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Association of dietary nitrate intake with primary open-angle glaucoma: a prospective analysis from the Nurses' Health Study and Health Professionals Follow-up Study

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Abstract

Importance—Nitric oxide (NO) signaling alterations in outflow facility and retinal blood flow autoregulation are implicated in primary open-angle glaucoma (POAG). NO donation has emerged as a POAG therapeutic target. An exogenous source of NO is dietary nitrates.

Objective—We evaluated the association between dietary nitrate intake, derived mainly from green leafy vegetables, and POAG.

Design, Setting, Participants—We followed biennially participants of the prospective cohorts, Nurses' Health Study (63,893 women; 1984-2012) and Health Professionals Follow-up

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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.

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Study (41,094 men; 1986-2012); at each 2-year risk period, eligible participants were 40+ years old, free of POAG, and reported eye examinations.

Exposure—Dietary nitrate intake. Information on diet and potential confounders were updated with validated questionnaires.

Main outcome measure—Incidence of POAG and POAG subtypes; 1,483 cases were confirmed with medical records and classified into subtypes defined by intraocular pressure (IOP) (≥ or < 22 mm Hg) or by visual field (VF) loss pattern at diagnosis (peripheral loss only or early paracentral loss). Cohort-specific and pooled multivariable rate ratios (MVRR) and 95% confidence intervals (CIs) were estimated.

Results—Compared with the lowest quintile of dietary nitrate intake (Q1;∼80 mg/day), the pooled MVRR for the highest quintile (Q5;∼240 mg/day) was 0.79 (95%CI, 0.66,0.93; p for trend [p-trend]=0.02). The dose-response was stronger (p for heterogeneity [p-het]=0.01) for POAG with early paracentral VF loss (433 cases; Q5 vs. Q1 MVRR=0.56; 95%CI, 0.40,0.79; p-trend=0.0003) than for POAG with peripheral VF loss only (835 cases; Q5 vs. Q1 MVRR=0.85; 95%CI, 0.68,1.06; p-trend=0.50). The association did not differ (p-het=0.75) by POAG subtypes defined by IOP (997 cases with IOP≥22 mm Hg: Q5 vs. Q1 MVRR=0.82; 95%CI, 0.67,1.01; p-trend=0.11 vs. 486 cases with IOP<22 mm Hg: Q5 vs. Q1 MVRR=0.71; 95%CI, 0.53,0.96; p-trend=0.12). Green leafy vegetables accounted for 56.7% of nitrate intake variation. Compared with consuming 0.31 servings/day, the MVRR for consuming 1.45+ servings/day was 0.82 for all POAG (95%CI, 0.69,0.97; p-trend=0.02) and 0.52 for POAG with paracentral VF loss (95%CI, 0.29,0.96; p-trend=0.0002).

Conclusion and relevance—Higher dietary nitrate and green leafy vegetable intake was associated with a lower POAG risk, particularly POAG with early paracentral VF loss at diagnosis.

Introduction

Elevated intraocular pressure (IOP) and impaired autoregulation of optic nerve blood flow are implicated in primary open-angle glaucoma (POAG).[1-10] Endothelial dysfunction, a key contributor to vascular regulatory impairment, is involved in both processes.[11] The vascular endothelium regulates the microcirculation via vasoactive factors; one potent factor is nitric oxide (NO). In the L-arginine-NO pathway, NO is formed from L-arginine and oxygen by NO synthases (NOS) such as endothelial NOS (NOS3).[12]

Abundant evidence supports NO's role in POAG pathogenesis.[13] With administration of a systemic NOS inhibitor, differences in ocular blood flow response was observed between POAG cases and controls.[14] Also, polymorphisms in *NOS3*, the gene for NOS3, were associated with lower blood NO levels,[15-19] and POAG.[16,20-22]

As an alternative to the L-arginine-NO pathway, under hypoxia[23,24] or when NOS may be dysfunctional,[14,16,20,21] as may occur in POAG, exogenous nitrate (NO_3-) can be reduced to nitrite (NO_2)[25-27] by commensal bacteria[28-30] and subsequently converted enzymatically or non-enzymatically to NO in tissues[26,31-33] in the nitrate-nitrite-NO pathway.[34-38] Evidence suggests that nitrate or nitrite, precursors for NO, is beneficial for blood circulation.[34,39-42] Dietary nitrate is predominately from green leafy vegetables,[43] which contribute ∼ 80% of

nitrate intake.[29] Although plasma nitrite levels[16,44] or intake of specific vegetables[45,46] have been associated with POAG; dietary nitrate intake as a specific nutrient has not been evaluated. Therefore, we evaluated dietary nitrate and incident POAG in a 25+ year prospective study of 63,893 women in the Nurses' Health Study (NHS) and 41,094 men in the Health Professionals Follow-up Study (HPFS).

Subjects and Methods

The NHS began in 1976 with 121,700 US registered female nurses (30-55 years old) who completed a mailed questionnaire.[47] The HPFS began in 1986 with 51,529 US male health professionals (dentists, veterinarians, pharmacists, optometrists, osteopaths, and podiatrists) aged 40 to 75 years.[48] Participants have been followed biennially with mailed questionnaires of health, diet, and diseases such as glaucoma. Follow-up rates were high (> 85%). The Human Research Committees of Brigham & Women's Hospital, Massachusetts Eye and Ear and the Harvard School of Public Health approved this study.

The first detailed assessment of diet with a semiquantitative food frequency questionnaire (SFFQ) was in 1984 for NHS and 1986 for HPFS, and thus are “baseline” years. Participants contributed person-time in ∼2-year units from the return date of one questionnaire to that of another until the earliest occurrence of a glaucoma report, cancer, death, loss to follow-up or the end of study in 2012. Eligible participants were aged 40+ years (when glaucoma risk increases) and reported an eye exam in the 2-year risk period (to minimize possible detection bias).

We excluded at baseline the following NHS and HPFS participants, respectively: 1) 47,512 and 1596 who did not respond to baseline SFFQs or had outlying total caloric intakes, 2) 4,011 and 1,927 with prevalent cancers excluding nonmelanoma skin cancer, as cancer diagnoses could alter diet, 3) 902 and 1,035 with prevalent glaucoma, 4) 450 and 1,874 lost to follow-up from 1976-1984 (NHS) or <2 years of baseline (NHS and HPFS), 5) 2,391 and 3,251 who never reported an eye exam during follow-up. After these exclusions, 66,435 and 41,846 were eligible; however, at the beginning of each 2-year risk period, we applied additional provisional exclusions for age and eye exam status. For example, for the 1984-'86 (NHS) and 1986-'88 (HPFS) risk periods, only 45,955 and 29,039 contributed person-time after we provisionally excluded participants (20,480 and 12,807) who were age<40 years and reported no eye exam. In later periods, those provisionally excluded were allowed in analyses if they met eligibility criteria during follow-up. Thus, over the study period, 63,893 and 41,094 ever contributed person-time.

Ascertainment of POAG cases and classification of POAG by IOP and visual field (VF) loss pattern

We included 1483 confirmed incident POAG (1000 women and 483 men). Glaucoma case ascertainment occurred biennially, when we asked about eye exams and physician-diagnoses of glaucoma. For those self-reporting glaucoma, we sought permission to contact eye care providers, who were requested to send all VFs with medical records or a completed glaucoma questionnaire with items on maximal IOP, status of the filtration apparatus, optic nerve structural information, ophthalmic surgery, and VF loss. Records were reviewed by a

glaucoma specialist (LRP), masked to participants' diet, to confirm POAG cases using standardized criteria.

Cases had to be appraised as either “definite” or “probable” POAG. For definite POAG cases (>70% of all cases), we required: (1) gonioscopy where the filtration angle was not occludable in either eye, (2) slit lamp biomicroscopy showed no signs in either eye of pigment dispersion syndrome, uveitis, exfoliation syndrome, trauma, or rubeosis, and (3) reproducible VF defects consistent with POAG on ≥2 reliable tests. For probable POAG cases, the slit lamp exam and VF criteria were also required, but documentation of pupil dilation without subsequent adverse events was accepted in lieu of gonioscopy. For VF defects, the type of perimetry was not restricted; however, full static threshold testing was documented in 95%, and kinetic VFs in <1%. For static threshold or suprathreshold tests, we used the following reliability definitions: fixation loss ≤ 33%, false positive rate ≤ 20% and false negative rate ≤ 20%. For kinetic VFs, a VF test was considered reliable unless the examiner noted to the contrary.

New glaucoma diagnoses were self-reported by the following NHS and HPFS participants, respectively: 8,611 and 3,786. These were confirmed as various types of glaucoma or glaucoma suspect in 64% and 54%: potential POAG with VF loss (27% and 26%), only elevated IOP or optic disc cupping (18% and 16%), and other types of glaucoma/glaucoma suspect (19% and 12%). The remaining (36% and 46%) were unconfirmed, as participants (8% and 15%), or eye care providers (5% and 5%) were unreachable, participants denied permission for record review (11% and 10%), participants indicated the report was erroneous (10% and 14%) or eye care providers refuted the glaucoma diagnosis (2% and 2%). Among those classified as potential POAG with VF loss, we included only the definite or probable POAG cases (1000 women and 483 men); other confirmed and unconfirmed self-reports were censored as of the diagnosis date.

For secondary analyses, we classified cases into subtypes by IOP and by VF loss pattern at diagnosis. We defined subtypes of “high-tension” (n=997; 651 and 346) and “normal-tension” POAG (n=486; 349 and 137) 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=835; 576 and 259) or early paracentral VF loss (n=433; 288 and 145) or undetermined VF loss (n=215; 136 and 79) with a method previously described.[49] For a case with peripheral VF loss only, nasal step, temporal wedge or Bjerrum scotoma was present with no paracentral loss. For a case with early paracentral loss, there was 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. We included the latter paracentral group as those with only paracentral loss were uncommon (∼21%) whereas those with clear paracentral loss frequently also showed peripheral loss. Cases (n=215) with undetermined VF loss (i.e., 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. The proportion of those with normal-tension POAG was 38% in those with early paracentral VF loss and 29% in those with peripheral VF loss only.

Measurement of intake of nitrate and vegetable sources of nitrate

Validated SFFQs were administered every 2-4 years. The 1984 NHS SFFQ included 116 items, and similar versions were used from 1986 in the NHS (126 items) and HPFS (131 items).

The SFFQ asks about the average intake of a serving of a food/beverage over the preceding year, with intake choices from "never or <1/ month" to "6+/day". To convert responses into average daily intakes of nitrate, nutrient content information of each food obtained from updated U.S. Department of Agriculture food-composition[50] was used and combined with frequency information.

For primary analyses, we examined daily intake of nitrate and vegetables. Vegetables included celery and others in 4 groups: green leafy vegetables (iceberg lettuce, romaine lettuce, kale/mustard/chard, cooked spinach, raw spinach), cruciferous vegetables (kale/mustard/chard, broccoli, cabbage/coleslaw, cauliflower, Brussels sprouts), root vegetables (beet, potato, onion, carrot, yam/sweet potato), and tomato-based foods (tomato, tomato sauce, tomato juice).

We evaluated updated cumulatively averaged intakes, which better represent long-term exposure and have less random measurement error.[51] With cumulative averaging, the average of all available information was used (e.g. in NHS in 1984, the 1984 nitrate value was used; in 1986, the average of 1984 and 1986 values was used, etc.). Intakes of other dietary factors (e.g., other antioxidants, caffeine, alcohol, folate, flavonoids) were similarly derived.

Validity of SFFQ assessment of nitrate and vegetable sources

The reproducibility and validity of the SFFQ have been reported previously.[52,53] In a biomarker study among 630 participants, being in the highest tertile of dietary nitrate intake based on the SFFQ was associated with a 3.18 mmol/L increase in plasma nitrate (p=0.1).[54] In 127 participants who completed both SFFQ and multiple weighed dietary records,[55] the SFFQ performed reasonably well, with a mean correlation with dietary record values of 0.46 across vegetables, from 0.25 for kale/mustard/chard greens to 0.73 for lettuce.

Statistical Analysis

For analyses of nitrate intake, intake values were total energy adjusted using the residual method.[56] For food analyses, cumulatively updated total calories were adjusted for.

We calculated incidence rates of POAG by dividing the incident cases by person-years accrued for each intake category (quintiles). For multivariable analyses, we conducted Cox proportional hazards analysis stratified by age in months and the specific 2-year period at risk,[57] while simultaneously controlling for potential glaucoma risk factors. We derived incidence rate ratios (RRs) and 95% confidence intervals (CIs). We conducted tests for trend by evaluating the significance of a variable representing quintile median values.

Potential covariates were updated biennially from baseline: glaucoma family history, African heritage, body mass index (kg/m^2), pack years of smoking, hypertension, diabetes, physical

activity (MET [metabolic equivalent]-hours/week), number of eye exams reported during follow-up, multi-vitamin use; and among women, age at menopause and postmenopausal hormone use. Also, main multivariable models ("model 1") included other dietary components: cumulatively updated intake categories of total calories, alcohol, and caffeine. In additional multivariable models ("model 2"), we further adjusted for intake of folate, vitamin A and antioxidants (α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, other carotenoids, flavonoids, vitamins C, and E).

We analyzed cohort-specific data separately and performed tests for heterogeneity to check for appropriateness of pooling the results. Then, we pooled the results using meta-analytic methods incorporating random effects.[58]

Secondary analyses

We performed several secondary analyses. First, we evaluated nitrate intake only as of baseline or as of the most recent questionnaire. Second, we separately analyzed the risks of high- vs. normal-tension POAG, and of POAG with peripheral VF loss only vs. early paracentral loss. For testing whether the associations between nitrate and one POAG subtype is different from that with another subtype, we combined the datasets into one, then conducted Cox regression analyses that further stratified on the 2 cohorts (to allow for differing hazard functions) and used the Lunn-McNeil approach[59] to derive the p for heterogeneity [p-het].

Results

During 1,678,713 person-years of follow-up, we identified 1483 incident cases. Highest consumers of dietary nitrate consumed more antioxidants (carotenoids, vitamin C, vitamin E), flavonoids, folate and vitamin A, exercised more and were more frequently African American (Table 1). They were also leaner and smoked less. These differences were adjusted for in multivariable analyses.

In nitrate analyses, cohort-specific results were not heterogeneous and thus were pooled. Age-adjusted and multivariable analyses showed similar associations. Compared with the lowest quintile (Q1) of ~80 mg of nitrate/day, the pooled multivariable relative risk (MVRR, 95%CI) of POAG in the main model ("model 1") was 0.81 (95%CI, 0.69, 0.96) for Q2, 0.88 (95%CI, 0.75, 1.04) for Q3, 0.90 (95%CI, 0.66, 1.23) for Q4 and 0.79 (95%CI, 0.66, 0.93) for Q5 (p for trend [p-trend]=0.02) (Table 2). When other dietary factors ("model 2") were also adjusted for, similar inverse associations were observed (pooled MVRR for Q5 vs. Q1 was 0.67 (95%CI, 0.52, 0.85); p-trend=0.01).

When we explored the timing of intake, nitrate intake just at baseline or at the most recent SFFQ was not associated: compared with Q1, the pooled MVRR for "model 1" was 0.88 (95%CI, 0.74, 1.04) for Q5 (p-trend=0.21) for baseline intake and 0.91 (95%CI, 0.76, 1.09) for Q5 (p-trend=0.13) for most recent intake.

When we evaluated nitrate intake with POAG subtypes characterized by IOP at diagnosis, we observed similar associations, and the p-het was 0.75. However, we observed differences

(p-het=0.01) in associations by VF subtypes: compared with Q1, the pooled MVRR for POAG with peripheral VF loss only was 0.85 (95%CI, 0.68, 1.06) for Q5 (p-trend=0.50) and that for POAG with early paracentral VF loss was 0.56 (95%CI, 0.40, 0.79) for Q5 (p-trend=0.0003) (Table 2).

When we examined specific foods and food groups (Table 3), compared with those consuming a median of 0.31 (Q1) servings/day of green leafy vegetables, the pooled MVRR for 1.45 servings/day (Q5) was 0.82 (95%CI, 0.69, 0.97; p-trend=0.02) for overall POAG and was 0.52 (95%CI, 0.29, 0.96; p-trend=0.0002) for POAG with early paracentral VF loss. Among green leafy vegetables, the pooled MVRR comparing Q5 versus Q1 ranged from 0.72 to 0.89 for overall POAG; for POAG with early paracentral VF loss, the pooled MVRRs were 0.69 (95%CI, 0.49, 0.97; p-trend=0.001) for iceberg lettuce, 0.71 (95%CI, 0.29, 1.75; p-trend=0.19) for romaine lettuce, and 0.33 (95%CI, 0.16, 0.69; p-trend=0.01) for kale/mustard/chard greens. Associations were not observed with other nitrate contributing food/food groups except root vegetables. For root vegetables, inverse associations were observed in men only (p-het<0.05): in men, the pooled MVRR for consuming 1.76 servings/day (Q5) compared to 0.5 servings/day (Q1) was 0.68 (95%CI, 0.48, 0.96; p-trend=0.04) for overall POAG and was 0.51 (95%CI, 0.27, 0.96; p-trend=0.04) for POAG with early paracentral VF loss.

Discussion

Greater intake of dietary nitrate and green-leafy vegetables was associated with 20-30% lower POAG risk; the relation was particularly strong (40-50% lower risk) for POAG with early paracentral VF loss at diagnosis, for which ocular vascular dysregulation has been implicated.[60]

Evidence suggests a key role of the NO system in POAG pathogenesis; alterations of this system may dysregulate ocular blood flow[14,61] and IOP.[62-68] Elevated IOP was observed in a murine POAG model after the gene for soluble guanylate cyclase, the NO intracellular receptor, was knocked out.[69] NO may regulate IOP by mediating aqueous humor outflow; in an *in vitro* study, glaucomatous Schlemm's canal cells produced negligible NO after shear stress compared to non-glaucomatous cells.[70] Thus, exogenous NO donators are emerging as new glaucoma therapeutics.[13]

The nitrate-nitrite-NO pathway may be an important alternative source of NO in POAG. One lettuce serving can yield more NO than that generated daily via the L-arginine-NO pathway.[71] Tissue NO bioavailability and cerebral blood flow can increase with nitrate salts[72,73] and nitrate-rich beet juice supplementation.[74-79] Therefore, dietary nitrate supplementation represents a practical method to increase NO levels. Indeed, across the two cross-sectional studies in all (95 cases among 1,155 total)[45] or only African-American (77 cases among 587 total)[46] women in the Study of Osteoporotic Fractures, the only vegetable that was consistently inversely associated with POAG was kale/collard greens: ≥ 1 serving/month of kale/collard greens was significantly associated with 55-70% reduced odds of POAG.

The stronger inverse association with POAG with early paracentral VF loss is consistent with evidence that this subtype is more strongly associated with vascular dysregulation.[69,80,81] The blood vessels for the inferior paracentral fibers are in the “macula vulnerability zone”[82] and make more acute arcuate turns than others, creating greater shear forces that could compromise local blood flow.[61] Also, among glaucoma patients with autonomic dysfunction or abnormal peripheral microcirculation, paracentral VF defects were more common;[80] one hypothesis is that central fibers may have relatively high oxygen demand and thus be more vulnerable to vascular dysregulation.[83,84] Furthermore, genetic loci related to the NO pathway (e.g., *CAV1/CAV2*[85] and *GUCY1A3/GUCY1B3* regions[69]) are most strongly associated with POAG with paracentral loss. Thus, further studies are warranted of exogenous nitrate and POAG with paracentral VF loss.

This was a large prospective study with 1483 incident cases identified from 63,893 women and 41,094 men followed for 25+ years, with high follow-up rates. With repeated questionnaires, we evaluated nitrate intake and POAG in various ways (i.e., baseline, recent, cumulative intake) and controlled for numerous updated POAG risk factors.

Our study had a few limitations. We could not conduct repeated eye exams, and thus, we relied on questionnaires and medical records for disease confirmation. Our case ascertainment method had low sensitivity; however, methodologically, incidence rate *ratios* can still be valid if the case definition is highly specific and the ascertainment method is unrelated to exposure.[86] Our case definition was highly specific with requirement of reproducible VF loss, the case ascertainment was unlikely to be related to diet, and we required eye exams at each follow-up cycle to minimize biases. Another limitation was residual confounding by other dietary factors, as nitrate-rich vegetables may have other nutrients. However, we adjusted for intake of other nutrients, and the inverse associations were robust. We may have had some misclassification of nitrate intake from errors in participants' recall and because vegetable nitrate content can vary by soil conditions, season and storage;[87,88] however, this would have biased associations towards the null. Also, as both cohorts are 90+% Caucasian, our results may not be generalizable; however, in a study of African-Americans, kale/collard intake was also associated with a lower POAG risk.[46] Finally, these data represent findings from the first population-based observational study, and thus, the association between dietary nitrate consumption and POAG should be interpreted cautiously and confirmed.

In summary, greater intake of dietary nitrate, an exogenous NO source, was associated with a lower risk of POAG, particularly POAG with early paracentral VF loss. These results, if confirmed in observational and intervention studies, could have important public health implications.

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Table 1

Age and age-adjusted characteristics by total nitrate intake (1st, 3rd and 5th quintiles) over the follow-up period in NHS (1984-2012) and in HPFS (1986-2012)

		Total Nitrate		
		Q1	Q3	Q5
Mean age (years [SD])	Women	61.0 [10.2]	62.3 [10.0]	63.4 [9.7]
	Men	61.5 [10.6]	62.7 [10.5]	63.7 [10.2]
Total nitrate (mg/day)	Women	77.3 [17.1]	141.9 [9.0]	261.0 [75.3]
	Men	78.6 [18.1]	148.2 [10.7]	279.5 [89.8]
Green leafy vegetables (servings/day)	Women	0.3 [0.2]	0.8 [0.2]	1.5 [0.6]
	Men	0.3 [0.2]	0.7 [0.2]	1.4 [0.6]
Cruciferous vegetables (servings/day)	Women	0.3 [0.2]	0.5 [0.2]	0.7 [0.4]
	Men	0.3 [0.2]	0.5 [0.3]	0.8 [0.5]
Root vegetables (servings/day)	Women	0.9 [0.4]	1.1 [0.5]	1.3 [0.6]
	Men	0.9 [0.5]	1.1 [0.5]	1.3 [0.7]
Tomato-based foods (servings/day)	Women	0.4 [0.2]	0.6 [0.3]	0.8 [0.4]
	Men	0.4 [0.3]	0.6 [0.3]	0.8 [0.5]
Celery (servings/day)	Women	0.1 [0.1]	0.2 [0.2]	0.4 [0.4]
	Men	0.1 [0.1]	0.2 [0.2]	0.3 [0.3]
Total caloric intake (kcal/day)	Women	1723.4 [450.3]	1781.0 [438.5]	1716.0 [444.1]
	Men	1967.2 [564.0]	2011.9 [540.0]	1946.4 [542.8]
Alcohol intake (g/day)	Women	5.2 [9.5]	6.1 [9.1]	6.2 [8.8]
	Men	10.8 [14.8]	11.4 [13.4]	10.3 [12.0]
Caffeine intake (mg/day)	Women	275.0 [200.0]	264.8 [185.4]	263.3 [193.0]
	Men	249.3 [225.2]	224.9 [207.7]	214.9 [209.3]
Total carotenoid intake (IU/day)	Women	6045.8 [2951.2]	9192.8 [3678.9]	13974.2 [6338.7]
	Men	6744.1 [3952.3]	10289.4 [5101.9]	16411.0 [9393.3]
Total folate intake (μg/day)	Women	393.7 [184.5]	447.9 [182.0]	530.3 [201.6]
	Men	472.9 [231.1]	536.1 [231.3]	639.3 [266.6]
Total flavonoid intake (mg/day)	Women	318.2 [283.7]	342.3 [251.0]	383.0 [272.0]
	Men	293.8 [246.5]	342.3 [237.6]	391.6 [255.4]
Vitamin A intake (IU/day)	Women	9931.5 [4712.9]	13368.4 [5380.1]	18618.7 [8092.1]
	Men	11460.8 [6350.9]	15142.8 [7555.8]	21672.4 [11477.7]
Vitamin C intake (mg/day)	Women	276.5 [279.0]	336.0 [301.1]	432.7 [368.0]

		Total Nitrate		
		Q1	**Q3**	**Q5**
	Men	350.6 [371.8]	429.4 [409.0]	545.1 [474.9]
Vitamin E intake (mg/day)	Women	46.2 [67.3]	53.3 [70.8]	65.5 [83.2]
	Men	55.8 [79.6]	64.3 [83.6]	78.4 [97.4]
Family history of glaucoma (%)	Women	13.1	13.5	13.8
	Men	11.0	11.5	11.6
African ancestry (%)	Women	0.7	0.8	1.8
	Men	0.5	0.6	1.1
Self-reported diabetes diagnosis (%)	Women	7.3	7.0	7.1
	Men	6.3	6.2	7.8
Self-reported hypertension diagnosis (%)	Women	42.1	41.8	42.1
	Men	36.6	36.2	37.1
≥ 30 pack-years of smoking (%)	Women	20.1	16.0	16.1
	Men	19.1	16.0	15.3
Body mass index (kg/m^2)) ≥ 30 (%)	Women	19.6	18.8	18.2
	Men	11.4	10.6	11.3
Physical activity (top 25 percentile) (%)	Women	16.8	25.3	34.5
	Men	20.2	25.7	31.7

Table 2

Age-adjusted and multivariable adjusted relative risk (95% confidence interval) of primary open-angle glaucoma, by quintiles of nitrate intake,[*] in Nurses' Health Study (1984-2012) and in Health Professionals' Follow-up Study (1986-2012)

			Quintiles of dietary nitrate intake					
			Q1	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
Cumulatively updated diet	Women	Median (mg/day)	80	114	142	175	238	
		Cases	210	173	207	199	211	
		Person-time	227,054	227,827	226,545	227,053	226,982	
		Age-adjusted RR (95% CI)	1.00 (ref)	0.79 (0.64, 0.96)	0.89 (0.73, 1.08)	0.82 (0.67, 0.99)	0.85 (0.70, 1.04)	0.28
		Model 1: Multivariable[†] RR(95% CI)	1.00 (ref)	0.77 (0.63, 0.95)	0.85 (0.70, 1.03)	0.78 (0.64, 0.95)	0.81 (0.67, 0.99)	0.12
		Model 2: Multivariable RR (95% CI)	1.00 (ref)	0.74 (0.59, 0.92)	0.77 (0.61, 0.97)	0.67 (0.51, 0.86)	0.67 (0.50, 0.90)	0.02
	Men	Median (mg/day)	81	117	148	185	254	
		Cases	98	89	101	111	84	
		Person-time	108,530	109,243	108,596	108,704	108,180	
		Age-adjusted RR (95% CI)	1.00 (ref)	0.92 (0.69, 1.24)	1.02 (0.76, 1.35)	1.11 (0.84, 1.47)	0.78 (0.57, 1.05)	0.21
		Model 1: Multivariable[†] RR(95% CI)	1.00 (ref)	0.90 (0.67, 1.22)	0.97 (0.72, 1.30)	1.08 (0.81, 1.43)	0.72 (0.53, 0.98)	0.09
		Model 2: Multivariable[‡] RR (95% CI)	1.00 (ref)	0.88 (0.64, 1.21)	0.94 (0.67, 1.32)	1.03 (0.71, 1.49)	0.66 (0.42, 1.03)	0.11
	Pooled[§]	**Model 1: Multivariable[†] RR (95% CI)**	**1.00 (ref)**	**0.81 (0.69, 0.96)**	**0.88 (0.75, 1.04)**	**0.90 (0.66, 1.23)**	**0.79 (0.66, 0.93)**	**0.02**
		Model 2: Multivariable[‡] RR (95% CI)	**1.00 (ref)**	**0.78 (0.65, 0.94)**	**0.82 (0.67, 0.99)**	**0.81 (0.53, 1.24)**	**0.67 (0.52, 0.85)**	**0.01**
Baseline[‖]	Pooled[§]	Model 1: Multivariable[†] RR (95% CI)	1.00 (ref)	0.96 (0.81, 1.13)	0.94 (0.77, 1.15)	1.04 (0.73, 1.47)	0.88 (0.74, 1.04)	0.21
	Pooled[§]	Model 2: Multivariable[‡] RR (95% CI)	1.00 (ref)	0.98 (0.82, 1.18)	1.00 (0.69, 1.44)	1.08 (0.59, 1.97)	0.90 (0.59, 1.36)	0.64
Most recent[‖]	Pooled[§]	Model 1: Multivariable[†] RR (95% CI)	1.00 (ref)	1.09 (0.92, 1.30)	1.03 (0.77, 1.37)	1.01 (0.85, 1.20)	0.91 (0.76, 1.09)	0.13
	Pooled[§]	Model 2: Multivariable[‡] RR (95% CI)	1.00 (ref)	1.05 (0.87, 1.26)	1.01 (0.66, 1.57)	1.00 (0.71, 1.40)	0.83 (0.65, 1.05)	0.08

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 (African-American, non-African heritage), family history of glaucoma, self-reported history of hypertension, diabetes, body mass index (22-23, 24-25, 26-27, 28-29, 30+ kg/m^2), cumulatively averaged intakes of total energy (kcal/day; quintiles), alcohol (g/day in categories of 0-4, 5-14, 15-29, 30+ g/day), and caffeine (mg/day; quintiles); pack-years of smoking (1-9, 10-19, 20-29, 30+ pack-years), physical activity (quartiles of MET-hours [metabolic equivalents] / week), number of eye exams reported during follow-up, multivitamin use (non-user, past-user, current-user) and in NHS only additionally adjusted for age at menopause (20-44, 45-50, 50-54, 54+ years) and postmenopausal hormone status (premenopausal, current user, past user, non-user).

[‡] To model 1, additionally adjusted for other nutrients: quintiles of α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein/zeaxanthin, other carotenoids, folate, flavonoid, vitamins A, C, and E.

[§] Pooled results were calculated using Dersimonian and Laird methods with random effects; p for heterogeneity between cohorts for all p for linear trend results were > 0.05.

[//] Baseline diet refers to diet as of 1984 in women and 1986 in men; most recent diet refers to the intake as of the food frequency questionnaire immediately before each 2-year period at risk

Table 3

Age-adjusted and multivariable adjusted relative risk (95% confidence interval) of subtypes of primary open-angle glaucoma, by quintiles of nitrate intake,* in Nurses' Health Study (1984-2012) and in Health Professionals' Follow-up Study (1986-2012)

			Quintiles of dietary nitrate intake						
SUBTYPES DEFINED BY INTRAOCULAR PRESSURE†			**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**	**p-heterogeneity¶**
High-tension glaucoma (intraocular pressure ≥ 22 mm Hg; n=998)	Women	Cases	133	117	138	121	142		0.75
		Multivariable RR(95% CI)‡	1.00 (ref)	0.83 (0.64, 1.06)	0.89 (0.70, 1.14)	0.75 (0.58, 0.96)	0.87 (0.68, 1.11)	0.29	
	Men	Cases	68	62	75	82	59		
		Multivariable RR (95% CI)‡	1.00 (ref)	0.89 (0.62, 1.27)	1.01 (0.72, 1.43)	1.11 (0.79, 1.56)	0.73 (0.51, 1.06)	0.20	
	Pooled§	**Multivariable RR (95% CI)**	**1.00 (ref)**	**0.85 (0.69, 1.04)**	**0.93 (0.76, 1.13)**	**0.90 (0.61, 1.32)**	**0.82 (0.67, 1.01)**	**0.11**	
Normal-tension glaucoma (intraocular pressure < 22 mm Hg; n=487)	Women	Cases	77	56	69	78	69		
		Multivariable RR(95% CI)‡	1.00 (ref)	0.68 (0.48, 0.96)	0.77 (0.55, 1.07)	0.82 (0.59, 1.13)	0.71 (0.51, 1.00)	0.22	
	Men	Cases	30	27	26	29	25		
		Multivariable RR (95% CI)‡	1.00 (ref)	0.90 (0.52, 1.56)	0.84 (0.48, 1.47)	0.98 (0.57, 1.70)	0.72 (0.41, 1.28)	0.34	
	Pooled§	**Multivariable RR (95% CI)**	**1.00 (ref)**	**0.73 (0.55, 0.98)**	**0.79 (0.59, 1.05)**	**0.86 (0.65, 1.13)**	**0.71 (0.53, 0.96)**	**0.12**	
SUBTYPES DEFINED BY INITIAL VISUAL FIELD LOSS PATTERN‖									
POAG with peripheral visual field loss only (n=836 cases)	Women	Cases	120	91	121	120	124		0.01
		Multivariable RR(95% CI)‡	1.00 (ref)	0.71 (0.54, 0.93)	0.86 (0.67, 1.12)	0.83 (0.64, 1.07)	0.84 (0.65, 1.09)	0.60	
	Men	Cases	47	47	58	62	45		
		Multivariable RR (95% CI)‡	1.00 (ref)	1.01 (0.67, 1.55)	1.20 (0.80, 1.80)	1.29 (0.86, 1.92)	0.87 (0.57, 1.34)	0.67	
	Pooled§	**Multivariable RR (95% CI)**	**1.00 (ref)**	**0.82 (0.58, 1.15)**	**0.98 (0.72, 1.34)**	**1.00 (0.65, 1.54)**	**0.85 (0.68, 1.06)**	**0.50**	

			Quintiles of dietary nitrate intake						
SUBTYPES DEFINED BY INTRAOCULAR PRESSURE†			**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**	**p-heterogeneity¶**
POAG with early paracentral visual field loss (n=433 cases)	Women	Cases	61	64	58	56	49		
		Multivariable RR (95% CI)‡	1.00 (ref)	0.95 (0.66, 1.35)	0.79 (0.55, 1.14)	0.74 (0.51, 1.07)	0.64 (0.43, 0.94)	0.01	
	Men	Cases	35	28	28	32	22		
		Multivariable RR (95% CI)‡	1.00 (ref)	0.79 (0.47, 1.34)	0.72 (0.42, 1.22)	0.84 (0.50, 1.39)	0.44 (0.25, 0.78)	0.01	
	Pooled§	**Multivariable RR (95% CI)**	**1.00 (ref)**	**0.89 (0.67, 1.20)**	**0.77 (0.57, 1.04)**	**0.77 (0.57, 1.04)**	**0.56 (0.40, 0.79)**	**0.0003**	

Abbreviations: RR = Relative Risk; CI = Confidence Interval; IOP=intraocular pressure; VF=visual field

*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).

†Based on the maximum untreated intraocular pressure at diagnosis.

‡All multivariable analyses were adjusted for the same variables as in Model 1 in Table 2.

§Pooled results were calculated using Dersimonian and Laird methods with random effects.

//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=216) 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.

¶For testing whether the associations between nitrate and one POAG subtype is significantly different from that with another subtype, we combined the two 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[59] to test for heterogeneity in associations and derived p for heterogeneity.

Table 4

Pooled multivariable relative risks (95% CI)[*] for quintiles of daily servings of foods high in nitrate in relation to all primary open-angle glaucoma (POAG) and POAG with early paracentral visual field loss ("Para-POAG") in Nurses' Health Study (1984-2012) and in Health Professionals' Follow-up Study (1986-2012)

			Quintiles of intake of foods [median servings per day]					
	% Variability in nitrate explained	**Outcome**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
Green leafy vegetables[†]	**56.7%**	Median	0.31	0.56	0.75	1.00	1.45	
		All POAG	1.00 (ref)	0.95 (0.81, 1.12)	0.90 (0.66, 1.22)	0.93 (0.61, 1.44)	0.82 (0.69, 0.97)	0.02
		Para-POAG	1.00 (ref)	0.89 (0.57, 1.41)	0.67 (0.49, 0.92)	0.69 (0.51, 0.93)	0.52 (0.29, 0.96)	0.0002
Iceberg lettuce	23.2%	Median	0.11	0.25	0.43	0.55	0.86	
		All POAG	1.00 (ref)	1.00 (0.83, 1.20)	1.03 (0.87, 1.23)	0.88 (0.69, 1.13)	0.89 (0.75, 1.06)	0.06
		Para-POAG	1.00 (ref)	1.03 (0.63, 1.70)	0.89 (0.56, 1.42)	0.72 (0.41, 1.26)	0.69 (0.49, 0.97)	0.001
Romaine lettuce	17.4%	Median	0.00	0.05	0.12	0.25	0.55	
		All POAG	1.00 (ref)	0.93 (0.77, 1.11)	0.98 (0.82, 1.15)	0.89 (0.76, 1.05)	0.87 (0.74, 1.03)	0.11
		Para-POAG	1.00 (ref)	1.10 (0.61, 1.97)	1.17 (0.48, 2.89)	1.01 (0.66, 1.55)	0.71 (0.29, 1.75)	0.19
Kale/mustard/chard greens[‡]	6.0%	Median	0.00	0.01	0.04	0.07	0.13	
		All POAG	1.00 (ref)	1.02 (0.84, 1.24)	0.96 (0.77, 1.19)	0.92 (0.61, 1.38)	0.72 (0.54, 0.95)	0.08
		Para-POAG	1.00 (ref)	0.97 (0.68, 1.39)	1.03 (0.70, 1.53)	1.09 (0.66, 1.80)	0.33 (0.16, 0.69)	0.01
Cruciferous vegetables[†]	**15.9%**	Median	0.16	0.29	0.42	0.58	0.90	
		All POAG	1.00 (ref)	1.28 (1.08, 1.52)	1.10 (0.87, 1.38)	1.10 (0.92, 1.32)	1.12 (0.94, 1.35)	0.93
		Para-POAG	1.00 (ref)	1.23 (0.90, 1.69)	1.04 (0.75, 1.45)	1.15 (0.83, 1.59)	1.02 (0.73, 1.43)	0.72
Root vegetables[†§]	**9.7%**	Median	0.50	0.77	1.00	1.29	1.76	
		All POAG	1.00 (ref)	0.88 (0.73, 1.05)	0.83 (0.63, 1.09)	0.94 (0.64, 1.40)	0.87 (0.56, 1.36)	0.77
		All POAG-women	1.00 (ref)	0.88 (0.71, 1.09)	0.93 (0.75, 1.15)	1.13 (0.91, 1.40)	1.07 (0.85, 1.35)	0.12
		All POAG-men	1.00 (ref)	0.88 (0.64, 1.21)	0.70 (0.50, 0.97)	0.76 (0.54, 1.05)	0.68 (0.48, 0.96)	0.04
		Para-POAG	1.00 (ref)	0.74 (0.48, 1.15)	0.83 (0.59, 1.15)	0.85 (0.27, 2.65)	0.84 (0.34, 2.08)	0.89
		Para-POAG-women	1.00 (ref)	0.88 (0.58, 1.35)	0.84 (0.55, 1.28)	1.48 (0.99, 2.20)	1.29 (0.84, 1.98)	0.03
		Para- POAG-men	1.00 (ref)	0.56 (0.31, 1.02)	0.80 (0.46, 1.37)	0.46 (0.25, 0.86)	0.51 (0.27, 0.96)	0.04
Celery	**6.5%**	Median	0.02	0.07	0.11	0.23	0.44	

			Quintiles of intake of foods [median servings per day]					
	% Variability in nitrate explained	**Outcome**	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-trend**
		All POAG	1.00 (ref)	0.88 (0.73, 1.06)	0.88 (0.63, 1.22)	0.92 (0.77, 1.10)	0.93 (0.78, 1.11)	0.74
		Para-POAG	1.00 (ref)	0.80 (0.56, 1.14)	0.76 (0.55, 1.05)	0.72 (0.47, 1.13)	0.93 (0.68, 1.27)	0.79
Tomato – based foods [†]	**4.0%**		0.21	0.37	0.53	0.71	1.02	
		All POAG	1.00 (ref)	0.97 (0.83, 1.14)	0.89 (0.75, 1.05)	0.86 (0.73, 1.02)	0.90 (0.75, 1.07)	0.14
		Para-POAG	1.00 (ref)	0.94 (0.69, 1.28)	0.89 (0.65, 1.22)	1.02 (0.57, 1.80)	0.84 (0.60, 1.17)	0.37

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). Pooled results were calculated using Dersimonian and Laird methods with random effects; p for heterogeneity between cohorts for all p for linear trend results were > 0.05. All multivariable analyses were adjusted for the same variables as in Model 1 in Table 2.

[†‡] Kale/mustard/chard greens were included in both the green leafy vegetable and cruciferous vegetable categories. Green leafy vegetables included iceberg lettuce, romaine lettuce, kale/mustard/chard greens, cooked spinach, raw spinach. Cruciferous vegetables included broccoli, cabbage/coleslaw, cauliflower, kale/mustard/chard greens, Brussels sprouts. Root vegetables included potato, beet, onion, carrot and yam / sweet potatoes. Tomato-based foods included whole tomato, tomato sauce and tomato juice.

[§] The results for women and men were heterogeneous (p for heterogeneity was 0.01 for all POAG, and it was 0.004 for all Para-POAG); thus, cohort specific results are also provided. For all other food groups, the p for heterogeneity were >0.5