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Dietary nitrate and brain health. Too much ado about nothing or a solution for dementia prevention?

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Abstract

Dementia is a significant public health priority with approximately 55 million cases worldwide, and this number is predicted to quadruple by 2050. Adherence to a healthy diet and achieving optimal nutritional status are vital strategies to improve brain health. The importance of this area of research has been consolidated into the new term 'nutritional psychiatry'. Dietary nitrate, closely associated with the intake of fruits and vegetables, is a compound that is increased in dietary patterns such as the Mediterranean and MIND diets and has protective effects on cognition and brain health. Nitrate is characterised by a complex metabolism and is the precursor of the nitrate–nitrite–nitric oxide (NO) pathway contributing to systemic NO generation. A higher intake of dietary nitrate has been linked to protective effects on vascular outcomes including blood pressure and endothelial function. However, the current evidence supporting the protective effects of dietary nitrate on brain health is less convincing. This article aims to provide a critical appraisal of the current evidence for dietary nitrate supplementation for improving brain health and provide suggestions for future research.

Keywords: **Dietary nitrate: Brain health: Cognitive function: Dementia**

The brain is an energetically expensive organ despite its small size (approximately 1·3–1·4 kg), accounting for approximately 20 % of resting energy expenditure[1]. From the moment of conception and through the various developmental stages, an optimal intake of energy and nutrients is essential for normal brain formation and neurocognitive development[2]. Environmental and/or genetic factors can affect nutritional status, especially if occurring during the early stages of development, and can often lead to various degrees of neurocognitive impairment[3]. Although rare, examples of genetic disorders include Prader–Willi syndrome, phenylketonuria and inherited metabolic disorders[4]. Examples of environmental factors include deficiencies of minerals (i.e. I, Fe) and vitamins (i.e., folic acid, vitamin A)[5]. This is particularly common in developing countries and still represents a public health concern.

Similar to other physiological parameters such as bone mass[6] and lung function[7], the trajectory of neurocognitive function is typically represented by the shape of a Maxwell–Boltzmann distribution curve[8]. This defines a maximum peak that is typically reached during early adulthood followed by progressive decline in performance with ageing[9]. Characterising the typical cognitive profile associated with a healthy ageing trajectory is fundamental for identifying key risk and protective factors and the development of intervention and risk reduction strategies. Indeed, factors influencing neurocognitive trajectories could be compared with vectorial forces shaping the direction and velocity of a given trajectory, which would represent the cumulative result of the forces applied by protective and risky factors at each time point during the life course of an individual. Fig. 1 provides a graphical model of this concept applied to a healthy (left graph) and abnormal (right graph) cognitive trajectory. The model illustrates the complex and dynamic interaction that may happen at any point in an individual's lifetime, which could shape the direction and velocity of the cognitive trajectory. The net balance between protective and modifiable/non-modifiable risk factors would determine the ascent rate and peak of the cognitive potential of an individual in early life; the subsequent rate of decline would be the result of the net effect of the ageing process, non-modifiable and modifiable protective and

Abbreviations: CBF, cerebral blood flow; MedDiet, Mediterranean Diet; NO, nitric oxide; NOS, nitric oxide synthase.

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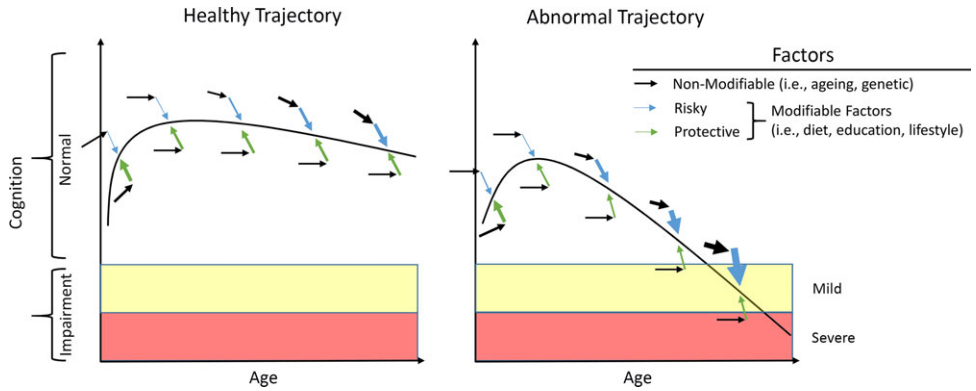

Fig. 1. This graph has been created based on the 'pendulum' model of disease risk[72]. The graph expands the concept by adding a vectorial dimension to the non-modifiable and modifiable factors that can shape the trajectories of cognition across the life course of an individual. First, a description of the key elements of the graph is needed. Arrows indicate vectorial forces resulting from the cumulative influences of protective (green) and risky (blue) modifiable risk factors. Black arrows indicate influence of modifiable risk factors on life course cognitive trajectories. The size of the arrows indicates the cumulative magnitude of the effects on the factors on the cognitive trajectories. The direction of the arrows indicates the applied cumulative force applied by factors to the cognitive trajectories. In a health trajectory, cognitive function achieves the greatest individual potential during the early life and starts to gradually decline as the influence of the ageing process (black arrows) progressively increases in magnitude but maintaining an overall normal cognitive function and staying well above the range of cognitive impairment (coloured areas). Influence of risky modifiable factors (blue arrows) may also increase later in life due to, for example, reduced physical mobility and diet quality. The abnormal trajectory on the right describes one of the possibly multiple scenarios leading to an accelerated cognitive decline that an individual may present during the life course with achievement of a lower cognitive potential followed by an accelerated cognitive decline due to greater net negative forces derived from the balance of non-modifiable and modifiable risky factors and modifiable protective factors. The result is an accelerated trajectory crossing into cognitive impairment and increasing the risk of developing severe cognitive impairment (i.e. dementia) within the lifetime of an individual.

risk factors. In the context of dementia risk, a greater downtrend of the trajectory would be given by a greater negative net balance leading to accelerated cognitive decline, cognitive impairment and, if protracted, development of dementia. The reversibility of the stages of cognitive impairment is a contentious area, but the general consensus is for interventions to follow the simple rule of thumb '*the earlier, the better*' as irreversibility may be difficult, if not impossible, once the onset of clinical dementia occurs[10].

Adherence to a healthy diet and achievement and maintenance of an optimal nutritional status are vital strategies to improve brain and cognitive health as captured in the term 'nutritional psychiatry'[11,12]. Research in this area has greatly expanded in the last two decades[13] with observational and interventional studies testing the influence of various nutrients (i.e. caffeine[14], polyphenols[15], PUFA[16], B vitamins[17], vitamin D[18], dietary nitrates[19]) and dietary patterns (i.e. Mediterranean Diet (MedDiet)[20], Dietary Approach to Stop Hypertension (DASH) and MIND diet[21]) on brain health alone or as part of multimodal interventions (i.e. Finger trial[22], Encore study[23]). While the evidence has been overall modest and conflicting on the protective effects of single nutrients, more convincing evidence has emerged from the investigation of holistic, nutritional approaches based on promoting a greater adherence to healthy dietary patterns[24]. Shannon *et al.*[25] demonstrated that a higher MedDiet adherence, defined by the Pyramid MedDiet score, was associated with better global cognition, memory and executive function in older (i.e. ≥ 60 years) UK adults recruited from the European Prospective Investigation into Cancer and Nutrition–Norfolk (EPIC-Norfolk). Further, the Predimed intervention trial showed that a MedDiet supplemented with olive oil or nuts was associated with improved composite measures of cognitive function after 4 years of follow-up in adults aged 55–80 years[26].

While these studies certainly have great potential for public health prevention, the mechanistic insights provided are limited as effects are likely to be derived from the synergy of different nutritional factors.

A compound that is increased in healthy dietary patterns, as it is closely associated with fruit and vegetable intake, is dietary nitrate[27]. It is estimated at the population level, in Western countries, that dietary nitrate intake is approximately 110 mg/d[28]. A previous review[27] estimated that the nitrate content of healthy dietary patterns, such as the MedDiet or DASH diet, could be 10-fold higher (approximately 1000–1200 mg/d) than the estimated average nitrate intake of Western populations (approximately 110 mg/d)[28] and considerably higher than the level of nitrate intake currently recommended by the WHO (3·7 mg/kg of body weight (corresponding to approximately 280 mg/d for a person with a body weight of 70 kg))[29]. The protective effects of higher levels of nitrate intake (approximately 400–800 mg/d) on cardiometabolic and neurocognitive health have been consistently reported in randomised trials[19,30]. Some studies have also suggested an interaction with ageing such that older individuals may need higher nitrate doses to elicit similar effects on vascular outcomes to those observed in younger groups[31,32].

Dietary nitrate and brain health

Inorganic nitrate is a water-soluble compound that can be found naturally in water and soil and is a fundamental component of

the nitrogen cycle[29]. Both nitrate and nitrite can be produced endogenously in humans via oxidation of nitric oxide (NO). Nitrate can be formed directly from the reaction between NO and oxy-Hb[33], while nitrite can be produced through auto-oxidation of NO, which is catalysed by plasma protein ceruloplasmin[34]. Both are considered end products of endogenous NO metabolism[35]. In animal studies, NO can be formed under acidotic conditions by the reduction of the large pool of systemic nitrite, and this formation is not blocked even after NO synthase (NOS) inhibition[36]. These findings have also been observed in humans after the infusion of 75 mg of sodium nitrite into the forearms of healthy individuals, resulting in blood flow increasing by 175 %. Interestingly, similar to the animal studies, the generation of NO was not blocked after NOS inhibition by the infusion of NG-monomethyl-L-arginine (NOS inhibitor). Therefore, it appears that systemic nitrite represents a storage pool for NO generation[37]. It has also been reported that nitrate can be used as a substrate for systemic nitrite formation after observing a significant increase in plasma nitrate and nitrite following a nitrate load[38]. All aforementioned studies have suggested that nitrate and nitrite can be recycled physiologically in tissues to synthesise NO independently of the enzymatic NOS pathway and are heavily dependent on the entero-salivary circulation of the nitrate pathway[35]. This pathway offers a backup system to promote NO production when endogenous NO generation via the NOS pathway is impaired[39].

NO is the biological effector of the putative protection that dietary nitrate exerts on brain function, and it has been found to be involved in learning and memory processes[40]. NO is a gaseous and highly reactive molecule that can diffuse quickly to surrounding tissues[41]. NO can be synthesised in neurons following the activation of N-methyl-D-aspartate receptors via the amino acid glutamate, and the first to observe this mechanism was Garthwaite *et al.*[42]. This activation leads to the influx of Ca^{++} into the nerve cell, thus activating NOS via Ca^{++}/calmodulin binding, which ultimately generates NO[43]. NOS is expressed in all brain cells, including vascular, neuronal and glial cells; thus, there is NO production in the brain[44], which has been implicated in cerebrovascular regulation. One of the mechanisms that underlie the regulation of cerebral blood flow (CBF) is neurovascular coupling[43]. There is also a growing body of evidence suggesting that NO plays substantial roles in various physiological processes, including the regulation of vascular resistance, neuromodulation and neurotransmission[45]. The neurotransmitter action of NO is achieved by stimulating soluble guanylate cyclase and forming a second messenger molecule, cyclic guanosine monophosphate[46]. NO is also involved in the modulation of synaptic functions, and the enhancement of synaptic activity has been shown to be mediated by the activation of soluble guanylate cyclase[47]. The loss of eNOS-generated NO via the NOS inhibitor has been shown to be related to the up-regulation of amyloid precursor protein expression, and an increase in Aβ, demonstrating the importance of endothelial NO in modulating amyloid precursor protein within the brain[48]. The NO-cyclic guanosine monophosphate pathway could be an essential therapeutic target in preventing neurocognitive impairment[48].

Dietary nitrate therefore has the mechanistic potential to influence brain functions; however, observational and clinical trials, overall, have contrasting results[19,49]. A meta-analysis conducted in 2018 exploring the effects of dietary nitrate supplementation on cognition and CBF[19] found a lack of evidence for the benefits of dietary nitrate on both outcomes. The review also highlighted the limitations of the studies (small sample size, short duration and use of healthy populations), which could have contributed to the limited efficacy of the dietary nitrate interventions. Since the publication of the review, additional studies[50,51,52,53,54,55,56] have been published on the topic; we have summarised in Fig. 2 a selection of studies that have investigated the effects of dietary nitrate on neurocognition and CBF following supplementation for at least 1 week and conducted in subjects at greater risk of cognitive impairment. Only two studies have concomitantly measured both neurocognition, CBF or brain metabolites[50,51], which was measured by magnetic resonance spectroscopy[51] and near-infrared spectroscopy[50]. Five studies[50,51,53,55,56] assessed changes in cognitive function, and three[51,55,56] reported significant changes in executive function, vigilance and motor skills. Four studies measured CBF or changes in brain metabolites[50,51,52,54] and the two studies reporting significant effects on CBF measured by MRI[54] and near-infrared spectroscopy[52] were conducted in participants with cardiovascular conditions suggesting greater benefits of dietary nitrate supplementation in individuals with reduced NO production. Nevertheless, while some promising results have been reported, the evidence is still contrasting. This could be because of the short study duration (longest duration was 13 weeks[50]), small sample size (largest sample size was sixty-two participants[50]) and recruitment of healthy individuals with no evidence of cognitive impairment.

Implications for research and future recommendations

Research into the potential applications of dietary nitrate as an aid to cognitive function is still in its infancy, and there is considerable scope for future investigation in this area. A schematic summary of the main priorities for future research is provided in Fig. 3. One approach which is starting to attract attention (see, e.g., Blekkenhorst *et al.*[57]), but could be further exploited, is the use of existing cohort studies to explore associations between nitrate intake with neurocognition and the risk of neurodegenerative diseases such as dementia. This relatively cost-effective approach could be applied to explore associations between dietary nitrate (including total nitrate intake and specific-nitrate-containing foods) and cognitive ageing in a real-world setting with longer follow-up durations and greater sample sizes than is typically feasibly in randomised clinical trials[58]. Such research may allow the identification of population sub-groups who may be particularly responsive to the effects of dietary nitrate and to identify potential effect moderators (e.g. genetic variants, age, sex, interactions with other dietary or lifestyle factors) which can then be used to inform the design of future randomised

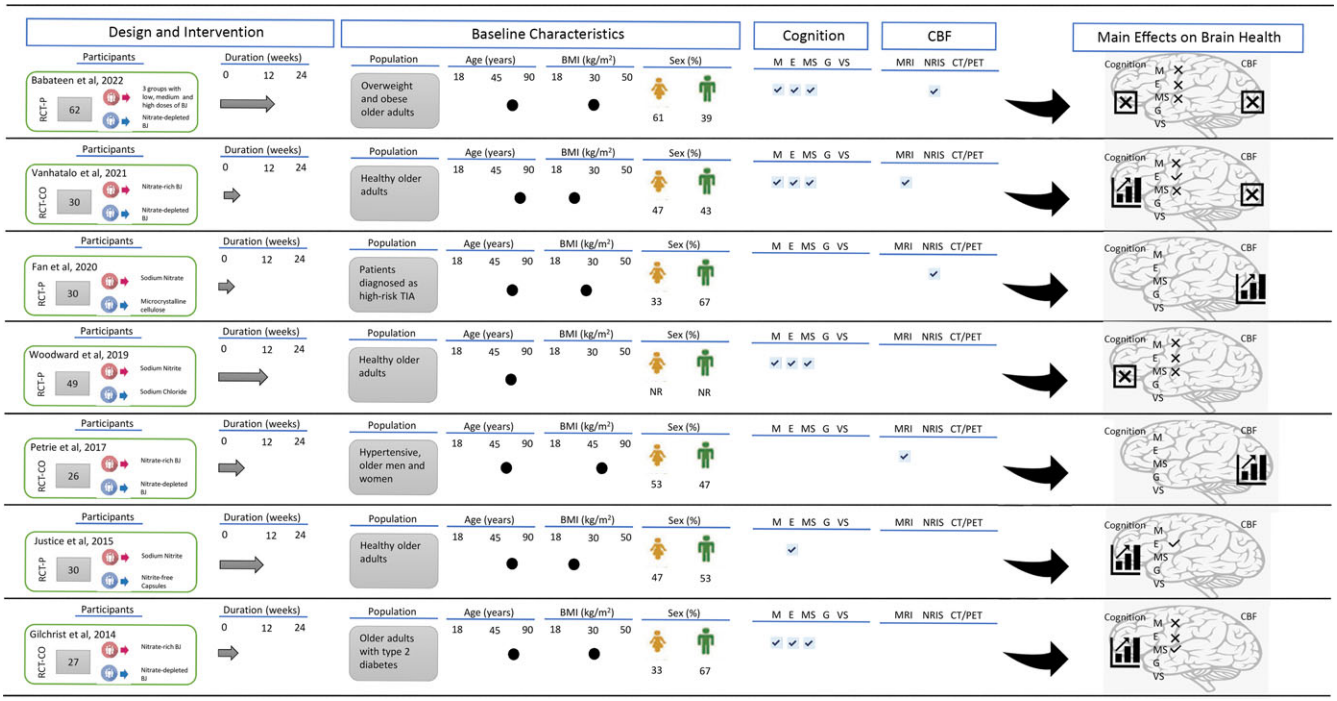

Fig. 2. GOfER diagram (Graphical Overview for Evidence Reviews) summarising main studies testing non-acute (duration of supplementation of at least 7 d) effects of dietary nitrate or nitrite on cognition and/or cerebral blood flow in humans. RCT, randomised clinical trial; P, parallel; CO, cross-over; BJ, beetroot juice; M, memory; E, executive function; MS, motor skills; G, global; VS, visuo-spatial; NRIS, near-infrared spectroscopy; CT, computerised tomography; PET, positron emission tomography; CBF, cerebral blood flow; TIA, transient ischaemic attack. The study by Vanhatalo *et al.* measured changes in brain metabolites using magnetic resonance spectroscopy.

clinical trials[58]. While results should be interpreted with some caution – observational studies do not allow us to infer cause and effect and may be subject to issues such as reverse causality and residual confounding – findings could complement those obtained from more labour-intensive randomised clinical trials[59].

Carefully designed randomised clinical trials are also needed to help better understand the efficacy of nitrate and mechanisms of action through which this polyatomic ion may influence neurocognitive function[19]. To date, most studies exploring the effects of nitrate on neurocognitive function are short in duration and use a small selection of cognitive tests which assess a limited set of cognitive domains (see Fig. 2). Larger trials with a longer duration of follow-up, ideally including multiple, comprehensive cognitive assessments over time to track cognitive trajectories, or assess hard clinical outcomes such as dementia incidence, would provide valuable insight. In this regard, it is possible that particularly demanding cognitive tasks are required to 'tease out' the potential benefits of nitrate on cognition. Future studies may wish to look at the potential additive or synergistic effects of administering nitrate as part of a combined intervention for improving cognitive ageing, whether alongside other dietary compounds which have shown promise in boosting cognition independently (e.g. *n*-3 fatty acids, sodium reduction[60]); dietary factors which may augment the effects of nitrate (e.g. polyphenols, vitamin C[61]) or parallel lifestyle interventions such as increased physical activity[62,63]. Most current trials use healthy participants, and studies are warranted in different populations, such as those with a degree of cognitive impairment or poor cardiovascular health (for whom nitrate could potentially improve cognition via direct effects on the brain and indirect effects via the improved cardiovascular function[64]), and individuals with low baseline NO status (e.g. older and obese individuals[65]). Such cohorts may be more responsive to the potentially beneficial effects of nitrate on cognition. Female participants are underrepresented in the nitrate literature, and future studies should seek to understand the effects of this polyatomic ion on cognition in both sexes rather than assuming similar responses in males and females[66].

Future studies may wish to exploit further use of novel imaging techniques (e.g. MRI, PET, near-infrared spectroscopy) to better understand the effects of nitrate on brain volume and function. Use of new 'omics' approaches (e.g. genomics, metabolomics, transcriptomics, proteomics), which provide insight into the cellular processes underpinning diet-related responses, could also provide valuable mechanistic insight[67], and so too could the measurement of biomarkers of neurodegenerative diseases such as β-amyloid deposition following prolonged nitrate supplementation. Animal model investigations have previously been used to explore physiological mechanisms of nitrate, particularly at the vascular and muscle levels[68,69], and may provide an opportunity to explicate brain-related changes occurring with nitrate supplementation. Nevertheless, results from animal studies of neurodegeneration should be treated with caution, as they do not fully account for the complexities of dementia in

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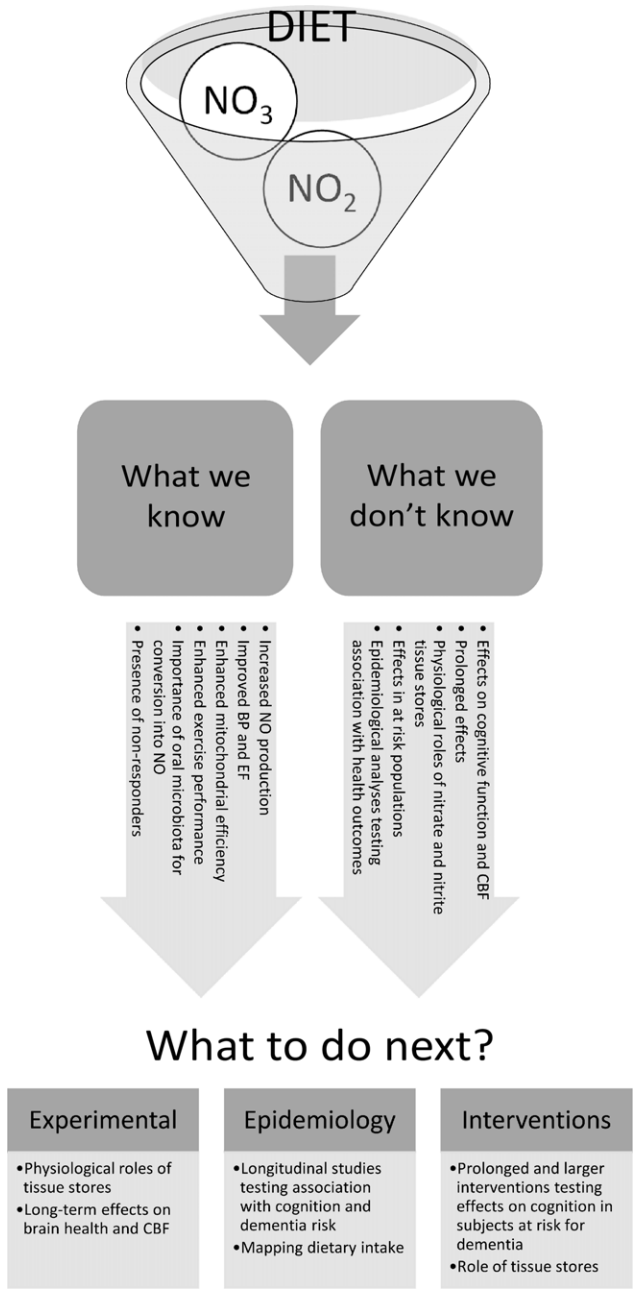

Fig. 3. Current evidence and proposal for a plan of action to conduct priority studies to advance knowledge on the effects of dietary nitrate (NO_3) and nitrite (NO_2) on brain health. NO, nitric oxide; BP, blood pressure; EF, endothelial function; CBF, cerebral blood flow.

humans[70]. Clearly, there is much work to do in this promising research area, and time will tell if consuming nitrate to improve cognition really is a 'NO brainer'.

Conclusions

In 2016, the NIH workshop on dietary nitrate[71] advocated for more epidemiological research and more robust randomised trials to better define the predictive role of dietary nitrate consumption in the prevention and treatment of chronic diseases. However, the impact on cognitive function and dementia risk was missing. The current evidence points towards the potential, protective role of dietary nitrate on brain health. However, the available evidence is limited. Most importantly, there are no data from large prospective studies on the association of dietary nitrate intake with cognitive impairment or dementia risk. Further, there is a lack of large and prolonged randomised trials conducted in subjects with or at risk of cognitive impairment. These studies are urgently needed, and for now, it is '*too much ado about nothing*' as there is still limited evidence.

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