Published in final edited form as:

Ophthalmic Epidemiol. 2016 ; 23(1): 32–39. doi:10.3109/09286586.2015.1130845.

Folic Acid, Vitamin B₆, and Vitamin B₁₂ in Combination and Age-related Cataract in a Randomized Trial of Women

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Abstract

Purpose—To examine incidence of cataract and cataract extraction in a trial of folic acid and vitamins B₆ and B₁₂.

Methods—In a randomized, double-masked, placebo-controlled trial, 5,442 female health professionals aged 40 years or older with preexisting cardiovascular disease (CVD) or 3 or more CVD risk factors were randomly assigned to receive a combination of folic acid (2.5mg/day), vitamin B₆ (50mg/day), and vitamin B₁₂ (1mg/day), or placebo. A total of 3,925 of these women did not have a diagnosis of cataract at baseline and were included in this analysis. The primary endpoint was age-related cataract, defined as an incident age-related lens opacity, responsible for a reduction in best-corrected visual acuity to 20/30 or worse, based on self-report confirmed by medical record review. Extraction of incident age-related cataract was a secondary endpoint of the trial.

Results—During an average of 7.3 years of treatment and follow-up, 408 cataracts and 275 cataract extractions were documented. There were 215 cataracts in the combination treatment group and 193 in the placebo group (hazard ratio, HR, 1.10, 95% confidence interval, CI, 0.90–1.33; P=0.36). For the secondary endpoint of cataract extraction, there were 155 in the combination treatment group and 120 in the placebo group (HR 1.28, 95% CI 1.01–1.63; P=0.04).

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Conflict of interest: The authors report no conflict of interest.

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

This submission has not been published anywhere previously and is not being considered for any other publication.

Trial Registration: NCT00000161, clinicaltrials.gov

Conclusions—In this large-scale randomized trial of women at high risk of CVD, daily supplementation with a combination of folic acid, vitamin B₆, and vitamin B₁₂ had no significant effect on cataract, but may have increased the risk of cataract extraction.

Keywords

Folic acid; vitamin B₆; vitamin B₁₂; supplements; cataract; randomized controlled trial; Women's Antioxidant Folic Acid Cardiovascular Study

INTRODUCTION

Nutritional factors, particularly nutrients with antioxidant capabilities, are believed to play a role in cataract development.^{1–3} Observational data from dietary and blood-based studies generally support an inverse relation between risk of cataract and a range of antioxidant nutrients.^{4–6} However, the results of randomized trials completed to date provide little evidence that supplements of 1 or several antioxidant nutrients, most notably vitamin E, vitamin C, and beta-carotene, can materially alter rates of cataract.^{7–15}

Folic acid and vitamins B₆ and B₁₂ have also been linked with lower rates of cataract in observational studies,^{16–24} but have not yet been tested in randomized trials of cataract prevention. In addition to their antioxidant capabilities,^{25–28} these nutrients exhibit other physiological actions including a well-established homocysteine-lowering effect,²⁹ and enhanced bioavailability of nitric oxide (NO),^{30,31} that might also affect cataract development

In this report, we present the results for cataract from the Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS). WAFACS was a randomized controlled trial that evaluated combined treatment with folic acid, vitamin B₆, and vitamin B₁₂ in the prevention of cardiovascular events among women at high risk of cardiovascular disease (CVD). The randomized trial results for age-related macular degeneration (AMD) in WAFACS were previously published.³²

MATERIALS AND METHODS

WAFACS was a randomized, double-blind, placebo-controlled trial funded by the National Heart, Lung, and Blood Institute (NHLBI) that tested whether a combination of folic acid, vitamin B₆, and vitamin B₁₂ could reduce cardiovascular events among women with preexisting CVD or 3 coronary risk factors.^{33–36} Funding to collect data for cataract and AMD in WAFACS was provided by the National Eye Institute (NEI). The WAFACS trial began in 1998 when the folic acid, vitamin B₆, and vitamin B₁₂ arm was added to the ongoing Women's Antioxidant Cardiovascular Study (WACS), a 2×2×2 factorial trial of 8,171 female health professionals at high risk of CVD, randomized to vitamin E, vitamin C, beta-carotene, or placebo (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, vitamin B₆, and vitamin B₁₂ arm of the trial. Of these, 5,442 women were willing and eligible to participate and were willing to forego the use of vitamin B supplements or multivitamins with greater than the recommended daily allowance of folic

acid, vitamin B₆, and vitamin B₁₂. In April 1998, these women were randomized in a retained factorial design to a daily combination of folic acid (2.5mg), vitamin B₆ (50mg), and vitamin B₁₂ (1mg). Of these, 3,925 women were without a diagnosis of cataract at baseline and are included in these analyses; 1,969 were in the combination treatment group and 1,956 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 cataract. Pill-taking was completed on July 31, 2005, at which point morbidity and mortality follow-up was 92.6% complete. Endpoint ascertainment for cataract 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 cataract on the baseline questionnaire were excluded. Information on new diagnoses of cataract was requested on annual questionnaires. Participants were asked "Since your last questionnaire, have you had any of the following?" with response options including "cataract right eye", "cataract extraction right eye", "cataract left eye", and "cataract extraction left eye". Participants who responded affirmatively were requested to provide the month and year of the ocular event and to complete a consent form granting permission to examine medical records pertaining to the event. Ophthalmologists and optometrists were contacted by mail and requested to complete a cataract questionnaire that asked about the presence of lens opacities, date of diagnosis, best-corrected visual acuity at diagnosis, and date when best-corrected visual acuity reached 20/30 or worse (if different from the date of diagnosis). The questionnaire also asked about cataract extraction, cataract type (eg, nuclear sclerosis, cortical, posterior subcapsular, PSC) and origin (including age-related, traumatic, congenital, inflammatory, or surgery- or steroid-induced), and whether there were other ocular abnormalities that could explain visual acuity loss. In the presence of other ocular abnormalities, ophthalmologists and optometrists were asked for their judgment as to whether the opacity, when considered alone, was of sufficient severity to reduce visual acuity to 20/30 or worse. Ophthalmologists and optometrists were given the option to provide the requested information by supplying copies of the relevant medical records. Medical record information was obtained for more than 92% of participants reporting cataract.

The primary study endpoint was incident cataract defined as a self-report confirmed by medical record review to be initially diagnosed after randomization but before July 31 2005, age-related in origin (congenital cataracts and those due to trauma, steroids, intraocular inflammation, or surgery were excluded), and responsible for a decrease in best-corrected visual acuity to 20/30 or worse. Cataract extraction was a secondary endpoint and was defined as the surgical removal of an incident cataract.

Data analysis

Cox proportional hazards regression was used to estimate the hazard ratio (HR) of cataract and cataract extraction among those in the combination treatment group compared with those in the placebo group after adjustment for age (years) at baseline and randomized vitamin C, vitamin E, and beta-carotene treatment assignments. Models were also fit separately within 3 pre-specified age groups (40–54, 55–64, 65+ years), and tests of trend were calculated by including a term for the interaction of combination treatment assignment and age (expressed as a continuous variable with values 1 to 3 corresponding to the 3 age groups) in a proportional hazards model. We tested the proportionality assumption throughout the follow-up period by including an interaction term of combination treatment assignment with the logarithm of time in the Cox models. The proportionality assumption was not violated for either cataract ($P=0.31$) or extraction ($P=0.83$). For each HR, we also calculated the 95% confidence interval (CI) and 2-sided P-value.

We analyzed subgroup data by categories of baseline variables that are possible risk factors for cataract, and explored possible modification of any effect of combination treatment by using interaction terms between subgroup indicators and combination treatment assignment, testing for trend when subgroup categories were ordinal.

We also considered the possibility that the apparent effect of treatment on cataract reflected, at least in part, the effect of treatment on AMD. To address this possibility, separate proportional hazards models were fitted to estimate the effect of the intervention on cataract while adjusting for a diagnosis of AMD as a time-varying covariate. We used similar analyses to assess the possibility that the previously reported effect of combination treatment on AMD reflected, at least in part, the effect of treatment on cataract.

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 the occurrence of cataract surgery or, in the absence of cataract surgery, by an earlier date of diagnosis.^{37,38} When both eyes had the same date of diagnosis, we designated the eye with the worse visual acuity at the most recent eye exam as the worse eye. When the worse eye was excluded because of visual acuity loss attributed to other ocular abnormalities or a cause which was not age-related, the fellow eye was considered for classification.

RESULTS

As expected in this large randomized trial, baseline characteristics of the participants were equally distributed between the combination treatment group and the placebo group (Table 1).

During an average of 7.3 years of treatment and follow-up, a total of 408 cataracts and 275 cataract extractions were documented. Participants assigned to the combination treatment group had a statistically non-significant 10% increased risk of cataract (215 cases versus 193 cases; HR 1.10, 95% CI 0.90–1.33; Table 2). Analyses of cataract subtypes indicated small, and statistically non-significant, increased risks of any nuclear sclerosis cataract (206 cases versus 180 cases; HR 1.13, 95% CI 0.92–1.37) and any cortical cataract (93 cases versus 74

cases; HR 1.24, 95% CI 0.91–1.68). For PSC cataract, there was a significant 60% increased risk in the combination treatment group (63 cases versus 39 cases; HR 1.60 95% CI 1.07–2.39). HRs did not vary significantly over the 3 age groups for cataract or any subtype.

For cataract extraction, the data indicated a significant 28% increased risk for women in the combination treatment group (155 cases versus 120 cases; HR 1.28, 95% CI 1.01–1.63). This included significantly elevated risks for all 3 subtypes; any nuclear sclerosis cataract (148 cases versus 112 cases; HR 1.31, 95% CI 1.03–1.68), any cortical cataract (76 cases versus 45 cases; HR 1.67, 95% CI 1.16–2.42), and any PSC cataract (53 cases versus 32 cases; HR 1.65, 95% CI 1.07–2.56; Table 3). HRs did not vary significantly over the 3 age groups for either cataract extraction[AU: confirm?] or subtypes.

Cumulative incidence rates of cataract and extraction according to year of follow-up are shown in Figure 2. For both cataract and extraction, curves appeared to diverge at about 3 years of follow-up, but attained statistical significance only for the extraction endpoint. For both cataract and extraction, the effect of combination treatment did not differ markedly within categories of known or possible risk factors listed in Table 1 (all P for interaction >0.10; data not shown).

HR estimates for cataract endpoints were not materially altered in analyses that accounted for a diagnosis of AMD as a time-varying covariate (cataract HR 1.09, 95% CI 0.90–1.33; extraction HR 1.28, 95% CI 1.01–1.63). Similarly, the previously reported beneficial effect of combined treatment on visually-significant AMD (HR 0.59, 95% CI 0.36–0.95) changed very little after adjustment for incident cataract (HR 0.58, 95% CI 0.36–0.94) or extraction (HR 0.57, 95% CI 0.35–0.93).

DISCUSSION

In this population of generally well-nourished women with existing CVD or risk factors, women assigned treatment with supplemental folic acid and B vitamins, compared to those assigned placebo, had a statistically non-significant 10% increased risk of the primary endpoint of cataract during 7.3 years of treatment and follow-up. Analyses of subtypes indicated a significant 60% increased risk of PSC cataract in the treated group. For the secondary endpoint of cataract extraction, women in the treated group had a significant 28% increased risk that included significant increases for all 3 subtypes.

To our knowledge, these are the first randomized trial data to assess the separate effect of combined treatment with folic acid, vitamin B₆, and vitamin B₁₂ in cataract prevention. 3 previous trials tested folic acid, vitamin B₆, and vitamin B₁₂ as components of a multivitamin/multimineral supplement, but the separate effects of these nutrients could not be determined in those trials.^{39–41} Previous observational data for these nutrients, derived from several small cross-sectional and case-control studies, generally indicate an inverse association between dietary or blood levels and risk of cataract.^{16–24} Thus, our finding of no significant effect overall, but significant increases in the PSC subtype, and extraction of all 3 subtypes, in the combined treatment group contrasts with previous observational data. Given the high dose of folic acid, vitamin B₆, and vitamin B₁₂ used in the WAFACS, one

interpretation of these findings is that the optimal dose for cataract prevention may be lower than the high doses tested in this trial.

The current findings for cataract also appear to contrast with the beneficial effect of treatment on AMD previously reported in these women.³² If real, the reasons for these contrasting effects are unclear. Folic acid and B vitamins are known to exert an antioxidant effect, and to lower plasma homocysteine levels. Indeed, in a substudy of 300 WAFACS participants (150 in each treatment group) who had blood samples collected at study entry in 1993 through 1995, and again at study completion in 2005, the geometric mean plasma homocysteine level was decreased by 18.5% (95% CI 12.5–24.1%) in the active arm compared with the placebo arm ($P < 0.001$).³³ However, it seems unlikely that lowering oxidation levels or plasma homocysteine levels would have an effect on cataract that was opposite to the effect on AMD. On the other hand, folic acid and B vitamins also increase the bioavailability of NO which could have dissimilar effects in the retina and in the lens. NO is a potent vasodilator and an important regulator of choroidal blood flow in the macular region of the retina.^{42–44} Thus, increased bioavailability of NO and enhancement of choroidal blood flow in the treated group in WAFACS may have contributed to our findings of benefit in AMD. On the other hand, there is growing evidence to suggest that increased levels of NO may be harmful to the human lens. Though the lens is avascular, NO is typically present at low concentrations in the aqueous humor that surrounds the lens.^{45,46} In vitro studies have shown that lenses bathed in a solution with a high concentration of NO are susceptible to biochemical and metabolic damage similar to changes seen in the aging lens and cataract.^{47–49} Moreover, nitrite, a stable metabolite of NO, has been found in higher concentration in human cataractous lenses compared to normal lenses.⁵⁰ It is also worth noting that nitrite levels are markedly increased by cigarette smoking,^{51–53} a well-established risk factor for cataract.^{54–57} Additional research is required to distinguish between these and other possible explanations for our findings.

Several possible limitations of our study need to be addressed. Misclassification of the cataract endpoint is a potential concern. Because our studies of cataract are conducted in large cohorts of men and women, it is not feasible to conduct eye examinations for all study participants. Instead, the cataract endpoint is based on participant reports and thus some degree of underascertainment of diagnosed cataract is plausible (underascertainment of cataract extraction seems less likely⁵⁸). However, underascertainment of disease is not associated with bias in randomized comparisons.⁵⁹ Random misclassification of reported cataract, which would tend to shift the relative risk estimate toward the null, was reduced by the use of medical records to confirm the participant reports. Non-random or differential misclassification was unlikely since medical records were reviewed without knowledge of combination treatment assignment, and study participants and treating ophthalmologists and optometrists were unaware of treatment assignment. Confounding is unlikely since, as expected, baseline characteristics were equally distributed between the combination treatment and placebo groups. Finally, it should be noted that this methodology has identified several important risk factors for cataract (eg, cigarette smoking,^{54,60} alcohol intake,⁶¹ and body weight^{62,63}) that have also been identified in examined populations using lens photographs and a standardized grading system which provides reassuring evidence for the construct validity of the methodology.

In summary, among women at high risk of CVD, daily supplementation with a combination of folic acid, vitamin B₆, and vitamin B₁₂ during an average of 7.3 years of treatment and follow-up had no significant overall effect on total cataract. However, women in the treated group did have a significant increased risk of PSC cataract, and a significant increased risk of extraction of all 3 cataract subtypes, which needs to be confirmed in other populations.

Acknowledgments

Supported by research grants HL46959 from the National Heart, Lung, and Blood Institute and EY 06633 from the National Eye Institute. Vitamin E and its placebo were provided by Cognis Corporation (LaGrange, IL). All other agents and their placebos were provided by BASF Corporation (Mount Olive, NJ). Cognis Corporation and BASF Corporation did not participate in the design and conduct of the study, in the collection, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

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Figure 1.

Enrollment and randomization scheme for the folic acid, vitamin B₆, and vitamin B₁₂ component of the Women's Antioxidant and Folic Acid Cardiovascular Study, USA (IU, international units; FA, folic acid; B₆, vitamin B₆; B₁₂, vitamin B₁₂)

Figure 2.
Cumulative incidence rates of cataract and cataract extraction in the Women's Antioxidant and Folic Acid Cardiovascular Study, USA
(y, years)

Table 1

Baseline characteristics in randomized folic acid, vitamin B₆, vitamin B₁₂ and placebo treatment groups in the Women's Antioxidant and Folic Acid Cardiovascular Study, USA

Characteristic	Folic acid/B ₆ /B ₁₂ (N=1,969)	Placebo (N=1,956)
Age, years		
Mean ±SD	60.5 ± 8.2	60.3 ± 8.1
40–54, %	27.7	28.6
55–64, %	42.6	40.5
65+, %	29.7	30.9
Cigarette smoking, %		
Current	12.4	12.8
Past only	42.6	44.8
Never	45.0	42.3
Alcohol use, %		
Daily	8.3	8.8
Weekly	25.8	24.7
Rarely/never	66.0	66.5
Body-mass index, [†] kg/m ²		
Mean ±SD	30.9 ± 6.7	31.2 ± 6.9
<25.0, %	20.9	18.5
25.0–29.9, %	27.7	28.2
30.0, %	51.4	53.3
Hypertension [‡] , %	85.3	85.1
Elevated cholesterol level [§] , %	76.9	77.8
Diabetes, %	18.9	18.9
Menopausal status, %		
Premenopausal	8.1	8.5
Postmenopausal/current HT	50.3	49.6
Postmenopausal/no HT	38.2	39.5
Dubious/unclear	3.3	2.4
Baseline strata, %		
Prior CVD	60.9	59.7
3 risk factors	39.1	40.3
Multivitamin use (current) , %	23.2	23.3
Aspirin use in past month [#] , %	61.7	60.4
Baseline dietary intake, mean ±SD		
Folic acid, mcg/day	491 ± 245.6	489 ± 241.7
Vitamin B ₆ , mg/day	4.9 ± 15.0	5.6 ± 17.3
Vitamin B ₁₂ , mcg/day	8.8 ± 8.4	8.9 ± 8.1
Randomized to receive vitamin E, %		
Yes	50.7	49.5

Characteristic	Folic acid/B ₆ /B ₁₂ (N=1,969)	Placebo (N=1,956)
No	49.3	50.5
Randomized to receive vitamin C, %		
Yes	49.5	50.5
No	50.5	49.5
Randomized to receive beta-carotene, %		
Yes	50.5	49.7
No	49.5	50.3

SD, standard deviation; HT, hormone therapy; CVD, cardiovascular disease.

[†] Calculated as weight in kg divided by the square of height in m.

[‡] Self-reported systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, self-reported physician-diagnosed hypertension, or reported treatment with medication for hypertension.

[§] Self-reported high cholesterol levels, cholesterol level ≥ 240 mg/dL, self-reported physician-diagnosed high cholesterol levels, or reported treatment with medication to lower cholesterol levels.

[¶] Any multivitamin use in the past month.

[#] Aspirin use at least 4 times per month.

Cases of cataract and cataract subtypes according to randomized treatment assignment in 3 age groups in the Women's Antioxidant and Folic Acid Cardiovascular Study, USA

Table 2

Cataract subtype	Folic acid/B ₆ /B ₁₂ , n (N=1,969)	Placebo, n (N=1,956)	RR* (95% CI)	P-value	P for trend [†]
Total cataract					
40–54 years	11	13	0.88 (0.39–1.96)	0.75	0.19
55–64 years	75	73	0.92 (0.67–1.27)	0.61	
65 years	129	107	1.27 (0.98–1.64)	0.07	
Total	215	193	1.10 (0.90–1.33)	0.36	
Nuclear sclerosis [‡]					
40–54 years	11	8	1.41 (0.57–3.50)	0.46	0.45
55–64 years	71	70	0.91 (0.65–1.27)	0.58	
65 years	124	102	1.28 (0.98–1.66)	0.07	
Total	206	180	1.13 (0.92–1.37)	0.25	
Cortical [‡]					
40–54 years	2	5	0.41 (0.08–2.10)	0.28	0.37
55–64 years	34	25	1.21 (0.72–2.04)	0.46	
65 years	57	44	1.36 (0.92–2.02)	0.12	
Total	93	74	1.24 (0.91–1.68)	0.17	
PSC [‡]					
40–54 years	5	6	0.89 (0.27–2.91)	0.84	0.92
55–64 years	28	14	1.80 (0.95–3.42)	0.07	
65 years	30	19	1.68 (0.95–2.99)	0.08	
Total	63	39	1.60 (1.07–2.39)	0.02	

RR, relative risk; CI, confidence interval; PSC, posterior subcapsular; B₆, vitamin B₆; B₁₂, vitamin B₁₂

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

[†] P test for trend of the effect of age on the association between folic acid/B₆/B₁₂ and cataract.

[‡] With or without other subtypes.

Table 3

Cases of extraction of cataract and cataract subtypes according to randomized treatment assignment in 3 age groups in the Women's Antioxidant and Folic Acid Cardiovascular Study, USA

Cataract subtype	Folic acid/B ₆ /B ₁₂ , n (N=1,969)	Placebo, n (N=1,956)	RR* (95% CI)	P value	P for trend [†]
Total extraction					
40–54 years	8	8	1.05 (0.39–2.80)	0.92	0.16
55–64 years	50	45	1.00 (0.67–1.50)	0.99	
65 years	97	67	1.53 (1.12–2.09)	0.008	
Total	155	120	1.28 (1.01–1.63)	0.04	
Nuclear sclerosis [‡]					
40–54 years	8	5	1.65 (0.54–5.05)	0.38	0.35
55–64 years	47	43	0.99 (0.66–1.50)	0.97	
65 years	93	64	1.54 (1.12–2.11)	0.008	
Total	148	112	1.31 (1.03–1.68)	0.03	
Cortical [‡]					
40–54 years	2	4	0.51 (0.09–2.80)	0.44	0.50
55–64 years	26	12	1.93 (0.97–3.83)	0.06	
65 years	48	29	1.75 (1.10–2.77)	0.02	
Total	76	45	1.67 (1.16–2.42)	0.006	
PSC [‡]					
40–54 years	5	4	1.35 (0.36–5.03)	0.66	0.62
55–64 years	22	13	1.52 (0.77–3.03)	0.23	
65 years	26	15	1.84 (0.97–3.47)	0.06	
Total	53	32	1.65 (1.07–2.56)	0.03	

RR, relative risk; CI, confidence interval; PSC, posterior subcapsular; B₆, vitamin B₆; B₁₂, vitamin B₁₂

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

[†] P test for trend of effect of age on the association between folic acid/B₆/B₁₂ and cataract.

[‡] With or without other subtypes.