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Original article

Habitual dietary nitrate intake and cognition in the Australian Imaging, Biomarkers and Lifestyle Study of ageing: A prospective cohort study☆

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SUMMARY

Background # aims: Dietary nitrate improves cardiovascular health via a nitric oxide (NO) pathway. NO is key to both cardiovascular and brain health. There is also a strong association between vascular risk factors and brain health. Dietary nitrate intake could therefore be associated with better cognitive function and reduced risk of cognitive decline. This is yet to be investigated. The aim of this study was to investigate the association between habitual intake of dietary nitrate from sources where nitrate is naturally present, and cognitive function, and cognitive decline, in the presence or absence of the apolipoprotein E (*APOE*) ε4 allele.

Methods: The study included 1254 older adult participants of the Australian Imaging, Biomarkers and Lifestyle Study of Ageing who were cognitively normal at baseline. Plant-derived, vegetable-derived, animal derived nitrate (not including meat where nitrate is an allowed additive), and total nitrate intakes were calculated from baseline food frequency questionnaires using comprehensive nitrate databases. Cognition was assessed at baseline and every 18 months over a follow-up period of 126 months using a comprehensive neuropsychological test battery. Multivariable-adjusted linear mixed effect models were used to examine the association between baseline nitrate intake and cognition over the 126 months (median [IQR] follow-up time of 36 [18–72] months), stratified by *APOE* ε4 carrier status.

Results: In non *APOE* ε4 carriers, for every 60 mg/day higher intake of plant-derived nitrate at baseline there was an associated higher language score [β (95% CI): 0.10 (0.01, 0.19)] over 126 months, after

☆ Data described in the manuscript, code book, and analytic code will be made available upon request pending approval of an Expression of Interest submitted to the AIBL Study Scientific Management Committee.

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multivariable adjustments. In *APOE* ε4 carriers, there was an associated better episodic recall memory [0.24 (0.08, 0.41)] and recognition memory [0.15 (0.01, 0.30)] scores. Similar associations were seen for the intakes of vegetable-derived and total nitrate. Additionally, in *APOE* ε4 carriers, for every 6 mg/day higher intake of animal-derived nitrate (excluding meat with nitrate as an allowed additive) at baseline there was an associated higher executive function score [β (95% CI): 1.41 (0.42, 2.39)]. We did not find any evidence of an association between dietary nitrate intake and rate of cognitive decline.

Conclusion: Our results suggest that habitual intake of dietary nitrate from sources where nitrate is naturally present impacts cognitive performance in an *APOE* genotype contingent manner. Further work is needed to validate our findings and understand potential mechanisms underlying the observed effects.

1. Introduction

Cognitive function comprises multiple intellectual functions including memory, perception, attention, language, visuoconstruction, and executive functions [1]. Cognitive decline has a global prevalence of approximately 40% in older adults [2] and is the earliest sign of dementia [3]. The scale of the dementia public health challenge is immense; there are over 50 million people living with dementia and this number is expected to rise to 152 million by 2050 [4]. Alzheimer's disease (AD) is the most common type of dementia, accounting for around 70% of cases [5]. The paucity of effective treatments highlights the pressing need for evidence-based approaches to reduce future risk of dementia. Both genetic and modifiable risk factors contribute to risk of dementia [6]. Regarding genetic risk factors, the ε4 allele of the apolipoprotein E gene (*APOE*) is the most common genetic risk factor for AD and is strongly associated with earlier age of disease onset [7]. The presence of a single ε4 allele increases risk of AD by 2- to 3-fold, whereas with two ε4 alleles, AD risk is 10–15 times higher [8–10]. Regarding modifiable risk factors, in 2020, the *Lancet Commission* into '*Dementia prevention, intervention, and care*' estimated that 40% of dementia cases could be prevented or delayed by modifying 12 risk factors, five of which are directly impacted by diet [11]. Our own work has also shown that diet can impact cognitive performance and rates of cognitive decline in an *APOE* genotype contingent manner [12,13], demonstrating an interplay between modifiable and non-modifiable dementia risk factors. Hence, it is important to identify key components of optimal diets to reduce the risk of cognitive impairment and delay or ideally prevent dementia onset, and consideration should also be given to the impact of non-modifiable risk factors, such as *APOE* genotype, on any such relationship.

A potential key component is dietary nitrate. The main sources of dietary nitrate are vegetables (~70–80%), meat (~10–15%, nitrate is found naturally in meat but both nitrate and nitrite are also highly regulated preservatives in processed meat products with potential detrimental effects) and drinking water (~1–10%, a contaminant). Nitrate is an exogenous source of an important biomolecule, nitric oxide (NO) [14]. NO is a signalling molecule in the cardiovascular system [15], central nervous system [16], and cerebrovascular system [17]. NO maintains vasomotor tone [18], coronary vascular tone [19], and inhibits platelet aggregation [20], thereby regulating blood pressure [21] and inhibiting thrombosis [22]. In the central nervous system, NO is a 'neuronal messenger' that has a role in both synaptic signalling and non-synaptic signalling events [23–25]. Thus, NO is important for learning, memory, and neurogenesis [26–28]. In addition, neurovascular coupling is a phenomenon that connects local neural activity and relative changes in the cerebrovascular system which is essential to maintain normal brain homeostasis [29–31]. A scientific statement from the American Heart Association and American Stroke Association highlighted the importance of vascular contributions, particularly vascular risk factors, to cognitive impairment and dementia [32]. Diminished NO is evident in most cardiovascular diseases that may also potentially affect brain homeostasis and cognition [17].

Robust evidence from randomized clinical trials shows that endogenous NO levels can be improved by intake of nitrate-rich vegetables [33]. For example, intake of 180 mg nitrate from 200 g spinach doubled levels of a biomarker of NO status within 2 h of intake [34]. This increase in endogenous NO, following the intake of nitrate-rich vegetables, is associated with improvement in validated vascular health markers such as blood pressure and endothelial function [35]. This effect is blunted when the pathway by which nitrate is converted to NO is interrupted [36]. Observational studies with a follow-up period ranging from 14 years to 26 years have demonstrated that a higher intake of nitrate from vegetables is associated with decreased cardiovascular disease risk compared to those with a lower intake [21,37–39]. A few clinical trials have demonstrated beneficial effects of vegetable-derived nitrate on cognitive function and cerebral blood flow [40–43], but the results from clinical trials are not consistent [44]. Conversely, the health consequences of naturally occurring nitrate in animal products are largely unexplored. The association between intake of nitrate from the diet and cognition in longitudinal observational studies in the presence or absence of genetic risk factors for AD is yet to be investigated.

Plant foods usually provide more than 80% of nitrate in the diet, with vegetables providing by far the largest contribution [45]. Consequently, the primary aim of this study was to investigate the association between habitual intake of plant-derived nitrate, cognitive function, and cognitive decline, in the presence or absence of *APOE* ε4, using data from the Australian Imaging, Biomarkers and Lifestyle Study. Secondary aims were to investigate the association between (i) vegetable-derived nitrate intake and cognition, (ii) animal derived nitrate (excluding meat where nitrate is an allowed additive) and cognition, (iii) total dietary nitrate intake and cognition.

2. Methods

2.1. Study population

The Australian Imaging, Biomarkers and Lifestyle (AIBL) Study of Ageing is a longitudinal, multicentre study that recruits older adult volunteers who are cognitively normal, have mild cognitive impairment (MCI), or AD [46]. Participants (n = 1112) were initially recruited between 2006 and 2008 with additional participants (n = 1247) recruited since 2011 to enrich the cohort.

This report describes data from 1254 participants who at baseline were cognitively normal, as determined via a comprehensive battery of neuropsychological measures, aged 60 years or older, who had completed the food frequency questionnaire (FFQ)

(Supplementary Fig. 1). Further details regarding recruitment, assessment, inclusion, and exclusion criteria are described in Ellis et al. [47] and Fowler et al. [46]. Ethics approval for AIBL was granted by the institutional ethics committees of St Vincent's Hospital, Austin Health, Hollywood Private Hospital, and Edith Cowan University [47]. Written informed consent was obtained from individuals prior to study participation.

2.2. Exposures

At baseline, participants completed the Cancer Council of Victoria Food Frequency Questionnaire (CCVFFQ) to assess habitual food and nutrient intake [48]. Participants reported their usual intakes of different food and beverage items over the previous 12 months. The CCVFFQ has been validated in relation to 7-day weighed diet records [49,50]. Intake of dietary nitrate from different sources where nitrate is naturally present (plant-derived nitrate, vegetable-derived nitrate, and animal-derived nitrate) and is an allowed additive (processed meat) was assessed using the FFQ and quantified in grams/day (g/d).

2.2.1. Plant- and vegetable-derived nitrate intake

Nitrate values of vegetables and all plant-derived foods (vegetables plus fruits, cereals, herbs, spices, pulses, and nuts) were calculated using a comprehensive plant-based food reference nitrate database which includes nitrate values from 304 plant-based foods from 64 countries [51]. The nitrate content of plant foods varies according to country of cultivation; therefore, the following strategy was employed. For each vegetable, if three or more entries were available in the database for Australia, the median of these values was used. If there were less than three entries in the database for Australia, the median of values for all Oceania (Australia, New Zealand, and surrounding islands) was used. If there were less than three entries available for Oceania, the median of values for all countries in the database was used. The estimated quantity of the vegetables and plant-based foods consumed (g/d) was multiplied by the median nitrate value (mg/g) of each vegetable or plant-based food, respectively [52]. A 50% reduction in the assigned nitrate value was applied to the cooked vegetables and plant-based foods to account for the effect of cooking [53]. The total vegetable nitrate and plant-based nitrate consumed per day was calculated by summing the nitrate values of each individual vegetable and plant-derived food, respectively.

2.2.2. Animal-derived nitrate intake

Animal-derived nitrate intake was calculated from red meat, dairy, seafood, and poultry. Meat where nitrate is an allowed additive was not included in this calculation as it is linked with negative health effects [54]. For the current study, a recently published animal source food product nitrate reference database, with data from 51 countries, was used to calculate animal-derived nitrate intake [55]. The same strategy for assigning animal-derived nitrate values was used as described for plant-based foods and vegetables. As there is inadequate data on the impact of cooking on nitrate content of animal-based foods no reduction in value was applied to account for the effect of cooking. The total animal-derived nitrate consumed (mg/d) was determined by multiplying the amount of the specific animal-based food (red meat, poultry, seafood, and dairy) consumed (g/d) by its median nitrate content (mg/g).

2.2.3. Total nitrate intake

Total nitrate intake (mg/d) was determined by calculating the sum of nitrate values/intake from all food items included in the FFQ. Nitrate intake (mg/d) was ascertained by multiplying the amount of food item consumed (g/d) by the assigned median nitrate value (mg/g) for that food item. Food items were assigned the value of zero if the nitrate value for that food item was not available in any of the above listed databases.

2.3. Study outcomes

A comprehensive neuropsychological test battery assessing six cognitive domains [episodic recall memory, language, recognition memory, attention and processing speed, executive function, and the AIBL Preclinical Alzheimer Cognitive Composite (AIBL PACC), previously shown to reliably measure the first signs of cognitive decline in at-risk cognitively normal populations [56], was administered by trained staff according to standard protocols [46,57]. The battery was administered at baseline then at 18-month intervals up to 126 months. The raw scores of individual cognitive tests were converted to sample-based Z scores based on baseline values of the complete AIBL cognitively normal sample. The standardized scores of various cognitive tests were clustered to form each of the six cognitive composite domain scores listed above [57], with tests assigned to the cognitive domains based on clinical and theoretical consensus of a panel of neuropsychologists, neurologists, and psychologists. Supplementary Table 1 lists the cognitive tests used to calculate each of the six cognitive domain composite scores.

2.4. Covariates

All demographic data including age, sex, education level, marital status, smoking status, and alcohol intake were self-reported via questionnaire administered by study staff, habitual physical activity was self-reported using the international physical activity questionnaire, and body mass index (BMI) was calculated as weight in kilograms divided by height in metered squared (height and weight were measured by study personnel) [47]. Specific TaqMan® (Thermo Fisher Scientific, Waltham, MA, USA) assays were used to determine Apolipoprotein E (*APOE*) genotype (rs7412, assay ID: C____904,973_10; rs429358, assay ID: C___3,084,793_20) from the DNA extracted from fasted blood samples, per standard protocols [46].

2.5. Statistical analysis

Statistical analyses were performed using Stata version 15 (StataCorp, College Station, Texas 77,845, USA). Given the different underlying risk of dementia, data were stratified by *APOE* ε4 allele carrier status. An array of linear mixed effect models was performed using multiple imputation estimation and independent covariance pattern to evaluate the association between habitual nitrate intake at baseline and cognitive function at all time points (baseline, 18 months, 36 months, 54 months, 72 months, 90 months, 108 months, and 126 months) to estimate the slope (rate of change) in cognitive function longitudinally.

We had more than 5% of cases with missing data in all the outcome variables and in the following covariates: BMI, physical activity, smoking status, and alcohol status. We employed multiple imputation (MI) to impute the missing data to reduce non-response bias, improve precision, and power [58–60]. We ran logistic regression to investigate the missingness mechanisms to determine whether any of the covariate or auxiliary variables can predict probability of missingness in any of the variables as per the Rubin's framework [61]. The missing values in the outcome variables and the covariates, namely BMI, physical activity, smoking status, and alcohol intake status at baseline, were imputed by multivariate imputation by chained equations (MICE) with 20 imputations.

To investigate longitudinal associations, multivariable-adjusted linear mixed models were carried out to examine associations between baseline intake of habitual plant-derived, vegetable-derived, animal-derived nitrate, excluding meat where nitrate is an allowed additive, and total nitrate, and both cognitive function and cognitive decline over a period of 126 months. As meat with nitrate as an allowed additive contributes ~0.45% to total dietary nitrate intake we did not examine associations for this exposure discretely. Models of adjustment used were as follows: Model 1 [age, sex, time (baseline and every 18 month follow-up timepoint until 126 months)], Model 2 [age, sex, time, BMI, physical activity, smoking status (never/former/current), education level (≤12 years and >12 years), marital status, total energy intake] and Model 3 [all the covariates adjusted in Model 2 plus the dietary confounders such as intakes (g/d) of red meat, fish, saturated fatty acids, polysaturated fatty acids, monosaturated fatty acids, and alcohol]. When exposures were modelled as a continuous linear variable, we present the beta estimates per 60 mg higher intake, because this equates to approximately 1 cup of raw green leafy vegetables. We chose this value as it has a clear public health translation message and is also the median vegetable derived nitrate intake in this cohort. For animal derived nitrate, we present the beta estimates per 6 mg higher intake as it is the median intake of animal derived nitrate (excluding meat with nitrate as an allowed additive) in this cohort. In addition to the abovementioned models of adjustment, we examined if there was a significant interaction between time and exposure term by including an interaction term [time*independent variable (dietary nitrate)] in all the models. Where the interaction term was not statistically significant, it was removed from the model. All covariates were added as fixed factors, participants as a random factor, and cognitive composite score as the dependent variable.

3. Results

The 1254 study participants had a median [IQR] age of 70 [66–76] years at study entry, and a median [IQR] follow-up time of 36 [18–72] months with a maximum follow-up of 126 months. The median [IQR] intake of plant-derived nitrate was 77 [58–103] mg/d, vegetable-derived nitrate intake was 61 [44–82] mg/d, and animal-derived nitrate intake was 6 [3–9] mg/d (Table 1). Of total nitrate intake, vegetable-derived nitrate intake contributed 66%, fruit-derived nitrate 16%, whole-grain-derived nitrate 2%, animal-derived nitrate 7%, and meat where nitrate is an allowed additive 0.45%. The main contributors to total vegetable-derived nitrate intake were lettuce (29%), spinach (14%), potato (8%), celery (6%), and cabbage (5%). The primary contributors to animal-derived nitrate in this cohort were yogurt (54%), tinned fish (13%), lamb (10%), beef (6%), and grilled fish (4%).

3.1. Baseline characteristics

Compared to participants in the lowest tertile of plant-derived nitrate intake over the previous twelve months, those in the highest tertile were more likely to be female, be more physically active, have a lower BMI, be married, >12 years of education, consume higher amounts of fish, and were less likely to be carriers of the *APOE* ε4 allele (Table 1). Baseline characteristics stratified by *APOE* ε4 allele carrier status is presented in Supplementary Table 2.

3.2. Longitudinal analyses between nitrate intake and cognition

Over 126 months (baseline, 18, 36, 54, 72, 90, 108, and 126 months) of follow-up, cognitive function scores decreased in both *APOE* ε4 carriers and non-carriers. However, no significant interaction was observed between baseline plant-derived nitrate intake and time for any cognitive outcome indicating no differential cognitive decline between high and low plant-derived nitrate consumers.

In *APOE* ε4 non-carriers, for every 60 mg/d higher intake of plant-derived nitrate at baseline there was an associated higher language score [β (95% CI): 0.10 (0.01, 0.19)] over 126 months, after multivariable adjustments (Model 2; Table 2), and participants in the highest tertile of plant-derived nitrate intake had a higher language score [0.13 (0.01, 0.25)] than those in the lowest intake tertile (Model 2; Table 2). Baseline plant-derived nitrate intake was not associated with other cognitive function domains in *APOE* ε4 non-carriers. These associations did not appear to be modified by the addition of dietary confounders.

In *APOE* ε4 carriers, for every 60 mg/d higher intake of plant-derived nitrate at baseline there were associated higher episodic recall memory [0.24 (0.08, 0.41)] and recognition memory [0.15 (0.01, 0.30)] scores over 126 months, after multivariable adjustments (Model 2; Table 2). These associations remained after adjustment for potential dietary confounders, and participants in the highest intake tertile had a higher episodic memory score [0.24 (0.01, 0.33)] than those in the lowest intake tertile (Model 2; Table 2). There was no association between habitual baseline intake of plant-derived nitrate and AIBL PACC score, attention and processing speed, executive function, or language in *APOE* ε4 carriers.

As seen for plant-derived nitrate, similar associations were observed for the higher intakes of vegetable-derived nitrate and total nitrate compared to lower intakes, except, 1) the participants in the highest vegetable-derived intake tertile had a higher recognition memory score [0.22 (0.04, 0.41)] compared to those in the lowest intake tertile amongst *APOE* ε4 carriers, 2) the association between total nitrate intake and recognition memory was not present in *APOE* ε4 carriers, and 3) there was no significant difference in language scores between the highest and lowest tertiles of total nitrate intake in *APOE* ε4 non-carriers (Model 2; Supplementary Tables 3 and 4).

Additionally, in *APOE* ε4 carriers, for every 6 mg/d higher intake of animal-derived nitrate at baseline there were associated higher executive function score [1.41 (0.42, 2.39)] over 126 months, after multivariable adjustments (Model 2; Table 2). These associations remained after adjustment for potential dietary confounders, and participants in the highest intake tertile had a higher episodic memory score [0.22 (0.03, 0.42)] than those in the lowest intake tertile (Model 2; Supplementary Table 5). There was no association between habitual baseline intake of animal-derived nitrate and cognition in *APOE* ε4 non-carriers.

4. Discussion

In this prospective cohort study of 1254 cognitively normal older men and women followed for 126 months, longitudinal analyses stratified by *APOE* ε4 allele carrier status showed that habitual intake of plant-derived dietary nitrate at baseline was associated with better performance in the cognitive domain of 'language' in *APOE* ε4 non-carriers and with better performance in the cognitive domains of 'episodic recall memory' and 'recognition memory' in *APOE* ε4 carriers over 126 months. Similar associations were observed for vegetable-derived and total nitrate intakes. Furthermore, habitual intake of animal derived nitrate (excluding meat with nitrate as an allowed additive) at baseline was associated with better performance in the cognitive domain of 'executive function' in *APOE* ε4 carriers.

To our knowledge, no prospective observational study has previously investigated the longitudinal relationship of dietary nitrate intake from all dietary sources to cognitive function assessed at

Table 1
Baseline characteristics of study population.

	Total population n = 1254	Plant nitrate intake tertiles		
		T1 n = 418	T2 n = 418	T3 n = 418
Plant-nitrate intake (mg/day)	77 [58, 103]	51 [42, 58]	77 [71, 86]	115 [103, 132]
Vegetable-nitrate intake (mg/day)	61 [44, 82]	37 [30, 46]	61 [54, 68]	93 [81, 109]
Animal-nitrate intake (mg/day)	6 [3, 9]	4 [2, 8]	6 [3, 9]	8 [4, 10]
Processed-meat nitrate intake (mg/day)	0.32 [0.13, 0.59]	0.28 [0.12, 0.53]	0.35 [0.15, 0.62]	0.34 [0.14, 0.63]
Total nitrate intake (mg/day)	94 [73, 121]	65 [54, 73]	93 [84, 102]	132 [119, 153]
Sex (male) n (%)	41.6 (522)	39.7 (166)	41.8 (175)	43.3 (181)
Age (years)	70 [66, 75]	70 [66, 75]	70 [66, 75]	70 [65, 75]
BMI	25 [23, 28]	25 [23, 29]	26 [23, 29]	25 [23, 28]
MET Score	3600 [1920, 6558]	2958 [1414, 5478]	3541 [1913, 6534]	4208 [2515, 7475]
Education status				
≤12 years	43.4 (543)	43.5 (181)	46.0 (192)	40.6 (170)
>12 years	56.5 (708)	56.4 (235)	53.9 (225)	59.3 (248)
Marital status				
Single	4.4 (55)	4.3 (18)	2 (12)	6 (25)
Married	72.6 (909)	70.5 (294)	74 (311)	72.9 (304)
Divorced/separated	11.1 (140)	12.2 (51)	9.5 (40)	11.7 (49)
Widowed	11.7 (147)	12.9 (54)	12.9 (54)	9.3 (39)
Smoking status				
Never	60.1 (662)	58.7 (209)	62.1 (231)	59.6 (222)
Former	36.3 (400)	37.3 (133)	33.8 (126)	37.9 (141)
Current	3.4 (38)	3.9 (14)	4.0 (15)	2.4 (9)
***APOE* ε4 carrier status (Yes)**	27.5 (345)	29.1 (122)	27.7 (116)	25.6 (107)
Dietary characteristics				
Energy (kj/d)	6481 [5184, 8201]	5684 [4624,7103]	6439 [5306,8023]	7345 [6020,9299]
Total fish intake (g/day)	31 [17, 52]	23 [13, 38]	31 [18, 49]	43 [25, 67]
Red meat intake (g/day)	51 [28, 79]	44 [25, 70]	50 [29, 76]	59 [36, 99]
Processed-meat intake (g/day)	6 [2, 13]	5 [2, 11]	6 [2, 13]	6 [2, 13]
Dietary fibre intake (g/day)	20 [16, 26]	17 [13, 20]	21 [17, 25]	25 [20, 31]
Saturated FA (g/day)	24 [17, 32]	21 [16, 29]	23 [17, 31]	26 [19, 34]
Polyunsaturated FA (g/day)	9 [6, 13]	8 [5, 12]	9 [6, 13]	11 [7, 15]
Monosaturated FA (g/day)	22 [16, 28]	19 [14, 25]	21 [17, 27]	25 [19, 33]
Fruit intake (g/day)	292 [192, 413]	217 [116, 312]	298 [202, 397]	383 [262, 510]
Vegetable intake (g/day)	147 [110, 196]	98 [77, 131]	147 [121, 181]	205 [169, 246]
Alcohol intake (g/day)	101 [14, 244]	104 [16, 252]	94 [12, 240]	103 [16, 254]

Median [IQR], %(n). Abbreviations: *APOE*, Apolipoprotein E; BMI, body mass index; FA, fatty acids; IQR, interquartile range; MET, metabolic equivalent of task; g/day, grams per day; kj/day, kilojoules per day; n, number; mg/day, milligrams per day; %, percentage; T, tertile.

multiple time points. Whilst we observed no relationship of dietary nitrate intake to cognitive decline, a prospective study of 960 participants, >58 years, from the Rush Memory and Aging Project (U.S.), followed for 4.7 years, observed that ~1 serving of green leafy vegetables per day was associated with slower cognitive decline, equivalent to being 11 years younger in age. Following nutrient analysis, the authors observed that specific bioactive compounds including nitrate, lutein, folate, phylloquinone, and α-tocopherol were also associated with slower cognitive decline [62]. In contrast to our findings and those of the Rush Memory and Aging Project, a cross-sectional analysis of 989 participants, >50 years, from the InCHIANTI cohort (Italy), observed no association between urinary nitrate excretion or dietary nitrate intake with cognitive function [63]. Furthermore, a cross-sectional analysis from the National Health and Nutrition Examination Survey (NHANES; U.S.) database observed no association between urinary nitrate and cognitive function in 1015 adults, >60 years [64]. It is important to note however, that urinary nitrate is not a reliable biomarker of habitual dietary nitrate intake [65]. Furthermore, the InCHIANTI study, utilised an older database to calculate dietary nitrate intake (mg/d) from 3498 to 2134 individual foods and beverages but did not detail how the authors calculated nitrate from different dietary sources. The mean intake of nitrate reported in the InCHIANTI study was 89.0 mg/d [45]. In addition, the NHANES study did not calculate dietary nitrate intake, relying instead on urinary nitrate concentration, and the Rush Memory and Aging Project did not report how the nitrate value of the green leafy vegetables used in the analysis was calculated, nor levels of nitrate intake [62]. Other potential reasons for the disparate findings between our study and those described here include the heterogenous nature of the study populations (e.g., epidemiological versus targeted cohort recruitment), the differing cognitive assessments employed, and differences in the evaluation of dietary nitrate intakes across studies.

While clinical trials consistently demonstrate short-term beneficial effects of dietary nitrate on measures of cardiovascular function, the effect of nitrate intake on cognitive function in clinical trials is inconsistent. Seven out of twelve clinical trials (duration ranging from one day – thirteen weeks) have shown that intake of dietary nitrate (beetroot juice and high nitrate diet including beetroot juice, vegetables, and bananas) is associated with an improvement in general cognitive function (mainly reaction time) and/or cerebral blood flow [40–43]. However, findings from other clinical trials of similar duration and equivalent doses have shown no statistically significant associations between dietary nitrate and cognitive function [66–69]. Like the observational studies described earlier, the inconsistent results among clinical trials could be attributed to shorter duration of clinical trials. Therefore, studies with longer duration (~six months to 1 year) are recommended to observe improvement in cognition [70]. Our study is an important addition to the literature as we are examining the association between baseline habitual dietary intake and cognitive function assessed for up to 126 months, which is of relevance to public health.

This is the first study to examine the association of habitual intake of animal derived nitrate (excluding meat with nitrate as an allowed additive) with cognitive function. The observed association

Table 2
Longitudinal association between baseline plant-derived nitrate intake (mg/day) and domains of cognitive function.

	APOE ε4 carriers				*APOE* ε4 non-carriers			
	Coef for 60 mg	Plant-derived nitrate intake tertiles			Coef for 60 mg	Plant-derived nitrate intake tertiles		
	n = 345	T1 n = 115	T2 n = 115	T3 n = 115	n = 909	T1 n = 303	T2 n = 303	T3 n = 303
Intake (mg/d) *	76 (57, 101)	48 (38, 57)	76 (70, 85)	112 (101, 127)	78 (59, 104)	52 (43, 59)	78 (71, 87)	116 (104, 133)
Cognitive composite								
AIBL PACC								
Model 1	0.09 [-0.03, 0.23]	Ref	0.04 [-0.14, 0.22]	0.06 [-0.10, 0.24]	0.02 [-0.04,0.09]	Ref	0.04 [-0.05, 0.14]	0.02 [-0.07, 0.12]
Model 2	0.13 [-0.00, 0.26]	Ref	0.07 [-0.08, 0.28]	0.09 [-0.08, 0.28]	0.02 [-0.04,0.10]	Ref	0.05 [-0.04, 0.14]	0.03 [-0.06, 0.13]
Model 3	0.13 [-0.01, 0.28]	Ref	0.07 [-0.11, 0.25]	0.10 [-0.08, 0.30]	0.03 [-0.04,0.11]	Ref	0.03 [-0.06, 0.13]	0.03 [-0.06, 0.14]
Episodic Recall Memory								
Model 1	0.20 [0.04, 0.36]	Ref	0.07 [-0.14, 0.29]	0.19 [-0.01, 0.40]	0.02 [-0.05, 0.11]	Ref	0.02 [-0.09, 0.13]	0.01 [-0.10, 0.13]
Model 2	0.24 [0.08, 0.41]	Ref	0.11 [-0.10, 0.33]	0.24 [0.01, 0.46]	0.02 [-0.05, 0.11]	Ref	0.02 [-0.08, 0.13]	0.02 [-0.09, 0.14]
Model 3	0.24 [0.06, 0.42]	Ref	0.10 [-0.12, 0.32]	0.24 [0.01, 0.47]	0.01 [-0.07, 0.11]	Ref	0.01 [-0.10, 0.13]	0.00 [-0.12, 0.12]
Recognition Memory								
Model 1	0.12 [-0.00, 0.25]	Ref	0.05 [-0.13, 0.24]	0.11 [-0.06, 0.29]	−0.01 [-0.09, 0.06]	Ref	0.01 [-0.09, 0.12]	−0.02 [-0.12, 0.08]
Model 2	0.15 [0.01, 0.30]	Ref	0.09 [-0.09, 0.28]	0.14 [-0.04, 0.33]	−0.00 [-0.08, 0.08]	Ref	0.02 [-0.08, 0.13]	0.00 [-0.11, 0.11]
Model 3	0.17 [0.02, 0.33]	Ref	0.11 [-0.07, 0.30]	0.16 [-0.03, 0.35]	−0.01 [-0.09, 0.07]	Ref	0.00 [-0.10, 0.10]	−0.01 [-0.12, 0.10]
Language								
Model 1	0.12 [-0.03, 0.27]	Ref	0.00 [-0.20, 0.22]	0.07 [-0.13, 0.29]	0.10 [0.01, 0.19]	Ref	0.04 [-0.07, 0.16]	0.12 [0.00, 0.24]
Model 2	0.12 [-0.03, 0.29]	Ref	0.01 [-0.20, 0.22]	0.06 [-0.15, 0.29]	0.10 [0.01, 0.19]	Ref	0.04 [-0.07, 0.17]	0.13 [0.01, 0.25]
Model 3	0.12 [-0.05, 0.31]	Ref	0.01 [-0.20, 0.24]	0.06 [-0.17, 0.30]	0.14 [0.04, 0.24]	Ref	0.05 [-0.07, 0.18]	0.17 [0.03, 0.30]
Attention & Processing Speed								
Model 1	0.05 [-0.06, 0.18]	Ref	−0.00 [-0.18, 0.18]	0.04 [-0.13, 0.21]	−0.01 [-0.10, 0.06]	Ref	0.04 [-0.07, 0.15]	−0.02 [-0.14, 0.08]
Model 2	0.07 [-0.05, 0.20]	Ref	0.02 [-0.15, 0.19]	0.06 [-0.12, 0.24]	−0.01 [-0.10, 0.07]	Ref	0.05 [-0.05, 0.16]	- 0.01 [-0.13, 0.10]
Model 3	0.07 [-0.07, 0.23]	Ref	0.01 [-0.16, 0.20]	0.06 [-0.13, 0.26]	−0.01 [-0.11, 0.07]	Ref	0.02 [-0.09, 0.14]	- 0.02 [-0.14, 0.10]
Executive Function								
Model 1	0.09 [-0.04, 0.22]	Ref	0.05 [-0.12, 0.24]	0.06 [-0.11, 0.24]	0.03 [-0.05, 0.12]	Ref	0.05 [-0.06, 0.17]	0.04 [-0.07, 0.16]
Model 2	0.09 [-0.05, 0.23]	Ref	0.06 [-0.12, 0.25]	0.05 [-0.14, 0.25]	0.04 [-0.04, 0.13]	Ref	0.06 [-0.05, 0.18]	0.06 [-0.05, 0.19]
Model 3	0.10 [-0.05, 0.26]	Ref	0.05 [-0.14, 0.24]	0.06 [-0.14, 0.27]	0.04 [-0.05, 0.14]	Ref	0.04 [-0.07, 0.16]	0.06 [-0.06, 0.19]

Unstandardized coefficients and 95% Confidence intervals were obtained from linear mixed models with the exposure fitted as a continuous variable. The coefficients for exposure fitted as tertiles are reported for the median intake in each tertile (Q) relative to the median intake in Q1. Model 1 adjusted for age, time, sex; model 2 adjusted for all covariates in model 1 plus physical activity levels, level of education, body mass index, smoking status, energy intake, marital status; model 3 adjusted for all covariates in model 2 excluding energy intake, plus intake (g/d) of alcohol, red meat, fish, processed meat, saturated fatty acids, polyunsaturated fatty acids, and monounsaturated fatty acids. Abbreviations: AIBL PACC, Australian Imaging, Biomarkers and Lifestyle Study of Ageing Preclinical Alzheimer Cognitive Composite; *APOE*, Apolipoprotein E; n, number; T, tertile, mg/d; milligram per day, median (Inter quartile range).

with better performance in the cognitive domain of 'executive function' in *APOE* ε4 carriers was unexpected and should be explored in other populations. Compared to plant derived nitrate, intakes of animal derived nitrate in this cohort were low. Furthermore, unlike sources of plant derived nitrate which have a wide variation in nitrate content, the range of estimated nitrate content across the different animal derived sources is much narrower. Thus, caution should be taken in attributing the observed associations to nitrate as they may also be due to other co-occurring components within the whole food.

It is complex to determine the underlying mechanisms to preserve cognitive function with aging, and to fully elucidate the causes of cognitive decline. However, there is evidence that blood flow is crucial for maintaining normal brain function and cognition. NO has a beneficial role in the cardiovascular system and makes a non-vascular contribution as a key signalling molecule in neurological function [16]. The observed benefits of habitual nitrate intake on cognitive function may be via effects of NO. Nitrate and NO are closely linked through two metabolic pathways. Nitrate is a stable end-product of NO metabolism and is also recycled back into source NO through a non-enzymatic pathway; the nitrate-nitrite-NO pathway. Through the nitrate-nitrite-NO pathway, dietary nitrate is now a recognised endogenous source of NO, with benefits observed after intake of dietary nitrate, primarily from vegetables. These effects are blunted when the nitrate-nitrite-NO pathway is interrupted [71]. We hypothesize that the observed beneficial effects of habitual plant-derived nitrate on cognitive function are through effects of NO.

Our results suggest there are differential effects of nitrate on cognitive function that are likely to be contingent on *APOE* ε4 allele carriage. Notably, this is not the first study to report differential effects of dietary factors on aspects of cognitive function that are contingent on *APOE* ε4 allele carriage. Our previous work has shown the relationships between Mediterranean and western diet adherence and cognitive decline are dependent on *APOE* genotype [12], and habitual carbohydrate intake has been shown to impact performance across multiple cognitive domains in an *APOE* ε4-dependent manner [13].

ApoE protein, encoded by the *APOE* gene, is an important lipoprotein which is abundantly released in the brain and primarily responsible for cholesterol transportation [72]. Previous studies have found that the ε4 allele is associated with higher risk of cardiovascular disease and AD compared to ε2 and ε3 alleles [73]. Moreover, ApoE isoforms differentially control amyloid beta deposition and clearance [74]; increased levels of amyloid beta protein in the brain are a hallmark of AD. Nonetheless, it is important to note that our results show associations between habitual dietary nitrate intake and better cognitive performance in both *APOE* ε4 carriers and non-carriers but in different cognitive domains. In *APOE* ε4 carriers, intake of habitual dietary nitrate was associated with better episodic recall memory and recognition scores whereas in non-carriers, intake of habitual dietary nitrate was associated with better language scores. The mechanisms underlying the differential associations of dietary nitrate with cognitive domains in *APOE* ε4 carriers and non-carrier are yet to be investigated. The differential associations between baseline dietary nitrate intake and cognition in *APOE* ε4 carriers and non-carriers could be related to a difference in underlying AD risk. Nevertheless, our findings need to be validated in additional cohorts with longitudinal data.

There are several limitations to be considered while interpretating the results of the current study. We cannot infer causality or disregard potential residual or unmeasured confounding factors from our observational findings. As this study utilised baseline dietary intake data, diet may have changed over the follow-up period of 126 months. However, any such misclassification would most likely have weakened the power to detect an association. The dietary data was self-reported, thus there is a possibility of recall bias. Therefore, dietary data are estimates rather than accurate measurements. We were unable to investigate the impact of antibacterial mouthwash and proton-pump inhibitors on our findings; factors which are known to inhibit the nitrate-nitrite-NO pathway [75,76]. Additionally, given the number of statistical tests, we only focus on clear and consistent associations. Lastly, the study cohort is comprised of mostly highly educated Caucasians, which limits the generalisability of our findings.

There are, however, several aspects of our study which provide confidence in our findings. We have utilised a well-characterised cohort, thereby increasing the internal validity of our results. We have taken a conservative approach by controlling for a wide range of demographic variables and potential dietary confounders. The dietary data were collected using an instrument previously validated in earlier epidemiological studies [50]. Moreover, the latest comprehensive nitrate databases were used to calculate dietary nitrate intake from different sources [52,55]. Finally, the mean nitrate intake in the current study is consistent with that observed in other cohort studies investigating associations of dietary nitrate and cardiovascular risk factors [21,22,37,38].

5. Conclusion

In this study, we observed an association between plant-derived nitrate intakes and cognitive performance in both *APOE* ε4 carriers and non-carriers. We found higher dietary nitrate intake to be associated with better episodic recall memory and recognition memory in *APOE* ε4 carriers and better language performance in non-ε4 carriers over 126 months. No effect on rates of decline across cognitive domains was observed. Further work is needed to understand the mechanisms underlying our results, and whether dietary intake of plant-derived nitrate could be incorporated into strategies aimed at preventing dementia.

Authors' contribution

AR, NPB, and CPB designed research, conducted research, analysed data, interpreted results and wrote the paper; SRRS, JMH, and SLG were involved in the study design, data analyses, interpreted results; AR, NPB, and KM were involved in the statistical analyses; LZ, CPB and LCB: were involved in nitrate calculation; DA, PM, and RNM were involved in study design and data collection, and all authors: edited and approved the final manuscript.

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Disclaimers

None.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.05.022.

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