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Prospective study of flavonoid intake and risk of primary open-angle glaucoma

Jae H. Kang[1], **Kerry L. Ivey**[2,3], **Tahani Boumenna**[4], **Bernard Rosner**[1,5], **Janey L. Wiggs**[4], and **Louis R. Pasquale**[1,4]

[1]Channing Division of Network Medicine, Department of Medicine, Brigham & Women's Hospital, and Harvard Medical School, Boston, MA 02115

[2]Department of Nutrition, Harvard T. H. Chan School of Public Health, Boston, MA 02115

[3]South Australian Health and Medical Research Institute, Infection and Immunity Theme, School of Medicine, Flinders University, Adelaide, Australia

[4]Glaucoma Service, Massachusetts Eye and Ear Infirmary, Boston, MA 02114

[5]Department of Biostatistics, Harvard T. H. Chan School of Public Health, Boston, MA 02115

Abstract

Purpose—To evaluate the association between flavonoid intake and incident primary open-angle glaucoma (POAG).

Methods—We followed 65,516 women from the Nurses' Health Study (from 1984) and 42,156 men from the Health Professionals Follow-up Study (from 1986) biennially to 2012, who were 40+ years old, free of POAG, and reported eye examinations. Dietary flavonoid intake was assessed with validated repeated semi-quantitative food frequency questionnaires. Incident POAG cases (n=1575) were confirmed with medical record review. Cohort-specific multivariable-adjusted relative risks (RRs) and 95% confidence intervals (CIs) were calculated and meta-analyzed.

Results—Total flavonoid intake was not associated with risk of POAG development (RR for highest [Q5: median ~645 mg/day] versus lowest quintile [Q1: ~130 mg/day]=0.91 [95%CI =0.77,1.08]; p for trend [p-trend]=0.19); the flavonoid subclasses of flavones, flavanones, polymeric flavanols or anthocyanidins were also not associated (Q5 vs. Q1 comparison p-values ≥0.05 and p-trends ≥0.09). Higher intakes of flavonols and monomeric flavanols were nominally associated with lower POAG risk, based on the Q5 vs. Q1 comparisons or p-trends. The Q5 vs. Q1 comparison RRs were: for flavonols, 0.82 (95%CI=0.69,0.97; p-trend=0.05; ~28 vs. ~8mg/day) and for monomeric flavanols, 0.86 (95%CI=0.72,1.02; p-trend=0.04; ~110 vs. 10 mg/day). The food/beverage that contributed most to both the variation of flavonols and monomeric flavanols was tea; consuming ~2 cups/day was associated with 18% lower POAG risk (RR=0.82; 95%CI=0.68,0.99; p-trend=0.02).

Correspondence and reprints: Jae H. Kang, ScD, Channing Division of Network Medicine, 181 Longwood Avenue, Boston, MA 02115, (TEL): 617-525-2022; (FAX) 617-525-2008, nhjhk@channing.harvard.edu.

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Conclusion—Total flavonoid intake was not associated with POAG risk. Greater intakes of flavonols and monomeric flavanols, and of tea showed suggestive modest associations with lower risk; these results need confirmation.

Keywords

epidemiology; flavonoids; glaucoma; cohort

INTRODUCTION

Primary open-angle glaucoma (POAG) is an age-related insidious blinding disease. Currently, regular eye exams represent the best form of prevention; however, with 80 million cases worldwide projected for 2040 (Tham et al., 2014), it is urgently important to identify modifiable risk factors, such as diet, for primary POAG prevention.

Flavonoids are polyphenolic compounds present in many common foods such as apples, berries, tea, and wine (Manach et al., 2004). Flavonoids can be grouped into the structural subclasses of flavanones, flavones, anthocyanidins, flavonols, monomeric flavanols, and polymeric flavanols (Kumar & Pandey, 2013). Flavonoids have multiple biological activities such as antioxidant defense (Heijnen et al., 2001) and modulation of key cellular signalling pathways (Williams et al., 2004) to affect processes that lead to reduced inflammation (Lee et al., 2014), improved endothelial function and nitric oxide homeostasis (Vauzour et al., 2008, Terai et al., 2014), protection against insulin resistance (Babu et al., 2013) and neuroprotection (Vauzour et al., 2008, Sokolov et al., 2013).

Flavonoids may play a role in POAG by 1) reducing oxidative stress (Milbury, 2012, Chu et al., 2010), which may be involved in elevating intraocular pressure (IOP) (De La Paz & Epstein, 1996) and may exacerbate neuronal damage (Ganapathy et al., 2011), and 2) improving ocular blood flow (Khoo et al., 2010) to minimize ischemia (Nickells, 1996) to retinal ganglion cells (RGCs). *In vitro* studies suggested that flavonoids may up-regulate RGC antioxidant mechanisms (Maher & Hanneken, 2005), and an animal study found that flavonoids may increase ocular blood flow (Park & Chiou, 2004). In humans, a meta-analysis of 6 clinical trials of various flavonoid supplements in glaucoma or ocular hypertension patients suggested that supplements slowed visual field (VF) loss progression and improved ocular blood flow (Patel et al., 2015). However, no prospective study has evaluated the relation between flavonoid intake and POAG risk. We hypothesized that flavonoid intake will be inversely associated with POAG risk. Therefore, we examined the association of intake of total flavonoids, flavonoid subclasses, and their food/beverage sources with POAG risk among 65,516 women of the Nurses' Health Study (NHS) and 42,156 men in the Health Professionals Follow-up Study (HPFS) followed for 26+ years.

SUBJECTS AND METHODS

The NHS began in 1976 with 121,700 US registered female nurses (30–55 years old) who completed mailed health and lifestyle questionnaires (Barton et al., 1980). The HPFS began in 1986 with 51,529 US male health professionals (40–75 years old) who returned similar questionnaires (Grobbee et al., 1990). Participants were followed biennially with mailed

questionnaires that asked about diet, health, and diseases such as glaucoma, and the follow-up has been high (>85% of total person-time). The Human Research Committees of Brigham & Women's Hospital, Massachusetts Eye and Ear and the Harvard School of Public Health approved this study, and the procedures followed were in accordance with the ethical standards of these institutions.

Because participants were first administered a detailed (116+ item) semi-quantitative food frequency questionnaire (FFQ) that allowed for a comprehensive evaluation of flavonoids in 1984 for NHS and in 1986 for HPFS (and every 2–4 years thereafter), these years are considered "baseline" for each cohort. Follow-up ended with the earliest occurrence of a glaucoma diagnosis, cancer, death, loss to follow-up, or 2012, which was the end of the study. Eligible participants were aged 40+ years (when glaucoma risk increases), provided baseline diet information and reported an eye exam (to minimize detection bias).

At baseline, we excluded the following women and men, respectively: 1) 29,233 women who did not complete the first 61-item FFQ in 1980, as the original purpose of the glaucoma substudy was to evaluate the relation between diet and glaucoma, 2) 18,279 and 1,596 who did not respond to the 116+ item FFQs that allowed for comprehensive evaluation of flavonoids in 1984 (NHS)/1986 (HPFS) FFQs or had outlying total calories (<600 or >3500 kcal for women; <800 or >4200 kcal for men), 3) 4,011 and 1,927 with cancer (except nonmelanoma skin cancer), as cancer diagnoses could alter diet, 4) 905 and 1,036 with prevalent glaucoma, 5) 455 and 956 whose last completed questionnaire was at baseline (thus lost-to-follow-up), and 6) 2,351 and 3,273 who never reported an eye exam. After these exclusions, 66,466 women and 42,741 men were eligible; however, every two years, we updated the provisional exclusions for age and eye exam status. For example, at 1984 (NHS) and 1986 (HPFS), only 45,945 women and 29,682 men were included after we excluded participants (20,521 women and 13,059 men) who were age<40 years or reported no eye exam. In later periods, those provisionally excluded were allowed in analyses if they became eligible (Kang et al., 2003). Thus, 65,516 women and 42,156 men contributed person-time to this analysis; of the 12 times that eye exams were asked about over the follow-up period in each cohort, the average number of times eligible participants reported positively was 7.5 in women and 5.9 in men.

POAG case ascertainment and POAG subtyping by IOP and VF loss pattern

We included 1,575 confirmed incident POAG (1,058 women and 517 men). We ascertained glaucoma cases biennially by asking participants to report physician-diagnoses of glaucoma. For those self-reporting glaucoma diagnoses, we obtained permission to contact eye care providers. We asked providers to send VFs with either medical records or a completed glaucoma questionnaire with items on maximal IOP, status of the filtration apparatus, optic nerve structural information, ophthalmic surgery, and earliest VF loss date. A glaucoma specialist (LRP), masked to participants' diet, reviewed records to confirm cases using standardized criteria.

For POAG case confirmation, we required: (a) gonioscopy indicating the filtration angle was not occludable in either eye (70% of cases) or slit lamp biomicroscopy demonstrating open angles plus pharmacological dilation (30% of cases), (b) slit lamp biomicroscopy showing

no signs, in either eye, of pigment dispersion syndrome, uveitis, exfoliation syndrome, trauma, or rubeosis; and (c) reproducible VF defects consistent with POAG on ≥2 reliable tests. For VF defects, the type of perimetry was not restricted. However, full static threshold testing was documented in 95% of cases, and kinetic VFs in <1% of cases. For static threshold or supra-threshold tests, we used the reliability definitions of fixation loss ≤ 33%, false positive rate ≤ 20%, and false negative rate ≤ 20%. Kinetic VFs were considered reliable unless there were examiners notes to the contrary.

Incident glaucoma was self-reported by 9,272 women and 4,088 men. Of these, for women and men, respectively, we found 26% and 25% with potential POAG with VF loss, 18% and 15% with only elevated IOP or optic disc cupping, and 19% and 12% with other types of glaucoma/glaucoma suspect. The remaining were unconfirmed, as participants (8% and 16%) or eye care providers (5% and 6%) were unreachable, participants denied permission for record review (12% and 9%), participants indicated the report was erroneous (10% and 15%) or eye care providers refuted the glaucoma diagnosis (2% and 2%). Among the 26% and 25% classified as potential POAG with VF loss, we included as analysis cases, a subset of 1,058 women and 517 men that met the above-mentioned POAG case-confirmation criteria; other self-reports were censored as of the diagnosis date in analyses.

We further classified cases into subtypes defined by IOP and by VF loss pattern at diagnosis. We defined subtypes of “high-tension” (HTG; n=1045; 680 women and 365 men) and “normal-tension” POAG (NTG; n=530; 378 women and 152 men) as those with maximum untreated IOP ≥ or <22 mm Hg, respectively. We defined subtypes by VF loss pattern: those with peripheral VF loss only (n=891; 609 women and 282 men) or early paracentral VF loss (n=454; 302 women and 152 men) or undetermined VF loss (n=230; 147 women and 83 men) with a method previously described (Kang et al., 2015). Briefly, for those with peripheral VF loss only, nasal step, temporal wedge or Bjerrum scotoma VF loss was present without any paracentral loss. Early paracentral loss was defined as either 1) paracentral loss only or 2) paracentral loss with VF loss in the Bjerrum area and/or nasal step area in the same hemifield, but without any temporal wedge loss (as those with only paracentral loss were uncommon (21%) whereas those with clear paracentral loss frequently also showed some peripheral loss). Cases (n=230) with undetermined VF loss (i.e., advanced VF loss, VF loss in the paracentral and any temporal wedge region in the same eye, or paracentral in one hemifield with peripheral loss only in the other hemifield) were censored as of diagnosis date in the VF loss subtype analyses.

Measurement of intake of flavonoids

Diet was assessed with detailed FFQs in 1984, 1986 and every 4 years thereafter in NHS and 1986 and every 4 years thereafter in HPFS. Intakes were calculated as the sum of the consumption frequency of each food multiplied by the specific flavonoid content for the stated portion size (Cassidy et al., 2011). We derived intakes of 6 flavonoid subclasses commonly consumed in the US: flavanones, anthocyanidins, flavonols, flavones, monomeric flavanols and polymeric flavanols.

Cumulatively averaged updated intakes (energy-adjusted) were calculated for a given questionnaire cycle by averaging the intake for the current and preceding FFQs (e.g., in NHS

in 1984, the 1984 flavonoid value was used; in 1986, the average of 1984 and 1986 values was used, etc.) to assess long-term flavonoid intake and minimize within-person variation (Hu et al., 1997). Intakes of other dietary factors (e.g., caffeine, alcohol) were similarly derived. We also conducted cumulatively averaged updated food/beverage analysis of the top two sources that accounted for the major variation for each flavonoid subclass: apples (monomeric [7.4% of monomeric flavanol variation] and polymeric flavanols [18.9%]); blueberries (anthocyanidins [40.0%]); onions (flavonols [27.5%]); oranges (flavones [13.6%], flavanones [23.2%]); orange juice (flavones [44.3%], flavanones [58.0%]); strawberries (anthocyanidins [18.4%]); and tea with caffeine (not herbal teas; flavonols [18.9%], monomeric [63.6%] and polymeric flavanols [43.2%]).

Validity of semi-quantitative FFQ assessment of flavonoid and vegetable sources

The validity and reproducibility of the FFQs have been reported previously, and correlations between major dietary sources of flavonoids, including fruits, vegetables, tea, and wine, measured by diet records and the FFQ were 0.70, 0.50, 0.77, and 0.83, respectively (Salvini et al., 1989, Feskanich et al., 1993, Hu et al., 1999).

Statistical Analysis

For flavonoid intake analyses, intake values were total energy adjusted using the residual method (Willett & Stampfer, 1986). For food analyses, cumulatively updated total calories were also included as a covariate.

We calculated POAG incidence rates by dividing incident cases by person-years for each intake category (quintiles). For multivariable analyses, we conducted cohort-specific Cox proportional hazards analysis (Cox & Oakes, 1984), while simultaneously controlling for potential glaucoma risk factors. To control as finely as possible for confounding by age, calendar time and any possible two-way interactions between these two time scales, we stratified the analysis jointly by age in months at start of follow-up and calendar year of the current questionnaire cycle. We derived relative risks (RRs) and 95% confidence intervals (CIs). We conducted tests for trend by evaluating the significance of a variable representing the medians of quintiles. All significance tests were 2-sided, and the significance level for all analyses was p-value <0.05. The SAS statistical software (version 9.4, SAS Institute Inc., Cary, NC, USA) was used for all statistical analyses.

In addition to age (in months) that was adjusted for, we also adjusted for potential covariates, which were updated biennially from baseline: body mass index (kg/m^2), cigarette smoking (pack-years), hypertension, diabetes, physical activity (MET [metabolic equivalent of task]-hours/week), number of eye exams reported during follow-up, cumulatively updated intake categories of alcohol, caffeine, and total calories (only for food analyses), glaucoma family history, race, and among women, age at menopause and postmenopausal hormone use. Missing indicators were used for the variables, family history of glaucoma, cigarette smoking, age at menopause, postmenopausal hormone use; for physical activity and body mass index, the median category was used for imputing the values of those with missing data as few cases occurred among those with missing values for these variables.

We performed tests for heterogeneity to check for appropriateness of pooling the cohort-specific results. Then, we pooled the results using meta-analytic methods with fixed effects (DerSimonian & Laird, 1986).

Secondary analyses

We performed several secondary analyses. As these secondary analyses were exploratory, correction for multiple comparisons was not performed, (Savitz & Olshan, 1998, Bender & Lange, 2001, Rothman, 2014, 1990), and therefore, reported p values should be read as descriptive and interpreted with caution. First, our primary exposure of interest was total flavonoids; thus, evaluation of the subclasses of flavonoids in relation to all POAG and POAG subtypes were secondary. Second, we separately analyzed the risks of HTG and NTG, and of POAG with peripheral VF loss only and of POAG with early paracentral loss. For testing the heterogeneity of association by POAG subtype, we combined the datasets, then conducted Cox regression analyses that further stratified on cohort (to allow for differing hazard functions) and used the Lunn-McNeil approach (Lunn & McNeil, 1995) to test for heterogeneity (p-heterogeneity). Third, we evaluated whether associations may differ by age or family history. For interaction testing, a product term of the effect modifier and median values of each quintile of flavonoid intake were added into models with the two main effects, and the statistical significance of this product term was evaluated with Wald tests.

RESULTS

During 1,706,804 person-years of follow-up, we identified 1575 incident POAG cases. High flavonoid consumers were leaner, exercised more, consumed less alcohol and smoked less. They were less likely to be African-American, and have diabetes and hypertension. Among women, high flavonoid consumers were also more likely to use postmenopausal hormones (Table 1).

We observed no heterogeneity by cohort (p-heterogeneity≥0.11); thus, results were pooled. Overall, we observed similar associations in the age-adjusted and multivariable analyses, Compared with the lowest quintile (Q1) of ~130 mg of total flavonoid/day, the pooled multivariable relative risk (RR, 95% CI) of POAG was 1.04 (95% CI=0.89, 1.23) for Q2, 1.00 (95% CI=0.85, 1.18) for Q3, 1.06 (95% CI=0.90, 1.25) for Q4 and 0.91 (95% CI=0.77, 1.08) for Q5 (p for trend [p-trend]=0.19) (Table 2).

In exploratory analyses of flavonoid subclasses, we observed modest inverse associations with higher intake of flavonols and monomeric flavanols, based on either the Q5 vs. Q1 comparisons or the p-trends: the pooled RR for flavonols of Q5 (~28 mg/day) versus Q1 (~8 mg/day) was 0.82 (95% CI=0.69, 0.97; p-trend=0.05) and for monomeric flavanols, the Q5 (~110 mg/day) versus Q1 (~10 mg/day) pooled RR was 0.86 (95% CI=0.72, 1.02; p-trend=0.04). We did not observe associations with polymeric flavanols (Q5 vs. Q1 RR=0.85; 95% CI=0.72, 1.00; p-trend=0.09), flavones (p-trend=0.42) or flavanones (p-trend=0.25). With anthocyanidins, we did not observe associations (p-trend=0.13), although a suggestive inverse association was observed with Q5 (26.6 mg/day) versus Q1 (2.9 mg/day) among men, with RR of 0.75 ([95% CI=0.56, 0.99]; p-trend=0.24).

We observed no significant differences in associations with the two POAG subtypes defined by IOP, with all p-heterogeneity ≥0.27 (Table 3). With flavonols (p-heterogeneity=0.60), we observed a suggestive stronger inverse association with HTG (for Q5 versus Q1, the pooled RR was 0.78 [95%CI=0.64, 0.97]; p-trend=0.05) but for NTG, the association was null (p-trend=0.43). With anthocyanidins (p-heterogeneity=0.27), we observed a suggestive stronger inverse association with NTG (for Q5 versus Q1, the pooled RR was 0.72 [95%CI=0.53, 0.96]; p-trend=0.07) but not with HTG (p-trend=0.55). We also did not observe different associations by pattern of VF loss with p-heterogeneity of ≥0.30; in general, the suggestive inverse associations were stronger for POAG with peripheral VF loss only. In secondary analyses, we observed no significant interactions by age or family history (data not shown).

Among major food/beverage sources (Table 4), we observed no strong associations (p-trend≥0.28) with 6 of the 7 selected foods: apples, blueberries, onions, oranges, orange juice, strawberries. The food/beverage that contributed most to the variations of both flavonols and monomeric flavanols was tea with caffeine (not herbal teas); compared to consuming no tea, consuming ~2 cups daily (or median of 13.1 cups/week) was associated with a significant 18% lower POAG risk (RR=0.82; 95% CI=0.68, 0.99; p-trend=0.02). For every 1 cup/day increase in tea (with caffeine, not herbal tea) intake, there was a borderline non-significant 6% lower POAG risk (RR=0.94; 95%CI=0.88, 1.00; p-trend=0.05). The association with tea did not differ by POAG subtypes defined either by IOP or pattern of VF loss (data not shown).

DISCUSSION

In this large long-term prospective study of flavonoid intake and POAG incidence, we observed no overall association with total flavonoids; however, in exploratory secondary analyses, we observed modestly lower POAG risk with higher intakes of flavonols and monomeric flavanols. Daily tea consumption was associated with an 18% lower POAG risk. No associations were observed with other flavonoid subclasses; also, associations with specific flavonoid subclasses or tea did not differ by POAG subtypes defined by IOP or VF loss pattern. As this was the first prospective study to evaluate these associations, the findings need confirmation.

In exploratory analyses, we observed that specific flavonoid subclasses, i.e., monomeric flavanols, flavonols and possibly also polymeric flavanols and anthocyanidins, may be associated with lower POAG risk. These subclasses did not differ in their associations by POAG subtypes defined by IOP or VF pattern (the POAG subtype with primarily paracentral VF loss is more strongly associated with vascular dysregulation (Park et al., 2012)); thus, the underlying biological mechanism of these associations (modulating IOP or blood flow) is not clear.

Tea is a rich source of monomeric (e.g., catechin, epigallocatechin (Kumar & Pandey, 2013, Manach et al., 2004)) and polymeric flavanols (e.g., theaflavins, thearubigins (Kumar & Pandey, 2013, Manach et al., 2004)), and is a source of flavonols (e.g., kaempferol, myricetin, quercetin (Kumar & Pandey, 2013, Manach et al., 2004)). Our results are consistent with studies that found that the monomeric flavanol epigallocatechin, which has

powerful antioxidant properties (Sichel et al., 1991), exhibits neuroprotective properties against various insults via modulating protein kinase signalling pathways, such as apoptosis (Zhang et al., 2008), and with a study that observed short-term beneficial effects on retinal function in patients with ocular hypertension and glaucoma (Falsini et al., 2009). While epigallocatechin is highest in green tea, the polymeric flavanols in black tea have equal antioxidant (Leung et al., 2001) and neuroprotective properties (Chaturvedi et al., 2006). In our FFQs, the one item of "tea with caffeine, not herbal teas" did not distinguish between black or green tea; however, tea consumption in our cohorts likely represents primarily intake of black tea, which is more commonly consumed (85% of US tea consumption was black, 14% was green tea) (Tea Association of the USA Inc.). Flavonols such as quercetin are common polyphenols in vegetables (Manach et al., 2004); quercetin induces the expression of antioxidant enzymes by trabecular meshwork cells, which would help maintain normal IOP levels (Miyamoto et al., 2011) Also, flavonols are in ginkgo biloba, which has been extensively studied for improving blood flow (Park et al., 2011) and neuroprotection (Shi et al., 2010). Ginkgo biloba extract has been shown in a meta-analysis (Patel et al., 2015) of 2 short-term (Quaranta et al., 2003, Guo et al., 2014) clinical trials in NTG patients to slow VF loss progression (but had no influence on IOP). Finally, we observed suggestively stronger inverse associations among men and for NTG with anthocyanidins; one 2-year trial (n=38) of black currant anthocyanidins on POAG patients observed significantly less VF loss progression and better ocular flow in the treatment versus placebo group (Ohguro et al., 2012), and other trials of a combination of bilberry extract anthocyanidins and pine bark extract monomeric flavanols in patients showed better ocular blood flow and IOP lowering (Steigerwalt et al., 2008, Steigerwalt et al., 2010). While there are relatively few studies of flavonoids and POAG, our results showing suggestive modest associations of flavonoid subclasses with lower POAG risk (modest associations possibly due to poor bioavailability of flavonoids (Costa et al., 2008, Kalt et al., 2008)) are consistent with previous studies. Thus, confirmatory studies are warranted.

Limitations of our study deserve mention. We could not administer repeated standardized eye exams, and we relied on questionnaires and medical records for disease confirmation. Thus, the case ascertainment method had low sensitivity; however, methodologically, RR estimates are still valid despite this low sensitivity if the case definition is highly specific (we required reproducible VF loss) and the case ascertainment is unrelated to exposure (we uniformly required an eye exam to minimize detection bias) (Rothman & Greenland, 1998). In addition, using our definition of POAG, we confirmed associations with established risk factors such as age, family history, African-heritage and diabetes, which further supports the validity of our POAG definition. We likely had misclassification of flavonoid intake from participants' recall errors compared to measuring blood levels of flavonoids; however, this would have biased associations towards the null. We performed multiple comparisons, and the significant associations in secondary analyses may have been due to chance; thus, we emphasize that our suggestive results need confirmation. Finally, as the study population were mostly healthy Caucasian participants trained as health professionals, our results may not be generalizable to other populations with different underlying risks of POAG.

Major strengths include the large number of cases, long follow-up, repeated dietary assessment using validated FFQs, and the availability of key covariates.

In summary, total flavonoid intake was not associated with POAG risk. However, greater consumption of flavanols and flavonols, both found in tea, showed suggestions of modest associations with lower POAG risks. These results, if confirmed, could have important public health implications.

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Table 1

Age-standardized characteristics of the total accrued person-time by quintiles of total flavonoid intake in the Nurses' Health Study (1984–2012) and the Health Professionals Follow-up Study (1986–2012)

	Quintile of total flavonoid intake					
	NHS			HPFS		
	Q1	Q3	Q5	Q1	Q3	Q5
Age, years	61.2 ± 9.8	62.5 ± 9.8	62.1 ± 9.9	61.7 ± 10.4	62.8 ± 10.5	63.3 ± 10.4
Total flavonoids, mg/day[1,2]	122.8 ± 34.6	267.5 ± 27.2	766.5 ± 304.5	131.8 ± 38.1	276.7 ± 29.8	721.4 ± 292.1
Flavonols, mg/day[1,2]	8.8 ± 3.7	13.8 ± 3.8	28.7 ± 9.4	10.3 ± 4.7	15.6 ± 5.2	29.2 ± 10.1
Monomeric flavanols, mg/day[1,2]	11.6 ± 5.4	30.8 ± 10.6	143.9 ± 73.3	13.6 ± 7.0	29.8 ± 10.9	123.6 ± 72.7
Polymeric flavanols, mg/day[1,2]	66.2 ± 24.1	156.6 ± 30.1	530.9 ± 235.5	68.0 ± 26.9	154.3 ± 37.2	487.7 ± 235.5
Anthocyanidins, mg/day[1,2]	6.7 ± 4.8	13.9 ± 9.4	14.9 ± 14.6	6.4 ± 5.0	14.3 ± 9.6	19.5 ± 22.8
Flavones, mg/day[1,2]	1.4 ± 0.8	2.3 ± 1.1	2.2 ± 1.2	1.6 ± 0.9	2.8 ± 1.3	3.1 ± 1.8
Flavanones, mg/day[1,2]	26.9 ± 19.6	48.2 ± 28.7	46.1 ± 32.2	29.9 ± 22.1	58.0 ± 33.7	64.6 ± 48.6
Total energy intake, kcal/day	1712.6 ± 448.5	1780.8 ± 444.9	1711.5 ± 438.0	1957.3 ± 557.4	2018.6 ± 551.5	1934.1 ± 528.2
Caffeine intake, mg/day[1,2]	296.0 ± 218.3	247.8 ± 182.9	285.1 ± 176.8	261.6 ± 243.4	204.1 ± 202.3	254.8 ± 201.8
Alcohol intake, g/day[2]	7.2 ± 11.1	6.0 ± 8.8	4.9 ± 7.9	12.7 ± 15.5	11.1 ± 13.2	9.4 ± 12.2
African American (%)	1.1	1.0	0.8	0.8	0.6	0.4
Family history of glaucoma (%)	13.7	13.0	13.2	11.2	11.7	11.7
Self-reported diabetes (%)	7.6	6.8	7.2	7.3	6.1	6.9
Self-reported hypertension (%)	43.3	41.4	41.4	38.1	36.0	36.9
Body mass index ≥30 kg/m^2 (%)	13.7	11.4	11.6	10.8	8.3	8.3
Cigarette smoking ≥30 pack-years (%)	27.8	13.4	14.7	25.0	14.2	14.5
Physical activity (top 25th percentile) (%)	19.6	29.8	27.3	21.3	30.9	29.2
Age at menopause <45 years (%)	12.5	11.7	12.5	n/a	n/a	n/a
Current postmenopausal hormone use (%)[3]	33.9	36.7	34.5	n/a	n/a	n/a

[1] Intake adjusted for total energy; means ± sd

[2] Cumulatively updated intake

[3] Among postmenopausal women only

Table 2

Cohort-specific and pooled relative risks (RRs) and 95% confidence intervals (CIs) for the association between total flavonoid and sub-class intakes and primary open-angle glaucoma in Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS)

	Quintiles of intakes					*P for trend*
	1	**2**	**3**	**4**	**5**	
Total flavonoids						
NHS: Median (mg/day)	125.6	196.5	267.5	376.8	674.7	
[Range (mg/day)]	[10.5–164.0]	[164.1–228.7]	[228.8–313.3]	[313.4–476.4]	[476.5–3110.0]	
Cases/Person-years	187/229,056	220/229,913	201/229,986	250/229,903	200/229,833	
Age-adjusted model	1.00 (ref)	1.10 (0.90, 1.34)	0.97 (0.79, 1.19)	1.20 (0.99, 1.46)	0.99 (0.81, 1.21)	0.78
Multivariable model[1]	1.00 (ref)	1.06 (0.87, 1.30)	0.93 (0.76, 1.14)	1.13 (0.93, 1.38)	0.95 (0.77, 1.16)	0.51
HPFS: Median (mg/day)	133.7	207.0	277.1	373.7	622.6	
[Range (mg/day)]	[10.8–174.6]	[174.7–239.9]	[240.0–317.1]	[317.2–456.1]	[456.2–2901.0]	
Cases/Person-years	95/110,879	104/111,575	121/111,880	103/111,979	94/111,801	
Age-adjusted model	1.00 (ref)	1.02 (0.77, 1.35)	1.19 (0.90, 1.56)	0.93 (0.70, 1.24)	0.89 (0.67, 1.20)	0.25
Multivariable model[1]	1.00 (ref)	1.01 (0.75, 1.34)	1.16 (0.88, 1.53)	0.92 (0.68, 1.23)	0.84 (0.62, 1.14)	0.15
POOLED: Multivariable model[1,2]	1.00 (ref)	1.04 (0.89, 1.23)	1.00 (0.85, 1.18)	1.06 (0.90, 1.25)	0.91 (0.77, 1.08)	0.19
Flavonols						
NHS: Median (mg/day)	7.5	11.0	14.0	18.1	27.2	
[Range (mg/day)]	[1.4–9.3]	[9.4–12.2]	[12.3–15.5]	[15.6–21.1]	[21.2–101.4]	
Multivariable model[1]	1.00 (ref)	0.89 (0.73, 1.09)	0.96 (0.79, 1.17)	1.14 (0.94, 1.38)	0.87 (0.71, 1.07)	0.47
HPFS: Median (mg/day)	8.2	12.3	15.6	20.0	28.9	
[Range (mg/day)]	[1.2–10.4]	[10.5–13.6]	[13.7–17.2]	[17.3–22.7]	[22.8–107.0]	
Multivariable model[1]	1.00 (ref)	1.01 (0.77, 1.33)	0.87 (0.65, 1.15)	0.89 (0.67, 1.18)	0.72 (0.53, 0.96)	0.01
POOLED: Multivariable model[1,2]	1.00 (ref)	0.93 (0.79, 1.10)	0.93 (0.79, 1.09)	1.05 (0.90, 1.23)	0.82 (0.69, 0.97)	0.05
Monomeric flavanols						
NHS: Median (mg/day)	10.0	18.1	30.4	54.9	124.1	
[Range (mg/day)]	[0.7–14.1]	[14.2–23.1]	[23.2–40.2]	[40.3–77.7]	[77.8–705.9]	
Multivariable model[1]	1.00 (ref)	1.02 (0.83, 1.24)	1.08 (0.89, 1.31)	1.11 (0.91, 1.35)	0.91 (0.74, 1.12)	0.19

	Quintiles of intakes					*P for trend*
	1	**2**	**3**	**4**	**5**	
HPFS: Median (mg/day)	11.1	19.2	28.9	47.1	103.0	
[Range (mg/day)]	[0.5–15.4]	[15.5–23.5]	[23.6–35.8]	[35.9–65.2]	[65.3–646.6]	
Multivariable model[1]	1.00 (ref)	0.94 (0.70, 1.24)	0.98 (0.74, 1.30)	1.00 (0.76, 1.32)	0.76 (0.56, 1.02)	0.05
POOLED: Multivariable model[1,2]	1.00 (ref)	0.99 (0.84, 1.16)	1.04 (0.89, 1.23)	1.07 (0.91, 1.26)	0.86 (0.72, 1.02)	0.04
Polymeric flavanols						
NHS: Median (mg/day)	62.4	107.9	157.8	238.3	461.7	
[Range (mg/day)]	[2.3–86.4]	[86.5–130.0]	[130.1–190.8]	[190.9–312.1]	[312.2–2410.9]	
Multivariable model[1]	1.00 (ref)	0.97 (0.80, 1.19)	0.97 (0.80, 1.18)	1.10 (0.90, 1.33)	0.88 (0.72, 1.09)	0.25
HPFS: Median (mg/day)	61.8	106.5	153.6	224.3	418.2	
[Range (mg/day)]	[0.9–85.7]	[85.8–128.2]	[128.3–182.9]	[183.0–287.3]	[287.4–2266.1]	
Multivariable model[1]	1.00 (ref)	0.81 (0.61, 1.08)	0.95 (0.72, 1.25)	0.84 (0.63, 1.11)	0.78 (0.58, 1.04)	0.16
POOLED: Multivariable model[1,2]	1.00 (ref)	0.92 (0.78, 1.08)	0.96 (0.82, 1.13)	1.00 (0.86, 1.18)	0.85 (0.72, 1.00)	0.09
Anthocyanidins						
NHS: Median (mg/day)	3.1	6.2	9.7	14.3	24.0	
[Range (mg/day)]	[0.0–4.7]	[4.8–7.8]	[7.9–11.8]	[11.9–17.8]	[17.9–221.5]	
Multivariable model[1]	1.00 (ref)	1.12 (0.92, 1.36)	1.08 (0.88, 1.32)	1.08 (0.88, 1.32)	0.96 (0.78, 1.17)	0.32
HPFS: Median (mg/day)	2.9	6.0	9.9	15.3	26.6	
[Range (mg/day)]	[0.0–4.8]	[4.9–8.1]	[8.2–12.6]	[12.7–19.6]	[19.7–425.5]	
Multivariable model[1]	1.00 (ref)	0.75 (0.57, 0.99)	0.77 (0.58, 1.01)	0.84 (0.64, 1.11)	0.75 (0.56, 0.99)	0.24
POOLED: Multivariable model[1,2]	1.00 (ref)	0.98 (0.83, 1.15)	0.96 (0.82, 1.13)	0.99 (0.84, 1.16)	0.88 (0.74, 1.04)	0.13
Flavones						
NHS: Median (mg/day)	0.8	1.4	1.9	2.5	3.4	
[Range (mg/day)]	[0.0–1.0]	[1.1–1.6]	[1.7–2.1]	[2.2–2.7]	[2.8–35.1]	
Multivariable model[1]	1.00 (ref)	1.09 (0.89, 1.33)	0.85 (0.69, 1.05)	0.98 (0.80, 1.19)	0.99 (0.81, 1.21)	0.74
HPFS: Median (mg/day)	1.0	1.7	2.4	3.1	4.3	
[Range (mg/day)]	[0.0–1.3]	[1.4–1.9]	[2.0–2.6]	[2.7–3.4]	[3.5–42.1]	
Multivariable model[1]	1.00 (ref)	1.06 (0.79, 1.41)	1.02 (0.77, 1.37)	1.02 (0.76, 1.37)	0.90 (0.66, 1.21)	0.40

	Quintiles of intakes					*P for trend*
	1	**2**	**3**	**4**	**5**	
POOLED: Multivariable model[1,2]	1.00 (ref)	1.08 (0.92, 1.27)	0.91 (0.77, 1.08)	0.99 (0.84, 1.17)	0.96 (0.81, 1.13)	0.42
Flavanones						
NHS: Median (mg/day)	10.4	24.5	38.2	53.4	78.9	
[Range (mg/day)]	[0.0–17.5]	[17.6–31.2]	[31.3–45.3]	[45.4–63.2]	[63.3–321.2]	
Multivariable model[1]	1.00 (ref)	1.05 (0.86, 1.29)	1.04 (0.85, 1.27)	0.99 (0.81, 1.21)	1.02 (0.83, 1.24)	0.89
HPFS: Median (mg/day)	11.7	29.9	47.4	66.1	98.7	
[Range (mg/day)]	[0.0–20.8]	[20.9–38.4]	[38.5–56.1]	[56.2–78.3]	[78.4–535.7]	
Multivariable model[1]	1.00 (ref)	1.07 (0.81, 1.43)	0.92 (0.68, 1.23)	0.91 (0.68, 1.21)	0.84 (0.62, 1.13)	0.11
POOLED: Multivariable model[1,2]	1.00 (ref)	1.06 (0.90, 1.25)	1.00 (0.85, 1.18)	0.96 (0.81, 1.13)	0.96 (0.81, 1.13)	0.25

[1]Stratified by age in months and calendar time, adjusted for race (Caucasian, African, Asian, Other), family history (yes, no), self-reported diabetes, body mass index (<22, 22–23, 24–25, 26–27, 28–29, 30+ kg/m^2), hypertension (yes, no), alcohol intake (0, 1–4, 5–14, 15–29, 30+ g/day), cigarette smoking (0, 1–9, 10–19, 20–29, 30+, pack-years), caffeine intake (quintiles of mg/day), physical activity (quartiles of MET-hours/week), number of eye exams reported during follow-up, and in NHS only additionally adjusted for age at menopause (20–44, 45–49, 50–53, 54+ years) and postmenopausal hormone status (premenopausal, current user, past user, non-user).

[2]p-heterogeneity based on the multivariable models of linear trends of the two cohorts ≥ 0.11 for all flavonoid and flavonoid sub-classes examined

Table 3

Pooled relative risks (RRs) and 95% confidence intervals (CIs) for the association between total flavonoid and sub-class intakes and subtypes of primary open-angle glaucoma defined by intraocular pressure at diagnosis and type of visual field loss in Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS)

	Intraocular pressure (IOP) at diagnosis			Visual field (VF) loss		
	IOP >21 mm Hg (n=1045)	**IOP ≤ 21 mm Hg (n=530)**	**p for heterogeneity[1]**	**Peripheral VF loss[2] (n=891)**	**Paracentral VF loss[2] (n=454)**	**p for heterogeneity[1]**
Total flavonoids						
RR[3] (95% CI)	0.96 (0.78, 1.18)	0.83 (0.61, 1.13)	0.46	0.86 (0.68, 1.08)	0.87 (0.64, 1.18)	0.61
p trend	0.51	0.17		0.09	0.57	
Flavonols						
RR[3] (95% CI)	0.78 (0.64, 0.97)	0.86 (0.64, 1.14)	0.60	0.75 (0.60, 0.93)	0.77 (0.56, 1.07)	0.98
p trend	0.05	0.43		0.04	0.13	
Monomeric flavanols						
RR[3] (95% CI)	0.86 (0.70, 1.06)	0.84 (0.62, 1.14)	0.66	0.78 (0.62, 0.98)	0.95 (0.69, 1.31)	0.86
p trend	0.15	0.12		0.05	0.26	
Polymeric flavanols						
RR[3] (95% CI)	0.85 (0.70, 1.05)	0.84 (0.62, 1.12)	0.75	0.76 (0.60, 0.95)	0.89 (0.66, 1.22)	0.51
p trend	0.23	0.21		0.03	0.47	
Anthocyanidins						
RR[3] (95% CI)	0.97 (0.79, 1.19)	0.72 (0.53, 0.96)	0.27	0.77 (0.61, 0.96)	0.99 (0.73, 1.35)	0.30
p trend	0.55	0.07		0.05	0.98	
Flavones						
RR[3] (95% CI)	0.96 (0.78, 1.17)	0.95 (0.72, 1.26)	0.84	0.90 (0.72, 1.12)	0.96 (0.71, 1.30)	>0.99
p trend	0.53	0.54		0.37	0.51	
Flavanones						
RR[3] (95% CI)	0.92 (0.75, 1.13)	1.01 (0.76, 1.34)	0.80	0.83 (0.67, 1.04)	1.15 (0.84, 1.57)	0.36
p trend	0.25	0.68		0.12	0.92	

[1] For testing whether the associations between flavonoid/flavonoid subclasses and one POAG subtype is significantly different from that with another subtype, we combined the NHS and HPFS datasets into one, then conducted Cox regression analyses that stratified on the 2 cohorts, which allowed for the baseline hazard function to be different in the cohorts; we then used the Lunn-McNeil approach to test for heterogeneity in associations and derived p for heterogeneity

[2] Based on visual field (VF) loss pattern as of the earliest reliable VF at diagnosis that was reproduced at the latest reliable VF. Cases (n=230) with advanced VF loss at diagnosis who could not be categorized based on initial presenting VF loss as either peripheral VF loss only or early paracentral VF loss were censored during analyses. See Methods for how cases were categorized according to initial presenting VF loss.

[3] Pooled relative risks across two cohorts comparing the highest quintile versus the lowest, stratified by age, 2-year period at risk, adjusted for the same variables as in multivariable model in Table 2.

Table 4

Pooled relative risks (RRs) and 95% confidence intervals (CI) for the association between intakes of flavonoid-rich food[1] and primary open-angle glaucoma in the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS)

Flavonoid source	**Categories of intake (servings per week)**					
Apples, unit=1 whole fruit/week (median)	0 − <0.5 (0.4)	0.5 − <1.2 (0.8)	1.2 − <2.5 (1.8)	2.5 − <3.9 (3.1)	3.9+ (5.3)	P for trend
No. of cases[2]	262	292	382	298	323	
POOLED RR[3] (95% CI)	1.00 (ref)	0.96 (0.81, 1.14)	0.91 (0.77, 1.07)	0.94 (0.79, 1.12)	1.00 (0.84, 1.20)	0.66
Blueberries, unit=½ cup/week (median)	0 (0)	0.1− <0.3 (0.1)	0.3 − <0.5 (0.4)	0.5 − <0.6 (0.5)	0.6+ (1.0)	
No. of cases	591	384	127	239	217	
POOLED RR (95% CI)	1.00 (ref)	0.98 (0.85, 1.12)	0.86 (0.70, 1.05)	0.98 (0.84, 1.15)	0.95 (0.80, 1.12)	0.45
Onions, unit=1 whole onion/week (median)	0 − <0.2 (0)	0.2 − <0.5 (0.4)	0.5 − <1.0 (0.7)	1.0 − <2.0 (1.3)	2.0+ (3.0)	
No. of cases	282	279	345	323	305	
POOLED RR (95% CI)	1.00 (ref)	0.90 (0.76, 1.07)	1.05 (0.89, 1.24)	0.99 (0.83, 1.17)	0.99 (0.83, 1.18)	0.87
Oranges, unit=1 whole fruit/week (median)	0 − <0.4 (0.1)	0.4 − <0.8 (0.6)	0.8 − <1.5 (1.1)	1.5 − <3.0 (2.2)	3.0+ (4.3)	
No. of cases	350	305	307	381	215	
POOLED RR (95% CI)	1.00 (ref)	1.02 (0.87, 1.20)	1.04 (0.89, 1.22)	0.97 (0.83, 1.13)	0.95 (0.79, 1.14)	0.28
Orange juice, unit=1 small glass/week (median)	0 − <0.5 (0.2)	0.5 − <1.8 (1.1)	1.8 − <3.5 (2.7)	3.5 − <6.8 (5.0)	6.8+ (7.0)	
No. of cases	361	276	304	352	262	
POOLED RR (95% CI)	1.00 (ref)	0.91 (0.77, 1.07)	0.89 (0.76, 1.04)	0.85 (0.73, 1.00)	0.93 (0.79, 1.10)	0.30
Strawberries, unit=½ cup/week (median)	0 – <0.1 (0)	0.1 − <0.5 (0.4)	0.5 − <0.6 (0.5)	0.6 − <0.9 (0.8)	0.9+ (1.4)	
No. of cases	267	282	315	335	359	
POOLED RR[2] (95% CI)	1.00 (ref)	0.97 (0.82, 1.16)	0.95 (0.80, 1.13)	1.07 (0.90, 1.28)	0.97 (0.82, 1.16)	0.91
Tea, unit=1 cup/week (median)	0 (0)	0.1 − <1.0 (0.5)	1.0 − <3.5 (2.1)	3.5 − <7.0 (5.3)	7.0+ (13.1)	
No. of cases	280	413	360	275	226	
POOLED RR[2] (95% CI)	1.00 (ref)	1.00 (0.86, 1.17)	1.00 (0.85, 1.18)	1.03 (0.87, 1.23)	0.82 (0.68, 0.99)	0.02

[1] The top two contributors to the variation in the intake of the individual flavonoid subclasses in 1998 were included.

[2] Total number across NHS and HPFS; number of cases are slightly different by food/beverage type due to missing values

[3] Pooled results of multivariable analyses stratified by age, 2-yr period at risk, adjusted for total caloric intake and the same variables as in multivariable model in Table 2