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Original Research Article

Dietary nitrate intake in relation to the risk of dementia and imaging markers of vascular brain health: a population-based study

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A B S T R A C T

Background: Nitric oxide is a free radical that can be produced from dietary nitrate and positively affects cardiovascular health. With cardiovascular health playing an important role in the etiology of dementia, we hypothesized a link between dietary nitrate intake and the risk of dementia.

Objectives: This study aimed to find the association of total, vegetable, and nonvegetable dietary nitrate intake with the risk of dementia and imaging markers of vascular brain health, such as total brain volume, global cerebral perfusion, white matter hyperintensity volume, microbleeds, and lacunar infarcts.

Methods: Between 1990 and 2009, dietary intake was assessed using food-frequency questionnaires in 9543 dementia-free participants (mean age, 64 y; 58% female) from the prospective population-based Rotterdam Study. Participants were followed up for incidence dementia until January 2020. We used Cox models to determine the association between dietary nitrate intake and incident dementia. Using linear mixed models and logistic regression models, we assessed the association of dietary nitrate intake with changes in imaging markers across 3 consecutive examination rounds (mean interval between images 4.6 y).

Results: Participants median dietary nitrate consumption was 85 mg/d (interquartile range, 55 mg/d), derived on average for 81% from vegetable sources. During a mean follow-up of 14.5 y, 1472 participants developed dementia. A higher intake of total and vegetable dietary nitrate was associated with a lower risk of dementia per 50-mg/d increase [hazard ratio (HR): 0.92; 95% confidence interval (CI): 0.87, 0.98; and HR: 0.92; 95% CI: 0.86, 0.97, respectively] but not with changes in neuroimaging markers. No association between nonvegetable dietary nitrate intake and the risk of dementia (HR: 1.15; 95% CI: 0.64, 2.07) or changes in neuroimaging markers were observed.

Conclusions: A higher dietary nitrate intake from vegetable sources was associated with a lower risk of dementia. We found no evidence that this association was driven by vascular brain health.

Keywords: nitrate, nitric oxide, dementia, cerebral perfusion, vascular brain disease

Introduction

Nitric oxide is a free radical that regulates vasodilation, has an inhibitory effect on platelet aggregation, and, thereby, improves blood circulation [1,2]. Nitric oxide can be produced endogenously by nitric oxide synthases through the oxidation of L-arginine [3,4]. Alternatively, dietary nitrate can be metabolized into nitric oxide through the nitrate-nitrite-nitric oxide pathway [5]. In particular, dietary nitrate from vegetable sources may convert easily to nitric oxide owing to the enhancing effects of accompanying bioactive compounds such as vitamin C and polyphenols [6,7].

Given these effects of nitric oxide on the vasculature, a higher dietary nitrate intake has been linked to a lower risk of various cardiovascular adverse health outcomes, such as hypertension and coronary artery disease [8–10]. With cardiovascular disease now established in the multifactorial etiology of dementia, a link between dietary nitrate intake and risk of dementia has been hypothesized, but direct evidence remains scarce [11,12].

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Previous randomized controlled trials have investigated the effect of nitrate or nitrite supplementation on cerebral blood perfusion and cognitive performance but yielded inconsistent results [13–20]. A recent meta-analysis concluded that this may be explained by the small sample sizes and short intervention timeframes of these trials [12]. Large and long-term experiments have not yet been conducted owing to challenges such as high attrition rates and high costs. This highlights the need to verify the link between nitrate intake and the risk of dementia using large observational cohort studies with long follow-up periods.

Therefore, we studied the association of total, vegetable, and non-vegetable dietary nitrate intake with the risk of dementia within a large prospective population-based cohort study. We further explored the association between dietary nitrate intake and imaging markers of vascular brain health, such as total brain volume, cerebral perfusion, white matter hyperintensity volume, microbleeds, and lacunar infarcts.

Methods

Study population

This study was embedded within the prospective population-based Rotterdam Study, details of which have been described elsewhere [21]. In briefly, the initial study was established in 1990 with 7983 participants aged 55 y or older, who were living in the district of Ommoord in Rotterdam, the Netherlands. In 2000, the study was enlarged with a second cohort including 3011 participants who turned 55 y or moved into the study area and, again, in the year 2005 with a third cohort comprising 3932 persons aged 45 y or older. Every 3 to 6 y, all participants are invited to undergo extensive follow-up examinations.

The Rotterdam Study has been approved by the Medical Ethics Committee of Erasmus Medical Center and by the board of the Netherlands Ministry of Health, Welfare, and Sports. Written informed consent was obtained from all participants.

At study entry of the 3 cohorts, dietary data were collected for 9737 participants. From this sample, we excluded participants who reported an unreliable energy intake of <500 or >5000 kcal/d ($n = 38$), participants who had prevalent dementia ($n = 38$), were insufficiently screened for dementia ($n = 58$), or did not sign the informed consent form to link the study database to medical records ($n = 60$). This resulted in a sample comprising 9543 participants who were eligible to be followed up for incident dementia (dementia sample) (Supplemental Figure 1A). From 2005 onward, magnetic resonance imaging (MRI) of the brain was implemented in the core protocol of the Rotterdam Study. Between 2006 and 2012, corresponding to the fifth examination round of the first cohort, the third examination round of the second cohort, and study entry of the third cohort, dietary data were collected for 5425 dementia-free participants, after which 4228 participants underwent ≥1 MRI scan. We excluded participants with insufficient segmentations ($n = 162$) and prevalent cortical infarcts ($n = 117$), resulting in an additional sample of 3949 participants (brain imaging sample) (Supplemental Figure 1B).

Dietary assessment

Dietary intake was assessed for all 3 subcohorts at study entry, which constituted the baseline for the dementia sample. For the first and second subcohorts, dietary intake was again assessed between 2009 and 2012, which constituted together with the study entry of the third subcohort as baseline for the brain imaging sample. The approach used to quantify dietary intake at study entry for the first and second cohort was slightly different compared with the method used at the other dietary assessment visits (Supplemental Figure 1). At study entry for the first and second cohort, participants first completed a self-administered food-frequency questionnaire (FFQ) including 170 food items, followed by a structured interview with a trained dietician to specify food items and preparation methods and to identify in which frequencies and amounts the food items were consumed. At the other dietary assessment visits, participants completed a self-administered semiquantitative 389-item FFQ. The FFQ includes questions on main food items, its subtypes, preparation methods, cooking fats, and condiments. Overall, the questionnaire is well-structured according to meals, with questions on frequency and portion sizes in household units as part of the food item instead of as a separate list. These FFQs have been validated against other dietary assessment methods, which showed that both FFQs can adequately rank individuals according to their intake of several nutrients [22–24]. Unfortunately, nitrate intake was not evaluated in these validation studies.

Dietary nitrate intake

In this study, all foods derived from plants, such as potatoes, but excluding fruits, nuts, and cereals, were defined as vegetables. Dietary nitrate content of vegetables obtained from the FFQs was calculated using a comprehensive vegetable nitrate database, which was developed by means of a systematic review including studies measuring nitrate concentrations in vegetables between 1980 and 2016 [25]. Measurements were mostly chemical analyzes performed in accredited laboratories. The final database summarizes data from 255 studies, with information on 178 vegetables. From these studies, 12 were conducted in the Netherlands, covering 557 entries from 26 different vegetables. Overall, dietary nitrate concentrations in vegetables vary substantially across and within countries, for instance, because of differences in agricultural practices, soil composition, farming methods, and fertilizer use [26]. Given that one entry per vegetable in the Netherlands presumably does not provide a reasonable proxy for the dietary nitrate content, we considered the median nitrate concentration of each vegetable that was measured in the Netherlands and surrounding countries. The median was taken as the distribution of the obtained dietary nitrate concentrations for each vegetable was quite skewed. For 30% of the vegetables from which 16% of the total calculated dietary nitrate was derived, <3 entries were available for the Netherlands and surrounding counties. For these vegetables, the median nitrate concentration of all European countries was used. For 12% of the vegetables from which 0.5% of the total calculated dietary nitrate was derived, <3 entries were available for all European countries. For these vegetables, the median nitrate concentration of all countries was used. The database included season-specific estimates because nitrate in vegetables varies between seasons [26]. However, given that we are interested in habitual nitrate intake, and we have previously shown that vegetable consumption across different seasons remains constant [27], mean nitrate concentrations throughout the year were used. Moreover, during the preparation processes boiling or cooking, ~50% of the nitrate content in vegetables will be lost [28]. Therefore, we divided the obtained nitrate concentration of the corresponding vegetable by 2 if such preparation method was used. This approach has been validated against urinary nitrate excretion samples in individuals from Australia, which showed that FFQs can adequately rank individuals according to their dietary nitrate intake [25]. The dietary nitrate content of non-vegetable food items obtained from the FFQs was determined based on a comprehensive database, which was developed by means of a systematic review including 26 studies that have used chemical analyses to measure nitrate concentrations of foods between 1967 and 2008 [29].

Data from Western countries only was used as dietary nitrate concentrations in nonvegetable foods differ substantially between high and middle income counties, but less variation is seen across high-income countries [30].

Follow-up for dementia

Participants were screened for dementia at baseline and every 3 to 6 y during follow-up examinations using the mini-mental state examination (MMSE) and the geriatric mental schedule organic level. Those with a MMSE score of <26 or a geriatric mental schedule organic level score of >0 were further examined using the Cambridge Examination for Mental Disorders in the Elderly diagnostic interview. Participants were also monitored for dementia on a continuous basis through an electronic link between the study database and medical records from general practitioners and the Regional Institute of Outpatients Mental health Care. The final diagnosis was established by a consensus panel led by a neurologist, according to standard criteria for dementia (DSM-III-R) and for subdiagnosis of Alzheimer disease (NINCDS-ADRDA). Follow-up was virtually completed until January 1, 2020, for 95.9% of the potential person-years.

Acquisition and postprocessing of brain MRI

MRI scanning of the brain was performed on a 1.5-Tesla MRI scanner (GE Signa Excite; General Electric Healthcare) according to standardized protocols [31] to determine total brain volume, global cerebral perfusion, white matter hyperintensity volume, microbleeds, and lacunar infarcts. There were no hardware or software updates over the study period. The scan protocol, sequence details, and processing of the data have been described in detail previously [31].

Covariables

Energy and alcohol intake were calculated from the obtained dietary data based on the Dutch Nutrient Database tables. Using structured interviews, data on educational attainment and smoking status were obtained. Education attainment was classified into 4 levels: primary, lower, intermediate, and higher. Smoking status was categorized as never, current, or former. Physical activity was measured using a validated adapted version of the Zutphen Physical Activity Questionnaire at study entry for the first and second cohort and the LASA Physical Activity Questionnaire at the other visits. A diet quality score reflecting adherence to the Dutch Dietary Guidelines was calculated by adding adherence scores for 14 food recommendations, as described in detail elsewhere [32]. A plant-based dietary index was derived from the dietary intake data as described in detail elsewhere [33]. A higher index reflects relatively higher consumption of plant-based and lower consumption of animal-based foods. Height and weight were measured at the research center and body mass index was calculated. *APOE* genotype was obtained using polymerase chain reaction of coded DNA samples for the first cohort and with bi-allelic TaqMan assay for the second and third cohorts.

Statistical analysis

Descriptive statistics are presented as mean (standard deviation) for normally distributed continuous variables, median (interquartile range) for nonnormally distrusted continuous variables and number (percentage) for categorical variables. The interquartile range is provided as a measure of spread, that is, the difference between the upper quartile and lower quartile [34].

Because the distribution of dietary nitrate intake was right-skewed, we performed a natural-log transformation to obtain a roughly normal distribution. Moreover, to consider energy intake, we applied the nutrient residual method to calculate energy-adjusted nitrate intake. However, given that both the natural-log transformation and nutrient residual method did not affect our results, effect estimates based on crude dietary nitrate intake variables are provided in the manuscript for interpretation purposes.

We determined the association between dietary nitrate intake at baseline and the risk of dementia and Alzheimer disease using Cox proportional hazard models. We verified that the proportional hazard assumption was met based on Schoenfeld residuals. Dietary nitrate intake was included in the model per 50-mg/d increase, equivalent to ~30 g of nitrate-rich vegetables and ~250 g of nitrate poor vegetables [25] and per quartile. To further explore potential nonlinear associations, we included dietary nitrate intake as a quadratic term in the models and tested whether this improved the fit of the models using ANOVA. All analyses were adjusted for age, sex, and energy intake (model I) and, additionally for educational attainment, diet quality, alcohol intake, physical activity, smoking status, body mass index, and *APOE* ε4 status (model II). To assess potential effect modification, we added an interaction term between dietary nitrate intake and sex, *APOE* ε4 status, dietary intake of vitamin C, and body mass index (as adiposity is associated with impaired nitric oxide availability [35]). Moreover, to evaluate the robustness of our findings, we performed several sensitivity analyses. First, to evaluate whether reverse causality accounted for potential associations, we repeated the analysis after excluding the first 5 y of follow-up. Second, given that for the first and second subcohorts, a different FFQ has been used to assess dietary nitrate intake than for the third subcohort, we repeated the analyses in the first and second subcohorts only. Third, to enhance comparability between the dementia risk results and brain imaging results, we also repeated the analyses within the brain imaging sample. Fourth, we repeated the analyses while additionally correcting for vegetable intake (grams per day) and after replacing the overall diet quality score in the models by a plant-based dietary index. Finally, we repeated the analyses for dietary nitrate intake derived from diverse vegetable groups separately.

We determined the association of dietary nitrate intake at baseline with total brain volume, cerebral perfusion, and white matter hyperintensity over time, using linear mixed models with a random intercept and slope. The natural logarithm of white matter hyperintensity volume was taken to reach an approximately normal distribution. Time in years between the consecutive MRI scans was used as underlying time scale. We included dietary nitrate intake in the model as fixed effect (main effect), representing overall differences in trajectories of imaging markers during follow-up. In addition, we included an interaction term between dietary nitrate intake and time (slope effect), allowing imaging markers to change differentially over time for different dietary nitrate intake levels. To correct for confounders, all aforementioned covariables were included in the models and intracranial volume as proxy of head size was additionally added. We further included an interaction term between age and time because changes in MRI markers are exponentially related to age [36]. To study the association between dietary nitrate intake with the prevalence and incidence of any microbleeds and lacunar infarcts, we used logistic regression models. All aforementioned covariables were included in the models and in the models assessing incidence, time between dietary nitrate intake assessment, and the MRI scan on which the incidence was detected was added.

Missing data on covariables were imputed using 5-fold multiple imputation. In the dementia sample, 19% of the physical activity data,

5% of the *APOE* ε4 status data, and <1% of all other covariables was missing. In the brain imaging sample, 6.5% of the *APOE* ε4 status data, 4.8% of the physical activity data, and <1% of all other covariables were missing. The distribution of covariables in the imputed data set was similar as in the nonimputed data set. All statistical analyses were conducted using R Statistical Software version 4.0.3.

Results

Baseline characteristics of the total study population are provided in Table 1 and per quartile of dietary nitrate intake in Supplemental Table 1. The mean age of the participants in the dementia sample was 64.1 y (standard deviation: 8.6 y), and 58% were female. Participants median dietary nitrate consumption was 85 mg/d (interquartile range, 55 mg/d), derived on average for 81% from vegetable sources. Total dietary nitrate and dietary nitrate from vegetable sources were positively correlated with total vegetable intake (r = 0.63 and 0.64, respectively). Overall, dietary nitrate was mainly derived from the category other vegetables, followed by dark green vegetables, starchy vegetables, red and orange vegetables, and beans and peas (Supplemental Table 2).

During a mean follow-up of 14.5 y, 1472 participants developed dementia (incidence rate 10.6 per 1000 person-years), of whom 1078 presented with Alzheimer disease. A higher intake of total and vegetable dietary nitrate intake was associated with a lower risk of dementia per 50-mg/d increase [hazard ratio (HR): 0.92; 95% confidence interval (CI): 0.87, 0.98; and HR: 0.92; 95% CI: 0.86, 0.97, respectively] (Table 2). No association between nonvegetable dietary nitrate intake and the risk of dementia was observed (HR: 1.15; 95% CI: 0.64, 2.07). Analyzing dietary nitrate intake into quartiles showed no indication for nonlinearity, neither did adding a quadratic term in the models (P > 0.4). No evidence for effect modification by sex, *APOE* ε4 status, dietary intake of vitamin C, or body mass index was found (P-interaction > 0.45). Furthermore, effect estimates were similar for Alzheimer disease, after excluding the first 5 y of follow-up, when restricting the analyses to the first and second subcohorts, and when repeating the analyses in the brain imaging sample (Supplemental Figure 2). Moreover, additional adjustment for vegetable consumption or replacing the diet quality score by a plant-based dietary index did not affect our results. In addition, effect estimates were somewhat stronger for dietary nitrate intake derived from dark green vegetables and starchy vegetables than for other vegetables (Supplemental Figure 3).

Participants in the brain imaging sample were on average aged 65.2 y (standard deviation: 10.8 y) and 57% were women (Table 1). Baseline characteristics per quartile of dietary nitrate intake are provided in Supplemental Table 3. Among the 3949 participants, 2433 participants underwent a second and 1107 participants a third MRI scan. The mean interval between scans was 4.6 y. Participants with second and third MRI scans were on average younger compared with those without an additional MRI scan, whereas other baseline characteristics were similar (Supplemental Table 4). Dietary nitrate intake was not associated with cerebral brain perfusion or white matter hyperintensity volume over time (Table 3). A higher nonvegetable, but not total or vegetable, dietary nitrate intake was associated with a higher total brain volume (mean difference in z-score of main effect per 50-mg/d increase: 0.11; 95% CI: 0.03, 0.20) but not with changes in total brain volume over time (mean difference in z-score of slope: 0.01; 95% CI: 0.00, 0.01). No association between dietary nitrate intake and either prevalent or incident microbleeds and lacunar infarcts was observed (Table 4).

TABLE 1
Baseline characteristics of the study population

Characteristics	Dementia sample (n = 9543)	Brain imaging sample (n = 3949)
Age (y)	64.1 (8.6)	65.2 (10.8)
Female sex	5530 (58)	2264 (57)
Energy intake (kcal/d)	2099 (595)	2144 (656)
Education attainment		
Primary	1463 (15)	328 (8)
Lower	3900 (41)	1473 (38)
Intermediate	2662 (28)	1178 (30)
Higher	1463 (15)	927 (24)
Diet quality score	6.7 (1.9)	6.9 (1.9)
Alcohol intake (g/d)	5 [17]	7 [17]
Physical activity (MET h/wk)	69 [63]	42 [65]
Smoking status		
Never	2231 (23)	701 (18)
Former	4152 (44)	1974 (50)
Current	3114 (33)	1268 (32)
Body mass index (kg/m^2)	26.8 (4.0)	27.3 (4.1)
APOE ε4 status		
No allele	6482 (72)	2656 (72)
1 allele	2353 (26)	957 (26)
2 allele	205 (2)	81 (2)
Total dietary nitrate intake (mg/d)	85 [55]	95 [75]
Vegetable dietary nitrate intake (mg/d)	70 [53]	78 [71]
Nonvegetable dietary nitrate intake (mg/d)	6 [7]	16 [10]
Total brain volume (mL)	—	934.5 (99.8)
Cerebral perfusion (mL)	—	524.7 (102.4)
White matter hyperintensity volume (mL)	—	3.2 [2.01]
Any microbleeds	—	808 (20)
Any lacunar infarcts	—	293 (7)

Data are shown for nonimputed data and are presented as mean (standard deviation) for normally distributed continuous variables, medium [interquartile range] for nonnormally distributed continuous variables, and number (percentages) for categorical variables.
MET, metabolic equivalent of task; N, number of participants.

Discussion

In this population-based cohort study, higher dietary nitrate intake from vegetable sources was associated with a lower risk of dementia, whereas no association was found for dietary nitrate intake from nonvegetable sources. Dietary nitrate intake was also not associated with cerebral perfusion or vascular pathology (i.e., white matter hyperintensity volume, microbleeds, and lacunar infarcts), but a higher dietary nitrate intake from nonvegetable sources was associated with a higher total brain tissue volume.

Although it has previously been hypothesized that dietary nitrate affects brain health [11], the direct link between dietary nitrate intake and the risk of dementia has not been studied before. However, few randomized controlled trials have studied the effect of nitrate or nitrite supplementation on cognitive performance. Some studies found an improvement in certain cognitive test performances after supplementation [13–15], but most studies found no effect [16–20]. These null findings may be explained by the relatively small sample sizes (i.e., maximum of 62 participants) and short study periods (i.e., <7 d in most studies). In particular, the hypothesis that nitrate becomes effective after long-term consumption seems likely because, particularly, the trail with the longest supplementation duration of 10

TABLE 2
Dietary nitrate intake and the risk of dementia

	n/N	Model I	Model II
Total dietary nitrate intake			
Per 50-mg/d increase	1472/9543	0.91 (0.86, 0.96)	0.92 (0.87, 0.98)
Per quartile			
Quartile $1_{(1.9–64.3\ mg/d)}$	434/2386	Reference	Reference
Quartile $2_{(64.3–85.4\ mg/d)}$	449/2386	0.98 (0.86, 1.12)	0.99 (0.87, 1.13)
Quartile $3_{(85.4–119.0\ mg/d)}$	383/2386	0.93 (0.80, 1.07)	0.93 (0.81, 1.08)
Quartile $4_{(119.1–1063.5\ mg/d)}$	206/2385	0.84 (071, 1.00)	0.88 (0.74, 1.05)
Vegetable dietary nitrate intake			
Per 50 mg/d increase	1472/9543	0.90 (0.85, 0.96)	0.92 (0.86, 0.97)
Per quartile			
Quartile $1_{(0.0–50.1\ mg/d)}$	443/2386	Reference	Reference
Quartile $2_{(50.1–70.4\ mg/d)}$	436/2386	0.95 (0.83, 1.09)	0.96 (0.84, 1.11)
Quartile $3_{(70.4–103.0\ mg/d)}$	381/2386	0.89 (0.77, 1.02)	0.91 (0.79, 1.05)
Quartile $4_{(103.1–1052.0\ mg/d)}$	212/2385	0.83 (0.70, 0.98)	0.87 (0.73, 1.03)
Nonvegetable dietary nitrate intake			
Per 50-mg/d increase	1472/9543	1.17 (0.66, 2.07)	1.15 (0.64, 2.07)
Per quartile			
Quartile $1_{(1.24–11.4\ mg/d)}$	384/2386	Reference	Reference
Quartile $2_{(11.4–14.3\ mg/d)}$	419/2386	1.03 (0.90, 1.19)	1.05 (0.91, 1.21)
Quartile $3_{(14.3–18.1\ mg/d)}$	393/2386	1.15 (0.99, 1.33)	1.13 (0.97, 1.32)
Quartile $4_{(18.1–82.0\ mg/d)}$	276/2385	1.06 (0.89, 1.25)	1.07 (0.90, 1.27)

Cox proportional hazards models were used to obtain hazard ratios and 95% confidence intervals. Model I is adjusted for age, sex, and energy intake. Model II is further adjusted for educational attainment, diet quality, alcohol intake, physical activity, smoking status, body mass index, and *APOE* ε4 status.
n, number of incident cases; N, number of participants at risk.

TABLE 3
Dietary nitrate intake and total brain volume, cerebral perfusion, and white matter hyperintensity volume ($n = 3949$)

	Total brain volume	Cerebral perfusion	White matter hyperintensity volume
Per 50-mg/d increase in	Main effect, mean difference in *z*-score (95% confidence interval)		
Total dietary nitrate intake	0.00 (−0.01, 0.01)	0.01 (−0.02, 0.04)	−0.01 (−0.03, 0.02)
Vegetable dietary nitrate intake	0.00 (−0.01, 0.01)	0.01 (−0.02, 0.04)	0.00 (−0.03, 0.02)
Nonvegetable dietary nitrate intake	0.11 (0.03, 0.20)	−0.14 (−0.38, 0.10	−0.02 (−0.24, 0.20)
Per 50-mg/d increase in	Slope, mean difference in *z*-score (95% confidence interval)		
Total dietary nitrate intake	0.00 (0.00, 0.00)	0.00 (−0.01, 0.00)	0.00 (0.00, 0.00)
Vegetable dietary nitrate intake	0.00 (0.00, 0.00)	0.00 (−0.01, 0.00)	0.00 (0.00, 0.00)
Nonvegetable dietary nitrate intake	0.01 (0.00, 0.01)	−0.03 (−0.08, 0.02)	0.00 (−0.01, 0.02)

Linear mixed models with a random intercept and slope were used to obtain mean differences of the main effect (i.e.,., dietary nitrate included in the models as fixed effect) and slope effect (i.e.,., interaction between dietary nitrate intake and time). Models are adjusted for age, sex, energy intake, educational attainment, diet quality, alcohol intake, physical activity, smoking status, body mass index, *APOE* ε4 status, and intracranial volume.

TABLE 4
Dietary nitrate intake and microbleeds and lacunar infarcts ($n = 3949$)

Per 50-mg/d increase in	Prevalent vascular brain disease	
	Any microbleeds ($n = 808$)	Any lacunar infarcts ($n = 293$)
Total dietary nitrate intake	0.97 (0.91, 1.05)	1.05 (0.94, 1.16)
Vegetable dietary nitrate intake	0.98 (0.91, 1.05)	1.04 (0.94, 1.16)
Nonvegetable dietary nitrate intake	0.65 (0.33, 1.26)	1.13 (0.43, 2.99)
Per 50-mg/d increase in	Incident vascular brain disease	
	Any microbleeds ($n = 279$)	Any lacunar infarcts ($n = 115$)
Total dietary nitrate intake	1.01 (0.91, 1.13)	0.96 (0.81, 1.15)
Vegetable dietary nitrate intake	1.01 (0.91, 1.13)	0.97 (0.82, 1.16)
Nonvegetable dietary nitrate intake	0.64 (0.21, 1.95)	0.34 (0.06, 1.93)

Logistic regression models were used to obtain odds ratios and 95% confidence intervals. Models are adjusted for age, sex, energy intake, educational attainment, diet quality, alcohol intake, physical activity, smoking status, body mass index, and *APOE* ε4 status. In the models assessing incidence, time between dietary nitrate intake assessment and MRI scan on which the incidence was detected was added.

wk reported robust improvement in executive functioning at the end of the trial [14]. We extended this evidence in a long-term cohort study by showing that dietary nitrate intake is also associated with a lower risk of dementia.

Our findings of an association between a higher dietary nitrate intake and a lower risk of dementia could be explained by the free radical nitric oxide. Oral bacteria metabolize dietary nitrate to nitrite, and in the acid environment of the stomach, nitrite can be metabolized

further into nitric oxide. This will be absorbed in the small intestine and subsequently released in the blood circulation [37]. In the blood circulation, nitric oxide regulates vasodilation and platelet aggregation. This maintains vascular health [1,2], which is a key determinant in the prevention of dementia [38]. However, our null findings with brain imaging markers could not confirm that vascular brain health drives the association between dietary nitrate intake and dementia. In line with these findings, a recent meta-analysis of randomized controlled trails found no strong evidence for an acute effect of dietary nitrate intake on cerebral perfusion [12], but other brain imaging markers have not previously been studied. An explanation for the observed null findings could be that nitric oxide mainly affects small blood vessels that cannot be detected on brain images. Alternative potential mechanisms underlying the association between dietary nitrate and dementia include a protective effects of nitric oxide on metabolic functions [30,39] and the regulation of reactive oxygen species homeostasis [40].

In this study, the association between dietary nitrate intake and the risk of dementia seems to be driven by dietary nitrate from vegetable sources. This may be explained by accompanying bioactive compounds in vegetables, such as vitamin C and polyphenols, which enhance the formation of nitrate into nitric oxide [6,7]. However, we found no evidence for interaction between dietary intake of nitrate and vitamin C, which could possibly suggest that enhancing effects require the presence of multiple bioactive compounds. However, it is also possible that bioactive compounds common in nitrate-rich vegetables drive the observed link between nitrate and dementia through pathways unrelated to nitrate [41].

That dietary nitrate from nonvegetable sources was not associated with the risk of dementia may be explained by the absence of enhancing effects of bioactive compounds present in vegetables. Without enhancing stimulus, nitrate can be converted into nitrosamines [42], chemical compounds that may adversely affect brain health [43]. Furthermore, nitrate from nonvegetable sources is mainly derived from animal-based foods that contain high levels of saturated fats and sodium, of which excessive consumption has been linked to an increased risk of dementia [44]. These adverse effects may have eclipsed the positive effects of dietary nitrate on brain health. However, observed null findings should be interpreted with caution because, on average, only 19% of the consumed dietary nitrate was derived from nonvegetable sources. Intake levels of nonvegetable dietary nitrate in this study, which are similar to those reported in other populations [26,45], may be too low to identify meaningful associations. In addition, the database used to assess dietary nitrate from nonvegetable sources was less extensive and up-to-date than the database from vegetable sources. Consequently, the relatively low precision of the nonvegetable dietary nitrate consumption levels may have diluted effect estimates toward the null.

Certain methodological considerations need to be considered when interpreting the findings. First, the FFQs used to determine dietary intake were not originally compiled to assess dietary nitrate intake. Consequently, intake of some nitrate-rich foods (i.e., radish and turnip) was not assessed in detail. Moreover, information on preparation methods was lacking for some foods, whereas most meal preparation methods reduce the nitrate content of the food [28]. Second, dietary nitrate concentrations in foods vary owing to environmental factors, such as season and temperature [26]. Therefore, the composition tables for the Netherlands are needed specifically to precisely determine dietary nitrate intake, and although we had access to an extensive database, for some food items, dietary nitrate content was not known for the Netherlands. Hence, misclassification, which is presumably differential, persists, and may have led to an underestimation of the true association. Third, nitrate concentrations in foods are slightly reduced over the past 30 y in the Netherlands [46]. Such temporal changes were not considered in the databases, which could have affected the precision of the calculated nitrate values. Fourth, data on food intake was self-reported and, thereby, sensitive to recall bias. Fifth, although we corrected for a wide range of potential confounders such as total energy intake and diet quality, residual confounding may persist. Sixth, this study includes individuals living in the Netherlands, an economic well-developed county, in which mean daily nitrate consumption levels are substantially lower compared with low economic developed countries [40]. Moreover, ~95% of our study population was of Caucasian ethnicity. This hampers generalizability of our results to study populations from lower economic developed counties and other ethnicities. Finally, dietary habits most likely change over time, but our data were too limited to consider this.

In conclusion, a higher dietary nitrate intake from vegetable sources was associated with a lower risk of dementia in the general population. We found no evidence for vascular brain health as an underlying mechanism. Further studies should verify our observations, elucidate the potential effects of nonvegetable dietary nitrate intake on brain health, and identify mechanisms underlying the association between dietary nitrate intake and the risk of dementia.

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Author disclosures

The authors report no conflicts of interest.

Author contributions

The authors' responsibilities were as follows—TOEC, LB, MWV, MKI, TV, MAI: design and conceptualization of the study; TOEC, LB, MWV, MKI, TV, MAI: acquisition of data; TOEC: analysis of data; TOEC, LB, MWV, MKI, TV, MAI: interpretation of data; TOEC: drafting the manuscript; LB, MWV, MKI, TV, MAI: revised the manuscript critically for important intellectual content; TOEC, MAI: had full access to all data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis; and all

authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data availability

Because of data protection standards of the informed consent procedure of the Rotterdam Study, data cannot be made freely available in publicly available repositories.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.05.027.

References

[1] S.K. Yao, J.C. Ober, A. Krishnaswami, J.J. Ferguson, H.V. Anderson, P. Golino, et al., Endogenous nitric oxide protects against platelet aggregation and cyclic flow variations in stenosed and endothelium-injured arteries, Circulation 86 (4) (1992) 1302–1309.
[2] M.J. Simmonds, J.A. Detterich, P. Connes, Nitric oxide, vasodilation and the red blood cell, Biorheology 51 (2–3) (2014) 121–134.
[3] M.A. Cinelli, H.T. Do, G.P. Miley, R.B. Silverman, Inducible nitric oxide synthase: regulation, structure, and inhibition, Med. Res. Rev. 40 (1) (2020) 158–189.
[4] C.D. Leaf, J.S. Wishnok, S.R. Tannenbaum, L-arginine is a precursor for nitrate biosynthesis in humans, Biochem. Biophys. Res. Commun. 163 (2) (1989) 1032–1037.
[5] E.L. Wightman, C.F. Haskell-Ramsay, K.G. Thompson, J.R. Blackwell, P.G. Winyard, J. Forster, et al., Dietary nitrate modulates cerebral blood flow parameters and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation, Physiol. Behav. 149 (2015) 149–158.
[6] A.W. Ashor, O.M. Shannon, A.D. Werner, F. Scialo, C.N. Gilliard, K.S. Cassel, et al., Effects of inorganic nitrate and vitamin C co-supplementation on blood pressure and vascular function in younger and older healthy adults: a randomised double-blind crossover trial, Clin. Nutr. 39 (3) (2020) 708–717.
[7] C. Pereira, N.R. Ferreira, B.S. Rocha, R.M. Barbosa, J. Laranjinha, The redox interplay between nitrite and nitric oxide: from the gut to the brain, Redox Biol 1 (1) (2013) 276–284.
[8] C.P. Bondonno, F. Dalgaard, L.C. Blekkenhorst, K. Murray, J.R. Lewis, K.D. Croft, et al., Vegetable nitrate intake, blood pressure and incident cardiovascular disease: Danish Diet, Cancer, and Health Study, Eur. J. Epidemiol. 36 (8) (2021) 813–825.
[9] F.J. Larsen, B. Ekblom, K. Sahlin, J.O. Lundberg, E. Weitzberg, Effects of dietary nitrate on blood pressure in healthy volunteers, N. Engl. J. Med. 355 (26) (2006) 2792–2793.
[10] A.J. Webb, N. Patel, S. Loukogeorgakis, M. Okorie, Z. Aboud, S. Misra, et al., Acute blood pressure lowering, vasoprotective, and antiplatelet properties of dietary nitrate via bioconversion to nitrite, Hypertension 51 (3) (2008) 784–790.
[11] M. Siervo, A. Babateen, M. Alharbi, B. Stephan, O. Shannon, Dietary nitrate and brain health. Too much ado about nothing or a solution for dementia prevention? Br. J. Nutr. 128 (6) (2022) 1130–1136.
[12] T. Clifford, A. Babateen, O.M. Shannon, T. Capper, A. Ashor, B. Stephan, et al., Effects of inorganic nitrate and nitrite consumption on cognitive function and cerebral blood flow: a systematic review and meta-analysis of randomized clinical trials, Crit. Rev. Food Sci. Nutr. 59 (15) (2019) 2400–2410.
[13] M. Gilchrist, P.G. Winyard, J. Fulford, C. Anning, A.C. Shore, N. Benjamin, Dietary nitrate supplementation improves reaction time in type 2 diabetes: development and application of a novel nitrate-depleted beetroot juice placebo, Nitric Oxide 40 (2014) 67–74.
[14] J.N. Justice, L.C. Johnson, A.E. DeVan, C. Cruickshank-Quinn, N. Reisdorph, C.J. Bassett, et al., Improved motor and cognitive performance with sodium nitrite supplementation is related to small metabolite signatures: a pilot trial in middle-aged and older adults, Aging (Albany NY) 7 (11) (2015) 1004–1021.
[15] C. Thompson, L.J. Wylie, J. Fulford, J. Kelly, M.I. Black, S.T. McDonagh, et al., Dietary nitrate improves sprint performance and cognitive function during prolonged intermittent exercise, Eur. J. Appl. Physiol. 115 (9) (2015) 1825–1834.
[16] A.M. Babateen, O.M. Shannon, G.M. O'Brien, E. Okello, E. Smith, D. Olgacer, et al., Incremental doses of nitrate-rich beetroot juice do not modify cognitive function and cerebral blood flow in overweight and obese older adults: a 13-week pilot randomised clinical trial, Nutrients 14 (5) (2022) 1052.
[17] J. Kelly, J. Fulford, A. Vanhatalo, J.R. Blackwell, O. French, S.J. Bailey, et al., Effects of short-term dietary nitrate supplementation on blood pressure, O2 uptake kinetics, and muscle and cognitive function in older adults, Am. J. Physiol. Regul. Integr. Comp. Physiol. 304 (2) (2013) R73–R83.
[18] C. Thompson, A. Vanhatalo, H. Jell, J. Fulford, J. Carter, L. Nyman, et al., Dietary nitrate supplementation improves sprint and high-intensity intermittent running performance, Nitric Oxide 61 (2016) 55–61.
[19] C.P. Bondonno, L.A. Downey, K.D. Croft, A. Scholey, C. Stough, X. Yang, et al., The acute effect of flavonoid-rich apples and nitrate-rich spinach on cognitive performance and mood in healthy men and women, Food Funct 5 (5) (2014) 849–858.
[20] W.K. Lefferts, W.E. Hughes, C.N. White, T.D. Brutsaert, K.S. Heffernan, Effect of acute nitrate supplementation on neurovascular coupling and cognitive performance in hypoxia, Appl. Physiol. Nutr. Metab. 41 (2) (2016) 133–141.
[21] M.A. Ikram, G. Brusselle, M. Ghanbari, A. Goedegebure, M.K. Ikram, M. Kavousi, et al., Objectives, design and main findings until 2020 from the Rotterdam Study, Eur. J. Epidemiol. 35 (5) (2020) 483–517.
[22] K. Klipstein-Grobusch, J.H. den Breeijen, R.A. Goldbohm, J.M. Geleijnse, A. Hofman, D.E. Grobbee, et al., Dietary assessment in the elderly: validation of a semiquantitative food frequency questionnaire, Eur. J. Clin. Nutr. 52 (8) (1998) 588–596.
[23] R.A. Goldbohm, P.A. van den Brandt, H.A. Brants, P. van't Veer, M. Al, F. Sturmans, et al., Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer, Eur. J. Clin. Nutr. 48 (4) (1994) 253–265.
[24] G.I. Feunekes, W.A. Van Staveren, J.H. De Vries, J. Burema, J.G. Hautvast, Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol, Am. J. Clin. Nutr. 58 (4) (1993) 489–496.
[25] L.C. Blekkenhorst, R.L. Prince, N.C. Ward, K.D. Croft, J.R. Lewis, A. Devine, et al., Development of a reference database for assessing dietary nitrate in vegetables, Mol. Nutr. Food Res. 61 (8) (2017).
[26] N.G. Hord, Y.P. Tang, N.S. Bryan, Food sources of nitrates and nitrites: the physiologic context for potential health benefits, Am. J. Clin. Nutr. 90 (1) (2009) 1–10.
[27] J.E. van der Toorn, M. Cepeda, J.C. Kiefte-de Jong, O.H. Franco, T. Voortman, J.D. Schoufour, Seasonal variation of diet quality in a large middle-aged and elderly Dutch population-based cohort, Eur. J. Nutr. 59 (2) (2020) 493–504.
[28] A.P. Keszei, L.J. Schouten, A.L. Driessen, C.J. Huysentruyt, Y.C. Keulemans, R.A. Goldbohm, et al., Vegetable, fruit and nitrate intake in relation to the risk of Barrett's oesophagus in a large Dutch cohort, Br. J. Nutr. 111 (8) (2014) 1452–1462.
[29] M. Inoue-Choi, M.K. Virk-Baker, B. Aschebrook-Kilfoy, A.J. Cross, A.F. Subar, F.E. Thompson, et al., Development and calibration of a dietary nitrate and nitrite database in the NIH-AARP Diet and Health Study, Public Health Nutr 19 (11) (2016) 1934–1943.
[30] L.D. Monti, C. Barlassina, L. Citterio, E. Galluccio, C. Berzuini, E. Setola, et al., Endothelial nitric oxide synthase polymorphisms are associated with type 2 diabetes and the insulin resistance syndrome, Diabetes 52 (5) (2003) 1270–1275.
[31] M.A. Ikram, A. van der Lugt, W.J. Niessen, P.J. Koudstaal, G.P. Krestin, A. Hofman, et al., The Rotterdam Scan Study: design update 2016 and main findings, Eur. J. Epidemiol. 30 (12) (2015) 1299–1315.
[32] T. Voortman, J.C. Kiefte-de Jong, M.A. Ikram, B.H. Stricker, F.J.A. van Rooij, L. Lahousse, et al., Adherence to the 2015 Dutch dietary guidelines and risk of non-communicable diseases and mortality in the Rotterdam Study, Eur. J. Epidemiol. 32 (11) (2017) 993–1005.
[33] Z.L. Chen, M.G. Zuurmond, N. van der Schaft, J. Nano, H.A.H. Wijnhoven, M.A. Ikram, et al., Plant versus animal based diets and insulin resistance, prediabetes and type 2 diabetes: the Rotterdam Study, Eur. J. Epidemiol. 33 (9) (2018) 883–893.
[34] D. Clark-Carter, Interquartile range, in: B.S. Everitt, D.C. Howell (Eds.), Encyclopedia of Statistics in Behavioral Science, John Wiley & Sons, Hoboken, N.J., 2005.

[35] N. Toda, T. Okamura, Obesity impairs vasodilatation and blood flow increase mediated by endothelial nitric oxide: an overview, J. Clin. Pharmacol. 53 (12) (2013) 1228–1239.
[36] E.J. Vinke, M. de Groot, V. Venkatraghavan, S. Klein, W.J. Niessen, M.A. Ikram, et al., Trajectories of imaging markers in brain aging: the Rotterdam Study, Neurobiol. Aging. 71 (2018) 32–40.
[37] J.O. Lundberg, E. Weitzberg, M.T. Gladwin, The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics, Nat. Rev. Drug Discov. 7 (2) (2008) 156–167.
[38] G. Tini, R. Scagliola, F. Monacelli, G. La Malfa, I. Porto, C. Brunelli, et al., Alzheimer's disease and cardiovascular disease: a particular association, Cardiol. Res. Pract. 2020 (2020), 2617970.
[39] M. Carlstrom, F.J. Larsen, T. Nystrom, M. Hezel, S. Borniquel, E. Weitzberg, et al., Dietary inorganic nitrate reverses features of metabolic syndrome in endothelial nitric oxide synthase-deficient mice, Proc. Natl. Acad. Sci. U. S. A. 107 (41) (2010) 17716–17720.
[40] J.O. Lundberg, M. Carlstrom, E. Weitzberg, Metabolic effects of dietary nitrate in health and disease, Cell Metab 28 (1) (2018) 9–22.
[41] M.C. Morris, Nutritional determinants of cognitive aging and dementia, Proc. Nutr. Soc. 71 (1) (2012) 1–13.
[42] I.T. Vermeer, D.M. Pachen, J.W. Dallinga, J.C. Kleinjans, J.M. van Maanen, Volatile N-nitrosamine formation after intake of nitrate at the ADI level in combination with an amine-rich diet, Environ. Health Perspect. 106 (8) (1998) 459–463.
[43] S.M. de la Monte, M. Tong, Brain metabolic dysfunction at the core of Alzheimer's disease, Biochem. Pharmacol. 88 (4) (2014) 548–559.
[44] F. Pistollato, R.C. Iglesias, R. Ruiz, S. Aparicio, J. Crespo, L.D. Lopez, et al., Nutritional patterns associated with the maintenance of neurocognitive functions and the risk of dementia and Alzheimer's disease: a focus on human studies, Pharmacol. Res. 131 (2018) 32–43.
[45] J.K. Jackson, A.J. Patterson, L.K. MacDonald-Wicks, C.P. Bondonno, L.C. Blekkenhorst, N.C. Ward, et al., Dietary nitrate and diet quality: an examination of changing dietary intakes within a representative sample of Australian women, Nutrients 10 (8) (2018) 1005.
[46] Ministry of Health, Welfare, and Sport, National Institute for Public Health and the Environment, Agricultural Practices and Water Quality in the Netherlands: Status (2016–2019) and Trends (1992–2019), 2020.