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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

2

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

36   intake.

37   **Conclusions:** These novel findings could have important implications, if the association

38   between total nitrate intake and vegetable nitrate intake and 15-year incidence of early AMD

39   is confirmed in other observational or intervention studies.

40

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-

66 examined, respectively. The University of Sydney and Western Sydney Area Human Ethics

67 Committees approved the study, and written informed consent was obtained from

68 participants.

69

70 *Assessment of AMD*

71 Detailed methodology of AMD ascertainment in this population has been previously

72 reported.[7] We took two 30° stereoscopic color retinal photographs of the macula of both eyes,

73 which were graded for presence of early and late AMD.[7] Early AMD was defined as the

74 absence of late AMD and presence of either: 1) large (>125-µm diameter) indistinct soft or

75 reticular drusen or 2) both large distinct soft drusen and retinal pigmentary abnormalities

76 (hyperpigmentation or hypopigmentation) in either eye. Late AMD was defined as the

77 presence of neovascular AMD or geographic atrophy in either eye.[7]

78

79 *Dietary assessment*

80 Dietary data were collected using a validated 145-item self-administered food frequency

81 questionnaire (FFQ). Foods listed in the FFQ were categorized into major food categories and

82 subcategories similar to those used for the 1995 Australian National Nutrition Survey.[8] A

83 comprehensive database which has nitrate data for 178 vegetables was applied to assess

84 nitrate levels for each vegetable.[9] Nitrate intake (mg/d) was then calculated by multiplying

85 the amount of daily vegetable consumption (g/d) by the median nitrate content (mg/g) of that

86 vegetable. Nitrate values from each individual vegetable were added up to get total daily

87 nitrate values.

88　　　Nitrate intake from all other food items listed on the FFQ was determined. Databases

89 applied to assess nitrate levels for each food item were from Inoue-Choi et al,[10] the Food

90 Standards Australia New Zealand (FSANZ) survey of nitrate and nitrite in food and

91 beverages in Australia,[11] and Griesenbeck et al.[12] Nitrate intake was calculated by

92 multiplying the daily consumption of each food item (g/d) by the assigned mean nitrate value

93 (mg/g). Total nitrate intake (mg/d) was determined by calculating the sum of daily nitrate

94 values from all other FFQ items together with vegetable items.

95

96 *Assessment of covariates*

97 Participants self-reported smoking status as: never smoked; past smoker; or current smoker.

98 We extracted separate data on the frequency of consuming fish (e.g. salmon, tuna and

99 sardines) and dietary intakes of lutein and zeaxanthin from the FFQ. Genotypic status was

100 available for the complement factor H (*CFH*) single nucleotide polymorphism (SNP)

101 *rs*1061170 in 2041 baseline participants who returned at BMES2 and for the age-related

102 maculopathy susceptibility gene 2 (*ARMS2*) SNP *rs*10490924 in 1893 baseline participants

103 who returned at BMES2.

104

105 *Statistical analysis*

106 Associations between energy-adjusted dietary nitrates and 15-year cumulative incidence of

107 AMD were examined in discrete logistic regression models, using SAS software (v9.4, SAS

108 Institute, Cary NC). Regression analysis adjusted for age, sex, current smoking, fish

109 consumption and presence of *complement factor H* (*CFH*) and *age-related maculopathy*

110 *susceptibility 2* (*ARMS2*) single nucleotide polymorphisms (SNPs), *rs*1061170 and

111 *rs*10490924, respectively.

112

113 **Results**

114 Complete AMD and dietary data were available in 2856 baseline participants, of these 2037

115 participants were re-examined 15 years later and included in incidence analysis. Baseline

116   characteristics of participants are shown in Table 1. There were 15.3% and 4.1% incident

117   early and late AMD cases, respectively. Participants in the third quartile compared to those in

118   the first quartile of energy-adjusted total nitrate (p=0.01) and total vegetable nitrate intake

119   (p=0.03) had reduced risk of incident early AMD: multivariable-adjusted OR 0.61 (95% CI

120   0.41-0.90) and OR 0.65 (95% CI 0.44-0.96), respectively (Table 2). Participants in the 2nd

121   and 4th quartiles of energy-adjusted total nitrate and total vegetable nitrate intake had reduced

122   odds of early AMD, but these associations were non-significant.

123

124   **Discussion**

125   Participants in the third versus first quartile of total nitrate intake and vegetable nitrate intake

126   had 39% and 35% reduced risk of incident early AMD, respectively, after adjustment for

127   AMD risk factors. Our data suggest a non-linear association or a possible threshold effect

128   between nitrate intake and incident early AMD because no additional risk reduction was

129   observed in the highest quartile of total nitrate intake (i.e. at ≥162 mg/day) or vegetable

130   nitrate intake (i.e. at ≥142 mg/day). Another study also indicated a non-linear association

131   between dietary nitrate and vascular disease mortality, with the largest benefits observed

132   among those with moderate nitrate intakes which is consistent with our data.[13] Alternatively,

133   the small number of incident AMD cases could have reduced power to detect a modest

134   association at the highest intake quartiles. Moreover, we observed no significant associations

135   with incidence of late AMD, again this is likely to be due to the small number of incident late

136   AMD cases (~4%), compared to ~15% incidence of early AMD cases. Additionally, we

137   observed no significant associations between non-vegetable nitrate intake and incidence of

138   early or late AMD, this is not surprising given that there is no clear consensus on the impacts

139   on health after consumption of non-vegetable sources of dietary nitrate.[13]

140   Inflammation, oxidative stress, and endothelial dysfunction are among the many factors

141   that are hypothesized to influence the incidence and progression of AMD.[2,6] Given that

142   nitrate intake, particularly from vegetable sources, was previously shown to improve

143   endothelial function and minimize oxidative stress,[4] this is a potential pathway by which

144   dietary nitrate intake could protect against early AMD. Further, dietary nitrates are shown to

145   beneficially influence cardiovascular function,[4, 14] as AMD and cardiovascular disease share

146   pathophysiologic pathways[15] this could potentially explain how nitrate intake prevents the

147   development of early AMD.

148   Our study had several strengths. These included the prospective study design, use of a

149   validated diet assessment tool, detailed information on AMD risk factors, as well as the

150   validated method of establishing AMD lesions,[7] which ensures negligible misclassification of

151   incident AMD. Hence, our findings are applicable to the general older Australian population

152   and could also be applicable to older adults in other Western countries. Several potential

153   limitations could be considered. First, increased nitrate intake could simply coincide with

154   other lifestyle or dietary patterns that are associated with the health of the macula. Although

155   we adjusted for several AMD risk factors as well as dietary factors (e.g. fish) in our analysis,

156   residual or unmeasured confounders cannot be disregarded. Finally, a causal relationship of

157   nitrate intake with incident early AMD cannot be established because of the observational

158   nature of this study.

159

160   **Conclusions**

161   In summary, our data are unique as they represent findings from the first population-based

162   study to demonstrate an association between dietary nitrate intake and incident early AMD

163   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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