non-users of multivitamins, increasing level of homocysteine was significantly associated with age- and treatment-adjusted risks of total (p for trend <0.0001) and visually-significant AMD (p for trend <0.001) (Table 4). Adjustment for other AMD risk factors slightly attenuated HR estimates but the trends remained significant (total AMD, p for trend <0.001; visually-significant AMD, p for trend <0.01). Among multivitamin users, there was no significant association with either AMD endpoint in age- and treatment-adjusted or fully-adjusted models. Tests for interaction between the association of homocysteine and AMD and multivitamin use were significant for total AMD (age- and treatment-adjusted, $p=0.003$; fully-adjusted, $p=0.006$) and borderline significant for visually-significant AMD (age- and treatment-adjusted, $p=0.08$; fully-adjusted, $p=0.10$).

We also examined two cut-points used in prior studies to mark the hyperhomocysteinemic range.²³ Woman with homocysteine level >15.0 $\mu\text{mol/L}$, as compared to women at or below this level, had a statistically non-significant 14% increased risk of total AMD (HR, 1.14; 95% CI, 0.89–1.46; $p = 0.30$). The HR was further reduced after adjustment for other AMD risk factors (HR, 1.09, 95% CI, 0.84–1.41; $p = 0.52$). For visually-significant AMD, woman with homocysteine level >15.0 $\mu\text{mol/L}$ had a significant 43% increased risk of AMD (HR, 1.43; 95% CI, 1.01–2.04; $p = 0.046$) in analyses adjusted for age and treatment assignment. Further adjustment for other AMD risk factors reduced the HR estimate to 1.25 (95% CI, 0.86–1.82; $p = 0.23$) and it was no longer significant. When we compared women above the 95th percentile for homocysteine level (19.0 $\mu\text{mol/L}$) to those at or below this level, HRs were somewhat higher, though non-significant, for both total (HR, 1.26; 95% CI, 0.87–1.81; $p = 0.22$) and visually-significant AMD (HR, 1.61; 95% CI, 0.97–2.65; $p = 0.06$) after adjustment for age and treatment assignment. HRs were reduced after adjustment for other AMD risk factors (total AMD: HR, 1.15; 95% CI, 0.79–1.69, $p = 0.46$; visually-significant AMD: HR, 1.38; 95% CI, 0.82–2.33; $p = 0.23$).

Discussion

In these prospective data from a large cohort of apparently-healthy female health professionals with no prior report of AMD and 10 years of follow-up, elevated plasma homocysteine level at baseline was associated with a modest, but statistically non-significant, increased risk of total and visually-significant AMD after adjustment for AMD risk factors. These findings do not support a major role for elevated plasma homocysteine as an independent risk factor for development of AMD in apparently healthy women.

Previous observational data have derived primarily from cross-sectional and case-control studies. Two cross-sectional studies indicated higher homocysteine levels in persons with advanced AMD (either neovascular AMD or geographic atrophy),^{8,15} while a third study, based on data from the National Health and Nutrition Examination Survey (NHANES), indicated no significant association between homocysteine and either early or late AMD (not

otherwise defined).¹¹ However, there were only 16 cases of late AMD in the NHANES study, and differences in the fasting state among participants may have introduced an additional source of variability in homocysteine concentration. Of nine case-control studies, seven indicated higher homocysteine levels in AMD cases than in matched controls.^{7,9,12–14,16,17} Cases were primarily patients with advanced AMD, either neovascular AMD^{7,9,12–14,16,17} or geographic atrophy,^{13,17} and the mean homocysteine level was typically 3–10 $\mu\text{mol/L}$ higher than the mean level in matched controls which ranged between 8.7 – 10.7 $\mu\text{mol/L}$. Two other case-control reports, based on data from the Blue Mountains Eye Study, indicated no significant difference in homocysteine level between AMD cases and controls.^{18,19} However, the case group in these two reports included both early and late AMD patients, and the mean homocysteine level in controls was markedly higher (12.8 $\mu\text{mol/L}$ ¹⁹ and 13.5 $\mu\text{mol/L}$ ¹⁸) than the level reported for controls in other case-control studies of AMD. Thus, the overall evidence from prior cross-sectional and case-control studies is broadly consistent with a positive association between elevated homocysteine level and risk of AMD, primarily advanced AMD.

Previous prospective data are limited to a recent report from the Blue Mountains Eye Study (BMES). Gopinath and colleagues examined the association of serum homocysteine, folate, and vitamin B₁₂ and 10 year incidence of AMD in an Australian cohort of 1,760 men and women aged 55 years and older.¹⁰ In analyses adjusted for age, sex, current smoking, white blood cell count, and fish consumption, each 1 standard deviation increase in serum homocysteine was associated with a modest 33% increased risk of early (OR, 1.33; 95% CI, 1.11 to 1.60) and any AMD (OR, 1.33; 95% CI, 1.09 to 1.63). There was no significant association with late AMD (geographic atrophy or neovascular AMD) (OR, 1.25; 95% CI, 0.93 to 1.69) in that study, although the number of cases was small (n=57).

Our findings in WHS appear broadly consistent with the prospective findings from the BMES. Both studies reported a modest increased risk of early AMD for elevated homocysteine levels, although in our study the trend was not significant. The BMES further reported no significant association with late AMD, while we had too few cases of late AMD (n=46) for analyses. Thus, available data from two prospective studies do not support a strong role for homocysteine in AMD occurrence. The prospective design of WHS and BMES precludes the possibility that AMD itself may have altered homocysteine levels at baseline. On the other hand, the elevated homocysteine levels observed for AMD cases in previous cross-sectional and case-control studies may have been distorted by the AMD disease process, and thus may have been a consequence of AMD, rather than a cause of the disease.

Alternatively, if elevated homocysteine is causally linked to AMD, we may have failed to observe an association for several reasons. Evidence from cross-sectional and case-control studies suggests an association primarily with advanced AMD, either neovascular AMD or geographic atrophy, and we had too few cases of advanced AMD to conduct meaningful analyses. It is also possible that the overall distribution of homocysteine levels in the generally healthy WHS population was insufficiently elevated to materially affect AMD occurrence. Our observation in subgroup analyses that homocysteine level was significantly associated with AMD among women who did not report multivitamin use at baseline, but

not among women who did, is consistent with this possibility; multivitamin non-users had higher median [IQR] homocysteine levels (11.1 [9.3, 13.6] $\mu\text{mol/L}$) than did multivitamin users (9.5 [8.0, 11.5] $\mu\text{mol/L}$). The further observation that HRs for visually-significant AMD increased as the cut-point marking the hyperhomocysteinemic range was increased (eg. age- and treatment-adjusted HRs: $>15.0 \mu\text{mol/L}$, 1.43, $p = 0.046$; $>19.0 \mu\text{mol/L}$, 1.61, $p = 0.06$) also appears consistent with this possibility.

Several limitations of our study need to be considered. Our analysis was based on a single baseline measure of homocysteine level; therefore, we were unable to account for changes in homocysteine level over time that may have occurred through medical treatment, changes in dietary habits, or folic acid fortification of the U.S. food supply²⁵ begun after blood collection in the WHS. Stability of blood samples during freezing is also a concern. However, prior work from other prospective studies has shown that correlations over time for several biochemical tests tend to be very stable with little or no sign of deterioration during storage.²⁶ Our study endpoint was based on participant self-report, thus, some degree of underascertainment of AMD is plausible, particularly for early AMD. However, underascertainment of disease does not bias prospective, non-randomized comparisons if specificity is high.^{27,28} High specificity of our case ascertainment for AMD has been demonstrated previously.^{29,30} Random misclassification of AMD would tend to shift the RR estimate toward the null, but was reduced by the use of medical records to confirm the self-reports. Surveillance bias was also a possibility since women with higher homocysteine levels were somewhat less likely to report an eye exam in the past 2 years, and thus may have been less likely to have existing AMD diagnosed. The likely effect of such bias would be to underestimate the true association between elevated homocysteine and AMD. We attempted to control for possible surveillance bias by including a term for a baseline report of an eye exam in the past 2 years in multivariate analyses

The demonstration of a causal link between homocysteine and AMD would have important public health implications. We recently reported that randomized treatment with folic acid, vitamin B₆, and vitamin B₁₂ for 5 years in the Woman's Antioxidant Folic Acid Cardiovascular Study (WAFACS) reduced the risk of total and visually-significant AMD by 35–40%.³¹ Additional analyses in a subsample of WAFACS participants ($n=300$) showed that homocysteine levels in the treated group were reduced by 18.5%.³² However, whether the reduced risk of AMD observed in WAFACS can be attributed to the homocysteine-lowering effect of treatment remains unclear. The present findings from WHS do not support an important causal connection between homocysteine and AMD in apparently healthy women. However, the WAFACS population was comprised of women with pre-existing CVD or 3 or more CVD risk factors, and thus was a higher risk population than that in the WHS. In the WAFACS subsample, median (IQR) homocysteine level was 12.3 (10.0, 15.0) $\mu\text{mol/L}$, compared to 10.5 [8.7, 12.9] $\mu\text{mol/L}$ in WHS, and an estimated 27.7% of participants in WAFACS had baseline homocysteine level $>15.0 \mu\text{mol/L}$ as compared with 13.2% of WHS participants. Analyses are ongoing to assess whether baseline homocysteine level predicts subsequent AMD in WAFACS. Such a finding could explain, at least in part, the beneficial effect of B vitamin supplementation on AMD in that high risk population.

In summary, our prospective data indicated modest and statistically non-significant increased risks of AMD for elevated levels of homocysteine and thus do not support an important role for elevated homocysteine as an independent risk factor for AMD in apparently healthy women.

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Figure 1. Cumulative incidence rates of total age-related macular degeneration (AMD) according to plasma homocysteine quartile in the Women’s Health Study. Abbreviations: AMD, age-related macular degeneration; Hcy, homocysteine; y, years.

Visually-significant AMD

Figure 2. Cumulative incidence rates of visually-significant age-related macular degeneration (AMD) according to plasma homocysteine quartile in the Women’s Health Study. Abbreviations: AMD, age-related macular degeneration; Hcy, homocysteine; y, years.

Table 1

Baseline Characteristics According to Quartile of Baseline Plasma Homocysteine in the Women's Health Study.*

Characteristics	Quartile of Plasma Hcy (μmol/L)					p-trend
	1 (1.56–8.70)	2 (8.71–10.46)	3 (10.47–12.88)	4 (12.89–166.36)		
Median (μmol/L)	7.7	9.6	11.5	15.2		
Mean age (y)	53.5	54.5	55.0	55.4		<.0001
Current smoking						
Current	6.3	9.7	12.0	18.6		<.0001
Past	35.6	36.9	37.7	36.4		
Never	58.1	53.4	50.3	45.1		
Alcohol use						
1 drinks/day	7.6	9.6	10.7	13.4		<.0001
1–6 drinks/week	32.6	33.4	32.6	30.6		
1–3 drinks/month	13.7	13.7	13.0	12.7		
Rarely/never	46.1	43.3	43.7	43.4		
Body mass index (kg/m ²)	25.5	25.8	26.2	26.2		<.0001
Diabetes mellitus	2.6	2.1	2.3	2.6		.83
Menopausal status/use of HRT						
Premenopausal	33.6	28.1	25.4	24.0		<.0001
Uncertain	19.2	18.2	17.2	18.1		
Postmenopausal/current HRT	33.4	33.3	31.3	26.3		
Postmenopausal/no HRT	13.7	20.4	26.1	31.6		
Hypertension [†]	21.6	23.5	25.4	29.2		<.0001
Hyperlipidemia [‡]	28.1	28.9	30.7	30.7		<.0001
Current multivitamin use	54.0	40.4	30.5	22.8		<.0001
Eye exam in past 2 years	85.4	83.4	82.6	82.1		<.0001

Abbreviations: Hcy, homocysteine; HRT, hormone replacement therapy.

* Data are given as the percentage of participants, unless otherwise indicated. Percentages may not total 100 because of rounding.

[†] Hypertension was defined as a systolic blood pressure of at least 140 mmHg, a diastolic blood pressure of at least 90 mmHg, or self-reported physician-diagnosed hypertension.[‡] Hyperlipidemia was defined as a total cholesterol of at least 240 mg/dl or self-reported physician-diagnosed high cholesterol levels.

Table 2

HRs and 95% CIs for diagnosis of total AMD according to quartiles of baseline plasma homocysteine.

Hcy quartile (median, $\mu\text{mol/L}$)	No. of participants	No. with AMD	Model 1 [*] HR (95% CI)	Model 2 [†] HR (95% CI)
Quartile 1 (7.7)	6898	86	1.0 (ref)	1.0 (ref)
Quartile 2 (9.6)	6843	104	1.02 (0.77–1.36)	1.00 (0.74–1.33)
Quartile 3 (11.5)	6874	116	1.03 (0.78–1.37)	1.03 (0.77–1.38)
Quartile 4 (15.2)	6864	146	1.24 (0.95–1.63)	1.23 (0.92–1.63)
P for trend			0.07	0.09

Abbreviations: HR, hazard ratio; CI, confidence interval; Hcy, homocysteine.

^{*} Adjusted for age and vitamin E, aspirin, and beta carotene treatment assignment.

[†] Adjusted for age, randomized treatment assignment, smoking (current, past, never), alcohol use (rarely/never, 1–3 drinks/month, 1–6 drinks/week, and 1 drinks/day), BMI (continuous), menopausal status and use of HT (premenopausal, uncertain, postmenopausal and current HT, postmenopausal and no HT), history of hypertension (ever diagnosis by physician or self-reported blood pressure 140/90; yes or no), history of hyperlipidemia (baseline history of cholesterol-medication use or a physician diagnosis of high cholesterol or a self-reported cholesterol of at least 240 mg/dL; yes or no), multivitamin use (current, past/never), history of eye exam in the last 2 years.

Table 3

HRs and 95% CIs for diagnosis of visually-significant AMD according to quartiles of baseline plasma homocysteine.

Hcy quartile (median, $\mu\text{mol/L}$)	No. of participants	No. with AMD	Model 1 [*] HR (95% CI)	Model 2 [†] HR (95% CI)
Quartile 1 (7.7)	6898	31	1.0 (ref)	1.0 (ref)
Quartile 2 (9.6)	6843	41	1.04 (0.65–1.66)	0.97 (0.61–1.56)
Quartile 3 (11.5)	6874	41	0.90 (0.56–1.44)	0.81 (0.50–1.31)
Quartile 4 (15.2)	6864	69	1.41 (0.92–2.17)	1.21 (0.77–1.90)
P for trend			0.052	0.25

Abbreviations: HR, hazard ratio; CI, confidence interval; Hcy, homocysteine.

^{*} Adjusted for age and vitamin E, aspirin, and beta carotene treatment assignment.

[†] Adjusted for age, randomized treatment assignment, smoking (current, past, never), alcohol use (rarely/never, 1–3 drinks/month, 1–6 drinks/week, and 1 drinks/day), BMI (continuous), menopausal status and use of HT (premenopausal, uncertain, postmenopausal and current HT, postmenopausal and no HT), history of hypertension (ever diagnosis by physician or self-reported blood pressure $\geq 140/90$; yes or no), history of hyperlipidemia (baseline history of cholesterol-medication use or a physician diagnosis of high cholesterol or a self-reported cholesterol of at least 240 mg/dL; yes or no), multivitamin use (current, past/never), history of eye exam in the last 2 years.

HRs and 95% CIs for Total and Visually-Significant AMD According to Plasma Homocysteine Quartiles and Reported Multivitamin Use at Baseline.*

Table 4

Multivitamin Users (n=10,011)				Multivitamin Non-users (n=17,098)			
<u>Hcy quartile (Median, $\mu\text{mol/L}$)</u>	<u>Model 1[†]</u> <u>HR (95% CI)</u>	<u>Model 2[‡]</u> <u>HR (95% CI)</u>	<u>Hcy quartile (Median, $\mu\text{mol/L}$)</u>	<u>Model 1[†]</u> <u>HR (95% CI)</u>	<u>Model 2[‡]</u> <u>HR (95% CI)</u>		
<u>Total AMD</u>							
Quartile 1 (7.1)	1.00 (referent)	1.00 (referent)	Quartile 1 (8.2)	1.00 (referent)	1.00 (referent)		
Quartile 2 (8.7)	1.49 (0.97–2.28)	1.47 (0.95–2.27)	Quartile 2 (10.2)	1.29 (0.85–1.94)	1.28 (0.85–1.93)		
Quartile 3 (10.4)	0.86 (0.54–1.36)	0.82 (0.51–1.32)	Quartile 3 (12.1)	1.47 (0.99–2.18)	1.38 (0.92–2.06)		
Quartile 4 (13.5)	0.93 (0.59–1.45)	0.89 (0.56–1.41)	Quartile 4 (15.9)	1.99 (1.37–2.89)	1.88 (1.28–2.76)		
P for trend	0.21	0.15	P for trend	<0.0001	<0.001		
<u>Visually-significant AMD</u>							
Quartile 1 (7.1)	1.00 (referent)	1.00 (referent)	Quartile 1 (8.2)	1.00 (referent)	1.00 (referent)		
Quartile 2 (8.7)	1.90(0.93–3.89)	1.81 (0.88–3.71)	Quartile 2 (10.2)	1.42 (0.70–2.87)	1.36 (0.67–2.75)		
Quartile 3 (10.4)	0.91 (0.41–1.99)	0.84 (0.38–1.86)	Quartile 3 (12.1)	1.44 (0.72–2.88)	1.21 (0.60–2.46)		
Quartile 4 (13.5)	1.18 (0.57–2.47)	1.02 (0.48–2.16)	Quartile 4 (15.9)	2.63 (1.39–4.97)	2.09 (1.10–4.00)		
P for trend	0.71	0.42	P for trend	<0.001	<0.01		

Abbreviations: HR, hazard ratio; CI, confidence interval; Hcy, homocysteine.

* Data are shown for the subgroups of women taking and not taking multivitamin supplements at baseline.

[†] Adjusted for age and vitamin E, aspirin, and beta carotene treatment assignment.

[‡] Adjusted for age, randomized treatment assignment, smoking (current, past, never), alcohol use (rarely/never, 1–3 drinks/month, 1–6 drinks/week, and 1 drinks/day), BMI (continuous), menopausal status and use of HT (premenopausal, uncertain, postmenopausal and current HT, postmenopausal and no HT), history of hypertension (ever diagnosis by physician or self-reported blood pressure 140/90; yes or no), history of hyperlipidemia (baseline history of cholesterol-medication use or a physician diagnosis of high cholesterol or a self-reported cholesterol of at least 240 mg/dL; yes or no), history of eye exam in the last 2 years.