***Reviewer #2***

We would like to thank the reviewer for reviewing our manuscript entitled “Dietary nitrate intake is associated with decreased incidence of open-angle glaucoma: The Rotterdam Study" (MS# nutrients-1760125). We appreciate the positive review and acknowledgement of these important findings. Enclosed please find our revised manuscript, which incorporates the comments and suggestions of both reviewers. In this letter we will address the comments of reviewer #2 point by point.

**The authors have conducted a case-control analysis based on a large epidemiological prospective study and investigated an association between dietary nitrate intake and incidence of OAG.**

**The study has been well planned and performed. Chapter Material and Methods contain all necessary information. The writing is of perfect quality and the results are clearly presented.**

**The results and conclusions of this study are important.**

We would like to thank the reviewer for the positive feedback on our study design and our final manuscript.

**I would only like to ask about one fact that I find intriguing. The authors have found that participants without OAG had a significantly higher BMI than those with OAG. Their mean BMI was found to be 27.1 kg/m2 which is considered overweight. Lower risk of OAG would be related to a higher total dietary nitrate intake. “Previous research has shown that a higher dietary nitrate intake was associated with significantly wider retinal arterioles.[46] Widening of retinal arteriolar caliber is not only associated with lower risk of cardiovascular and cerebrovascular diseases [47,48], but also with lower risk of glaucoma”.**

**On the other hand, “compared with individuals with a normal BMI (defined as a BMI of 18.5 to 24.9), lifetime risks for incident CVD [cardiovascular disease] were higher in middle-aged adults in the overweight and obese groups” Khan SS, Ning H, Wilkins JT, et al. Association of Body Mass Index With Lifetime Risk of Cardiovascular Disease and Compression of Morbidity. JAMA Cardiol. 2018;3(4):280–287. doi:10.1001/jamacardio.2018.0022**

**I wonder if the authors could comment on this in the Discussion.**

We would like to thank the reviewer for this interesting comment. In the Rotterdam Study we indeed find an inverse association between BMI and OAG incidence (Table 1). When we divide the participants in three categories based on their BMI (BMI < 18.5, BMI 18.5-24.9, or BMI >= 24.9), the difference between cases and controls is still (borderline) significant (p=0.054), with more people being overweight (68.7%) in the control group than in the glaucoma group (59.3%).

The finding that BMI is inversely associated with OAG is in line with previous studies. Lin et al. [1] found that lower BMI was associated with increased odds of OAG, especially in younger females. Ramdas et al. found similar results, with an association between obesity and a lower risk of developing OAG, although only present in women. [2] In line with these findings, underweight was associated with increased risk of primary OAG in diabetes patients. [3] Moreover, in a group of patients in which visual field loss progressed despite treatment with eye drops, lower BMI was associated with progression of visual field loss in normal-tension glaucoma patients. [4] One possible explanation for this is that cerebrospinal fluid pressure (CSFP) has a positive, linear relationship with BMI. [5] As reduced CSFP may be a risk factor for primary OAG, individuals with lower BMI may have an increased risk for developing primary OAG. [6] Again, a higher BMI may be protective.

We acknowledge that BMI is an important confounder in our study, as BMI is associated with both our outcome (OAG) and exposure (diet). We have therefore included BMI as covariate in our main model. Adding or removing BMI from the multivariate model (model 1) did not change the results, as can be seen in the table below.

|  |  |  |
| --- | --- | --- |
|  | OR (95%CI) | P-value |
| Total dietary nitrate intake (per 10 mg/day) |  |  |
| Model 1 (including BMI) | 0.95 (0.91-0.98) | .002 |
| *Model 1 (excluding BMI)* | *0.94 (0.91-0.97)* | *<.001* |
| Vegetable dietary nitrate intake (per 10 mg/day) |  |  |
| Model 1 (including BMI) | 0.95 (0.91-0.98) | .004 |
| *Model 1 (excluding BMI)* | *0.94 (0.91-0.98)* | *.001* |
| Nitrate intake from non-vegetable food sources (per 10 mg/day) |  |  |
| Model 1 (including BMI) | 0.63 (0.41-0.96) | .03 |
| *Model 1 (excluding BMI)* | *0.61 (0.40-0.93)* | *.02* |

That the association did not change after adjustment for BMI suggests that the association between dietary nitrate intake and OAG cannot be explained by BMI, although residual confounding may persist. To account for a possible effect of BMI on the association between dietary nitrate intake and OAG, we performed additional matching. We returned to the original dataset and matched cases and controls based on age (3-years range) and sex, as done previously, and additionally for BMI (2 kg/m2 range). Please see below for the “new” baseline characteristics table.

|  |  |  |  |
| --- | --- | --- | --- |
|  | No iOAG (N=845) | iOAG (N=169) | P-value |
| Age, years, mean (SD) | 65.1 (6.8) | 65.6 (6.7) | .42 |
| Sex, female, N (%) | 455 (53.8) | 91 (53.8) | 1.00 |
| Education, N (%)  Primary education  Lower education  Intermediate education  Higher education | 113 (13.5)  367 (43.8)  248 (29.6)  109 (13.0) | 21 (12.4)  77 (45.6)  53 (31.4)  18 (10.7) | .81 |
| Smoking status, N (%)  Non-smoker  Former smoker  Current smoker | 252 (30.0)  389 (46.3)  199 (23.7) | 52 (30.8)  80 (47.3)  37 (21.9) | .88 |
| Hypertension, N (%) | 462 (54.7) | 91 (54.2) | .89 |
| SBP, mmHg, mean (SD) | 137.1 (20.6) | 136.6 (21.1) | .74 |
| DBP, mmHg, mean (SD) | 74.8 (10.5) | 75.2 (12.6) | .67 |
| BMI, kg/m2, mean (SD) | 26.1 (3.2) | 25.9 (3.3) | .52 |
| Total energy intake, kcal/day, mean (SD) | 2047.0 (510.2) | 2053.9 (507.4) | .87 |
| Diet quality, mean (SD) | 6.6 (1.9) | 6.9 (1.9) | .07 |
| Physical activity, MET hours/week, mean (SD) | 0.0 (0.9) | 0.1 (0.9) | .22 |
| IOP, mmHg, mean (SD) | 14.2 (2.8) | 16.3 (3.8) | **<.001** |
| Follow-up time, years, mean (SD) | 12.6 (5.3) | 10.9 (5.3) | **<.001** |
| Total dietary nitrate intake, mg/day, mean (SD) | 100.2 (69.0) | 92.7 (47.3) | .18 |
| Nitrate intake from vegetables, mg/day, mean (SD) | 85.0 (66.5) | 77.4 (45.4) | .15 |
| Nitrate intake from non-vegetable food sources, mg/day, mean (SD) | 15.2 (13.8) | 15.3 (11.0) | .90 |

Once the participants have been matched on age, sex and BMI, you can see that none of the covariates included in the models are significant between iOAG cases and controls. Cases only have a significantly higher IOP, which is expected, and somewhat shorter follow-up (not surprising, since they are excluded one they are diagnosed with iOAG). Diastolic blood pressure, BMI, and the dietary nitrate intakes are no longer different in the univariate analyses between cases and controls.

We have created two supplementary tables (Table S4 and Table S5), to show that the associations found between dietary nitrate intake and iOAG or IOP, were largely similar in the analyses where we matched on BMI additionally, as compared to including BMI as covariate into model 1.

Only the association between dietary nitrate intake from non-vegetable food sources and iOAG is no longer significant, but the change in effect estimates was minimal and they remained far below 1 (Table S4). For the analyses of IOP, the association between dietary nitrate intake from non-vegetable food sources goes from borderline significant to significant (Table S5).

**Table S4. Multivariable adjusted odds ratio (95% confidence interval) of open-angle glaucoma by nitrate intake**

|  |  |  |
| --- | --- | --- |
|  | ORa per 1 unit increase in nitrate intakec | P-value |
| Total dietary nitrate intake (per 10 mg/day) |  |  |
| Model 1a | 0.95 (0.91-0.98) | .002 |
| *Model 1b* | *0.96 (0.92-0.99)* | *.03* |
| Vegetable dietary nitrate intake (per 10 mg/day) |  |  |
| Model 1a | 0.95 (0.91-0.98) | .004 |
| *Model 1b* | *0.96 (0.92-0.99)* | *.03* |
| Nitrate intake from non-vegetable food sources (per 10 mg/day) |  |  |
| Model 1a | 0.63 (0.41-0.96) | .03 |
| *Model 1b* | *0.76 (0.49-1.17)* | *.22* |

a Model 1: adjusted for body mass index, total energy intake, diet quality, physical activity, and follow-up time. Analysis performed in participants matched on age and sex.

b Model 1: adjusted for total energy intake, diet quality, physical activity, and follow-up time. Analysis performed in participants matched on age, sex and BMI.

c Odds Ratios (95%CI) for open-angle glaucoma by total dietary nitrate intake, nitrate intake from vegetables, and nitrate intake from non-vegetable food sources (as continuous variables) analyzed using conditional logistic regression.

**Table S5. Multivariable adjusted beta (95% confidence interval) of intraocular pressure by nitrate intake**

|  |  |  |
| --- | --- | --- |
|  | Betaa per 1 unit increase in nitrate intakec | P-value |
| Total dietary nitrate intake (per 10 mg/day) |  |  |
| Model 1a | 0.02 (-0.02-0.06) | .35 |
| *Model 1b* | *0.00 (-0.06-0.05)* | *.89* |
| Vegetable dietary nitrate intake (per 10 mg/day) |  |  |
| Model 1a | 0.02 (-0.02-0.06) | .29 |
| *Model 1b* | *0.00 (-0.05-0.05)* | *.97* |
| Nitrate intake from non-vegetable food sources (per 10 mg/day) |  |  |
| Model 1a | -0.45 (-0.96-0.06) | .09 |
| *Model 1b* | *-0.67 (-1.30- -0.05)* | *.04* |

a Model 1: adjusted for body mass index, total energy intake, diet quality, physical activity, and follow-up time. Analysis performed in participants matched on age and sex.

b Model 1: adjusted for total energy intake, diet quality, physical activity, and follow-up time, Analysis performed in participants matched on age, sex and BMI.

c Betas (95%CI) for intraocular pressure by total dietary nitrate intake, nitrate intake from vegetables, and nitrate intake from non-vegetable food sources (as continuous variables) analyzed using linear regression.

By displaying these results, we hope to have shown that the associations described in the original manuscript are most likely true associations and explained by dietary nitrate intake rather than age, sex, or BMI.

Although an association between glaucoma and cardiovascular disease has been described in the literature [7-9], we believe this is more linked to their shared pathology, impaired auto regulatory capacity of glaucomatous eyes and arterial stiffness of CVD [7], or even genetics [10], than mediated by BMI.

Apart from the two additional supplementary figures, we have added some information regarding this comment in lines 301-304 of the manuscript.

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