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Association of dietary nitrate intake with the 15-year incidence of age-related macular degeneration

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Research Snapshot

Research Question: Is there an independent association between dietary nitrate intake (from vegetable and non-vegetable sources) and the 15-year incidence of age-related macular degeneration (AMD)?

Key Findings: In this prospective cohort study of 2037 participants (aged 49+ years at baseline), total nitrate intake and vegetable nitrate intake was significantly associated with incident early AMD, but not late AMD. Dietary intake of non-vegetable nitrate was not significantly associated with the 15-year incidence of early and late AMD. .

Abstract

Background: Dietary nitrate, found predominantly in green leafy vegetables and beetroot, is a precursor of nitric oxide. Under- or over-production of nitric oxide is implicated in the etiology of several eye diseases. However, the potential influence of dietary nitrate intake on age-related macular degeneration (AMD) risk has not been assessed.

Objective: To investigate the temporal association between dietary nitrate intake (from both vegetable and non-vegetable sources) and the 15-year incidence of AMD, independent of potential confounders.

Design: A longitudinal cohort study conducted from 1992-1994 to 2007-2009.

Participants/ Setting: The Blue Mountains Eye Study is a population-based study of adults aged 49+ at baseline, from a region west of Sydney, Australia. At baseline, 2856 participants with complete dietary data and AMD information were examined and of these, 2037 participants were re-examined 15 years later and thus included in incidence analysis.

Main Outcomes Measured: Incidence of AMD (main outcome) was assessed from retinal photographs. Dietary intake was assessed using a semi-quantitative food-frequency questionnaire. Nitrate intake from vegetables and non-vegetable sources were calculated by use of a validated comprehensive database.

Results: After adjusting for age, sex, smoking, energy-intake, fish consumption and AMD risk alleles (complement factor H and age-related maculopathy susceptibility-2 single nucleotide polymorphisms), participants in the third quartile compared to those in the first quartile (reference group) of total nitrate and total vegetable nitrate intake had reduced risk of incident early AMD: OR 0.61 (95% CI 0.41-0.90) and OR 0.65 (95% CI 0.44-0.96), respectively. Significant associations were not observed between the fourth versus first quartile of total nitrate and vegetable nitrate intake with incident early AMD: OR 0.74 (95% CI 0.51-1.08) and OR 0.69 (95% CI 0.47-1.00), respectively. Non-significant associations

were also observed with 15-year incidence of late AMD and total non-vegetable nitrate intake.

Conclusions: These novel findings could have important implications, if the association between total nitrate intake and vegetable nitrate intake and 15-year incidence of early AMD is confirmed in other observational or intervention studies.

Introduction

Age-related macular degeneration (AMD) is a leading cause of visual impairment and blindness.[1] Endothelial dysfunction in the choroidal vessels supplying affected ocular structures could play a key role in the development of AMD.[2] Nitric oxide (NO) plays a critical role in the maintenance of normal endothelial cell function and vascular tone; influencing blood pressure and blood flow.[3] Low bioavailability and/or bioactivity of NO results in endothelial dysfunction. One source of NO is the enterosalivary nitrate-nitrite-NO pathway. Nitrate and nitrite, precursors for NO, play a role in maintaining optimal endothelial function.[4] Vegetable-derived nitrate, predominantly found in beetroot and leafy green vegetables contributes to ~80% of total nitrate intake.[4] Although consumption of dark green leafy vegetables is protective against AMD[5] and there is a role for endothelial dysfunction and NO in AMD pathogenesis,[2,6] the influence of dietary nitrate intake on AMD risk has not been assessed. We therefore investigated the association between dietary nitrate intake (from vegetable and non-vegetable sources) and 15-year incidence of AMD in adults aged 49+ years.

Methods

Study population

The Blue Mountains Eye Study (BMES) is a population-based study of adults aged >49 years from Sydney, Australia. Study methods and procedures have been described elsewhere.[7] Baseline examinations of 3654 residents were conducted during 1992-4. Selection bias at baseline was minimized after multiple call-back visits, including door-knocking, telephone reminders and letters at recruitment. Surviving baseline participants were invited to attend examinations after 5- (1997-9), 10- (2002-4), and 15 years (2007-9) at which 2334 (75.1% of survivors), 1952 (75.6% of survivors) and 1149 (55.4% of survivors) participants were re-

examined, respectively. The University of Sydney and Western Sydney Area Human Ethics Committees approved the study, and written informed consent was obtained from participants.

Assessment of AMD

Detailed methodology of AMD ascertainment in this population has been previously reported.[7] We took two 30° stereoscopic color retinal photographs of the macula of both eyes, which were graded for presence of early and late AMD.[7] Early AMD was defined as the absence of late AMD and presence of either: 1) large (>125-μm diameter) indistinct soft or reticular drusen or 2) both large distinct soft drusen and retinal pigmentary abnormalities (hyperpigmentation or hypopigmentation) in either eye. Late AMD was defined as the presence of neovascular AMD or geographic atrophy in either eye.[7]

Dietary assessment

Dietary data were collected using a validated 145-item self-administered food frequency questionnaire (FFQ). Foods listed in the FFQ were categorized into major food categories and subcategories similar to those used for the 1995 Australian National Nutrition Survey.[8] A comprehensive database which has nitrate data for 178 vegetables was applied to assess nitrate levels for each vegetable.[9] Nitrate intake (mg/d) was then calculated by multiplying the amount of daily vegetable consumption (g/d) by the median nitrate content (mg/g) of that vegetable. Nitrate values from each individual vegetable were added up to get total daily nitrate values.

Nitrate intake from all other food items listed on the FFQ was determined. Databases applied to assess nitrate levels for each food item were from Inoue-Choi et al,[10] the Food Standards Australia New Zealand (FSANZ) survey of nitrate and nitrite in food and

beverages in Australia,[11] and Griesenbeck et al.[12] Nitrate intake was calculated by multiplying the daily consumption of each food item (g/d) by the assigned mean nitrate value (mg/g). Total nitrate intake (mg/d) was determined by calculating the sum of daily nitrate values from all other FFQ items together with vegetable items.

Assessment of covariates

Participants self-reported smoking status as: never smoked; past smoker; or current smoker. We extracted separate data on the frequency of consuming fish (e.g. salmon, tuna and sardines) and dietary intakes of lutein and zeaxanthin from the FFQ. Genotypic status was available for the complement factor H (*CFH*) single nucleotide polymorphism (SNP) *rs*1061170 in 2041 baseline participants who returned at BMES2 and for the age-related maculopathy susceptibility gene 2 (*ARMS2*) SNP *rs*10490924 in 1893 baseline participants who returned at BMES2.

Statistical analysis

Associations between energy-adjusted dietary nitrates and 15-year cumulative incidence of AMD were examined in discrete logistic regression models, using SAS software (v9.4, SAS Institute, Cary NC). Regression analysis adjusted for age, sex, current smoking, fish consumption and presence of *complement factor H* (*CFH*) and *age-related maculopathy susceptibility 2* (*ARMS2*) single nucleotide polymorphisms (SNPs), *rs*1061170 and *rs*10490924, respectively.

Results

Complete AMD and dietary data were available in 2856 baseline participants, of these 2037 participants were re-examined 15 years later and included in incidence analysis. Baseline

characteristics of participants are shown in Table 1. There were 15.3% and 4.1% incident early and late AMD cases, respectively. Participants in the third quartile compared to those in the first quartile of energy-adjusted total nitrate (p=0.01) and total vegetable nitrate intake (p=0.03) had reduced risk of incident early AMD: multivariable-adjusted OR 0.61 (95% CI 0.41-0.90) and OR 0.65 (95% CI 0.44-0.96), respectively (Table 2). Participants in the 2nd and 4th quartiles of energy-adjusted total nitrate and total vegetable nitrate intake had reduced odds of early AMD, but these associations were non-significant.

Discussion

Participants in the third versus first quartile of total nitrate intake and vegetable nitrate intake had 39% and 35% reduced risk of incident early AMD, respectively, after adjustment for AMD risk factors. Our data suggest a non-linear association or a possible threshold effect between nitrate intake and incident early AMD because no additional risk reduction was observed in the highest quartile of total nitrate intake (i.e. at ≥162 mg/day) or vegetable nitrate intake (i.e. at ≥142 mg/day). Another study also indicated a non-linear association between dietary nitrate and vascular disease mortality, with the largest benefits observed among those with moderate nitrate intakes which is consistent with our data.[13] Alternatively, the small number of incident AMD cases could have reduced power to detect a modest association at the highest intake quartiles. Moreover, we observed no significant associations with incidence of late AMD, again this is likely to be due to the small number of incident late AMD cases (~4%), compared to ~15% incidence of early AMD cases. Additionally, we observed no significant associations between non-vegetable nitrate intake and incidence of early or late AMD, this is not surprising given that there is no clear consensus on the impacts on health after consumption of non-vegetable sources of dietary nitrate.[13]

Inflammation, oxidative stress, and endothelial dysfunction are among the many factors that are hypothesized to influence the incidence and progression of AMD.[2,6] Given that nitrate intake, particularly from vegetable sources, was previously shown to improve endothelial function and minimize oxidative stress,[4] this is a potential pathway by which dietary nitrate intake could protect against early AMD. Further, dietary nitrates are shown to beneficially influence cardiovascular function,[4, 14] as AMD and cardiovascular disease share pathophysiologic pathways[15] this could potentially explain how nitrate intake prevents the development of early AMD.

Our study had several strengths. These included the prospective study design, use of a validated diet assessment tool, detailed information on AMD risk factors, as well as the validated method of establishing AMD lesions,[7] which ensures negligible misclassification of incident AMD. Hence, our findings are applicable to the general older Australian population and could also be applicable to older adults in other Western countries. Several potential limitations could be considered. First, increased nitrate intake could simply coincide with other lifestyle or dietary patterns that are associated with the health of the macula. Although we adjusted for several AMD risk factors as well as dietary factors (e.g. fish) in our analysis, residual or unmeasured confounders cannot be disregarded. Finally, a causal relationship of nitrate intake with incident early AMD cannot be established because of the observational nature of this study.

Conclusions

In summary, our data are unique as they represent findings from the first population-based study to demonstrate an association between dietary nitrate intake and incident early AMD but not late AMD. If our findings are confirmed, incorporating a range of foods that are rich

in dietary nitrate such as green leafy vegetables and beetroot could represent a simple strategy to enhance NO status, thereby potentially minimizing AMD risk.

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