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Anjana Rajendra
Edith Cowan University

Nicola P. Bondonno
Edith Cowan University

Stephanie R. Rainey-Smith

Samantha L. Gardener

Jonathan M. Hodgson
Edith Cowan University

See next page for additional authors

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POTENTIAL ROLE OF DIETARY NITRATE IN RELATION TO CARDIOVASCULAR AND CEREBROVASCULAR HEALTH, COGNITION, COGNITIVE DECLINE AND DEMENTIA: A REVIEW

Anjana Rajendra BDS, MS [1], Nicola P. Bondonno PhD [1,2], Stephanie R Rainey-Smith PhD [3,4,5,6,7] Samantha L Gardener PhD [4,5,6], Jonathan M. Hodgson PhD [1,8], Catherine P. Bondonno PhD [1,8]

[1] Nutrition & Health Innovation Research Institute, School of Medical and Health Sciences, Edith Cowan University, Perth, Australia.

[2] Danish Cancer Society Research Center, Copenhagen, Denmark.

[3] Centre for Healthy Ageing, Health Futures Institute, Murdoch University, Murdoch, Western Australia, Australia.

[4] Australian Alzheimer's Research Foundation, Perth, Western Australia, Australia.

[5] Centre of Excellence for Alzheimer's Disease Research & Care, School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia.

[6] Lifestyle Approaches Towards Cognitive Health Research Group, Murdoch University, Murdoch, Western Australia, Australia.

[7] School of Psychological Science, University of Western Australia, Perth, Western Australia, Australia.

[8] Medical School, The University of Western Australia, Royal Perth Hospital Research Foundation, Perth, Western Australia, Australia.

Correspondance author : Dr Catherine P. Bondonno

Nutrition & Health Innovation Research Institute, School of Medical and Health Sciences, Edith Cowan University, Level 3, Royal Perth Hospital Research Foundation, Rear 50 Murray St, Perth Western Australia, Australia WA 6000

Tel: +61 8 6304 4601

Email: c.bondonno@ecu.edu.au

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Abstract

There is currently no effective treatment for dementia, of which Alzheimer's disease (AD) is the most common form. It is, therefore, imperative to focus on evidence-based preventive strategies to combat this extremely debilitating chronic disease. Nitric oxide (NO) is a key signalling molecule in the cardiovascular, cerebrovascular, and central nervous systems. Vegetables rich in nitrate, such as spinach and beetroot, are an important source of NO, with beneficial effects on validated markers of cardiovascular health and an association with a lower risk of cardiovascular disease. Given the link between cardiovascular disease risk factors and dementia, together with the important role of NO in vascular health and cognition, it is important to determine whether dietary nitrate could also improve cognitive function, markers of brain health, and lower risk of dementia. This review presents an overview of NO's role in the cardiovascular, cerebrovascular, and central nervous systems; an overview of the available evidence that nitrate, through effects on NO, improves cardiovascular health; and evaluates the current evidence regarding dietary nitrate's potential role in cerebrovascular health, cognitive function, and brain health assessed via biomarkers.

Keywords: Dietary nitrate, Cognition, Alzheimer's Disease, Dementia, Nitric Oxide

Statement of Significance: Evidence is accumulating for improved cardiovascular health with dietary nitrate intake. This review identifies and discusses the potential for dietary nitrate to improve cognitive function and markers of brain health, and to reduce risk of dementia.

Introduction

Dementia is a progressively debilitating condition, the incidence of which is growing at an alarming rate across the globe. Currently, there are over 50 million people living with dementia and this number is expected to rise to 152 million by 2050 [1]. In the current absence of any effective treatment or cure, reducing or preventing the development of risk factors for dementia is the only viable approach to lower the prevalence of this disease [2]. Risk factors for dementia, including hypertension and hypercholesteremia [3,4,5] are shaped by both genetic factors and lifestyle factors, with the latter having by far the greatest impact. Alzheimer's disease (AD) is the most common form of dementia, accounting for around 70% of cases. The preclinical phase of dementia due to AD is lengthy [6]. Abnormal deposits of beta-amyloid (Aβ) and tau tangles within the brain, and the subsequent neuronal damage, are believed to commence 15-20 years before objective cognitive decline is evident [7]. The majority of therapeutic trials to date have involved the initiation of treatments late in the course of AD development, when significant symptoms are apparent [8]. As these trials have failed, there has been a shift towards targeting the preclinical phase of the disease, before overt symptoms manifest, and towards understanding modifiable risk factors and the subsequent development of preventive strategies. Highlighting the importance of this notion is a message from the Lancet Commission for "Dementia Prevention, Intervention, and Care" [4] to "be ambitious about prevention".

Cognitive health is greatly impacted by vascular health and cerebrovascular blood flow [9]. The importance of vascular contributions to cognitive impairment and dementia has been highlighted in the scientific statement from the American Heart Association and American Stroke Association [10]. Randomized clinical trials and longitudinal studies show a clear link between vascular risk factors and a higher risk of dementia, particularly dementia due to AD and vascular dementia [9, 11]. Vascular health is preserved in part by nitric oxide (NO), a key vascular signalling molecule that also functions as a potent vasodilator [12, 13]. A deficiency in NO can lead to vascular dysfunction and

alterations in cerebral blood flow [14-16]. NO also functions as a neurotransmitter, participates in several synaptic signalling events, and plays an important role in memory and learning [17]. Additionally, NO has been shown to prevent tau phosphorylation, a pathological hallmark of AD, in animal studies [18].

NO is synthesised via two pathways: The L-arginine-NOS pathway and the nitrate-nitrite-NO pathway [19-21]. There is strong evidence from randomized clinical trials that endogenous NO levels can be increased through the consumption of nitrate-rich vegetables [22]. Nitrate enhances endogenous NO through the nitrate-nitrite-NO pathway; this is associated with concomitant improvements in validated measures of vascular health [23]. Whether this increase in NO is associated with better cognitive function has been investigated in small clinical trials, but no prospective cohort studies have yet investigated associations with cognitive decline and dementia later in life. Increasing intake of nitrate-rich vegetables could potentially be a preventative approach to reduce risk factors for cognitive impairment and dementia onset.

This review presents an overview of NO and its role in cardiovascular and cerebrovascular health. Furthermore, this review discusses nitrate as an alternate source of NO, presents the current evidence of nitrate's role in cardiovascular health, and for the first time evaluates the potential role of nitrate in improving cognitive health and reducing the risk of cognitive decline and dementia.

1. Nitric Oxide

Nitric oxide (NO) is a key signalling molecule in the cardiovascular system, central nervous system (CNS), and immune system [24]. NO is considered a bio-essential molecule at the cellular level to maintain homeostasis across many physiological processes. It is a highly reactive soluble gas with lipophilic properties and a half-life of less than 2 seconds [25].

1.1 Pathways to NO

1.1.1 L-arginine Nitric Oxide Synthase Pathway

The L-arginine-NOS pathway (**Figure 1** is the primary source of NO, yielding approximately 70%, and has been reviewed extensively [26-28]. NO is produced during the conversion of the semi-essential amino acid L-arginine to L-citrulline by the group of enzymes known as nitric oxide synthases (NOS) [24]. There are three isoforms of NOS: NOS1 widely referred to as neuronal NOS (nNOS), NOS2 referred to as inducible NOS (iNOS), and NOS3 referred to as endothelial NOS (eNOS) [29]. All three isoforms are encoded by different genes and are regulated by distinct signalling pathways[30], possess varied functions, and are structurally different [31]. The L-arginine and NOS reaction is a synchronized catalytic reaction where NOS co-ordinates the binding of several substrates and co-factors to produce NO [32, 33]. Nitrate and nitrite are formed as end-products of NO synthesis and are recycled back to NO through the nitrate-nitrite-NO pathway [27].

1.1.2 Nitrate-Nitrite-NO Pathway

Dietary nitrate has been identified as an alternative source of NO, through the nitrate-nitrite-NO pathway [21, 34] (**Figure 2**). After intake of dietary nitrate, nitrate is absorbed in the small intestine and enters the circulation. Approximately 75% of nitrate is excreted through the kidneys and approximately 25% of plasma nitrate is taken up by salivary glands and secreted into the saliva [35]. The oral facultative anaerobic bacteria present in deep clefts on the dorsum of the tongue reduce nitrate to nitrite through a range of bacterial nitrate reductase enzymes [36-39]. Once swallowed, the low pH of the stomach and enteric bacterial nitrite reductases reduce nitrite to NO, with localised anti-inflammatory and anti-microbial effects [39]. The remaining nitrite is absorbed in the small intestine and enters the circulation. In the circulation, nitrite can be reduced to NO [34].

1.2 Role of NO in Vascular Health

NO, was first identified as an endothelium-derived vasorelaxing factor that acts as a physiologic vasodilator [40]. It is now known that endothelial derived NO performs other protective and regulatory roles in the vascular system, detailed in **Table 1**. These include maintaining vasomotor tone [41],

92 coronary tone [42], and inhibiting platelet aggregation [43], thereby regulating blood pressure [44],
93 modulating myocardial contraction [42], and preventing thrombosis [45]. Reduced NO bioavailability has
94 been associated with detrimental vascular effects such as atherosclerosis [46]. Impaired function of
95 endothelium and diminished NO has been observed in several pathophysiological conditions
96 including systemic hypertension, diabetes, congestive heart failure, hypercholesterolemia, pulmonary
97 hypertension, estrogen deficiency, and the ageing process itself [15, 47].

98 **1.3 Role of NO in Cognition, Brain Function, and Cerebrovascular Blood Flow**

99 NO is a key regulatory molecule in maintaining brain homeostasis as it plays an important role in
100 cerebrovascular blood flow thereby contributing to preservation of cognitive function. NO maintains
101 this homeostasis by activating NO-sensitive guanylyl cyclase, controlling gene transcriptase and
102 mRNA translation, and importantly, NO also acts as a neurotransmitter [57, 58].

103 **1.3.1 Role of NO in Cerebrovascular Blood Flow**

104 Cerebrovascular blood flow (CBF) modulates the preservation of brain function and cognition[48].
105 Inadequate blood flow to the brain can cause irreversible damage to brain parenchyma thereby
106 negatively impacting cognition and brain function. Approximately 15% of cardiac output is supplied
107 to the brain, which itself makes up only 2% of total bodyweight [48]. There is a body of evidence
108 which supports the idea that NO is essential in the regulation of CBF during hypercapnia [59], focal
109 brain ischemia [60, 61], and global brain ischemia [62, 63]. Constitutive NOS (eNOS and iNOS) plays a
110 major role in regulating basal CBF and is essential throughout the hypercapnic CBF response to
111 improve perfusion of the brain parenchyma and to avoid prolonged ischemia of the brain (**Figure 3**)
112 [64]. Thus, any variation in NO synthesis could interfere with brain homeostasis, potentially leading to
113 hypoperfusion of brain parenchyma and formation of brain lesions and pathological hallmarks such
114 as those which manifest in age-related dementias such as AD.

115 **1.3.2 Role of NO in Cognition and Social behaviour**

Experimental studies have shown that NO plays a significant role in cognitive function and social behaviour. de La Torre and Aliev [65] investigated the effect of NOS isoforms on spatial memory function in an ageing rat model with chronic brain hypoperfusion, to mimic human mild cognitive impairment. Inhibition of eNOS significantly decreased the performance in the Morris water-maze, which reflects spatial memory impairment. Their results suggested that eNOS may be essential in spatial memory function during chronic brain hypoperfusion, potentially by maintaining cerebrovascular tone and optimum blood flow to the brain. This implies that alteration in the activity of eNOS may result in spatial memory dysfunction and other neurological impairments. Moreover, it has been shown that a decrease in vascular NO in the brain parenchyma may lead to pathogenesis of AD in late middle-aged mice [66]. Compared to wild type mice, loss of eNOS in late middle-aged mice was associated with higher levels of Aβ in the brain, elevated mean systolic blood pressure (115 ± 8 Vs 149 ± 14 mmHg), cholesterol (87 ± 20 Vs 117 ± 25 mg/dL), triglyceride levels (59 ± 21 Vs 101 ± 39 mg/dL), and glucose levels (154 ± 46 Vs 237 ± 43 mg/dL) [66]. Additionally, animal studies have illustrated NO's role as a behaviour modulator [67] and findings indicate that nNOS plays a role in social behaviour, anxiety, and empathy in a rat model [68, 69] (**Figure 3**).

NO is proposed to modulate these processes through its role in synaptic signalling, and via participation in a novel form of inter-neuronal communication, i.e., non-synaptic communication without receptors [70]. Indeed, the strength of excitatory output may be conveyed to the surrounding neurons by means of this NO-mediated non-synaptic communication. Numerous experimental studies have acknowledged NO's role in the pre-synaptic terminal [71], post-synaptic [72, 73], long-term potentiation [57], and synaptic plasticity [74, 75], which are required for learning and memory [76].

2. Dietary Nitrate

2.1 Sources of Dietary Nitrate

Vegetables are the primary source of dietary nitrate accounting for ~70-80% of intake [77]. Particular groups of vegetables, such as green leafy vegetables and root vegetables such as beetroot, are rich in nitrate whereas other vegetables such as potatoes, onion, and peas, contain a low level of nitrate [78]. A list of dietary sources categorised according to their nitrate content is presented in **Table 2.** Factors which determine the level of nitrate in vegetables include soil type, nitrate content in fertilizers and water, the intensity of sunlight, transport methods, storage conditions, and cooking procedures [79-82]. For these reasons, nitrate intake differs between individuals and geographical regions; an individual's nitrate intake may range from less than 20 mg to more than 400 mg per day [79, 80].

Another source of nitrate is drinking water [27]. The level of nitrate in drinking water is regulated in multiple countries due to health concerns [83]. Nitrate content in water is influenced by bacterial nitrogen fixation, as well as manure from livestock production, and fertilizer usage [84]. Meat is an additional dietary source of nitrate. Nitrate and nitrite are used as additives by the meat industry to enhance food quality and impede microbial contamination and chemical changes [85].

2.2 Guidelines and Acceptable Daily Intakes

A concern about nitrate and health has persisted since 1970 when the potential for nitrate to form carcinogenic *N*-nitrosamines was discovered. The International Agency for Research on Cancer has supported the hypothesis that ingested nitrate increases risk of cancer based on the results of early studies [89-91]. On the contrary, recent studies did not support the results of earlier studies, reporting null association between dietary nitrate and cancer risk [92]. Furthermore, a recent meta-analysis has evidenced that the nitrate reduces the risk of gastric cancer [93]. It is still debatable if the different sources of nitrate could offer similar benefit or harm. For example, plant-derived foods are packed with antioxidants like polyphenols and vitamin C which impede the formation of carcinogens [94], unlike meat and water. However, due to ongoing health concerns, the WHO has established the Acceptable Daily Intake of nitrate as 0-3.7 mg/kg body weight [95] and this was reviewed and accepted by The European Food Safety Authority [96].

3. Nitrate and Cardiovascular Disease

There is now robust evidence from clinical trials and observational studies that dietary nitrate has beneficial effects on cardiovascular disease (CVD) [97]. There is an established link between vascular risk factors and dementia [10, 11]. The beneficial effects of NO on vascular risk factors and CVD risk could potentially decrease the risk of dementia.

3.1 Overview of Cardiovascular Disease-burden of disease and impact

CVD is the leading cause of global mortality and morbidity, impacting quality of life [98, 99], and economic burden. Globally, around 17.9 million deaths in the year 2019 were reportedly due to CVD, accounting for 32 % of all global deaths [100]. On average, an individual dies of CVD every 36 seconds in the US, totalling approximately 2,400 deaths every day [101]. In 2014-2015, USD 351.3 billion was spent on treatment of people with CVD in the US alone, and the total cost of treating CVD is expected to reach USD 1.1 trillion by 2035 [102]. Treatment for CVD often includes extensive surgical intervention, a prolonged recovery period, loss of independence, and loss of income, thereby imparting significant personal and economic burden [103].

3.2 Beneficial Effects of Nitrate on Vascular Health and Risk of CVD – Evidence from Clinical Trials

There is a growing body of evidence that dietary nitrate may play a significant role in improving cardiovascular risk factors with beneficial effects observed on endothelial function (as measured by flow mediated dilatation) [21, 23, 104-107] and a reduction in systolic blood pressure [21, 104, 105, 108-113], diastolic blood pressure [104, 105, 107, 109, 111, 112, 114], and arterial stiffness [105, 115], as well as platelet reactivity and reduced platelet aggregation [21, 105, 116]. These clinical trials were conducted in healthy individuals as well as participants with pre-existing CVD risk factors such as hypertension and obesity and were inclusive of all adult age groups. Intake of dietary nitrate was in the form of spinach, spinach juice, beetroot juice, arugula juice, beetroot breads and nitrate salts over 2 hrs to 42 days. Dose of dietary nitrate ranged from as low as 68 mg/day to 1395 mg/day in the reported

clinical trials. While not all clinical studies have reported consistent findings [105, 117-120], a meta-analysis examining all CVD risk factors reported a significant reduction in systolic blood pressure (-4.8 mmHg), diastolic blood pressure (-1.7 mmHg), endothelial function (as measured by flow mediated dilatation, 0.6%), arterial stiffness (as measured by pulse wave velocity, -0.23 m/s) and platelet aggregation (-19%) [121]. The results described above are encouraging as a 2 mmHg lower systolic blood pressure is associated with reductions in coronary heart disease events of 17.9 per 100,000 person-years, stroke events by 9.6 per 100,000 person-years, and heart failure events by 26.6 per 100,000 person-years, as reported in the Atherosclerosis Risk in Communities Study [122]. Moreover, a 2-mmHg reduction in diastolic blood pressure has been shown to decrease risk of coronary heart disease by 6% and stroke by 15% in both male and females aged 35-64 years, as observed by Framingham Heart Study investigators [123]. Presley et al.,[124] observed a significant improvement in regional cerebral perfusion one hour after consumption of a high nitrate meal (769 mg) in older adults with a mean age of 75 ± 7 years. Therefore, it can be hypothesized that the beneficial effects of dietary nitrate on vascular health may lead to improvement in regional cerebral perfusion.

3.3 Beneficial Effects of Nitrate on Risk of CVD – Evidence from Observational Studies

To date, five observational studies have investigated the association between vegetable nitrate intake and long-term effects on cardiovascular health. The Perth Longitudinal Study of Aging in Women, reported a 21% lower risk of atherosclerotic vascular disease mortality [Hazard Ratio (HR): 0.79 (95% CI: 0.68, 0.93)] per standard deviation (SD) (~ 30 mg/day) higher intake of nitrate rich vegetable, and a 17% lower risk of an ischemic cerebrovascular episode [HR: 0.83 (95% CI: 0.70, 0.97)] per SD (~ 29 mg/day) higher intake of vegetable nitrate [44, 125]. The Australian Blue Mountains Eye Study reported a 27% lower hazard for CVD mortality [HR: 0.63 (95% CI: 0.41, 0.95)] among participants in quartile 4 whose dietary nitrate intake was > 137.8 mg/day, as compared to participants in quartile 1 (< 69.5 mg/day) [126]. Moreover, the Australian Longitudinal Study on

Women's Health described a 27% lower risk of self-reported incidents of CVD-related health complications [Odds Ratio: 0.73 (95% CI: 0.61, 0.88)] in women in the highest quartile of vegetable nitrate intake (> 64.4 mg/day) compared to women in the lowest quartile (< 34.8 mg/day) [127]. Outside of Australia, in the American Nurses' Health cohort study, risk of coronary heart disease in women in the highest quintile of vegetable nitrate intake was 9% lower, compared to those in the lowest intake quintile [Risk Ratio: 0.91 (95% CI: 0.80, 1.04)]. However, this association was no longer evident following adjustment for age, smoking, physical activity, body mass index and race [128]. Most recently, the Danish Diet, Cancer and Health (DDCH) study with 23 years of follow-up reported that moderate vegetable nitrate intake (~60 mg/day = 1 cup of green leafy vegetables) was linked with 12%, 15%, 17% and 26% lower risk of ischemic heart disease, heart failure, ischemic stroke, and peripheral artery disease hospitalizations, respectively [129]. The disparate results of the American Nurses' Health cohort study compared to the other three Australian cohort studies and the Danish cohort study could potentially be explained by the nitrate databases used to quantify intakes. Specifically, the nitrate database used by the American Nurses' Health cohort study was less comprehensive [128] than the newer version utilised by the other four studies [86]. However, all such measures come with a number of inherent limitations such as the variability of the nitrate content of food, which is dependent on a number of environmental factors, the tool used to measure intakes in these studies (food frequency questionnaire) as well as physiological and lifestyle factors that influence the bioavailability of nitrate.

Nevertheless, most evidence comes from clinical trials which are supported by findings from recent observational studies. Future research should focus on longer-term randomized controlled trials to establish causality of habitual intake of vegetable nitrate and reduction of CVD risk factors. Given the link between, cardiovascular system and cognition, the role of nitrate in cognition and dementia warrants investigation.

4. Nitrate and Dementia

4.1 Overview of Dementia - Burden of Disease and Impact

Over 50 million people are currently living with dementia globally, with this number expected rise to 152 million by 2050 [95]. Every 3 seconds an individual is diagnosed with dementia and the present annual cost of dementia is about USD 1 trillion worldwide and expected to increase twofold by 2030 [1].

Dementia is a progressive neurological disorder that affects cognition [130]. Dementia is primarily classified into four types namely, dementia due to AD, vascular dementia, frontotemporal dementia, and dementia with Lewy bodies [130]. AD is the most common form of dementia accounting for 60-70% of cases [131]. AD is characterized by initial memory loss and subsequent cognitive dysfunction which ultimately impairs speech, motor system function, and visuospatial orientation [131], making day-to-day life challenging for patients, families, and caregivers. Mild cognitive impairment (MCI) is a symptomatic predementia stage which often precedes AD [132]. Carriage of an ε4 allele of the Apolipoprotein E (*APOE*) gene is the strongest genetic risk factor for AD [133-135]. There is currently no cure for AD, although a disease-modifying drug for AD has recently been approved for use in the United States of America [136]. AD is currently recognised by the World Health Organisation as a global health priority [137]. Consequently, research focussed on AD prevention is gaining momentum. Thus far, three multidomain lifestyle-based intervention trials to prevent cognitive decline in the older population have been conducted: the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) [138], the Dutch Prevention of Dementia by Intensive Vascular Care (PreDIVA) [139] and the French Multidomain Alzheimer Preventive Trial (MAPT) [140]. Whilst the results of these studies have been encouraging, there is a need to further understand the role of specific protective components of a healthy diet, such as nitrate, in relation to cognitive decline in different populations.

262 **4.2 Nitrate, CVD, and Dementia**

263 There is a well-established multifaceted link between the cardiovascular system and all-cause
264 dementia. As detailed above, cerebrovascular blood flow is crucial to maintain normal brain function
265 and cognition [141, 142]. Blood flow is a function of the cardiovascular system; therefore, brain function
266 and cognition are dependent on the cardiovascular system. Cerebral blood flow is regulated primarily
267 by functional hyperaemia [143], cerebral autoregulation [144], endothelial cell regulation [145, 146], and the
268 blood-brain barrier [147]. A multifactorial data-driven analysis conducted on the Alzheimer's Disease
269 Neuroimaging Initiative (ADNI) cohort suggested that vascular dysregulation in the brain is first to
270 manifest before other hallmarks of AD pathology such as Aβ deposits and hyperphosphorylated tau
271 accumulation [148]. Reflecting this finding, a hypothetical model of *in vivo* AD dynamic biomarker
272 manifestation has been suggested to include the role of vascular changes in the brain and cerebral
273 blood flow **(Figure 4)** [149]. Consistent with this model, a growing body of evidence shows that there is
274 a reduction in cerebral blood flow, as revealed by arterial spin labelling, in non-demented patients [150-
275 156], indicating vascular dysregulation manifests before other disease biomarkers including Aβ
276 changes in cerebrospinal fluid, cerebral Aβ deposits, hyperphosphorylated tau tangles, and brain
277 atrophy; all of which appear before objective cognitive impairment. In 2018, Kapasi et al., [157]
278 presented an inverse association between multiple microinfarcts in cortical watershed regions, global
279 cognition, and cognitive function in specific domains of working memory and visuospatial abilities.
280 Furthermore, Lane et al.,[11] showed a strong association between mid-life vascular risk factors and
281 late-life brain health, and such risk factors have been cited in the recent Lancet Commission into
282 'Dementia prevention, intervention, and care' as requiring action for reduction of dementia risk.
283 Consistent with this message, most cases of AD have mixed pathology with both vascular pathology
284 and phosphorylated tau evident [158, 159]. There is additionally strong evidence that the vascular
285 endothelium plays a vital role in functional neurovascular coupling [160]. Endothelial cells regulate
286 vascular resistance by releasing NO to maintain vascular homeostasis and brain health. Apart from

vascular regulation, as stated earlier, endothelial-derived NO plays a significant role in the prevention of tau phosphorylation – hyperphosphorylation of which is a hallmark of AD pathology. Indeed, deficiency of NO decreases nitrosylation of neuronal caplain which activates enzyme cyclin dependent kinase 5 responsible for tau phosphorylation [18].

4.3 Nitrate and Cognitive Function

Despite the well-established beneficial effects of nitrate on cardiovascular health, evidence of a beneficial effect of nitrate intake on cognitive function from clinical trials is inconsistent (**Table 3**). Seven out of twelve clinical trials have shown that intake of dietary nitrate is associated with an improvement in cognitive function and cerebral blood flow [162-166]. On the contrary, findings from other clinical trials have shown no effect on cognitive function following the intake of dietary nitrate [111, 167-170]. The nitrate dose ranged from 310 mg to 775 mg in acute studies and 397 mg to 800 mg in chronic studies. The intervention length was just a single occasion in acute studies and ranged from two days to thirteen weeks in chronic studies. The sample size ranged from 10 to 24 participants (mean age below 30 years) in acute studies and from 12 to 62 participants in chronic studies with mean age above 60 years in three studies out of five. Possible reasons for the observed differences in the effect of nitrate on cognitive function measurements include dose of nitrate, cognitive status, and age of the participants (only five studies were in older adults), background diet, number of participants in the study (all studies had a relatively small sample number), as well as the timing and sensitivity of the cognitive function measures used. To date, no comprehensive cognitive battery has been used to assess a range of cognitive domains in clinical trials of nitrate intake.

Notably, a meta-analysis of randomized clinical trials conducted before 2017 evaluated the effect of inorganic nitrate and nitrite on cerebral blood flow and cognitive function. The authors reported that due to the small sample sizes and short duration of the studies they were unable to draw any conclusions and highlighted the need for larger, adequately powered studies [171]. A cross-sectional

evaluation in 1,015 older adults observed no association between urinary nitrate concentration and cognition [172], however urinary nitrate is a poor biomarker of habitual dietary nitrate intake [173]. A further cross-sectional study in 989 older adults also reported no association between cognitive function and nitrate (urinary nitrate and nitrate intake assessed using a food frequency questionnaire) [174]. To date, no prospective observational studies have investigated a long-term relationship between habitual intake of dietary nitrate and cognitive function. The long-term association of habitual dietary nitrate and cognition can only be examined by such prospective observational studies due to the long pre-clinical phase of AD. Hence, this is an area that warrants further investigation to characterise the long-term relationship between habitual dietary nitrate and cognition, cognitive decline, and AD.

5. Biomarkers of Dementia

The disease course leading to dementia is protracted, with a long preclinical phase which precedes the prodromal and clinical stages, where cognitive dysfunction is evident. Given that the hallmarks of dementia begin to accumulate in the brain during the preclinical phase, to investigate the potential role of habitual intake of dietary nitrate in relation to cognition, cognitive decline, and AD, we need longitudinal data on habitual dietary intake, biomarker profiles, and incident dementia. As mentioned earlier, AD is characterized by histopathological changes in the brain along with progressive atrophy of brain parenchyma. The neuropathological hallmarks of AD are deposition of plaques formed of Aβ protein and neurofibrillary tangles of hyperphosphorylated tau, a filamentous protein. As shown in **Figure 4**, cerebral Aβ deposition and tau aggregates accumulate before other structural and functional changes such as loss of hippocampal volume (brain region associated with memory), white matter hyperintensities, reduced grey matter integrity, cerebral atrophy, and reduced glucose metabolism [175, 176]. In addition, neuropil threads, reactive astrocytes, eosinophilic Hirano Bodies, granulovacuolar degeneration and cerebral amyloid angiopathy are also present [177, 178] . These lesions result in loss of synapses and neurons leading to the symptoms generally associated with AD. The histopathological diagnosis of AD mandates both Aβ plaques and neurofibrillary tangles.

In animal studies it has been demonstrated that NO appears to confer some protection against the development of AD associated with Aβ accumulation [179]. Previously, Austin at al., found that loss of eNOS in a murine model of AD is linked with an increase in cyclin dependent kinase 5 enzyme required for tau phosphorylation in neuronal tissue [180]. Furthermore, a recent study by Faraco et al., reported that NO deficiency can lead to tau-hyperphosphorylation in mice [18]. The authors showed that the NO deficiency decreased neuronal caplain nitrosylation, which activates enzyme cyclin-dependent kinase 5 responsible for tau phosphorylation. Moreover, other animal studies have shown that inhibition of NO results in impaired synaptic plasticity, memory formation, and cognitive performance [181-183]. Thus, we hypothesize that dietary nitrate, by augmenting NO, may have a potential role in reducing risk of dementia by positively modulating the formation of pathological hallmarks responsible for the decline in cognition that occurs downstream.

Future directions

To date, no clinical trials have examined the long-term effects of habitual intake of dietary nitrate, of minimum one-year duration, on cognitive function, cognitive decline, risk of dementia, and biomarkers of AD. Observational studies are also required to understand the association of mid-life dietary habits such as intake of dietary nitrate on late-life cognition. Such investigation requires prospective observational studies with comprehensive data on diet, cognitive function, and AD biomarkers. These studies should evaluate different sources of nitrate (i.e., plant-derived nitrate, animal source-derived nitrate and water-derived nitrate), dose of nitrate, and other synergistic compounds (such as vitamin C and polyphenols) on late-life cognition. The results of such studies will enhance our understanding of NO's role in the pathogenesis of AD and dementia and could form an important part of a multi-domain lifestyle prevention approach (diet, physical exercise, sleep, cognitive training etc.) aimed at reducing risk of cognitive decline and dementia.

Conclusion

NO has a crucial role in maintaining cardiovascular health. Animal studies have demonstrated that NO plays a role in behaviour, and spatial memory, and that NO deficiency has been linked with pathogenesis of AD. There is strong evidence that habitual intake of dietary nitrate, through effects on NO, can have beneficial effects on cardiovascular biomarkers such as blood pressure, endothelial function, arterial stiffness, and platelet function. Several studies have demonstrated a strong association between cardiovascular disease risk factors and a decline in cognitive function and increased risk of dementia. There are inconsistent results from clinical trials investigating nitrate intake and cognitive function, a significant knowledge gap. The long-term role of dietary nitrate in brain and cognitive health still needs to be investigated. Due to the prolonged pre-clinical phase of AD and other forms of dementia, studies in cohorts with longitudinal data are essential to investigate whether dietary nitrate could be an effective strategy to boost cognitive health and in doing so prevent dementia.

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Figure 1. L-arginine-Nitric Oxide Synthase pathway in vasculature (Created with BioRender.com).

O_2, oxygen; NADPH, nicotinamide adenine dinucleotide phosphate; eNOS, endothelial nitric oxide synthase; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; BH_4, (6R-)5,6,7,8-tetrahydroL-biopterin; NAD; nicotinamide adenine dinucleotide; NO, nitric oxide. Image created with BioRender.com.

Figure 2. Nitrate-nitrite-nitric oxide pathway (Created with BioRender.com)

The nitrate–nitrite–nitric oxide (NO) pathway. (1) Ingested dietary nitrate is absorbed through small intestine and approximately 75% of nitrate is excreted via the kidneys (2) Nitrate enters circulation after absorption of nitrate through small intestine (3) Nitrate in the circulation from food and NO metabolism (4) Active uptake of the nitrate from blood by the salivary glands (5) The nitrate reducing bacteria found on the dorsum of tongue converts nitrate to nitrite (6) Salivary nitrite is swallowed (7) Salivary nitrite is converted to NO in the acidic environment of the stomach (8) The remaining salivary nitrite is absorbed in the small intestine (9) Nitrite enters the circulation (10) Circulating nitrite is a source of NO (11) Nitrate and nitrite are end products of systemic NO metabolism (12) Nitrate thus formed could enter the cycle together with ingested nitrate

Figure 3. Possible role of nitric oxide in neuronal function and behavioural outcomes (Created with BioRender.com).

Figure 4. Updated hypothetical model of Alzheimer's disease biomarker manifestation. Figure adapted from Sweeney MD et al., 2018 [161].

Hypothetical model of AD biomarker changes illustrating that initial vascular dysregulation in cerebral blood flow and the blood brain barrier may contribute to the early stages of AD pathophysiological progression from no cognitive impairment to mild cognitive impairment to AD. The initial vascular dysregulation is later followed by beta-amyloid and abnormal tau biomarkers in

cerebrospinal fluid and brain. All the biomarkers converge at the top right-hand corner of the plot, that is the point of maximum abnormality. The cognitive response is illustrated as the blue zone with low and high-risk borders. Subjects with high-risk of cognitive impairment due to genetic predisposition, AD pathology, and low cognitive reserve are shown with a cognitive response curve shifted to the left. Conversely, the cognitive response curve for the low-risk subjects with protective genetic profile, high cognitive reserve, and the absence of comorbid brain pathologies is shifted to the right.

Abbreviations: Aβ, beta-amyloid in cerebrospinal fluid; amyloid, brain amyloid plaques; CBF, cerebral blood flow; BBB, blood brain barrier; NCI, no cognitive impairment; MCI, mild cognitive impairment; AD, Alzheimer's disease.

Table 1. Roles of nitric oxide in the cardiovascular system

Regulatory Functions	**Protective Functions**
Endothelium-dependent vasodilation [40, 41]	Free radical scavenger [48, 49]
Reduces oxidation of LDL [50]	Inhibits variety of immunomodulatory cytokines [51]
Reduces platelet aggregation and adhesion [52]	Pro-angiogenic effects of VEGF and thereby affects healing processes and tumour growth[53]
Reduces stickiness of monocytes[54]	
Regulates mast cell reactivity [55]	
Regulates both basal epicardial and arteriolar dilator tone [56]	

Abbreviations: LDL, low density lipoprotein; VEGF, vascular endothelial growth factor

Table 2. Classification of dietary sources based on their nitrate content

Source	**Nitrate content**	**Items**

Vegetables [86]	Very high > 2000 mg / kg fw	Chinese flat cabbage, Mustard greens, Sea beet, Chinese broccoli, Swiss Chard
	High 1000 to < 2000 mg / kg fw	Lettuce, Kale, Spinach, Beet, New Zealand Spinach, Celery, Kohlrabi
	Medium 500 to 1000 mg / kg fw	Endive, Fenugreek, Turnip
	Low ≤ 500 mg/ kg fw	Pumpkin, Okra, Onion, Pea, Sweet potato
Meat [87]	Very low < 20 mg / 100 g	Meat, Processed Meat
Water [88]	Very low 5 mg/100 g	

Abbreviations: fw, fresh weight; kg, kilogram; mg, milligram; g, gram.

Table 3. Clinical trials of nitrate and cognitive function

First author, year [Ref]	Nitrate source	Nitrate dose	Duration	Participants (mean ± SD age years)	Cognitive tests / CBF	Results
Acute						

Shannon et al., 2017 [168]	Beetroot Juice	775 mg, once	120 minutes	10 M (23 ± 3), healthy	Attention Switching task	No effect
					RVIP	No effect
					Spatial span task	No effect
Lefferts et al., 2015 [169]	Nitrate Bolus	400–450 mg, once	120 minutes	24 M (23 ± 3), healthy, active	Sensorimotor	No effect
					Memory	No effect
					Social cognitive domains	No effect
					Attention	No effect
					Executive Function	No effect
Bond et al., 2013 [108]	Beetroot Juice	750 mg, once	120 minutes	12 F (20.7 ± 0.3)	Transcranial Doppler Ultrasonography	Improved systemic and cerebral haemodynamics
Thompson et al., 2014 [167]	Organic Beetroot Juice	310 mg, once	100 minutes	16 M (24 ± 4), healthy, active	RVIP	No effect
					Stroop test	No effect
Wightman et al., 2015 [164]	Beetroot Juice	341 mg, once	90 minutes	12 M, 28 F (21 yrs), healthy	A 9-minute battery consisting of 4 min serial subtractions	Improved CBF and performed better on serial 3 subtractions.
					5 min RVIP	No effect
					A mental fatigue analogue scale, the three Bond-Lader mood factors	No effect
Chronic						
Thompson et al., 2015 [162]	Organic Beetroot Juice	794 mg/day	7 days, 150 minutes before the test	16 M (24 ± 5), team-sport players	Stroop test	Significant improvement in reaction time of response to the cognitive tests
					Decision reaction task	No effect
Kelly et al., 2013 [111]	Beetroot Juice	595 mg/day	2.5 days before the tests	6 M (64 ± 4), 6 F (63 ± 2), healthy	Serial Subtraction	No effect
					RVIP	No effect
					Number Recall	No effect
Gilchrest et al., 2014 [163]	Beetroot Juice	465 mg/day	Two weeks	18 M, 9 F (67.2 ±4 .9), Type 2 Diabetes Mellitus	Reaction time	Improved reaction time
					Decision reaction time	No effect
					Rapid processing	No effect
					Shape memory	No effect
					Spatial memory	No effect
Thompson et al., 2016 [165]	Beetroot Juice	397 mg/day	5 days, 2.5 hours before the test	36 M (24 ± 4), team-sport players	Stroop test	Improved RT
Presley et al., 2011 [124]	High Nitrate Diet	769 mg/day	2 days, 1 hour before the test	14 M (74.7 ± 6.9)	Arterial spin labelling magnetic resonance images to assess CBF	Improved perfusion in frontal lobe

Vahatalo et al., 2021 [166]	Nitrate Rich Beetroot Juice	750 mg/day	10 days	13 M, 17 F (73 ± 3)	RVIP, Stroop test, choice reaction time	Improved sustained attention (RVIP), no effect on Stroop and choice reaction time
Babateen et al., 2022 [170]	High Nitrate, Medium Nitrate, Low Nitrate	400 mg twice daily, 400 mg/day, 400 mg every other day	13 weeks	24 M, 38 F (66 ± 3), overweight and obese participants	Near infrared spectroscopy to assess CBF, COMPASS software to assess cognitive domains	No effect

Abbreviations: M, male; Mg, milligram; F, female; RT, reaction time; RVIP, rapid visual information processing; CBF, cerebral blood flow; SD, Standard Deviation; COMPASS, computerised mental performance assessment system.