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Prospective Study of Plasma Homocysteine, Its Dietary Determinants, and Risk of Age-related Macular Degeneration in Men

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Abstract

Purpose: Cross-sectional and case-control studies generally support a direct association between elevated plasma homocysteine and age-related macular degeneration (AMD), but data from prospective studies are limited. We examined the prospective relation of plasma homocysteine level, its dietary determinants, and risk of AMD in a large cohort of apparently healthy male physicians.

Methods: During a mean follow-up of 11.2 years, we identified 146 incident cases of visually-significant AMD (responsible for a reduction of visual acuity to 20/30 or worse), and 146 controls matched for age, smoking status, and time of blood draw. We measured concentration of homocysteine in blood samples collected at baseline using an enzymatic assay. and we assessed dietary intake of B vitamins and related compounds betaine and choline with a food frequency questionnaire administered at baseline.

Results: AMD was not associated with plasma level of homocysteine; the multivariable-adjusted odds ratio (OR) of AMD comparing the highest and lowest quartile of homocysteine was 1.09 (95% confidence interval [95% CI], 0.52–2.31; p for trend=0.99). However, AMD was inversely associated with quartile of intake of total folate (OR, 0.55, 95% CI, 0.24–1.23; p for trend=0.08), vitamin B₆ from food (OR, 0.39, 95% CI, 0.17–0.88; p for trend=0.01), and betaine (OR, 0.53, 95% CI, 0.22–1.27; p for trend=0.048).

Conclusions: These prospective data from a cohort of apparently-healthy men do not support a major role for homocysteine in AMD occurrence, but do suggest a possible beneficial role for higher intake of several nutrients involved in homocysteine metabolism.

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Introduction

Age-related macular degeneration is a complex degenerative condition of the central retina that is estimated to affect 9 million American adults.¹ The pathophysiology of AMD is incompletely understood, but is thought to involve multiple mechanisms^{2–4} with a final common pathway of impaired choroidal circulation and retinal ischemia.⁵

Elevated plasma homocysteine has been linked to vascular complications in basic research studies,⁶ and to increased risks of vascular disease in epidemiologic studies,^{7,8} but its significance in AMD remains unclear. Cross-sectional and case-control studies generally indicate a direct association with AMD, particularly advanced AMD,⁹ but data from limited prospective studies are less supportive.^{10,11} Moreover, homocysteine levels can be elevated due to inadequate intake of nutrients involved in homocysteine metabolism (eg. folate, vitamin B₆, vitamin B₁₂, choline, betaine),^{12,13} which may themselves be more directly associated with risks of AMD.

In this report, we examined the prospective relation of plasma homocysteine, its dietary determinants, and risk of AMD in apparently-healthy United States male physicians.

Materials and Methods

The Physicians' Health Study II Cohort

Study participants were members of Physicians' Health Study II (PHS II), a randomized, double-blind, placebo-controlled $2 \times 2 \times 2 \times 2$ factorial trial of vitamin E, vitamin C, β -carotene, and a multivitamin in the prevention of cardiovascular disease (CVD), cancer, and age-related eye disease.^{14–20} The PHS II population comprised 14,641 U.S. male physicians aged 55 years who were enrolled between 1997 and 2001, and included 7,641 original participants in PHS I, a completed trial of aspirin and β -carotene in the primary prevention of CVD and cancer in men.^{21,22} Dietary data were ascertained at baseline through a self-administered food frequency questionnaire [FFQ]. Participants also completed annual questionnaires providing information on compliance with pill taking, potential adverse events, updated risk factors, and the occurrence of any new study endpoints including AMD. Treatment and follow-up in PHS II continued in blinded fashion through June 1, 2011. Each study participant gave written informed consent, and the Brigham and Women's Hospital Institutional Review Board approved the study protocol. This research adhered to the tenets of the Declaration of Helsinki.

Blood collection

Blood samples were obtained from 11,133 (76%) of the 14,641 PHS II participants between 1995 and 2001. Participants were sent blood collection kits containing 3 EDTA and 3 citrate tubes; a gel-filled freezer pack; a completed overnight courier air bill, and written instructions and other supplies needed for venipuncture. Specimens were returned to our laboratory in freezer packs within 24 hours of blood draw. Upon receipt, samples were fractionated into plasma, red blood cells and buffy coat, and frozen at -170°C .

Homocysteine measurement

The concentration of homocysteine was determined in EDTA blood samples collected at baseline (and frozen at -170°C) using an enzymatic assay on the Hitachi 917 analyzer (Roche Diagnostics) using reagents and calibrators from Catch, Inc.

Dietary assessment

Information on dietary folate, vitamin B₆, vitamin B₁₂, choline, and betaine consumption was obtained by using an FFQ administered between 1997 and 2001. Participants were asked how frequently, on average, during the past year they consumed one standard serving of a specific food item in 9 categories ranging from “never or less than once per month” to “6 or more times per day.” Responses on frequencies of a specified serving size for each food item were converted to average daily intakes. The FFQ also contained specific questions about the use of multivitamins and several individual vitamin supplements. Men were asked to report whether they currently took multivitamins, how many they took per week (2, 3–5, 6–9, or 10 tablets), and the specific brand used. Men were also asked to specify their use and dose of individual vitamin B₆ supplements (<10, 10–39, 40–79, or 80 mg/d). Additional questions on regular use of folic acid and B-complex supplements (yes or no) were also included.

Nutrient intake was computed using the food composition database from the Harvard School of Public Health and manufacturers’ information. We calculated total intake of folate, vitamin B₆, and vitamin B₁₂ by summing intakes from food and supplemental sources. Folate consumption included intake from unfortified and fortified foods, taking into account mandatory fortification since 1998. The choline and betaine composition of individual foods was added to the FFQs nutrient database with the use of values published by Zeisel et al²³ in 2003, and from the U.S. Department of Agriculture’s choline database.²⁴ Choline intake was calculated as the sum of intake from water-soluble compounds free choline, phosphocholine, glycerophosphocholine, and lipid-soluble compounds phosphatidylcholine (lecithin), and sphingomyelin. Because supplements contributed little to total intake of choline and betaine, intake from food only is presented. The validity and reproducibility of FFQs has been demonstrated in other cohorts.^{25,26} For example, Pearson correlation comparing an FFQ and 14 day average from diet records (two 7-day records administered approximately 6 months apart) for total folate, total vitamin B₆, and total vitamin B₁₂ were 0.77, 0.85, and 0.56, respectively, after adjustment for total energy intake.²⁵ Support for the validity of choline and betaine assessments made by use of the FFQ in similar populations has also been presented.^{27,28}

Ascertainment of Incident AMD

Participants reporting a new diagnosis of AMD were asked to provide written consent to obtain medical records, and ophthalmologists/optometrists were contacted by mail and asked to complete an AMD questionnaire. The questionnaire asked about AMD diagnosis date, best-corrected visual acuity at diagnosis, date when best-corrected visual acuity reached 20/30 or worse, and signs of AMD observed when visual acuity was first noted to be 20/30 or worse. The questionnaire also asked whether there were other ocular abnormalities and, if so, whether the AMD, by itself, was significant enough to reduce best-corrected visual

acuity to 20/30 or worse. Ophthalmologists/optometrists could also provide the requested information by supplying copies of relevant medical records.

The study endpoint was visually-significant AMD, defined as a self-report confirmed by medical record evidence of an initial diagnosis after randomization but before June 1, 2011, with best-corrected visual acuity loss to 20/30 or worse attributable to AMD.

Participants were classified according to the status of the worse eye as defined by disease severity.²⁹ When the worse eye was excluded because of visual acuity loss attributed to other ocular abnormalities, the fellow eye was considered for classification.

Case-control selection

During a mean of 11.2 years of follow-up, visually-significant AMD was documented in 146 men who had provided a baseline blood sample and had complete dietary information for folate, vitamin B₆, vitamin B₁₂, choline and betaine. Controls were randomly selected to match cases, in a 1:1 ratio, according to age (within 1 year), time of blood draw, and cigarette smoking.

Data analysis

Distributions for plasma homocysteine and dietary nutrients were skewed to the right and thus were log-transformed to normalize them. After log-transformation, we used the residual method to adjust nutrients for energy intake.³⁰ Analyses comparing distribution of risk factors between case-control pairs used McNemar's test for categorical variables and paired t-test for continuous variables. Correlation of plasma homocysteine and dietary nutrients was summarized using Spearman correlation coefficient.

Participants were categorized into quartiles of plasma homocysteine concentration and dietary intake of nutrients based on the distribution in controls. Conditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for AMD. For analyses of folate, vitamin B₆, and vitamin B₁₂, we separately evaluated total intake from food and supplements, and intake from food sources only. Initial models adjusted for matching factors (age, time of blood draw, smoking). We then adjusted the models for alcohol use (rarely/never, 1 drink/month), BMI (kg/m²), current exercise (1 time/wk), history of high cholesterol (self-reported total cholesterol level of at least 240 mg/dL, or past or current treatment for high cholesterol), history of diabetes, and randomized treatment assignment (vitamin E, vitamin C, beta carotene, multivitamins). Analyses of nutrient intake further adjusted for plasma homocysteine concentration. Tests for linear trend were performed by fitting the median intake for each quartile as a continuous variable in the model. We also examined dietary intakes as continuous variables (per estimated standard deviation [SD]) and, given the inter-correlation of intakes, selected an optimal model using stepwise logistic regression models adjusting for matching factors (age, time of blood draw, smoking). Variable entry and retention criterion was set at <0.20. Finally, we examined the possibility of nonlinear relationships between dietary intake and AMD non-parametrically using restricted cubic splines with 4 knots positioned at the 5th, 35th, 65th, and 95th percentiles of the intake distribution.³¹ We tested for non-linearity using the likelihood ratio test comparing the model with only the linear term to the model with the

linear and cubic spline terms. The results were adjusted for the same potential confounding factors as the fully-adjusted logistic regression models. We used SAS statistical software (version 8.2; SAS Institute, Cary, NC) for all analyses. All p values were two sided.

Results

Selected characteristics of AMD cases and matched controls are presented in Table 1. Compared to controls, AMD cases were less likely to report a personal history of high cholesterol. Plasma homocysteine levels were similar in cases and controls, with nearly 30% in each group having levels that exceeded 15 μ mol/L. Cases had lower intake of folate and betaine than did controls.

Plasma homocysteine levels were only weakly associated with intake of the various nutrients among the combined sample, and among the separate case and control groups (Table 2). Dietary intakes of the various nutrients were moderately associated with each other among cases, controls, and the combined sample.

Table 3 presents multivariable-adjusted ORs of AMD for quartiles of plasma homocysteine. Men in the highest quartile, compared to those in the lowest, had a modest and statistically non-significant, 29% increased risk of AMD (OR, 1.29, 95% CI, 0.66–2.55; p for trend=0.78) after adjustment for matching factors. This estimate was further reduced after additional adjustment for other possible AMD risk factors and randomized treatment assignment (OR, 1.09, 95% CI, 0.52–2.31; p for trend=0.99).

For dietary nutrients, in models adjusting for matching factors, we found inverse trends between AMD risk and intake of total folate (OR comparing highest to lowest quartile, 0.53, 95% CI, 0.25–1.13; p for trend=0.04), vitamin B₆ from food only (OR, 0.39, 95% CI, 0.18–0.81; p for trend=0.005), and betaine (OR, 0.60, 95% CI, 0.27–1.30; p for trend=0.08) (Table 4). OR estimates changed little after further adjustment for other possible AMD risk factors and for homocysteine (Table 4).

Because nutrient intakes were inter-correlated, we examined the independent contribution of nutrients to AMD risk using forward stepwise conditional logistic models that included adjustment for matching factors. Candidate variables included total folate, folate from food, total vitamin B₆, vitamin B₆ from food, total vitamin B₁₂, vitamin B₁₂ from food, choline, and betaine examined as continuous variables (per SD of log-transformed intake). Variable entry and retention criterion was set at <0.20. Vitamin B₆ from food was the only nutrient selected in the final regression model; a 1 SD increase was independently associated with a 49% decrease in AMD risk (OR, 0.51, 95% CI, 0.32–0.81; p=0.005).

We examined the possibility of nonlinear associations between nutrient intake and AMD using restricted cubic splines. There was no evidence of a nonlinear relation between intake of any of the nutrients examined and risk of AMD.

Discussion

In this study of United States male physicians, elevated plasma homocysteine at baseline was not associated with risk of incident visually-significant AMD after multivariable adjustment. However, intake of several nutrients involved in homocysteine metabolism was inversely associated with AMD risk. These prospective data do not support a major role for homocysteine as an independent risk factor for AMD in apparently healthy men, but do suggest a possible beneficial role for higher intake of several nutrients involved in homocysteine metabolism.

Previous cross-sectional and case-control studies generally indicate a direct association between elevated homocysteine and AMD, primarily advanced AMD.⁹ However, because of inherent uncertainty about the timing of exposure and disease in these study designs, it is often unclear whether elevated homocysteine is a potential cause of AMD, or simply a consequence of AMD and its underlying mechanisms. Prospective studies can provide more reliable evidence to evaluate a possible causal connection, but are limited to two recent reports. In the Blue Mountains Eye Study (BMES) of 1,390 Australian men and women followed for 10 years, higher homocysteine level at baseline was associated with a modest 33% (per 1 SD) increased risk of early AMD, and a smaller, statistically non-significant, 25% increased risk of late (advanced) AMD, after adjustment for age, sex, current smoking, white blood cell count, and fish consumption.¹⁰ In a second prospective study, conducted among 27,479 Women's Health Study (WHS) participants followed for 10 years, women in the highest quartile of plasma homocysteine at baseline, compared to those in the lowest, had a slightly elevated, and statistically non-significant, 21% increased risk of visually-significant AMD in multivariable-adjusted analysis. Our finding in male physicians of no material increased risk of visually-significant AMD for those with elevated homocysteine appears broadly consistent with the findings in BMES and WHS. Taken together, these prospective data indicate that elevated homocysteine is unlikely to be a major, independent risk factor for AMD in apparently healthy men and women.

In contrast, our data for nutrients indicated that intakes of folate, vitamin B₆ from food, and betaine were inversely associated with risk of AMD when assessed in separate, fully-adjusted regression models for each nutrient. After accounting for total intake of each nutrient in stepwise logistic models, only vitamin B₆ from food was found to be independently associated with AMD.

Previous observational data for B vitamins and AMD are limited and inconclusive,^{10,32-37} and only two studies have been prospective. In one, the BMES reported that high intake of folate from food and supplements (high vs low tertile) was associated with a lower risk of developing late AMD (neovascular AMD or geographic atrophy), but was not associated with early AMD.¹⁰ High blood levels of folate (> 11 nmol/L) were inversely associated with both early and late AMD in that study. The BMES also reported no association between tertiles of vitamin B₁₂ intake and AMD, although high blood levels (> 185 pmol/L) were inversely associated with both early and late AMD.¹⁰ More recently, analysis of prospective data from the Age-Related Eye Disease 2 (AREDS2) cohort indicated a significant inverse association between quintiles of dietary folate from food sources only (no supplements) and

progression to geographic atrophy in fully adjusted models.³⁶ Dietary intake of other B vitamins (thiamin, riboflavin, niacin, vitamin B₆, vitamin B₁₂) from food sources only were not significantly associated with progression to geographic atrophy.

Our finding in PHS II of an inverse relation between dietary intake of folate and AMD, and no association between vitamin B₁₂ intake and AMD, appears consistent with the findings in BMES and AREDS2. Our observation of an inverse association of AMD with intake of vitamin B₆ from food differs with the findings for this nutrient in AREDS2 (which examined progression to geographic atrophy) and needs to be confirmed in other populations. Finally, our finding of possible benefit for higher intake of betaine appears consistent with the only previous report for this nutrient in AMD, a case series of monozygotic twin pairs with discordant AMD phenotypes.³⁸

All nutrients examined in this study are cofactors in the enzymatic pathway of homocysteine metabolism. However, the finding that several nutrients remained inversely associated with AMD, even after adjustment for homocysteine level, suggests mechanisms unrelated to homocysteine-lowering. Other plausible mechanisms that could potentially mediate a beneficial effect of these nutrients in AMD include increased antioxidant activity,³⁹ increased bioavailability of nitric oxide (a potent vasodilator and an important regulator of choroidal blood flow in the macular region of the retina),⁴⁰ reduced inflammation,^{41,42} and DNA methylation and gene expression.^{38, 43, 44} Further investigation is required to distinguish between these and other possibilities.

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 or changes in dietary habits. 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 including total homocysteine tend to be very stable with little or no sign of deterioration during storage.^{45,46} Nutrient intake data were obtained from FFQs which may be subject to measurement error. Because we examined multiple nutrients, one or more of the associations observed in this study may have been due to chance. In addition, the inter-correlation among nutrients made it difficult to determine the independent contribution of each nutrient to risk of AMD, and stepwise logistic models may only partially remove the influence of other nutrients. Our study was also relatively underpowered to detect modification of associations by other dietary factors (eg. low folate levels, alcohol use). As with all other observational studies, there remains the possibility of residual confounding due to unmeasured or poorly measured confounding. Finally, as the PHS II population is generally well-nourished, these findings may not apply to less well-nourished populations.

Strengths of the current study include the comprehensive and simultaneous assessment of risk factors, dietary intake, and plasma homocysteine in the same study population. Of particular importance, information on dietary intake and plasma homocysteine was collected at baseline, prior to any diagnosis of AMD, and thus the prospective study design allowed us to minimize biases due to recall, and to largely eliminate the possibility of temporal bias and reverse causality, which is a common limitation of cross-sectional and case-control studies

making it difficult to determine whether increased concentrations of homocysteine precede AMD or vice versa.

In summary, our prospective data from a cohort of apparently-healthy men do not support an important role for homocysteine level in AMD occurrence. However, the data do suggest a possible beneficial role for higher intake of several nutrients involved in homocysteine metabolism. These findings need to be confirmed in other prospective studies of men and women.

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Table 1.

Selected characteristics of AMD cases and controls in Physicians' Health Study II.

	AMD Cases (n=146)	No AMD (n=146)	P value
Age (mean [SD], y)	73.6 (6.3)	72.8 (6.0)	Matched
Cigarette smoking, %			Matched
Current	1.4	1.4	
Past	59.6	59.6	
Never	39.0	39.0	
Alcohol intake, %			0.57
Rarely/never	18.5	21.2	
1 drink/month	81.5	78.8	
Body mass index (kg/m ²) (mean [SD])	25.5 (3.2)	25.5 (3.3)	0.97
Current exercise 1 time/wk, %	65.8	65.8	1.00
Reported history, %			
High cholesterol *	32.9	44.5	0.04
Diabetes	6.9	7.5	0.82
Plasma homocysteine, µmol/L (median [IQR])	14.0 (12.7–17.1)	14.0 (12.2–16.2)	0.67
Total dietary folate, µg/d (median [IQR])	401.7 (334.2–597.6)	462.4 (344.8–698.5)	0.027
Total dietary vitamin B ₆ , mg/d (median [IQR])	2.0 (1.7–2.9)	2.2 (1.8–3.5)	0.99
Total dietary vitamin B ₁₂ , µg/d (median [IQR])	5.8 (4.2–10.6)	6.3 (4.4–12.7)	0.30
Total dietary choline, mg/d (median [IQR])	301.6 (264.5–327.8)	306.0 (268.3–336.7)	0.40
water soluble	134.2 (116.0–152.1)	135.8 (116.9–162.0)	0.17
lipid soluble	162.4 (132.6–191.0)	160.2 (130.0–193.5)	0.84
Total dietary betaine, mg/d (median [IQR])	89.8 (68.0–123.5)	105.8 (71.5–144.3)	0.04

Abbreviations: AMD, age-related macular degeneration; SD, standard deviation; IQR, interquartile range.

* History of high cholesterol was defined as self-reported total cholesterol level of at least 240 mg/dL or past or current treatment for high cholesterol.

Table 2.

Spearman correlation coefficients* among plasma homocysteine level and dietary intake (food + supplements) of folate, vitamin B₆, vitamin B₁₂, choline, and betaine in Physicians' Health Study II.

	Plasma tHcy	Dietary folate	Dietary B ₆	Dietary B ₁₂	Dietary choline	Dietary betaine
a) cases + controls, n=292						
Plasma tHcy	1.0					
Dietary folate	-0.10	1.0				
Dietary B ₆	-0.09	0.83 [§]	1.0			
Dietary B ₁₂	-0.19 [‡]	0.53 [§]	0.66 [§]	1.0		
Dietary choline	-0.07	0.02	0.19 [‡]	0.36 [§]	1.0	
Dietary betaine	-0.10	0.27 [§]	0.21 [‡]	0.06	0.06	1.0
b) cases, n=146						
Plasma tHcy	1.0					
Dietary folate	-0.05	1.0				
Dietary B ₆	-0.06	0.83 [§]	1.0			
Dietary B ₁₂	-0.24 [‡]	0.46 [§]	0.60 [§]	1.0		
Dietary choline	-0.05	0.08	0.23 [‡]	0.36 [§]	1.0	
Dietary betaine	-0.02	0.23 [‡]	0.16	0.02	0.16	1.0
c) controls, n=146						
Plasma tHcy	1.0					
Dietary folate	-0.13	1.0				
Dietary B ₆	-0.08	0.82 [§]	1.0			
Dietary B ₁₂	-0.14	0.60 [§]	0.72 [§]	1.0		
Dietary choline	-0.07	-0.04	0.14	0.36 [§]	1.0	
Dietary betaine	-0.16	0.30 [‡]	0.24 [‡]	0.09	-0.02	1.0

Abbreviations: tHcy, homocysteine

1000>d
8
100>d
+
10>d
/
Age of patients
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Table 3. Multivariable-adjusted OR (95% CI) of AMD according to quartile of plasma homocysteine (146 case-control pairs) in Physicians’ Health Study II.

	Quartile				P (trend)
	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	
Median (range), $\mu\text{mol/L}$	10.9 (9.5–12.1)	13.0 (12.2–13.9)	14.6 (14.0–16.0)	19.3 (16.2–66.7)	
Cases/Controls	30/36	42/35	31/38	43/37	
Model 1, OR (95% CI)	1.0	1.41 (0.71–2.82)	0.88 (0.44–1.77)	1.29 (0.66–2.55)	0.78
Model 2, OR (95% CI)	1.0	1.10 (0.51–2.35)	0.78 (0.36–1.68)	1.09 (0.52–2.31)	0.99

Abbreviations: OR, odds ratio; CI, confidence interval; AMD, age-related macular degeneration.

Model 1 – Control for matching factors (age, smoking, time of blood draw).

Model 2 – Model 1 plus control for alcohol use (rarely/never, 1 drink/month), BMI (kg/m2), current exercise (1 time/wk), history of high cholesterol (self-reported total cholesterol level of at least 240 mg/dL, or past or current treatment for high cholesterol), history of diabetes, and randomized treatment assignment (vitamin E, vitamin C, beta carotene, multivitamins).

Table 4.

Multivariable-adjusted OR (95% CI) of AMD according to quartile of dietary intake of folate, vitamin B₆, vitamin B₁₂, choline, and betaine (146 case-control pairs) in Physicians' Health Study II.

	Quartile				P (trend)
	1	2	3	4	
Total Folate, µg/d					
Median (range)	305.6 (214.7–338.6)	387.8 (344.8–461.2)	555.6 (463.5–698.5)	924.5 (698.7–1,821.9)	
Cases/Controls	45/36	47/37	28/37	26/36	
Model 1, OR (95% CI)	1.0	0.90 (0.46–1.77)	0.52 (0.25–1.08)	0.53 (0.25–1.13)	0.04
Model 2, OR (95% CI)	1.0	0.82 (0.39–1.71)	0.51 (0.23–1.14)	0.55 (0.25–1.22)	0.08
Model 3, OR (95% CI)	1.0	0.80 (0.37–1.71)	0.48 (0.21–1.09)	0.55 (0.24–1.23)	0.08
Folate (food only), µg/d					
Median (range)	201.4 (146.1–223.0)	255.5 (225.6–271.3)	290.6 (272.3–312.1)	355.6 (312.9–713.8)	
Cases/Controls	37/37	38/36	31/37	40/36	
Model 1, OR (95% CI)	1.0	1.15 (0.59–2.21)	0.80 (0.41–1.56)	1.15 (0.58–2.30)	0.97
Model 2, OR (95% CI)	1.0	1.18 (0.59–2.34)	0.85 (0.41–1.75)	1.30 (0.62–2.72)	0.68
Model 3, OR (95% CI)	1.0	1.19 (0.59–2.41)	0.84 (0.40–1.77)	1.29 (0.61–2.74)	0.70
Total Vitamin B ₆ , mg/d					
Median (range)	1.6 (1.2–1.8)	2.0 (1.8–2.1)	2.6 (2.2–3.5)	4.6 (3.5–94.7)	
Cases/Controls	45/36	42/37	30/37	29/36	
Model 1, OR (95% CI)	1.0	1.04 (0.55–1.96)	0.61 (0.31–1.19)	0.67 (0.33–1.37)	0.14
Model 2, OR (95% CI)	1.0	1.02 (0.52–2.00)	0.56 (0.28–1.15)	0.68 (0.32–1.45)	0.17
Model 3, OR (95% CI)	1.0	1.00 (0.51–1.99)	0.53 (0.26–1.10)	0.67 (0.31–1.44)	0.16
Vitamin B ₆ (food only), mg/d					
Median (range)	1.6 (1.2–1.8)	1.9 (1.8–2.1)	2.2 (2.1–2.5)	3.2 (2.5–5.5)	
Cases/Controls	53/37	46/36	30/37	17/36	
Model 1, OR (95% CI)	1.0	0.98 (0.50–1.91)	0.52 (0.26–1.06)	0.39 (0.18–0.81)	0.005
Model 2, OR (95% CI)	1.0	0.98 (0.48–2.00)	0.51 (0.24–1.10)	0.40 (0.18–0.89)	0.01
Model 3, OR (95% CI)	1.0	0.97 (0.47–1.99)	0.49 (0.23–1.06)	0.39 (0.17–0.88)	0.01
Total Vitamin B ₁₂ , µg/d					
Median (range)	3.4 (0.2–4.4)	5.3 (4.4–6.2)	7.6 (6.3–12.5)	16.2 (12.7–156.3)	

	Quartile				P (trend)
	1	2	3	4	
Cases/Controls	38/37	42/36	39/36	27/37	
Model 1, OR (95% CI)	1.0	0.99 (0.52–1.90)	0.97 (0.49–1.94)	0.68 (0.34–1.38)	0.28
Model 2, OR (95% CI)	1.0	0.95 (0.47–1.89)	1.08 (0.52–2.27)	0.66 (0.30–1.44)	0.34
Model 3, OR (95% CI)	1.0	0.92 (0.46–1.85)	1.06 (0.50–2.26)	0.67 (0.30–1.46)	0.35
<u>Vitamin B₁₂ (food only) µg/d</u>					
Median (range)	3.5 (0.2–4.0)	4.7 (4.0–5.8)	6.5 (5.9–8.7)	13.0 (8.7–35.3)	
Cases/Controls	33/37	54/36	29/36	30/37	
Model 1, OR (95% CI)	1.0	1.45 (0.78–2.71)	0.85 (0.41–1.78)	0.90 (0.46–1.76)	0.36
Model 2, OR (95% CI)	1.0	1.49 (0.77–2.87)	0.92 (0.43–1.98)	1.01 (0.50–2.07)	0.63
Model 3, OR (95% CI)	1.0	1.48 (0.75–2.90)	0.88 (0.40–1.93)	1.01 (0.48–2.10)	0.59
<u>Choline, mg/d</u>					
Median (range)	245.2 (163.7–268.3)	280.3 (268.4–305.7)	323.5 (306.3–336.7)	369.0 (337.1–487.7)	
Cases/Controls	39/37	43/36	35/37	29/36	
Model 1, OR (95% CI)	1.0	1.00 (0.51–1.94)	0.81 (0.40–1.65)	0.68 (0.34–1.39)	0.23
Model 2, OR (95% CI)	1.0	0.98 (0.49–1.97)	0.81 (0.38–1.71)	0.70 (0.33–1.51)	0.30
Model 3, OR (95% CI)	1.0	0.97 (0.48–1.96)	0.80 (0.38–1.69)	0.69 (0.32–1.50)	0.29
<u>Choline, water soluble, mg/d</u>					
Median (range)	101.4 (74.1–116.7)	128.2 (116.9–135.7)	143.8 (135.9–162.0)	179.0 (162.5–241.2)	
Cases/Controls	37/36	42/37	45/37	22/36	
Model 1, OR (95% CI)	1.0	1.04 (0.51–2.11)	1.02 (0.51–2.05)	0.40 (0.17–0.96)	0.08
Model 2, OR (95% CI)	1.0	1.01 (0.48–2.13)	1.01 (0.49–2.10)	0.40 (0.16–1.01)	0.11
Model 3, OR (95% CI)	1.0	1.12 (0.53–2.38)	1.12 (0.54–2.31)	0.41 (0.16–1.05)	0.08
<u>Choline, lipid soluble, mg/d</u>					
Median (range)	117.1 (73.5–129.9)	147.0 (130.0–160.0)	174.7 (160.4–192.9)	216.1 (193.5–365.4)	
Cases/Controls	35/36	34/37	43/36	34/37	
Model 1, OR (95% CI)	1.0	0.86 (0.42–1.79)	1.25 (0.63–2.50)	0.92 (0.45–1.87)	0.94
Model 2, OR (95% CI)	1.0	0.91 (0.42–2.01)	1.34 (0.64–2.80)	0.94 (0.44–2.01)	0.87
Model 3, OR (95% CI)	1.0	1.04 (0.48–2.25)	1.35 (0.66–2.79)	1.01 (0.48–2.12)	0.80
<u>Betaine, mg/d</u>					
Median (range)	57.2 (37.7–70.4)	85.4 (71.5–105.3)	119.2 (106.4–144.3)	182.4 (147.2–416.7)	

	Quartile				P (trend)
	1	2	3	4	
Cases/Controls	41/36	53/37	27/37	25/36	
Model 1, OR (95% CI)	1.0	1.32 (0.72–2.42)	0.65 (0.33–1.27)	0.60 (0.27–1.30)	0.08
Model 2, OR (95% CI)	1.0	1.24 (0.63–2.44)	0.49 (0.23–1.05)	0.56 (0.24–1.32)	0.05
Model 3, OR (95% CI)	1.0	1.19 (0.59–2.37)	0.46 (0.21–1.00)	0.53 (0.22–1.27)	0.048

Abbreviations: OR, odds ratio; CI, confidence interval; AMD, age-related macular degeneration.

Model 1 – Control for matching factors (age, smoking, time of blood draw).

Model 2 – Model 1 plus control for alcohol use (rarely/never, 1 drink/month), BMI (kg/m2), current exercise (1 time/wk), history of high cholesterol (self-reported total cholesterol level of at least 240 mg/dL, or past or current treatment for high cholesterol), history of diabetes, and randomized treatment assignment (vitamin E, vitamin C, beta carotene, multivitamins).

Model 3 – Model 2 plus control for homocysteine quartiles.