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## Folic Acid, Vitamin B<sub>6</sub>, and Vitamin B<sub>12</sub> in Combination and Age-related Macular Degeneration in a Randomized Trial of Women

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### Abstract

**Context**—Observational epidemiologic studies indicate a direct association between homocysteine concentration in the blood and risk of age-related macular degeneration (AMD), but randomized trial data to examine the effect of homocysteine-lowering in AMD are lacking.

**Objective**—To examine incidence of AMD in a trial of folic acid/vitamin B<sub>6</sub>/vitamin B<sub>12</sub>.

**Design**—Randomized, double-masked, placebo-controlled trial.

**Participants**—5,442 female health professionals aged 40 years or older with preexisting cardiovascular disease (CVD) or 3 or more CVD risk factors. A total of 5,205 of these women did not have a diagnosis of AMD at baseline and were included in this analysis.

**Intervention**—Participants were randomly assigned to receive a combination of folic acid (2.5 mg/d), vitamin B<sub>6</sub> (50 mg/d), and vitamin B<sub>12</sub> (1 mg/d), or placebo.

**Main Outcome Measures**—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**—After an average of 7.3 years of treatment and follow-up, there were 55 cases of AMD in the folic acid/B<sub>6</sub>/B<sub>12</sub> group and 82 in the placebo group (relative risk [RR], 0.66; 95% confidence interval [CI], 0.47–0.93; p=0.02). For visually-significant AMD, there were 26 cases in the folic acid/B<sub>6</sub>/B<sub>12</sub> group and 44 in the placebo group (RR, 0.59; 95% CI, 0.36–0.95; p=0.03).

**Conclusions**—These randomized trial data from a large cohort of women at high risk of CVD indicate that daily supplementation with folic acid/B<sub>6</sub>/B<sub>12</sub> may reduce the risk of AMD.

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Study concept and design: Manson.

Acquisition of data: Christen, Manson.

Analysis and interpretation of data: Christen, Glynn, Chew, Albert, Manson.

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Study supervision: Christen, Manson.

## Introduction

Age-related macular degeneration (AMD) is the leading cause of severe irreversible vision loss in older Americans (1). An estimated 1.75 million individuals in the U.S. suffer from advanced AMD (i.e. geographic atrophy and 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 (2,3), but does increase the risk of developing advanced AMD (4,5). Current treatment options are limited to a minority of persons with late-stage, neovascular AMD (6–11), or intermediate AMD (12). For the large majority of persons with early or no AMD, there is no method of disease prevention other than avoidance of cigarette smoking (13–15). Accordingly, the National Eye Institute has designated the development of new treatments for AMD as an important program goal for vision research (16).

Recent cross-sectional (17–19) and case-control (20–24) studies indicate a direct association between homocysteine concentration in the blood and risk of AMD, suggesting that homocysteine may be a modifiable risk factor for AMD. Homocysteine is an intermediary amino acid formed during the metabolism of methionine, an essential amino acid derived from protein (25). Hyperhomocysteinemia, defined as plasma homocysteine concentration  $>15$   $\mu\text{mol/L}$  (26,27), induces vascular endothelial dysfunction (28–30) and is considered to be an independent risk factor for atherosclerosis and cardiovascular disease (CVD) (31,32). Treatment with folic acid, vitamin B<sub>6</sub> (pyridoxine), and vitamin B<sub>12</sub> (cyanocobalamin) has been shown to reduce homocysteine levels in intervention studies (33), and to reverse endothelial dysfunction independent of its homocysteine-lowering effect (34,35). However, trials of homocysteine-lowering among persons with pre-existing vascular disease provide little support for a benefit of supplemental folic acid and B vitamins in reducing cardiovascular events (36). Nonetheless, given the recent evidence supporting a link between homocysteine and AMD, and other data suggesting an etiologic role for atherosclerosis and endothelial dysfunction in AMD (37–40), it is reasonable to propose that homocysteine-lowering with folic acid and B vitamins may help to decrease risks of AMD. At present, there are no previous data from large randomized trials to examine this hypothesis.

In this report we present the results for AMD from the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS), a randomized trial that evaluated whether combined treatment with folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> could prevent cardiovascular events among women at high risk of CVD.

## Methods

### Study design

WAFACS was a randomized, double-blind, placebo-controlled trial that evaluated whether a combination of folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> could reduce cardiovascular events among women with preexisting CVD or 3 or more coronary risk factors (41–43). The WAFACS trial began in 1998 when the folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub> arm was added to the ongoing Women's Antioxidant Cardiovascular Study (WACS), a 2×2×2 factorial trial of 8,171 women at high-risk of CVD randomized between June, 1995, and October, 1996 to vitamin E, vitamin C, beta carotene, or placebos (Figure 1). Between August, 1997, and January, 1998, all 8,171 women participating in WACS were sent invitations and consent forms for participation in the folic acid/B<sub>6</sub>/B<sub>12</sub> arm of the trial. Of the total cohort, 5,442 women were willing and eligible to participate in this arm of the trial and were willing to forego the use of vitamin B supplements or multivitamins with greater than the RDA of folic acid, vitamin B<sub>6</sub>, and vitamin B<sub>12</sub>. In April, 1998, these women were randomized in a retained factorial design to a daily combination of folic acid (2.5 mg/d), vitamin B<sub>6</sub> (50 mg/d), and vitamin B<sub>12</sub> (1 mg/d).

d). Five thousand, two hundred five of these women were without a diagnosis of AMD at baseline and are included in these analyses: 2,607 were in the folic acid/B<sub>6</sub>/B<sub>12</sub> group and 2,598 were in the placebo group. Informed consent was obtained from all participants, and the research protocol was reviewed and approved by the institutional review board at Brigham and Women's Hospital in Boston.

Annual questionnaires were sent to all participants to monitor their compliance with pill-taking and the occurrence of any relevant events including AMD. Pill-taking was completed on July 31, 2005, at which point morbidity and mortality follow-up was 92.6% complete. Endpoint ascertainment for AMD was ended in November, 2005. Overall, approximately 84% of women reported taking at least 2/3 of their study pills over the course of the study with no significant difference between active and placebo groups.

### Ascertainment and definition of endpoints

Women who reported a diagnosis of AMD on the baseline questionnaire were excluded. Information on new diagnoses of AMD was requested on annual questionnaires. Participants were asked "Since your last questionnaire, have you had any of the following?" with response options including "macular degeneration right eye" and "macular degeneration left eye". If yes, participants were requested 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 requested to complete an AMD questionnaire that asked about 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). Information was also requested about signs of AMD observed (drusen, retinal pigment epithelium [RPE] hypo/hyperpigmentation, 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 disease, if present, was first noted (defined by presence of RPE detachment, subretinal neovascular membrane, or disciform scar). Ophthalmologists and optometrists were also asked whether there were other ocular abnormalities that could explain or contribute to the vision loss and, if so, whether the AMD, by itself, was significant enough to cause the best-corrected visual acuity to be reduced to 20/30 or worse. Alternatively, they could provide the requested information by supplying photocopies of the relevant medical records. Medical records were obtained for 94% 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 July 31, 2005, and 2) visually-significant AMD, defined as above, but with best-corrected visual acuity loss to 20/30 or worse attributable to AMD.

### Data analysis

Cox proportional hazards regression was used to estimate the relative risk (RR) of AMD among those assigned to receive folic acid/B<sub>6</sub>/B<sub>12</sub> compared with those assigned to receive placebo after adjustment for age (years) at baseline and randomized vitamin C, vitamin E, and beta carotene assignments (44). Models were also fit separately within three prespecified age groups; 40–54, 55–64, 65+ years. The proportionality assumption throughout the follow-up period was tested by including an interaction term of folic acid/B<sub>6</sub>/B<sub>12</sub> with the logarithm of time in the Cox models. For each RR, we also calculated the 95% confidence interval (CI) and two-sided *P* value.

We also analyzed subgroup data by categories of baseline variables that are possible risk factors for AMD. We explored possible modification of any effect of folic acid/B<sub>6</sub>/B<sub>12</sub> by using

interaction terms between subgroup indicators and folic acid/B<sub>6</sub>/B<sub>12</sub>, testing for trend when subgroup categories were ordinal.

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 (45,46). When the worse eye was excluded because of visual acuity loss attributed to other ocular abnormalities, the fellow eye was considered for classification.

## Results

The baseline characteristics of participants in the folic acid/B<sub>6</sub>/B<sub>12</sub> and placebo groups are shown in Table 1. As expected, characteristics were equally distributed between the two treatment groups.

During an average of 7.3 years of treatment and follow-up, a total of 137 cases of AMD were documented, including 70 cases of visually-significant AMD. Most of the visually-significant cases were characterized by some combination of drusen and RPE changes at the time vision was first noted to be 20/30 or worse, reflecting an early stage of AMD development (Table 2).

For the endpoint of total AMD, there were 55 cases in the folic acid/B<sub>6</sub>/B<sub>12</sub> group and 82 in the placebo group (relative risk [RR], 0.66; 95% confidence interval [CI], 0.47–0.93;  $p=0.02$ ) (Table 3). For visually-significant AMD, there were 26 cases in the folic acid/B<sub>6</sub>/B<sub>12</sub> group and 44 in the placebo group (RR, 0.59; CI, 0.36–0.95;  $p=0.03$ ). For both endpoints, RRs did not vary significantly over the three age groups ( $p$  interaction, both  $> 0.2$ ).

Cumulative incidence rates of total AMD and visually significant AMD according to the year of follow-up are shown in Figures 2 and 3. A beneficial effect of folic acid/B<sub>6</sub>/B<sub>12</sub> on total AMD began to emerge at approximately 2 years of treatment and follow-up and persisted throughout the trial (Figure 2). For visually-significant AMD, the curves appeared to diverge later in the trial, at approximately 4 years (Figure 3). For both endpoints, the rate differences appeared to increase with longer follow-up. During the first three years of follow-up, RRs were 0.87 (CI, 0.54–1.42;  $p=0.59$ ) for total AMD and 0.84 (CI, 0.39–1.78;  $p=0.65$ ) for visually-significant AMD. During the remaining 4.3 years of follow-up, RRs were 0.72 (CI, 0.44–1.18;  $p=0.19$ ) for total AMD and 0.52 (CI, 0.27–0.98;  $p=0.04$ ) for visually-significant AMD. Tests of proportionality throughout the follow-up period, however, indicated that the proportionality assumption for treatment was not violated for either endpoint (total AMD,  $P=0.47$ ; visually-significant AMD,  $P=0.42$ ).

There was no evidence that the effect of folic acid/B<sub>6</sub>/B<sub>12</sub> on either AMD endpoint was modified by any AMD risk factor considered. The results for visually-significant AMD are shown in Table 4.

## Discussion

This is the first randomized trial to investigate supplemental use of folic acid and B vitamins in the prevention of AMD. The results, based on an average of 7.3 years of treatment and follow-up of women at increased risk of CVD, indicate that those assigned to active treatment had a statistically significant 35–40% decreased risk of AMD. The beneficial effect of treatment began to emerge at approximately 2 years of follow-up and persisted throughout the trial.

Support for the hypothesis that folic acid and B vitamins could lower the risk of AMD has derived largely from observational evidence of a direct association between homocysteine level in the blood and risk of AMD (17–24), and the demonstration in intervention studies that treatment with folic acid and B vitamins could lower homocysteine levels (33). Further support

has been provided by laboratory evidence that the damaging sequelae of elevated homocysteine (e.g. endothelial dysfunction [28,30,47], impaired vascular reactivity [29,48,49], promotion of inflammatory processes leading to atherosclerosis [50–52]), thought to underlie the increased risk of vascular disease, may also contribute to the pathophysiology of AMD (37–40). The trial findings reported here are the strongest evidence to date in support of a possible beneficial effect for folic acid and B vitamins in AMD prevention. Moreover, because these findings apply to the early stages of AMD development (most cases were characterized by a combination of drusen and RPE changes) in persons without a prior diagnosis of AMD, they appear to represent the first identified means, other than avoidance of cigarette smoking, of reducing risks of AMD in persons at usual risk. From a public health perspective, this is particularly important since persons with early AMD are at increased risk of developing advanced AMD, the leading cause of severe irreversible vision loss in older Americans.

Whether the reduced risk of AMD observed in WAFACS is due to homocysteine-lowering by the folic acid, B vitamin combination, or is independent of homocysteine lowering, is an important question to be investigated. We examined the impact of the intervention on homocysteine levels in a sub-study of 300 WAFACS participants (150 in each treatment group) who had blood samples collected at study entry in 1993–1995, and again at study completion in 2005. Details of the sub-study are presented elsewhere (43). In short, the geometric mean plasma homocysteine level was decreased by 18.5% (95% CI, 12.5–24.1;  $P < 0.001$ ) in the active arm compared to the placebo arm, a difference of 2.27  $\mu\text{mol/L}$  (95% CI, 1.54–2.96). These sub-study findings indicate that the reduced risk of AMD we observed in the treated group may have been due, at least in part, to homocysteine-lowering. However, a treatment benefit independent of homocysteine-lowering is also possible. Plausible mechanisms include a direct antioxidant effect of folic acid and B vitamins, and enhancement of endothelial nitric oxide levels in the choroidal vasculature, with an associated increase in vascular reactivity (53–55). Further study is required to distinguish between these and other possibilities.

Our findings for AMD are in sharp contrast to the null findings for CVD observed in WAFACS (43) and other completed trials of homocysteine-lowering in persons with pre-existing vascular disease, despite substantially lowered homocysteine concentrations by study treatment in those trials (56–66). While our findings could be due to chance and need to be confirmed in other populations, it may be worthwhile to consider whether the discordant findings for AMD and CVD reflect important differences between the choroidal and systemic vasculature with respect to responsiveness to homocysteine-lowering. AMD is a disease that likely involves damage to the small vessels of the choroid (67,68), and some evidence suggests that homocysteine may be a more potent risk factor for small vessel disease than for large vessel disease (69–71). If so, then small vessel diseases such as AMD, and perhaps some subtypes of stroke (e.g. lacunar brain infarcts, cerebral white matter lesions), may be more amenable to benefit from homocysteine-lowering. Of note, a recent meta-analysis of completed trials of homocysteine-lowering indicated that folic acid supplementation had little impact on CVD (pooled RR 0.95; 95% CI, 0.88–1.03) or coronary heart disease (pooled RR 1.04; 95% CI, 0.92–1.17), but was associated with a non-significant 14% reduced risk for total stroke (pooled RR 0.86; 95% CI, 0.71–1.04) (36). Further detailed analyses of etiologic subtypes of stroke in these trials may suggest a beneficial effect of folic acid supplementation that is observable primarily in diseases of small vessels.

In summary, daily supplementation with folic acid/B<sub>6</sub>/B<sub>12</sub> over seven years reduced the risk of AMD in women at increased risk of vascular disease. Because there are currently no recognized means to prevent the early stages of AMD development other than avoidance of cigarette smoking, these findings could have important clinical and public health implications and need to be confirmed in other populations of men and women.



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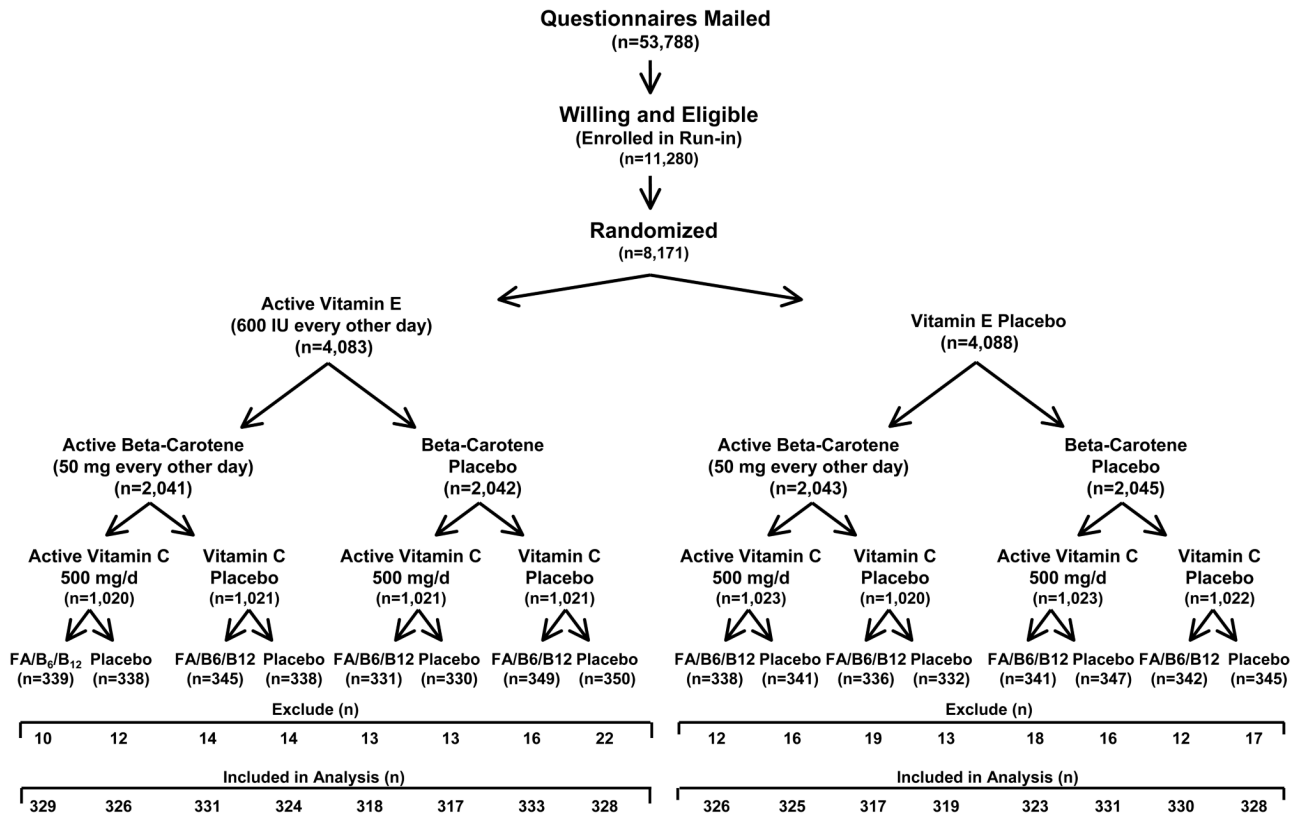
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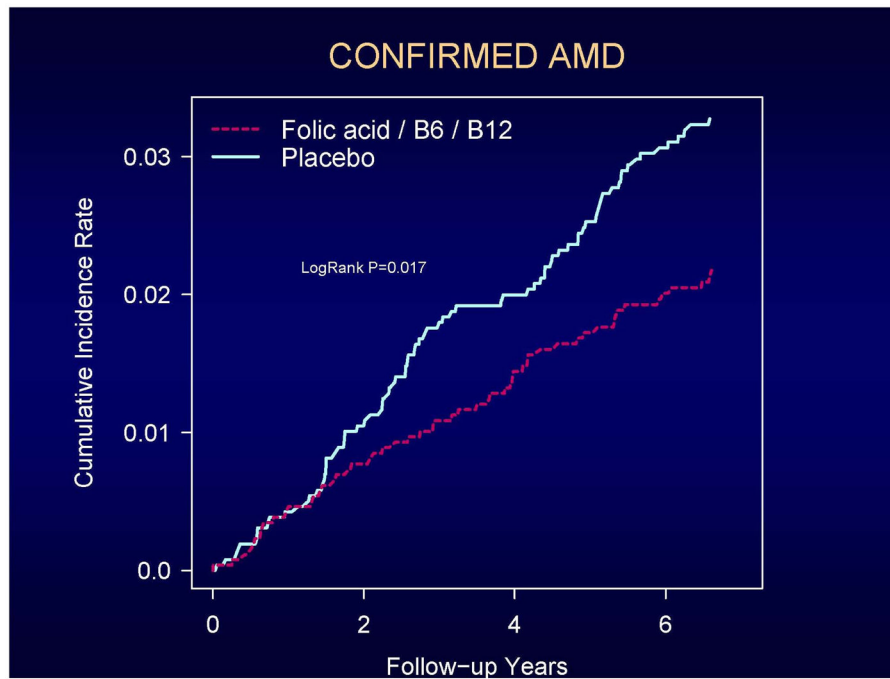
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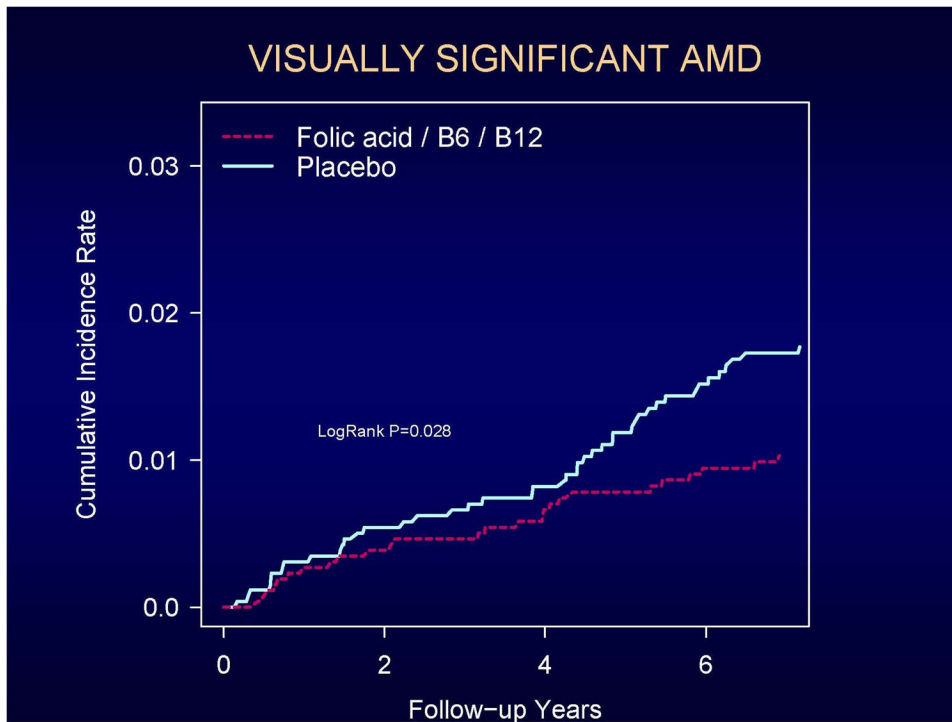
# Randomization Scheme



**Figure 1.**  
Randomization scheme



**Figure 2.**  
Cumulative incidence rates of confirmed AMD.



**Figure 3.**  
Cumulative incidence rates of visually-significant AMD.



**Table 1**  
Baseline Characteristics in Randomized Folic acid/B<sub>6</sub>/B<sub>12</sub> and Placebo Treatment Groups.

Characteristics	Folic acid/B <sub>6</sub> /B <sub>12</sub> (n = 2,607)	Placebo (n = 2,598)
Mean age, y	62.6	62.6
40–54	22.0	22.1
55–64	37.1	36.3
65+	40.9	41.7
Cigarette smoking (%)		
Current	11.4	12.2
Past only	43.6	45.0
Never	45.0	42.7
Alcohol use (%)		
Daily	33.2	32.7
Weekly	12.2	12.4
Rarely/Never	54.6	54.9
Body-mass index*		
Mean √ SD	30.6 (6.7)	30.7 (6.7)
<25.0 (%)	22.5	20.3
25.0–29.9 (%)	27.9	29.5
≥30.0 (%)	49.6	50.2
Hypertension (%) <sup>†</sup>	86.6	85.7
Elevated cholesterol (%) <sup>‡</sup>	77.6	78.8
Diabetes (%)	21.3	21.6
Prior cardiovascular disease (%)	64.4	62.6
Postmenopausal (%)		
Premenopausal	6.3	6.5
Postmenopausal/current HRT	48.9	49.3
Postmenopausal/non HRT	42.3	42.2
Dubious/unclear	2.5	2.0
Multivitamin use (current) (%) <sup>§</sup>	22.5	23.1
Aspirin use in past month (%) <sup>//</sup>	62.4	62.1

Abbreviation: HRT, hormone-replacement therapy.

\* Body-mass index is the weight in kilograms divided by the square of the height in meters.

<sup>†</sup> Self-reported systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg; self-reported physician-diagnosed hypertension; or reported treatment with medication for hypertension.

<sup>‡</sup> Self-reported high cholesterol, cholesterol level ≥240 mg/dl; self-reported physician diagnosed, high cholesterol levels; or reported treatment with cholesterol lowering medication.

<sup>§</sup> Any multivitamin use in the past month.

<sup>//</sup> Aspirin use at least 4 times per month.

**Table 2**  
Retinal Signs\* Noted for 70 Cases of Visually-Significant AMD

Signs of AMD	N	%
Drusen only	13	18.6
RPE changes only	18	25.7
Drusen and RPE changes	19	27.1
Geographic atrophy	2	2.9
Exudative changes*	17	24.3
Information missing	1	<u>1.4</u>
Total	70	100.0

Abbreviations: AMD, age-related macular degeneration; RPE, retinal pigment epithelium.

\* Signs of AMD observed when visual acuity was first noted to be 20/30 or worse due to AMD.

† Includes RPE detachment, subretinal neovascular membrane, and disciform scar.

**Table 3**  
RRs and 95% CIs for Diagnosis of AMD According to Folic acid/B<sub>6</sub>/B<sub>12</sub> Treatment Assignment

Endpoint	Folic acid/B <sub>6</sub> /B <sub>12</sub> (N=2,607)	Placebo (N=2,598)	RR <sup>*</sup>	(95% CI)	P-value
Total AMD	55	82	0.66	0.47–0.93	0.02
Visually significant AMD	26	44	0.59	0.36–0.95	0.03

Abbreviations: AMD, age-related macular degeneration; RR, relative risk; CI, confidence interval.

\* Adjusted for age and vitamin C, vitamin E, and beta carotene treatment assignment.

**Table 4**  
RRs and 95% CIs for Diagnosis of Visually-Significant AMD According to Folic acid/B<sub>6</sub>/B<sub>12</sub> Treatment Assignment, as Modified by Other Risk Factors.

	Number of AMD		RR*	95% CI	P (test of interaction)
	Folic acid/B <sub>6</sub> /B <sub>12</sub> (N= 2,607)	Placebo (N= 2,598)			
Age					0.24
40-54	0/574	2/573	—	—	
55-64	4/968	9/942	0.42	0.13-1.38	
65+	22/1,065	33/1,083	0.67	0.39-1.15	
Smoke Cigarettes					0.47
Current	2/296	7/318	0.32	0.07-1.55	
Past only	12/1,137	19/1,170	0.61	0.30-1.26	
Never	12/1,174	18/1,110	0.66	0.32-1.37	
Alcohol Use					0.54
Daily	8/866	17/850	0.45	0.20-1.05	
Weekly	7/318	5/321	1.33	0.42-4.21	
Rarely/Never	11/1,423	22/1,427	0.50	0.24-1.04	
Body-mass index					0.76
<25.0 (%)	8/586	11/528	0.63	0.25-1.58	
25.0-29.9 (%)	9/727	16/767	0.62	0.27-1.40	
≥30.0 (%)	9/1,294	17/1,303	0.53	0.24-1.19	
Hypertension					0.16
Yes	20/2,258	39/2,227	0.50	0.29-0.86	
No	6/349	5/371	1.21	0.37-3.99	
Hyperlipidemia					0.97
Yes	20/2,023	34/2,046	0.59	0.34-1.02	
No	6/584	10/552	0.70	0.25-1.97	
Diabetes					0.19
Yes	1/554	7/560	0.14	0.02-1.12	
No	25/2,053	37/2,038	0.65	0.39-1.09	
Prior cardiovascular disease					0.57
Yes	21/1,678	33/1,627	0.64	0.37-1.09	

	Number of AMD		RR*	95% CI	P (test of interaction)
	Folic acid/B <sub>6</sub> /B <sub>12</sub> (N=2,607)	Placebo (N=2,598)			
No	5/929	11/971	0.45	0.16–1.30	
HRT use (current) <sup>‡</sup>					0.92
Yes	12/1,274	21/1,280	0.60	0.29–1.22	
No	14/1,102	23/1,096	0.57	0.29–1.11	
Multivitamin use (current)					0.92
Yes	6/587	9/599	0.72	0.26–2.03	
No	20/2,020	35/1,997	0.56	0.32–0.97	
Aspirin use in past month					0.30
Yes	18/1,626	36/1,614	0.51	0.29–0.90	
No	8/980	8/984	0.91	0.34–2.44	

Abbreviations: AMD, age-related macular degeneration; HRT, hormone-replacement therapy.

\* Adjusted for age and vitamin C, vitamin E, and beta carotene treatment assignment.

<sup>‡</sup> Analysis restricted to post-menopausal women.