

12-1-2018

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

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[10.1016/j.jand.2018.07.012](https://ro.ecu.edu.au/ecuworkspost2013/5715)

This is an Author's Accepted Manuscript of: Gopinath, B., Liew, G., Kifley, A., Lewis, J. R., Bondonno, C., Joachim, N., ... Mitchell, P. (2018). Association of dietary nitrate intake with the 15-year incidence of age-related macular degeneration. *Journal of the Academy of Nutrition and Dietetics*, 118(12), 2311-2314. Available [here](#)

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1 **Research Snapshot**

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

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

9

10 **Abstract**

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

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

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

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

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

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

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

41 **Introduction**

42 Age-related macular degeneration (AMD) is a leading cause of visual impairment and
43 blindness.¹ Endothelial dysfunction in the choroidal vessels supplying affected ocular
44 structures could play a key role in the development of AMD.² Nitric oxide (NO) plays a
45 critical role in the maintenance of normal endothelial cell function and vascular tone;
46 influencing blood pressure and blood flow.³ Low bioavailability and/or bioactivity of NO
47 results in endothelial dysfunction. One source of NO is the enterosalivary nitrate-nitrite-NO
48 pathway. Nitrate and nitrite, precursors for NO, play a role in maintaining optimal endothelial
49 function.⁴ Vegetable-derived nitrate, predominantly found in beetroot and leafy green
50 vegetables contributes to ~80% of total nitrate intake.⁴ Although consumption of dark green
51 leafy vegetables is protective against AMD⁵ and there is a role for endothelial dysfunction
52 and NO in AMD pathogenesis,^{2,6} the influence of dietary nitrate intake on AMD risk has not
53 been assessed. We therefore investigated the association between dietary nitrate intake (from
54 vegetable and non-vegetable sources) and 15-year incidence of AMD in adults aged 49+
55 years.

56

57 **Methods**

58 *Study population*

59 The Blue Mountains Eye Study (BMES) is a population-based study of adults aged >49 years
60 from Sydney, Australia. Study methods and procedures have been described elsewhere.⁷
61 Baseline examinations of 3654 residents were conducted during 1992-4. Selection bias at
62 baseline was minimized after multiple call-back visits, including door-knocking, telephone
63 reminders and letters at recruitment. Surviving baseline participants were invited to attend
64 examinations after 5- (1997-9), 10- (2002-4), and 15 years (2007-9) at which 2334 (75.1% of
65 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 *rs1061170* in 2041 baseline participants who returned at BMES2 and for the age-related

102 maculopathy susceptibility gene 2 (*ARMS2*) SNP *rs10490924* 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), *rs1061170* and

111 *rs10490924*, 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 pathways¹⁵ 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

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

166

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