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A prospective study of folate, vitamin B6 and vitamin B12 in relation to exfoliation glaucoma or exfoliation glaucoma suspect

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Abstract

IMPORTANCE—Effective strategies for primary prevention are lacking for exfoliation glaucoma, which is the most common type of secondary glaucoma.

OBJECTIVE—We examined the association of B vitamin intake and exfoliation glaucoma or exfoliation glaucoma suspect (EG/EGS) risk.

DESIGN—Prospective cohort study using 20+ years follow-up data from Nurses' Health Study (NHS; all female registered nurses) and the Health Professionals Follow-up Study (HPFS; all male health professionals) from 1980 (NHS) / 1986 (HPFS) to 2010.

SETTING—United States.

PARTICIPANTS—We included a subset of 78,980 NHS women and 41,221 HPFS men who were 40+ years of age, free of glaucoma, had completed diet questionnaires and reported eye exams (follow-up rate >85%).

EXPOSURE—Cumulatively updated intake of B vitamins (folate, vitamin B6 and vitamin B12) as ascertained by repeated administration of validated questionnaires.

MAIN OUTCOME MEASURE—Incident cases of EG/EGS, totaling 399 cases (329 women and 70 men), were first identified with the questionnaires and were subsequently confirmed with medical records. Multivariate rate ratios (MVRRs) for EG/EGS were calculated in each cohort and then pooled with meta-analysis.

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Dr. Jae Hee Kang 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.

Dr. Jae Hee Kang (Assistant Professor of Medicine, Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital / Harvard Medical School, Boston, MA) conducted and was responsible for data analysis.

None of the authors have conflicts of interest, including financial interests, activities, relationships and affiliations.

RESULTS—Vitamin B6 and vitamin B12 intake were not associated with EG/EGS risk in pooled analyses (p for linear trend was 0.52 and 0.99, respectively). However, a suggestive trend of a reduced risk was observed with higher intake of folate: compared with the lowest quintile (Q1) of cumulatively averaged updated total folate intake, the MVRR of EG/EGS for the highest quintile (Q5; ≥ 654 μg/day) was 0.75 (95% Confidence Interval[CI]: 0.54 - 1.04; p for linear trend=0.02). These results were not materially altered after adjustment for vitamin B6 and vitamin B12. An association was observed for supplemental folate intake but not for folate from diet only (p for linear trend was 0.03 and 0.64, respectively). Greater frequency of multivitamin use showed a modest suggestive inverse association (current multivitamin use of 6+times / week versus non-use MVRR= 0.84, 95% CI, 0.64-1.11; p for linear trend=0.06).

CONCLUSIONS AND RELEVANCE—Higher total folate intake was associated with a suggestive lower risk of EG/EGS, supporting a possible etiologic role of homocysteine in EG/EGS.

INTRODUCTION

Exfoliation glaucoma (EG), associated with exfoliation syndrome (ES), is the most common secondary open-angle glaucoma.[1] In EG, IOP may become elevated due to narrowing and increased pigmentation in the filtration angle,[2] protein leakage into the anterior chamber[3] and exfoliation material build-up in the trabecular meshwork.[4] Established EG risk factors include older age,[5,6] lysyl oxidase like 1 variants (*LOXL1*)[7] and northern latitude residence.[7-9] Effective strategies for EG primary prevention are lacking.

One possible EG risk factor that has received substantial research attention is homocysteine (Hcy). Elevated Hcy may enhance exfoliation material formation by contributing to vascular damage,[10] oxidative stress,[11,12] and extracellular matrix alterations.[13] Indeed, Hcy levels in plasma,[14-19] aqueous humor[20] and tears[21] have been consistently elevated with ES/EG. Lowering Hcy levels may be an attractive target for intervention, as this can be achieved by increasing intakes of vitamin B6, vitamin B12, and most importantly, folate.[22] Several case-control studies have reported lower plasma folate levels with ES,[23-27] but did not find differences in vitamin B6 or vitamin B12 levels.[28] Major limitations of these studies are the small sample size and cross-sectional design. To date, no prospective studies have evaluated B vitamin intake and risk of EG; thus, we conducted a prospective study among 78,980 women and 41,221 men followed for 20+ years of the relation between intake of folate, vitamin B6 and vitamin B12 and risk of exfoliation glaucoma or glaucoma suspect (EG/EGS).

METHODS

Study population

In 1976, the NHS began when 121,700 US registered female nurses aged 30 to 55 years responded to a mailed questionnaire.[29] In 1986, the HPFS began with 51,529 male health professionals who responded to a similar questionnaire. In these studies, participants have been completing biennial questionnaires about their diet, lifestyle and newly diagnosed diseases, such as glaucoma. The follow-up rate was high (> 85%). The Human Research

Committees of Brigham & Women's Hospital, Harvard School of Public Health and Massachusetts Eye and Ear Infirmary approved this study.

The study period was from "baseline" (1980 in NHS and 1986 in HPFS) to 2010. A participant contributed person-time, if s/he was aged 40+ years (as glaucoma risk increases > 40 years) and indicated an eye exam in the 2-year risk period (to minimize detection bias). Participants contributed person-time in approximate 2-year units based on biennial questionnaire responses, from baseline until the earliest occurrence of glaucoma, cancer, death, loss to follow-up or 2010 (study end).

Of the original cohort members, participants were excluded at baseline for the following reasons: 1) did not complete the initial semiquantitative food frequency questionnaire (SFFQ); 2) the SFFQ dietary data was inadequate (adequate dietary information was defined as > 50 of 61 items completed, yielding total calories of 500-3500 kcal/day in women and >60 out of 131 items completed, yielding 800-4200 kcal/day for men); 3) prevalent cancer (excluding nonmelanoma skin cancer); 4) prevalent glaucoma or glaucoma suspect; 5) permanently lost to follow-up within 2 years after baseline; 6) no report of any eye exam during follow-up; and 7) prevalent cataract extraction, which makes EG/EGS diagnosis difficult. By 2010, a total of 78,980 women and 41,221 men contributed person-time.

Case identification

From participants self-reporting glaucoma, we obtained permission to retrieve medical information. Diagnosing eye care providers were sent a glaucoma questionnaire about maximum IOP, optic nerve features, filtration apparatus status and presence of exfoliation material or other secondary causes for elevated IOP and were asked to send all visual field (VF) reports; alternatively, they were asked to send complete medical records. A glaucoma specialist (LRP), masked to dietary data, evaluated the available medical information in a standardized manner to confirm diagnoses.

We defined EG/EGS based on documentation ≥1 of the following in the eye with exfoliation material: 1) IOP > 21 mm Hg, 2) cup-disc ratio ≥ 0.6 or 3) VF loss consistent with glaucoma on at least 1 reliable test. Patients with ES only who did not meet these criteria were excluded from analysis, as we initially asked all participants about any glaucoma diagnoses, and our intent was to identify different types of glaucoma cases.

During the study period, 8,029 women and 3,422 men reported new glaucoma diagnoses. This was confirmed in 64% of women and 56% of men as follows: EG/EGS (5% of total women, 3% of total men), POAG (26% women, 27% men), only elevated IOP or optic disc cupping (19% women, 16% men), and other types of glaucomas or glaucoma suspect (14% women, 10% men). The remaining were unconfirmed (36% women, 44% men), as the participants (7% women, 14% men), or eye care providers (5% women, 4% men) could not be contacted, participants did not give permission for record review (12% women, 10% men), participants indicated the initial report was erroneous (10% women, 14% men) or eye care providers refuted the glaucoma diagnosis (2% women, 2% men). Ultimately, we included 329 women and 70 men who met the criteria for incident EG/EGS.

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Measurement of intake of folate, vitamin B6 and vitamin B12

We collected dietary intake data repeatedly using the SFFQ every 2-4 years from 1980 in NHS and from 1986, in HPFS.[30,31] The 1980 SFFQ included 61 food / beverage items; the 1984 SFFQ included 116 items, and similar versions were used from 1986 (126 items) onward in the NHS and HPFS (131 items). The SFFQ lists foods/beverages, each with a serving size, and asks about the average intake over the past year (with nine responses for intake frequency from “never or less than once per month” to “6 or more times per day”). Questions were included on the brands of breakfast cereal used (as these foods are an important source of B vitamins) as well as on the brands and duration of use of multivitamins used.

Nutrient intakes were computed by multiplying the consumption frequency of each food by the nutrient content of the portion specified. Food nutrient contents were obtained from the Harvard University Food Composition Database, derived mainly from US Department of Agriculture sources[32] and other sources; these sources have been continually updated, with incorporation of changes in food folate content after grain folate fortification.[33] All nutrient values were total-energy-adjusted with the residuals method.[34] In a validation study of the SFFQ compared with detailed 1-week diet records,[31,35] the correlation coefficient between the SFFQ estimates, and the dietary record estimates was 0.77 for folate, 0.85 for vitamin B6 and 0.56 for vitamin B12. Furthermore, the correlation between folate intake and red cell folate level was 0.56,[36,37] and vitamin B6 intake predicted plasma pyridoxal phosphate levels (r=0.52).

Statistical Analysis

For each cohort, we calculated the cumulatively updated intakes for each B vitamin by averaging intakes from all available dietary assessments up to the start of each 2-year risk period. Because glaucoma develops slowly, we chose to study cumulatively averaged intakes as they best represent long-term diet; also, cumulatively averaged intake estimates have inherently less measurement error than estimates from single assessments.[38]

For age-adjusted and multivariable analyses that controlled for potential EG/EGS risk factors simultaneously, we used Cox proportional hazards analysis stratified by age in months and the specific 2-year risk period[39] to estimate incidence rate ratios (RR) and their 95% confidence intervals (CIs). We conducted tests for trend by including the median values within each intake category of quintiles.

We first analyzed each cohort’s data separately and then pooled the results using meta-analytic methods incorporating random effects with testing for any heterogeneity across cohorts.[40]

We included the following covariates in our multivariable models: family history of glaucoma (glaucoma in biologic parents, siblings or children), major ancestry (Scandinavian Caucasian, Southern-European Caucasian, other Caucasian, other ancestry), body mass index (kg/m^2), cigarette smoking (pack-years), cumulatively updated intakes of alcohol (g/day), total calories (kcal/day) and caffeine (mg/day), self-report (yes/no) of hypertension, diabetes, high cholesterol and myocardial infarction, and lifetime average continental US residence categorized by latitude (Northern:> 42° N; Middle: 37°-42° N; Southern <37° N).

For secondary analyses, we evaluated timing of exposure by investigating baseline intake only and the most recent intake only. Also, we separately evaluated the intakes from different sources, i.e., diet only or from supplements only – this may be most pertinent for vitamin B12[41] and folate, which are more bioavailable in supplemental form (e.g., supplemental folate in the form of folic acid is more bioavailable than natural folate (1 μg of food folate = 1.0 μg of dietary folate equivalents (DFEs) but 1 μg of folate supplement = 2.0 μg of DFEs).[42]

To investigate effect modification by other factors for folate, we examined associations separately by family history of glaucoma, lifetime residential history[43] and alcohol intake (as alcohol can interfere with folate metabolism).[22] We tested for effect modifications by testing interaction terms in Cox regression models.

Because the source of supplemental B vitamins in this population was mostly from multivitamins, we evaluated the association between multivitamin use and risk of EG/EGS.

RESULTS

During follow-up with 1,347,004 person-years in NHS and 494,822 person-years in HPFS, we identified 329 incident EG/EGS cases in the NHS and 70 cases in HPFS. As expected, at diagnosis, EG/EGS cases were likely to be > 65 years old (mean age of 68 (SD=7) years in women and 71 (SD=7) in the men), had high IOP (28-29 mmHg (SD=7)) and had one affected eye (53-64%).

Participants with the highest B vitamin intakes generally consumed less alcohol, caffeine, and cigarettes, were likely to have lower body mass index and less likely to have lived in the Northern tier states compared with the lowest consumers (Table 1). All these differences were accounted for in multivariable analyses.

Age-adjusted and multivariable analyses were similar, as were the results in men and women (Table 2). In pooled results, there was an inverse trend between higher total folate intake and risk of EG/EGS (p-value for linear trend [p-trend] =0.02; Table 2). Compared with the lowest quintile (Q1; median of 217 μg/day in women and 282 μg/day in men), the multivariable relative risk (MVRR, 95% Confidence Interval (CI)) of EG/EGS was 0.75 (95% CI, 0.54 −1.04) for the highest quintile of total folate intake (Q5; 654 μg/day in women and 839 μg/day in men). These results were minimally changed when we additionally adjusted for vitamin B6 and vitamin B12: MVRR for Q5 vs. Q1 was 0.75 (95% CI, 0.52 −1.07), and p-trend was 0.03.

To evaluate folate intake by source, we simultaneously included dietary folate and supplemental folate in multivariable models. We did not observe trends with dietary intake (p-trend=0.64); compared with Q1 of dietary folate intake (197 μg/day in women and 259 μg/day in men), the MVRR of EG/EGS was 0.97 (95% CI, 0.70 −1.35) for Q5 (377 μg/day in women and 496 μg/day in men). However, we observed (p-trend=0.03) inverse trends with greater supplemental folate intake: compared with Q1 of supplementary folate intake (0 μg/day in women and men), the MVRR of EG/EGS was 0.83 (95% CI, 0.54 −1.28) for Q5 (335 μg/day in women and 434 μg/day in the men).

For timing of exposure, the association between total folate intake at baseline was null, while the association with the most recent diet was similar to that with the cumulatively averaged diet (main analysis): MVRR for Q5 vs. Q1 was 0.73 (95% CI, 0.53 −1.02), and p-trend was 0.02.

We did not observe interactions of folate intake with alcohol intake, family history or geographic location (p-interaction>0.60) (Table 3). Nonetheless, suggestive stronger inverse associations were observed for those with higher intake of alcohol (p-trend=0.03 with MVRR for Q5 vs. Q1=0.63, 95% CI, 0.33-1.21), those without a family history (p-trend=0.09 with MVRR for Q5 vs. Q1=0.72, 95% CI, 0.49-1.08) and those who have lived mostly in the northern tier (p-trend=0.02 with MVRR for Q5 vs. Q1=0.73, 95% CI, 0.47-1.15). We did not observe interactions between supplemental and dietary intakes (p-interaction=0.29); however, inverse trends were observed for greater supplemental folate intake among those with dietary folate intake less than the median (p-trend=0.01 with MVRR for Q5 vs. Q1=0.65, 95% CI, 0.33-1.27).

Overall, we did not observe associations between higher total vitamin B6 intake and risk of EG/EGS (Table 4). Compared with Q1 (1 mg/day in women and 2 mg/day in men), the MVRR of EG/EGS was 0.86 (95% CI, 0.53 −1.41) for Q5 (14 mg/day in women and men). The point estimate was closer to the null when we additionally adjusted for folate and vitamin B12: MVRR for Q5 vs. Q1 was 1.05 (95% CI, 0.67 −1.62), and p-trend was 0.53. There was little variation in dietary vitamin B6, and associations were null; for supplemental B6, the p-trend was 0.45, and the MVRR for Q5 vs. Q1 was 0.77 (95% CI, 0.50-1.18). Analysis results of baseline intake or the most recent intake of vitamin B6 were similar to the main analysis results.

Similarly, we did not observe a trend (p-trend=0.99) between higher total vitamin B12 intake and risk of EG/EGS (Table 5). Compared with Q1 (5 μg/day in women and 6 μg/day in the men), the MVRR of EG/EGS was 0.88 (95% CI, 0.64 −1.21) for Q5 (16 μg/day in women and 22 μg/day in the men). The point estimate was closer to the null when we additionally adjusted for folate and vitamin B6: MVRR for Q5 vs. Q1 was 1.11 (95% CI, 0.75 −1.63), and p-trend was 0.25. The association with dietary vitamin B12 was null. For supplemental B12, the p-trend was 0.47, and MVRR for Q5 vs. Q1 was 0.59 (95% CI, 0.36-0.98); the point estimate with was closer to the null with adjustment for folate and vitamin B6 (MVRR for Q5 vs. Q1= 0.68, 95% CI, 0.39-1.18; p-trend=0.81). Analysis results of baseline intake or the most recent intake of vitamin B12 were similar to the main analysis results.

Greater frequency of multivitamin use showed a modest suggestive inverse association (current use of 6+times / week versus non-use: MVRR= 0.84, 95% CI, 0.64-1.11; p for linear trend=0.06).

DISCUSSION

We observed a suggestive trend of a lower risk of EG/EGS with higher total folate intake, supporting the possible role of homocysteine in the etiology of EG/EGS. Inverse associations were stronger with supplemental folate (which is more bioavailable than natural folate[42]), especially among those with low dietary folate intake. For folate, no interactions were observed with alcohol, family history or residential latitude. Associations were not observed with baseline intakes, indicating that consistent long-term and more recent intakes of B vitamins may be more etiologically relevant. We did not observe associations with intake of vitamin B6 or vitamin B12. Consistent with our previous study showing associations between EG/EGS and higher coffee intake,[44] which also increases Hcy,[45,46] the inverse association with Hcy lower folate intake further implicate Hcy in the etiology of EG/EGS. Because this is the first prospective study to evaluate long-term B vitamin intake and risk of EG/EGS, our results must be interpreted cautiously.

The association of lower risk of EG/EGS with higher folate only and not with vitamin B6 or vitamin B12 is consistent with a recent meta-analysis of case control studies of plasma levels of Hcy, folate, vitamin B6 and vitamin B12.[25] It reported that compared with levels in controls, the mean blood Hcy in cases was higher and that blood levels in cases of folate, but not vitamin B12 and vitamin B6 levels, was lower.[25] While all these vitamins are likely important for lowering Hcy, the vast majority of participants had intakes of vitamin B6 and vitamin B12 that exceeded the recommended daily allowance (RDA) of ~1 mg and 2.4 µg, respectively, while >50% of participants had low folate intake (RDA of 400 µg),[42] thus, we had more variability in folate intake that allowed for detection of associations. From the diet, folate is commonly found in fruits and vegetables, and in a study from Europe where supplement use and folate fortification is less common than in the US, the Reykjavik Eye Study showed that higher consumption of fruits and vegetables were protective against ES,[47] consistent with our findings. Future studies should assess these possible associations with B vitamin intake, evaluate biomarkers in a prospective design and evaluate gene-environment interactions. Although a recent meta-analysis[25] of genetic variants of Hcy metabolism enzymes showed no overall associations, the studies have been few, and no studies have evaluated gene-environment interactions.

Our study's strengths include the prospective design where diet was assessed before disease onset and the long-term large study with high follow-up rates. Other strengths include repeated updating of diet and lifestyle risk factors that allowed us to examine intake at various periods and the evaluation of nutrients from different sources. Finally, with the wealth of follow-up data, we had information on major potential confounders that were adjusted for to minimize confounding bias.

A major limitation was that it was not feasible to conduct in-person eye exams, and EG/EGS cases were identified by self-report then confirmed by medical records. However, using this approach, we previously confirmed that incident EG/EGS is a strongly age-related condition that produces higher IOPs at diagnosis than incident POAG[9] and that northern latitude residence is a risk factor[9]– this indicates our ability to validly detect established risk factors of EG/EGS.[6] Our study could not identify people with only ES without any signs of glaucoma, and we likely had substantial under-ascertainment of EG/EGS. However, our intent was not to ascertain absolute incidence rates but to evaluate the relative rates by categories of potential risk factors. For such a goal, it has been methodologically established that a low sensitivity for disease identification does not cause biases if the disease definition has high specificity and the under-ascertainment is similar across exposure groups, which applies to our study.[48] One limitation is that higher B-vitamin intake may be a marker of another unmeasured risk factor, and we may have had some residual confounding. Finally, this population was predominately Caucasian and well-nourished, limiting the generalizability of results more broadly.

In conclusion, in this large prospective study, higher intake of folate was associated with a lower risk of EG/EGS. This further supports the hypothesis that Hcy may play an etiologic role in EG/EGS.

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Table 1

Age and age-adjusted characteristics by total folate, vitamin B6 and vitamin B12 intake (1st, 3rd and 5th quintiles) over the follow-up period in NHS (1980-2010) and in HPFS (1986-2010)

		Total Folate			**Total Vitamin B6**			**Total Vitamin B12**		
		Q1	**Q3**	**Q5**	**Q1**	**Q3**	**Q5**	**Q1**	**Q3**	**Q5**
Mean age(years[SD])	Women Men	58 [9] 60 [10]	59 [9] 61 [10]	60 [10] 63 [10]	58 [10] 59 [10]	60 [9] 62 [10]	59 [9] 62 [10]	58 [9] 60 [10]	59 [9] 61 [10]	60 [9] 63 [10]
Folate from diet (μg/d)	Women Men	218 [51] 287 [59]	303 [56] 398 [71]	339 [104] 448 [139]	240 [62] 320 [79]	309 [76] 406 [98]	311 [93] 404 [120]	267 [75] 363 [99]	292 [78] 379 [102]	314 [93] 407 [119]
Folate from supplements (μg/d)	Women Men	12 [24] 12 [26]	74 [72] 87 [93]	373 [215] 470 [227]	13 [29] 13 [38]	110 [100] 121 [121]	267 [253] 359 [281]	22 [42] 24 [53]	113 [114] 141 [147]	282 [256] 344 [288]
Vitamin B6 from diet (mg/d)	Women Men	1 [0.3] 2 [0.4]	2 [0.3] 2 [0.4]	2 [0.5] 3 [0.7]	1 [0.3] 2 [0.3]	2 [0.3] 2 [0.5]	2 [0.4] 2 [0.6]	2 [0.3] 2 [0.5]	2 [0.4] 2 [0.5]	2 [0.4] 2 [0.6]
Vitamin B6 from supplements (mg/d)	Women Men	2 [8] 1 [9]	4 [11] 4 [14]	12 [24] 20 [36]	0 [0.1] 0 [0.1]	1 [0.6] 1 [0.7]	23 [27] 32 [39]	2 [8] 1 [10]	3 [10] 4 [14]	14 [25] 22 [35]
Vitamin B12 from diet (μg/d)	Women Men	6 [3] 8 [4]	7 [3] 9 [4]	7 [3] 9 [5]	6 [3] 8 [4]	7 [3] 9 [4]	7 [3] 8 [5]	4 [1] 5 [1]	7 [2] 8 [2]	9 [4] 12 [7]
Vitamin B12 from supplements (μg/d)	Women Men	1 [6] 1 [6]	4 [10] 5 [13]	13 [29] 20 [38]	1 [5] 1 [5]	3 [6] 4 [9]	16 [31] 24 [41]	0 [1] 0 [1]	3 [2] 3 [3]	18 [33] 27 [43]
Total caloric intake(kcal/day)	Women Men	1635 [450] 1921 [559]	1729 [432] 2045 [553]	1610 [415] 1892 [520]	1607 [443] 1865 [533]	1745 [434] 2076 [556]	1632 [425] 1929 [534]	1651 [445] 1928 [541]	1737 [442] 2049 [556]	1606 [423] 1898 [527]
Alcohol intake(g/day)	Women Men	6 [10] 12 [16]	6 [9] 11 [13]	6 [9] 10 [12]	6 [10] 12 [14]	6 [9] 11 [13]	6 [9] 11 [14]	7 [10] 11 [15]	6 [9] 11 [13]	5 [8] 10 [13]
Caffeine intake(g/day)	Women Men	359 [227] 280 [244]	312 [208] 221 [203]	293 [211] 206 [211]	358 [226] 279 [243]	309 [207] 217 [202]	294 [209] 210 [212]	336 [220] 236 [223]	316 [210] 231 [212]	303 [214] 225 [221]
Residence in the northern tier(%)	Women Men	27 16	27 17	24 15	29 17	26 16	23 15	29 16	26 16	23 15
Scandinavian ancestry(%)	Women Men	6 12	7 11	8 10	6 11	8 12	8 11	7 10	7 12	7 11
Family history of glaucoma(%)	Women Men	13 11	14 12	14 12	13 11	14 12	14 12	13 11	14 12	14 11
Self-reported diabetes diagnosis(%)	Women Men	6 6	6 6	5 6	5 6	6 6	6 6	5 5	6 6	6 6
Self-reported hypertension diagnosis(%)	Women Men	36 35	36 34	36 34	35 34	37 34	37 35	35 34	36 34	37 35
Self-reported high cholesterol diagnosis(%)	Women Men	41 40	40 42	39 42	39 39	40 43	41 43	40 42	40 41	40 41

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		Total Folate			Total Vitamin B6			Total Vitamin B12		
		Q1	Q3	Q5	Q1	Q3	Q5	Q1	Q3	Q5
Self-reported myocardial infarction(%)	Women Men	3 6	2 7	2 6	2 6	2 7	2 6	3 7	2 6	2 6
≥ 30 pack-years of smoking(%)	Women Men	23 19	15 13	14 13	22 18	14 13	15 13	19 14	16 15	16 15
Body mass index(kg/m^2[SD]) ≥ 30 (%)	Women Men	20 13	18 9	15 8	19 12	18 10	17 9	17 9	18 10	18 10
Mean number of eye exams reported (out of 10)	Women Men	5 [3] 5 [3]	6 [3] 6 [3]	6 [3] 6 [3]	5 [3] 5 [3]	6 [3] 6 [3]	6 [3] 6 [3]	6 [3] 5 [3]	6 [3] 6 [3]	6 [3] 6 [3]

Table 2

Quintiles of folate intake in relation to the risks of exfoliation glaucoma / glaucoma suspect in Nurses' Health Study (1980-2010) and in Health Professionals' Follow-up Study (1986-2010)

				Quintiles of folate intake					
SOURCE	***Total Folate****			**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
		Women	Median (μg/day)	217	284	352	452	654	
			Cases	61	84	64	58	62	
			Person-time	267,427	269,761	269,874	270,185	269,757	
			Age-adjusted RR (95% CI)	1.00 (ref)	1.18 (0.85, 1.65)	0.86 (0.60, 1.22)	0.79 (0.55, 1.13)	0.77 (0.54, 1.09)	0.02
			Multivariable† RR(95% CI)	1.00 (ref)	1.18 (0.84, 1.65)	0.85 (0.60, 1.22)	0.78 (0.54, 1.13)	0.77 (0.53, 1.10)	0.02
		Men	Median (μg/day)	282	365	454	586	839	
			Cases	16	15	14	14	11	
			Person-time	98,085	98,962	99,613	99,813	99,371	
			Age-adjusted RR (95% CI)	1.00 (ref)	0.69 (0.33, 1.47)	0.78 (0.38,1.63)	0.74 (0.36, 1.54)	0.57 (0.26, 1.25)	0.26
			Multivariable RR (95% CI)	1.00 (ref)	0.68 (0.32, 1.48)	0.80 (0.37, 1.71)	0.72 (0.33, 1.54)	0.64 (0.29, 1.46)	0.44
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.01 (0.62, 1.63)	0.84 (0.61, 1.17)	0.77 (0.55, 1.07)	0.75 (0.54, 1.04)	0.02
	Folate from diet*	Women	Median (μg/day)	197	241	274	311	377	
			Multivariable RR(95% CI)	1.00 (ref)	0.95 (0.65, 1.38)	1.10 (0.77, 1.58)	0.95 (0.66, 1.36)	0.95 (0.66, 1.37)	0.69
		Men	Median (μg/day)	259	314	357	404	496	
			Multivariable RR (95% CI)	1.00 (ref)	1.34 (0.62, 2.93)	1.09 (0.50, 2.40)	0.88 (0.37, 2.05)	1.07 (0.47, 2.46)	0.80
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.01 (0.72, 1.42)	1.10 (0.79, 1.52)	0.93 (0.67, 1.31)	0.97 (0.70, 1.35)	0.64
	Folate from supplements*	Women	Median (μg/day)	0	4	47	165	335	
			Multivariable RR(95% CI)	1.00 (ref)	1.22 (0.75, 1.98)	1.18 (0.71, 1.95)	1.11 (0.68, 1.81)	0.85 (0.52, 1.40)	0.03
		Men	Median (μg/day)	0	5	37	219	434	
			Multivariable RR (95% CI)	1.00 (ref)	0.81 (0.33, 1.96)	0.60 (0.23, 1.58)	0.53 (0.21, 1.36)	0.75 (0.31, 1.84)	0.61

				Quintiles of folate intake					
SOURCE	***Total Folate*** *			**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.11 (0.73, 1.70)	0.96 (0.52, 1.76)	0.86 (0.43, 1.71)	0.83 (0.54, 1.28)	0.03
TIMING	Baseline//	Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	0.90 (0.64, 1.26)	1.33 (0.98, 1.81)	1.01 (0.73, 1.41)	0.96 (0.69, 1.33)	0.61
	Most recent//	Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.05 (0.77, 1.44)	0.78 (0.56, 1.09)	0.65 (0.42, 1.01)	0.73 (0.53, 1.02)	0.02

Abbreviations: RR = Relative Risk; CI = Confidence Interval

* Intake calculated using cumulative average (i.e., average of all available intake data from food frequency questionnaires completed before each two-year period at risk).

† All multivariable analyses were stratified by age in months and period at risk, and they were adjusted for the following variables: ancestry (Scandinavian Caucasian, Southern European Caucasian, All others), family history of glaucoma, self-reported history of high cholesterol, hypertension, diabetes, myocardial infarction, body mass index (22-23, 24-25, 26-27, 28-29, 30+ kg/m^2), cumulatively averaged total energy intake (kcal/day), cumulatively averaged alcohol intake (g/day), cumulatively averaged caffeine intake (mg/day), pack-years of smoking (1-9, 10-19, 20-29, 30+ pack-years), lifetime cumulative residential history (northern [41-49° latitude], northern or middle tier [37-49° latitude], middle tier only [37-40° latitude], middle or southern tier [24-40° latitude], southern tier only [24-36° latitude]). For analyses of intake from diet, intake from supplements was adjusted for and vice versa.

‡ Pooled results were calculated using Dersimonian and Laird methods with random effects; p for heterogeneity between cohorts for all results were > 0.05.

// Baseline diet refers to diet as of 1980 in women and 1986 in men; most recent diet refers to the intake as of the food frequency questionnaire immediately before the 2-year period at risk

Table 3

Pooled multivariable relative risks (95% CI)* for quintiles of folate intake in relation to the risks of exfoliation glaucoma / glaucoma suspect by various factors in Nurses' Health Study (1980-2010) and in Health Professionals' Follow-up Study (1986-2010)

	Quintiles of total folate						
By alcohol intake	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**	**p-interaction**
< ***median of alcohol intake***†	1.00 (ref)	1.26 (0.79, 2.02)	0.91 (0.55, 1.51)	0.91 (0.55, 1.53)	0.84 (0.50, 1.41)	0.96	
≥ ***median of alcohol intake***†	1.00 (ref)	0.96 (0.64, 1.46)	0.77 (0.50, 1.20)	0.70 (0.44, 1.09)	0.63 (0.33, 1.21)	0.03	0.69
By family history of glaucoma							
Without family history	1.00 (ref)	1.04 (0.64, 1.68)	0.91 (0.62, 1.33)	0.80 (0.54, 1.19)	0.72 (0.49, 1.08)	0.09	
With family history	1.00 (ref)	1.24 (0.56, 2.72)	0.64 (0.06, 7.30)	1.07 (0.48, 2.36*	1.36 (0.62, 2.99)	0.67	0.96
By geographic location							
Northern tier	1.00 (ref)	1.06 (0.69, 1.60)	0.78 (0.50, 1.21)	0.61 (0.38, 0.98)	0.73 (0.47, 1.15)	0.02	
Middle or southern tier	1.00 (ref)	1.27 (0.75, 2.17)	0.98 (0.49, 1.98)	1.47 (0.31, 6.94)	0.89 (0.42, 1.87)	0.92	0.62

	Quintiles of supplementary folate intake						
By dietary folate intake	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**	**p-interaction**
< ***median of dietary intake***	1.00 (ref)	1.20 (0.63, 2.32)	0.89 (0.24, 3.25)	0.79 (0.18, 3.52)	0.65 (0.33, 1.27)	0.01	
≥ ***median of dietary intake***	1.00 (ref)	1.08 (0.58, 2.01)	1.10 (0.59, 2.07)	0.89 (0.47, 1.65)	0.96 (0.53, 1.75)	0.39	0.29

*Pooled results were calculated using Dersimonian and Laird methods with random effects; p for heterogeneity between cohorts for all results were > 0.05.. All multivariable analyses were stratified by age in months and period at risk, and they were adjusted for the following variables: ancestry (Scandinavian Caucasian, Southern European Caucasian, All others), family history of glaucoma, self-reported history of high cholesterol, hypertension, diabetes, myocardial infarction, body mass index (22-23, 24-25, 26-27, 28-29, 30+ kg/m^2), cumulatively averaged total energy intake (kcal/day), cumulatively averaged alcohol intake (g/day), cumulatively averaged caffeine intake (mg/day), pack-years of smoking (1-9, 10-19, 20-29, 30+ pack-years), lifetime cumulative residential history (northern [41-49° latitude], northern or middle tier [37-49° latitude], middle tier only [37-40° latitude], middle or southern tier [24-40° latitude], southern tier only [24-36° latitude]). For analyses of intake from diet, intake from supplements was adjusted for.

†Median alcohol intake in women is 2.15 g/day, and median alcohol intake in men is 6.31 g/day

Table 4

Quintiles of vitamin B6 intake in relation to the risks of exfoliation glaucoma / glaucoma suspect in NHS (1980-2010) and in HPFS (1986-2010)

				Quintiles of vitamin B6					
SOURCE	***Total Vitamin B6*** *			**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
		Women	Median, mg/d	1	2	2	4	14	
			Cases	59	83	60	79	48	
			Person-time	282,852	263,205	266,999	266,687	267,262	
			Age-adjusted RR (95% CI)	1.00 (ref)	1.22 (0.87, 1.71)	0.84 (0.58, 1.20)	1.07 (0.76, 1.50)	0.70 (0.48, 1.03)	0.03
			Multivariable† RR(95% CI)	1.00 (ref)	1.26 (0.90, 1.77)	0.87 (0.61, 1.26)	1.13 (0.80, 1.59)	0.74 (0.50, 1.09)	0.05
		Men	Median, mg/d	2	2	3	4	14	
			Cases	11	17	17	10	15	
			Person-time	100,722	100,737	96,863	98,500	99,020	
			Age-adjusted RR (95% CI)	1.00 (ref)	1.41 (0.65, 3.09)	1.32 (0.61, 2.84)	0.82 (0.35, 1.96)	1.16 (0.52, 2.58)	0.78
			Multivariable RR (95% CI)	1.00 (ref)	1.44 (0.64, 3.26)	1.45 (0.65, 3.25)	0.91 (0.37, 2.23)	1.30 (0.56, 2.99)	0.44
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.29 (0.94, 1.76)	0.99 (0.64, 1.52)	1.10 (0.79, 1.51)	0.86 (0.53, 1.41)	0.60
	Vitamin B6 from diet *	Women	Median, mg/d	1	2	2	2	2	
			Multivariable RR(95% CI)	1.00 (ref)	1.37 (0.93, 2.02)	1.33 (0.91, 1.95)	1.24 (0.85, 1.83)	1.25 (0.85, 1.83)	0.59
		Men	Median, mg/d	2	2	2	3	3	
			Multivariable RR (95% CI)	1.00 (ref)	0.83 (0.37, 1.87)	0.95 (0.42, 2.14)	1.02 (0.47, 2.25)	0.96 (0.42, 2.19)	0.99
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.22 (0.80, 1.85)	1.25 (0.89, 1.77)	1.20 (0.85, 1.69)	1.19 (0.84, 1.68)	0.64
	Vitamin B6 from supplements *	Women	Median, mg/d	0	0.1	1	2	12	
			Multivariable RR(95% CI)	1.00 (ref)	0.96 (0.60, 1.52)	0.92 (0.57, 1.47)	0.90 (0.56, 1.42)	0.72 (0.45, 1.16)	0.26
		Men	Median, mg/d	0	0.1	1	2	10	
			Multivariable RR (95% CI)	1.00 (ref)	1.02 (0.40, 2.65)	0.80 (0.29, 2.20)	0.82 (0.31, 2.18)	1.00 (0.38, 2.59)	0.61
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	0.97 (0.64, 1.47)	0.90 (0.58, 1.37)	0.88 (0.58, 1.34)	0.77 (0.50, 1.18)	0.45

				Quintiles of vitamin B6					
SOURCE	***Total Vitamin B6*** *			**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
TIMING	***Baseline*** //	Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.01 (0.63, 1.60)	1.06 (0.78, 1.45)	1.11 (0.81, 1.52)	0.83 (0.59, 1.16)	0.20
	Most recent //	Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.56 (0.62, 3.89)	0.99 (0.70, 1.39)	0.98 (0.70, 1.37)	0.85 (0.57, 1.26)	0.52

Abbreviations: RR = Relative Risk; CI = Confidence Interval

* Intake calculated using cumulative average (i.e., average of intake data from all food frequency questionnaires completed before each two-year period at risk).

† All multivariable analyses were stratified by age in months and period at risk, and they were adjusted for the following variables: ancestry (Scandinavian Caucasian, Southern European Caucasian, All others), family history of glaucoma, self-reported history of high cholesterol, hypertension, diabetes, myocardial infarction, body mass index (22-23, 24-25, 26-27, 28-29, 30+ kg/m^2), cumulatively averaged total energy intake (kcal/day), cumulatively averaged alcohol intake (g/day), cumulatively averaged caffeine intake (mg/day), pack-years of smoking (1-9, 10-19, 20-29, 30+ pack-years), lifetime cumulative residential history (northern [41-49° latitude], northern or middle tier [37-49° latitude], middle tier only [37-40° latitude], middle or southern tier [24-40° latitude], southern tier only [24-36° latitude]). For analyses of intake from diet, intake from supplements was adjusted for and vice versa.

‡ Pooled results were calculated using Dersimonian and Laird methods with random effects; p for heterogeneity between cohorts for all results were > 0.05.

// Baseline diet refers to diet as of 1980 in the women and 1986 in the men; most recent diet refers to the intake as of the food frequency questionnaire immediately before the 2-year period at risk

Table 5

Quintiles of vitamin B12 intake in relation to the risks of exfoliation glaucoma / glaucoma suspect in NHS (1980-2010) and in HPFS (1986-2010)

				Quintiles of vitamin B12					
SOURCE	***Total Vitamin B12*** *			**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
		Women	Median (μg/day)	5	7	9	11	16	
			Cases	65	67	71	65	61	
			Person-time	292,077	253,407	270,527	270,107	260,887	
			Age-adjusted RR (95% CI)	1.00 (ref)	0.98 (0.70, 1.39)	1.00 (0.71, 1.40)	0.86 (0.61, 1.21)	0.79 (0.56, 1.12)	0.43
			Multivariable† RR(95% CI)	1.00 (ref)	1.01 (0.72, 1.43)	1.03 (0.73, 1.45)	0.91 (0.65, 1.29)	0.86 (0.60, 1.22)	0.66
		Men	Median (μg/day)	6	8	10	14	22	
			Cases	14	13	16	13	14	
			Person-time	104,819	96,427	100,950	96,650	96,991	
			Age-adjusted RR (95% CI)	1.00 (ref)	1.08 (0.50, 2.33)	1.04 (0.51, 2.16)	0.75 (0.34, 1.68)	0.93 (0.43, 1.97)	0.76
			Multivariable RR (95% CI)	1.00 (ref)	1.13 (0.51, 2.52)	1.05 (0.50, 2.24)	0.79 (0.35, 1.80)	0.98 0.44, 2.17)	0.58
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.03 (0.75, 1.42)	1.03 (0.76, 1.41)	0.89 (0.65, 1.23)	0.88 (0.64, 1.21)	0.99
	Vitamin B12 from diet *	Women	Median (μg/day)	4	5	6	7	10	
			Multivariable RR(95% CI)	1.00 (ref)	0.68 (0.47, 0.99)	0.79 (0.55, 1.13)	1.05 (0.76, 1.46)	1.03 (0.73, 1.43)	0.21
		Men	Median (μg/day)	5	6	7	9	14	
			Multivariable RR (95% CI)	1.00 (ref)	1.32 (0.59, 2.96)	1.32 (0.59, 2.96)	0.91 (0.39, 2.14)	1.05 (0.46, 2.40)	0.68
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	0.86 (0.46, 1.59)	0.89 (0.58, 1.36)	1.03 (0.76, 1.40)	1.03 (0.75, 1.40)	0.41
	Vitamin B12 from supplements *	Women	Median (μg/day)	0	0.2	1	3	7	
			Multivariable RR(95% CI)	1.00 (ref)	0.76 (0.45, 1.27)	0.78 (0.46, 1.33)	0.81 (0.48, 1.37)	0.52 (0.30, 0.88)	0.05
		Men	Median (μg/day)	0	0.2	1	4	9	
			Multivariable RR (95% CI)	1.00 (ref)	1.06 (0.40, 2.84)	1.18 (0.43, 3.25)	1.29 (0.49, 3.37)	0.94 (0.34, 2.56)	0.73
		Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	0.81 (0.51, 1.29)	0.86 (0.54, 1.37)	0.90 (0.57, 1.43)	0.59 (0.36, 0.98)	0.47

				Quintiles of vitamin B12					
SOURCE	***Total Vitamin B12*** *			**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
TIMING	Baseline//	Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.02 (0.74, 1.40)	1.01 (0.75, 1.35)	1.01 (0.75, 1.36)	0.88 (0.63, 1.23)	0.52
	Most recent//	Pooled‡	Multivariable RR (95% CI)	1.00 (ref)	1.30 (0.94, 1.79)	1.31 (0.95, 1.80)	0.86 (0.61, 1.22)	0.91 (0.64, 1.28)	0.76

Abbreviations: RR = Relative Risk; CI = Confidence Interval

* Intake calculated using cumulative average (i.e., average of all available intake data from food frequency questionnaires completed before each two-year period at risk).

† All multivariable analyses were stratified by age in months and period at risk, and they were adjusted for the following variables: ancestry (Scandinavian Caucasian, Southern European Caucasian, All others), family history of glaucoma, self-reported history of high cholesterol, hypertension, diabetes, myocardial infarction, body mass index (22-23, 24-25, 26-27, 28-29, 30+ kg/m^2), cumulatively averaged total energy intake (kcal/day), cumulatively averaged alcohol intake (g/day), cumulatively averaged caffeine intake (mg/day), pack-years of smoking (1-9, 10-19, 20-29, 30+ pack-years), lifetime cumulative residential history (northern [41-49° latitude], northern or middle tier [37-49° latitude], middle tier only [37-40° latitude], middle or southern tier [24-40° latitude], southern tier only [24-36° latitude]). For analyses of intake from diet, intake from supplements was adjusted for and vice versa.

‡ Pooled results were calculated using Dersimonian and Laird methods with random effects; p for heterogeneity between cohorts for all results were > 0.05, except for the analysis of most recent intake of vitamin B12 (p-value for heterogeneity=0.03)

// Baseline diet refers to diet as of 1980 in the women and 1986 in the men; most recent diet refers to the intake as of the food frequency questionnaire immediately before the 2-year period at risk