IN SYMPHONY

Nutrient combinations for healthy brain ageing

Annick P. M. van Soest

Propositions

1. The WHO's advice against recommending nutrient supplementation for dementia prevention[1] is premature. (this thesis)

2. The preferred diet for healthy brain ageing depends on geographical region. (this thesis)

3. Publishing over 30 scientific papers per year conflicts with the ICMJE[2] recommendations for authorship.

4. PhD candidates require adequate didactic training prior to start teaching.

5. The default option for catered meals should be vegetarian.

6. The societal cultural bias towards extraversion hurts both introverts and extraverts.

Propositions belonging to the thesis, entitled

IN SYMPHONY: Nutrient combinations for healthy brain ageing.

Annick Pauline Marie van Soest
Wageningen, 24 June 2024

[1] World Health Organization. (2019). Risk reduction of cognitive decline and dementia: WHO guidelines. Geneva, Switzerland.
[2] International Committee of Medical Journal Editors

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NUTRIENT COMBINATIONS FOR HEALTHY BRAIN AGEING

Annick P. M. van Soest

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NUTRIENT COMBINATIONS FOR HEALTHY BRAIN AGEING

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Thesis

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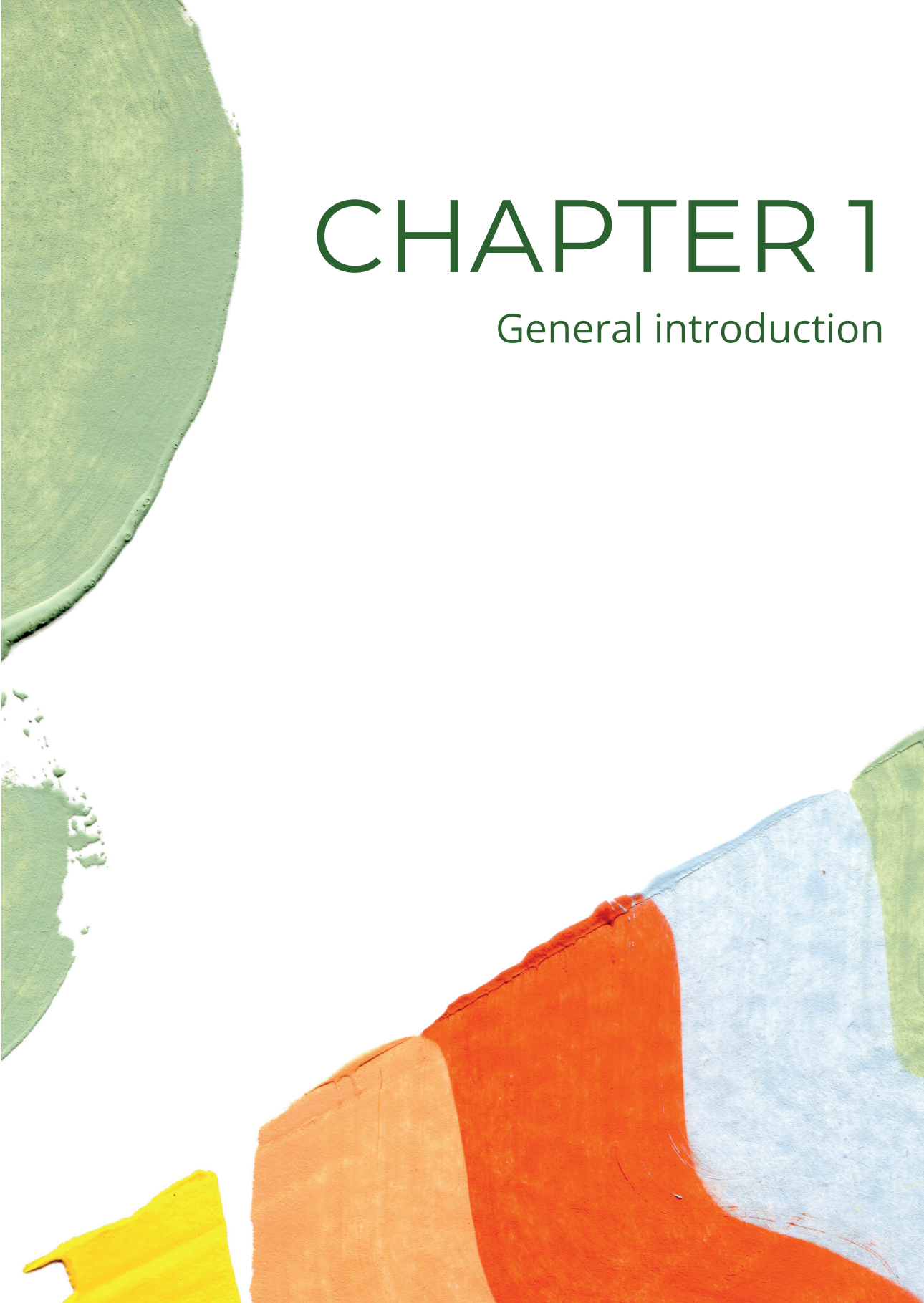
CHAPTER 1

General introduction



LONGEVITY

We are living longer than ever. Global life expectancy reached 72.8 years in 2019, an improvement of 9 years since 1990. The latest projections suggest further improvements in longevity for the coming 30 years, reaching an average global lifespan of 77.2 years in 2050 [1]. The fact that we are much more likely to live longer than our ancestors can be attributed to advances in healthcare, improvements in living conditions, and changes in lifestyle choices [1]. This increase in longevity should be considered as a great achievement of humanity. However, it also brings challenges: as longevity increases, so does the prevalence of age-related conditions.

THE AGEING BRAIN

One of the organs affected by ageing is the brain. With increasing age, the brain shrinks in volume, a process known as atrophy. This shrinkage is particularly pronounced in regions crucial for cognition, such as the frontal and temporal lobes, and the hippocampus [2]. The decline in brain volume is accompanied by several physiological changes: there is a decrease in the number of neurons and synapses, misfolded proteins called β-amyloid and tau accumulate in the brain, and the blood flow to the brain is reduced. Additionally, oxidative stress and inflammation increase [3]. Collectively, these alterations in brain physiology result in a decline in cognitive functioning [4].

Broadly, the age-related cognitive decline trajectory comprises four stages (**figure 1**) [5]. The first stage is normal cognitive ageing. Individuals who age healthily experience some degree of cognitive decline, such as mild forgetfulness, a slight decline in information processing, or problem solving, but within the normal range for their age. The second stage is called the preclinical stage, or 'subjective memory complaints'. This is when individuals subjectively perceive their cognitive abilities to decline at a faster rate than normal, but these changes cannot be captured with objective tests. This preclinical stage can further progress into mild cognitive impairment (MCI). The decline in cognitive function that is experienced in this phase can be captured with tests and is more pronounced than that typically seen with normal ageing. At the same time this is not severe enough to interfere with daily life. Finally, the stage 'dementia' is reached once the impairment in cognition gets even more severe and progressive, leading to impaired daily living.

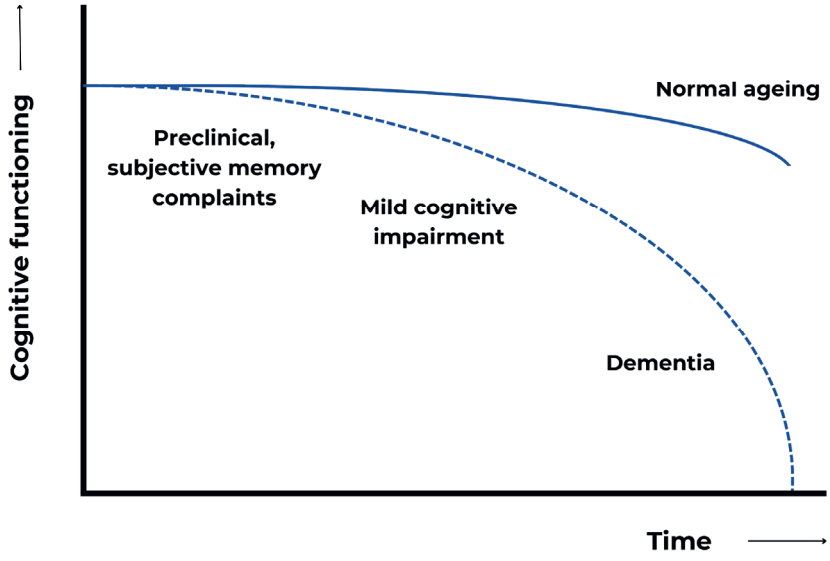


Figure 1: The continuum of brain ageing, adapted from Sperling 2011 [5].

Dementia is not a disease, but rather an umbrella term used to describe a set of symptoms relating to impaired brain functioning. These symptoms are progressive and include loss of memory, decline in language and problem solving, and the ability to perform everyday activities [6]. These symptoms have major social consequences: they are a cause of disability and dependency, burdening both the individuals living with dementia, as well as their family and caregivers [6]. Dementia also poses major economic consequences for society. In 2015, the worldwide costs for dementia were estimated at 818 billion US dollars. This amount is projected to double to 2 trillion US dollars in 2030, overwhelming health and social services [7]. Despite continuous efforts of the research community, there is currently no cure for dementia [8]. This lack of effective treatment options, in combination with the great social and economic burden, shows the urgent need to find strategies to prevent cognitive decline and dementia. One strategy to achieve this is by targeting modifiable risk factors.

RISK FACTORS

Risk factors for cognitive decline leading to dementia can be divided into two categories, non-modifiable and modifiable risk factors (**figure 2**).

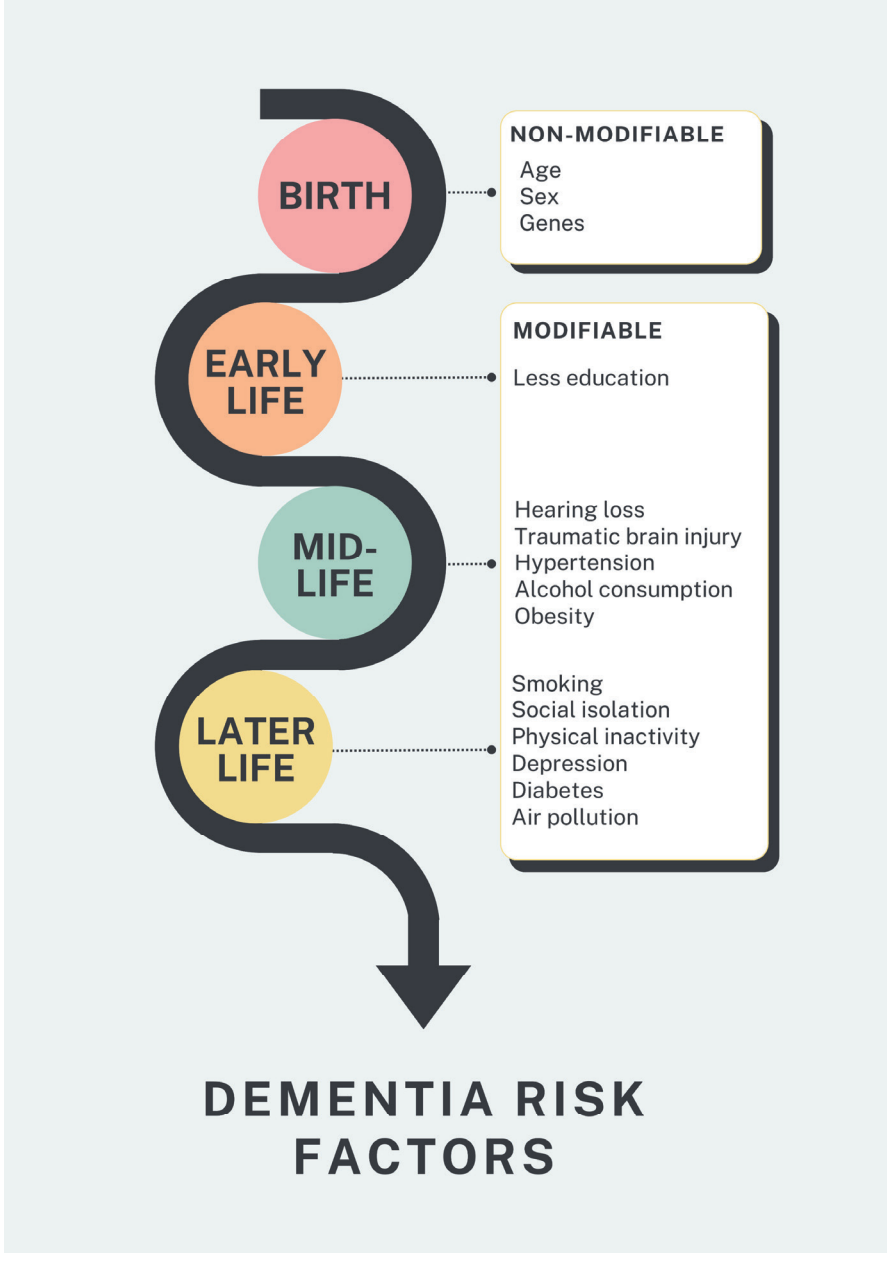


Figure 2: Non-modifiable and modifiable risk factors for cognitive decline leading to dementia, adapted from Livingston 2020 [9].

NON-MODIFIABLE RISK FACTORS

Non-modifiable risk factors are estimated to be responsible for 60% of dementia cases. Age is considered the most important risk factor for dementia, with the risk of dementia doubling with every 5 year increase in age [10, 11]. Also sex is a risk factor for dementia: women are twice as likely to develop dementia during their lifetime compared to men. The main driver for this greater risk is the longer lifespan of women [12]. Finally, genes influence dementia risk. A major risk factor for late onset dementia is the Apolipoprotein E (ApoE) gene, which is involved in cholesterol and triglyceride metabolism. The gene comes in three allelic variants, ε2, ε3 and ε4, of which the ε2 variant is protective and the ε4 variant increases dementia risk [13]. In addition to the ApoE gene, there are three genes that increase risk of early onset dementia: amyloid precursor protein, presenilin 1, and presenilin 2 [14].

MODIFIABLE RISK FACTORS

According to the Lancet commission on dementia prevention, intervention and care, the remaining 40% of dementia cases are caused by modifiable risk factors, and are therefore to a certain extent preventable [9]. Preventive factors for dementia are relevant already during early life, as higher levels of education during childhood and higher overall educational attainment could reduce dementia risk by 7%. In midlife, five factors are associated with dementia risk: hearing impairment, traumatic brain injury, hypertension, alcohol consumption (>21 units per week) and obesity (body mass index ≥30 kg/m^2). In total, modification of these factors is estimated to prevent 15% of dementia cases. Finally, still 18% of dementia cases can be prevented in later life, after the age of 65 years. Risk factors that influence dementia risk in later life comprise lifestyle factors (smoking, social isolation, and physical inactivity), diseases (depression and diabetes), and the environmental factor air pollution [9].

Except for alcohol consumption, the Lancet commission on dementia prevention, intervention and care does not consider nutrition a stand-alone modifiable risk factor. Yet, nutrition is a determinant of several of the modifiable risk factors, including obesity, depression and diabetes. Thereby, nutrition may indirectly influence dementia risk.

NUTRITION FOR HEALTHY BRAIN AGEING

Numerous studies have demonstrated a possible link between various nutrients and healthy brain ageing [15]. Relevant nutrients include B-vitamins, omega-3 fatty acids, vitamin D, anti-oxidants and polyphenols.

B-VITAMINS

The B-vitamins B6, B12 and folic acid/folate have been extensively studied in relation to healthy brain ageing, primarily because of their involvement in homocysteine metabolism. Homocysteine is an amino acid, and an elevated level of this compound is a risk factor for cognitive decline and dementia [16]. B-vitamins reduce homocysteine levels via two pathways: 1) vitamin B12 and folic acid are involved in the conversion of homocysteine into methionine, and 2) vitamin B6 is important for the transformation of homocysteine into cysteine [17]. There is no consensus on the biological mechanism via which homocysteine affects brain ageing. Hypotheses include vascular mechanisms and the inhibition of methylation reactions [18].

The associations of homocysteine with brain ageing are well-established. Higher homocysteine levels have been associated with a wide variety of brain-ageing outcomes, including faster rates of cognitive decline and increased risk of dementia [16, 18], brain atrophy, white matter hyperintensities, and density of neurofibrillary tangles [18]. However, associations of B-vitamin status and intake with these outcomes are less pronounced. For example, a meta-analysis of 11 cohort studies showed a protective association between serum folate and dementia risk, but folate intake, and vitamin B6 and B12 intake and status were not associated with lower risk of dementia [19].

While it seems a straightforward solution to supplement with B-vitamins to lower homocysteine levels and thereby slow cognitive decline, interventional studies on the effect of B-vitamin supplementation have mostly shown null-results. A large meta-analysis combining data from 11 trials showed that although B-vitamin supplementation was effective in lowering homocysteine levels, it did not impact cognitive function [20]. Three other meta-analyses confirm these results, further suggesting that the reduction in homocysteine levels through B-vitamins does not translate into measurable cognitive benefits [21-23].

OMEGA-3 FATTY ACIDS

Another group of nutrients that has been widely investigated for its neuroprotective properties are omega-3 fatty acids, with primary interest in the two types of omega-3 fatty acids found in fatty fish: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids are involved in different mechanisms shown to be important to maintain brain health [24]. Importantly, DHA is a major structural component of neuronal cell membranes and plays an important role in the

maintenance of integrity and fluidity of cell membranes. Also, omega-3 fatty acids possess anti-inflammatory properties. Chronic low-grade inflammation is an important mechanism contributing to cognitive decline leading to dementia, and omega-3 fatty acids, particularly EPA, have anti-inflammatory effects to mitigate (neuro)inflammation. Furthermore, omega-3 fatty acids also possess anti-oxidant effects, protecting brain cells from oxidative stress. Finally, omega-3 fatty acids may promote brain health via their vascular health promoting properties, as EPA and DHA may help regulate blood pressure, improve lipid profile, and improve endothelial function [24].

Observational research is generally in line with these proposed mechanisms. For example, a higher intake of fish rich in omega-3 fatty acids and of DHA, but not of EPA, was associated with reduced risk of dementia in a meta-analysis of 21 cohort studies. This same meta-analysis did not demonstrate associations between the status of DHA or EPA separately with risk of cognitive decline or dementia [25], but studies that used the omega-3 index (EPA + DHA) generally demonstrated protective associations [26]. Furthermore, higher omega-3 levels were associated with larger hippocampal, grey matter, and total brain volume, and fewer white matter hyperintensities [27].

In contrast with these mechanistic roles and the observational evidence, intervention studies investigating the protective effects of omega-3 fatty acid supplementation on brain ageing are largely negative. A meta-analysis that combined data from 10 intervention studies demonstrated no overall effect of omega-3 supplementation on various cognitive outcomes, including global cognition, various forms of memory, executive functioning and attention & processing speed. Only subgroup analyses revealed possible benefits for cognitively impaired individuals without dementia diagnosis on immediate recall and attention & processing speed [28]. Another meta-analysis of 17 interventions showed a small benefit of omega-3 supplementation on memory in non-demented adults, but not for global cognition or other cognitive domains [29].

VITAMIN D

Another nutrient of prime interest to the ageing brain is vitamin D. The neuroprotective effects of vitamin D are likely mediated by the vitamin D receptor, which is present in various regions in the brain important for cognition [30]. Binding of vitamin D to this receptor initiates several mechanisms. For instance, binding can trigger anti-inflammatory and anti-oxidant responses, and regulate calcium

homeostasis in the brain which is crucial for proper neuronal function. Also, vitamin D receptor activation can influence β-amyloid accumulation, by decreasing production and increasing clearance [31, 32].

In line with the proposed mechanisms of action, adequate vitamin D status is associated with favourable brain ageing outcomes. Various meta-analyses demonstrated that low 25-(OH) vitamin D concentrations were associated with worse cognitive functioning and steeper rates of cognitive decline [33], increased risk of cognitive impairment [34] and dementia [35, 36], and smaller brain volume [37].

However, intervention studies on the effect of vitamin D supplementation on brain ageing all produced negative results. Six large intervention studies (n=184 to 4,019) demonstrated no effect of vitamin D supplementation on various domains of cognitive functioning [38-41] and generic screening test performance (MMSE, TICS) [41-43]. Similarly, MCI and dementia incidence were not affected by long term (mean 7.8 years) supplementation of vitamin D combined with calcium in another large intervention study (n=4,143) [41].

ANTIOXIDANT NUTRIENTS

The antioxidant nutrients, vitamins C, E and carotenoids, have also been extensively studied for their potential benefits for brain ageing. This is because of their ability to counteract oxidative stress: a condition in which there is an imbalance between the production of reactive oxygen species and the body's ability to neutralize these compounds [44]. Oxidative stress is harmful to human health in general, but especially detrimental to the brain. This is because of the brain's high metabolic activity, and the abundance of polyunsaturated fatty acids that are highly susceptible to oxidative damage [44]. Indeed, oxidative stress is considered an important mechanism underlying brain ageing and it has been implicated to play a role in the pathogenesis of dementia [45].

Despite the strong mechanical basis for benefits of antioxidants on brain ageing, observational research shows mixed outcomes.

Most observational research on **vitamin C** intake reported no benefits for cognition [46, 47], risk of dementia [48, 49] or Alzheimer's disease (AD) [48, 50, 51]. One study reported that higher vitamin C intake was associated with lower AD incidence after 6 years [52] but this was no longer apparent after 10 years [49]. For vitamin C status, however, beneficial associations were demonstrated. A large meta-analysis

combining data from 16 studies reported that AD had lower plasma vitamin C levels compared to cognitively healthy individuals [53].

The observational evidence for **carotenoids** is similar, with null-associations for intake and protective associations for status. Higher intake of carotenoids was not associated with cognition [46], or risk of dementia [48, 49] or AD [48, 50-52]. With respect to carotenoid status, a recent meta-analysis combining data from 23 observational studies showed that dementia patients had lower lycopene, α-carotene, β-carotene, zeaxanthin and β-cryptoxanthin levels compared to controls [54]. Evidence for AD is limited to two carotenoid subgroups: higher levels of lutein and zeaxanthin, but not α-carotene, β-carotene, lycopene and β-cryptoxanthin, were associated with a decreased risk of AD according to a meta-analysis pooling data from 16 observational studies [55].

Observational evidence for **vitamin E** is largely positive. Higher intake of vitamin E was associated with lower AD risk in 7 out of 10 studies [56] and lower vitamin E status was associated with an increased risk of developing AD in 3 out of 3 studies [56]. Finally, a meta-analysis pooling data from 31 case-control studies revealed lower plasma vitamin E levels in AD patients compared to cognitively healthy individuals [53].

The results from intervention studies on the effect of supplementation of antioxidants on brain ageing are mixed. Short term supplementation (<3 years) with β-carotene did not impact cognition, but 15 years of supplementation did produce beneficial effects on cognition [57]. The outcomes of intervention studies with vitamin E are mixed, with one demonstrating no effect of supplementation on cognition [58], and another intervention showing a decline in disease progression in AD patients [59]. Furthermore, supplementation with vitamin E in combination with vitamin C did not affect cognition [60], and supplementation with a combination of vitamin E, C and β-carotene was not effective in slowing cognitive decline in three [61-63] of four studies [64].

POLYPHENOLS

Polyphenols have been widely explored for their neuroprotective effects. These plant metabolites are involved in many different mechanisms important to maintain brain function during ageing. Similar to several of the nutrients discussed before, polyphenols also possess anti-inflammatory, anti-oxidant and vascular-health promoting properties [65]. In addition, some polyphenols may have indirect

neuroprotective effects via the gut-brain axis, via bi-directional interactions with the gut microbiome [66].

Observational research points towards benefits for the ageing brain for some specific classes of polyphenols. Higher intake of flavonoids was associated with better cognition, slower rates of cognitive decline and lower dementia risk [67]. Among the flavonoid classes, higher intakes of anthocyanidins, flavan-3-ols, flavonols and flavones were associated with lower dementia risk. Evidence for other classes of flavonoids and polyphenols is too limited to draw conclusions [67].

Intervention studies overall confirm the mechanistic and observational evidence. A recent meta-analysis on the effects of flavonoids on cognition demonstrated small benefits in middle-aged adults (n=22, hedge's g=0.112) and older adults (n=29; hedge's g=0.176) [68]. A systematic review on this same topic overall confirms these findings, but also notes that more research is needed due to high heterogeneity between studies and their relatively small sample sizes [69].

FROM SINGLE- TO MULTIPLE-NUTRIENT APPROACHES

Considering the collective body of evidence, a clear pattern becomes evident. Mechanistic studies provide a solid basis for the effects of various single nutrients on brain ageing, acting via various mechanisms including inflammation, oxidative stress, or vascular dysfunction. Hypotheses that can be derived from these mechanistic studies are generally supported by the large majority of observational research, showing proof for associations between better intake and/or status of the nutrients and healthier brain ageing. Interventional research, however, has so far generally failed to demonstrate positive effects of supplementation with single nutrients on maintaining brain health during ageing. This raises the question of which factors are responsible for the failing translation from mechanical and observational research to interventional research in single-nutrient research. Our overarching hypothesis to explain this gap is based on the fact that, thus far, too little attention has been paid to nutrient interactions. People do not consume single nutrients in isolation, but rather consume a diet that is rich in a combination of nutrients. Through synergistic and antagonistic interactions between these nutrients, the 'whole' can differ from the sum of its parts. The importance of considering these interactions between nutrients is evidenced by the fact that the mechanisms underlying nutrition and brain ageing are multifactorial [70]. In addition, observational evidence for dietary patterns rich in nutrients of interest is

stronger compared to evidence for single nutrients [15] and interventional studies investigating the long-term effect of adopting a healthy dietary pattern are largely positive [71].

AIM AND OUTLINE OF THIS THESIS

Mechanistic and observational research suggests a promising role for several nutrients in slowing brain ageing, though interventional studies cannot confirm the positive effects. This discrepancy between observational and interventional research demands for a shift of focus from single- to multi-nutrient strategies. To this end, the overall aim of this thesis is to investigate to what extent combinations of specific nutrients are beneficial for healthy brain ageing. This thesis is divided into two parts. In the first part we will study the effect of interactions between nutrients on brain ageing. The focus of the second part will be on dietary patterns that combine different nutrients relevant to maintaining brain function during ageing. Eventually, this thesis aims to give insight into the interactive properties between nutrients on the brain during ageing.

Chapter 2 and **3** explore the interaction between folic acid and vitamin B12 and omega-3 fatty acids in relation to cognitive ageing in two different cohorts of cognitively healthy older adults. **Chapter 4** describes how combined suboptimal status of omega-3 fatty acids, homocysteine and vitamin D relate to dementia incidence. **Chapter 5** investigates how intake of foods is related to microbiota composition and cognitive functioning in healthy older adults. In the next two chapters, adherence to a plant-based diet (**chapter 6**) and the EAT-Lancet diet (**chapter 7**) are studied in relation to cognition and cognitive decline. **Chapter 8** presents a systematic literature review on the current evidence on the MIND diet and the ageing brain. Finally, **chapter 9** provides an overall reflection on the findings of this thesis, and proposes directions for further research.

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CHAPTER 2

Positive effects of folic acid supplementation on cognitive ageing are dependent on omega-3 fatty acid status

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ABSTRACT

Background: Although epidemiological studies suggest a protective role of B-vitamins and omega-3 fatty acids in cognitive decline, findings from intervention studies are conflicting. Mechanistic studies suggest that omega-3 fatty acid status can modulate effects of B-vitamins on cognitive decline.

Objective: We investigated the interaction between baseline omega-3 fatty acid status and folic acid treatment on cognitive decline.

Design: We used data from the FACIT trial, a randomized placebo-controlled trial investigating the effect of folic acid supplementation on cognition. A total of 791 older adults aged 50-70 years with plasma total homocysteine ≥13μmol/L and ≤26μmol/L and serum vitamin B12 ≥200pmol/L received 800μg folic acid or placebo daily for three years. Global cognitive functioning and domain-specific functioning (episodic memory, information processing speed, executive functioning) was assessed at baseline and after three years. The effect of the folic acid supplementation was analyzed according to tertiles of baseline omega-3 fatty acid concentrations using linear multiple regression.

Results: Mean age of the study population was 60.2±5.6y, and mean Mini-Mental State Examination score was 28.6±1.5. The treatment effect of folic acid was significantly larger in participants in the low compared to high omega-3 fatty acid tertile for global cognition (difference in Z-score 0.163±0.059 (mean±SE), $p<0.01$). Regarding domain-specific functioning, similar results were observed for information processing speed (0.167±0.068, $p=0.01$). There was no overall interaction between folic acid treatment and omega-3 fatty acid tertile for episodic memory ($p=0.14$) and executive functioning ($p=0.21$).

Conclusion: This post-hoc analysis revealed that the efficacy of folic acid treatment on cognitive functioning is dependent on omega-3 fatty acid status. Individuals with lower omega-3 fatty acid status at baseline benefited from folic acid treatment, while individuals with higher omega-3 fatty acid status did not. The results potentially explain the inconsistency in outcomes of B-vitamin supplementation trials and emphasize the importance of a personalized approach.

Keywords: B-vitamins, cognitive functioning, folic acid, omega-3 fatty acids, older adults, elderly, healthy ageing

INTRODUCTION

Dementia and cognitive decline are a public health priority. Due to population ageing, the number of people suffering from dementia is expected to triple the upcoming 30 years to 150 million in 2050. This steep increase in prevalence will pose a great social and economic impact on caregivers, families and society [1]. The great burden and impact, along with the lack of effective treatment options, create an urgent need for strategies to prevent or slow down disease progression at an early stage. Nutritional interventions are considered promising strategies, with special interest for B-vitamins and omega-3 fatty acids [2].

Inadequate levels of the B-vitamins folic acid, vitamin B6 and B12 result in accumulation of the amino acid homocysteine, which is a risk factor for cognitive decline and dementia [3, 4]. Supplementation with these B-vitamins has been shown to lower homocysteine levels, yet studies regarding the effectiveness of B-vitamin supplementation on cognitive decline show conflicting results [5]. Similarly, there is no consensus on the role of omega-3 fatty acids in cognitive decline and dementia. Although epidemiological studies support their protective role in cognitive decline [6, 7], intervention studies on omega-3 fatty acid supplementation are inconsistent [8].

The extent of cognitive impairment and baseline nutrient and homocysteine status are important factors explaining these mixed results [9-11], but more factors are thought to be involved. Interestingly, mechanistic studies suggest a link between omega-3 fatty acids and homocysteine at the level of phospholipid metabolism [12]. Therefore, it is hypothesized that sufficient availability of both omega-3 fatty acids and B-vitamins is crucial to inhibit the neuropathology underlining age-related cognitive decline and dementia.

Preliminary proof supporting this hypothesis comes from two studies showing that supplementation with a combination of vitamin B6, B12 and folic acid was more effective in slowing down cognitive decline [13] and brain atrophy [14] in subjects with mild cognitive impairment (MCI) having a high compared to a low omega-3 fatty acid status. In line with this, Jernerén and colleagues showed that positive effects of omega-3 fatty acid supplementation on cognitive functioning in Alzheimer's disease (AD) subjects were only present in individuals who had lower homocysteine levels at baseline [15]. The current analysis was undertaken to further investigate this interaction and to study whether it also occurs in healthy older adults without cognitive complaints. To this end, we investigated the interaction between baseline

omega-3 fatty acid status and folic acid treatment on cognitive decline in healthy older adults with elevated homocysteine levels in the FACIT trial.

METHODS

STUDY DESIGN AND PARTICIPANTS

Data used for this post-hoc analysis are from the FACIT study, a randomized, double-blind placebo-controlled trial investigating the effect of folic acid supplementation on carotid-intima-media thickness. Cognitive functioning was measured as a secondary outcome. Data were collected between 2000 and 2004 in the Netherlands. The trial has been registered at clinicaltrials.gov with NCT00110604. The study has been approved by the Medical Ethics committee from Wageningen University and Research and participants have given written informed consent. Details on recruitment and participants have been described previously [16]. Briefly, 819 men and post-menopausal women aged 50-70 years with elevated plasma total homocysteine concentrations (≥13μmol/L) were randomized to either supplementation with 800μg folic acid or placebo once daily for three years. Participants with plasma homocysteine concentrations >26μmol/L or serum vitamin B12 concentrations <200pmol were excluded. Our analysis included data of 791 participants. Data from 28 participants were excluded due to missing fatty acid (n=11), cognitive testing (n=1) or follow-up (n=16) data (**supplementary figure 1**).

COGNITIVE TESTING

Cognitive performance was assessed at baseline and after three years of intervention with a battery of five cognitive tests derived from the Maastricht Ageing Study [17].

CONCEPT SHIFTING TEST (CST)

The CST [18] assesses the ease of concept switching and is a measure of executive functioning. The test comprised four subtests, in all of which the participant was presented with a screen containing 16 small circles arranged in a large circle. In the four consecutive subtests, the small circles were either empty, contained a letter, a number, or both letters and numbers. Participants were instructed to cross out the numbers in chronological order (part A), the letters in alphabetical order (part B), to alternate crossing out numbers and letters in chronological and alphabetical order (part C) and to cross out all empty circles in any random order (subtest blank). The time to complete each of the four subtasks was documented.

Stroop Color-Word test

The Stroop Color-Word test [19] assesses cognitive interference and is a measure of executive functioning. The test consisted of three subtests, in which the participant was presented with stimuli, either written color names or colored blocks. The participant was asked to name the color names printed in black ink (subtest words), the color of the blocks (subtest colors) and the color of the ink while this color was incongruent with the written word (subtest colored words). Time to complete each of the three subsets was measured.

Word Learning Test (WLT)

The WLT [20] is a measure of memory. A total of 15 monosyllabic words were shown in a fixed sequence for two seconds each. Immediately after presentation, the participants were asked to recall the words. This procedure was repeated three times (immediate recall). Twenty minutes after the last presentation of the words, the participant was asked to recall the words again (delayed recall). The number of correctly recalled words was recorded.

Letter Digit Substitution Test (LDST)

The LDST [21] assesses information processing speed. Nine different letters are paired with the numbers 1 to 9. The participants were shown a random series of letters and were asked to match the corresponding number to the letter as fast as possible. The number of correctly matched numbers and letters in 90 seconds was documented.

Verbal Fluency Test

The Verbal Fluency Test [22] measures verbal functioning. The participants were given one minute to name as many animals as possible. The number of uniquely named animals was documented.

BIOCHEMICAL ASSAYS

At baseline, blood was obtained via venipuncture after an overnight fast and collected in Vacutainer® EDTA tubes. Following centrifuging, plasma was obtained and stored within two hours at -80 degrees Celsius until analysis. Concentrations of fatty acids were measured in plasma cholesteryl esters by gas chromatography using a modified version of a previously described protocol [6]. In short, 650 µL EDTA plasma was extracted using hexane, followed by isolation of the cholesteryl fraction by solid phase extraction using silica columns. Subsequently, fatty acids in cholesteryl esters were trans-methylated using sulphuric acid in methanol. After extraction with hexane, individual fatty acid methyl-esters were separated by gas

chromatography and detected by flame ionization. Peaks were identified based on comparison of retention times to known standards. Data on omega-3 fatty acids as methyl esters are presented in relative concentrations of total fatty acid methyl esters. Total relative plasma omega-3 fatty acids was derived by adding the relative concentrations of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The inter-assay coefficients of variability for EPA and DHA were 4.35% and 4.62%, respectively.

Apolipoprotein genotype was determined by polymerase chain reaction of DNA with the restriction enzyme HhaI [23]. Plasma total homocysteine and vitamin B6 were measured using high-performance liquid chromatography [24, 25]. Chemiluminescent immunoassays were used for the determination of vitamin B12 and folate levels (Immulite 2000, Diagnostic Products Corporation).

STATISTICAL ANALYSIS

Cognitive composite scores were created to limit the number of dependent outcomes and reduce the chance of type I error. Z-scores for each cognitive test at baseline and after three years of follow-up were standardized to the mean and standard deviation at baseline. Z-scores for CST and Stroop Color-Word test were reversed, as lower outcomes for these tests represent better cognitive functioning. The individual tests Z-scores were clustered into global cognition and three domain-specific Z-scores:

$$Global\ cognition = (zWLT_{total} + zWLT_{max} + zWLT_{delayed} + -zCST_{numbers\&letters} + -zStroop_{words\&colors} + zLDST + -zStroop_{interference} + -zCST_{shift} + zVerbal\ Fluency)/9$$

$$Episodic\ memory = (zWLT_{immediate\ total} + zWLT_{immediate\ max} + zWLT_{delayed})/3$$

$$Information\ processing\ speed = (-zCST_{numbers\&letters} + -zStroop_{words\&colors} + zLDST)/3$$

$$Executive\ functioning = (-zStroop_{interference} + -zCST_{shift} + zVerbal\ Fluency)/3$$

Stroop interference, Stroop words & colors, CST shift and CST numbers & letters were calculated as follows:

$$Stroop_{interference} = Stroop_{colored\ words} - \frac{Stroop_{words} + Stroop_{colors}}{2}$$

$$Stroop_{words\&colors} = \frac{Stroop_{words} + Stroop_{colors}}{2}$$

$$CST_{shift} = CST_{partC} - CST_{blank} - \frac{CST_{part\ A} - CST_{blank} + CST_{part\ B} - CST_{blank}}{2}$$

$$CST_{numbers\&letters} = \frac{CST_{part\ A} - CST_{blank} + CST_{part\ B} - CST_{blank}}{2}$$

Data are expressed as n (%) or mean (SD) unless otherwise stated. Differences in baseline characteristics between groups were analyzed using independent sample t-test and ANOVA for continuous variables and chi-square tests for categorical variables. Multiple linear regression was used to investigate if omega-3 fatty acid status modifies the treatment effect of folic acid. The change in Z-score between baseline and after three years of follow up for each specific domain was modelled as a function of treatment (folic acid or placebo), baseline omega-3 fatty acid status (low, middle, high) and their interaction. Omega-3 fatty acid status was based on tertiles of baseline omega-3 fatty acid concentrations. To investigate whether the interaction was driven by either EPA or DHA, additional analyses were run using baseline EPA and DHA status separately instead of baseline omega-3 fatty acid status. Moreover, additional analysis including ApoE4 status in the interaction were run to determine the role of ApoE4 carrier status in explaining the interaction. The final model was adjusted for the covariates baseline cognitive Z-score, age, sex, level of education (divided into three groups), ApoE4 status, baseline homocysteine concentration and baseline body mass index. The covariates physical activity, cardiovascular disease and diabetes mellitus were assessed but are not included in the model as this did not lead to improvement. Tukey correction was used for multiple testing of the treatment effects within the omega-3 fatty acid tertiles. Three strongly deviating individual test Z-scores with values of ≤-10 were removed from the analysis to warrant normality of residuals and heterogeneity of variance. Running the analysis including these values did not alter conclusions. A p-value of <0.05 was considered significant, with exception of p-values for interactions where p<0.1 was considered significant. All statistical analyses were performed using RStudio Version 1.1.463 [26].

RESULTS

PARTICIPANT CHARACTERISTICS

Baseline characteristics of the 791 participants are presented in **table 1**. The mean age of the total study population was 60.2±5.6 years, 71.4% of the participants was male. Mean baseline homocysteine level was 13.3±2.9 µmol/L and mean Mini-Mental State Examination (MMSE) score was 28.6±1.5. A larger proportion of the

participants in the folic acid group had received low education (p=0.021) and suffered from cardiovascular disease (p=0.035) compared to participants in the placebo group. There were no further differences in baseline characteristics between intervention groups. With respect to omega-3 fatty acid status, participants in the middle omega-3 fatty acid tertile were more physically active (p<0.01) and higher baseline vitamin B12 levels were observed in participants in the high omega-3 fatty acid tertile (respectively, p=0.023; p=0.012) (**supplementary table 1**). Mean baseline cognitive scores for global cognition and all three cognitive domains (episodic memory, information processing speed and executive functioning) did not differ between either intervention or baseline omega-3 fatty acid status groups.

Table 1: Baseline characteristics per treatment group in the FACIT study[1]

Characteristic	Overall (n=791)	Folic acid (n=391)	Placebo (n=400)	p-value
Age (years)	60.2±5.6	60.0±5.5	60.4±5.7	0.35
Sex n (%)				
Male	565 (71.4%)	282 (72.1%)	283 (70.8%)	0.73
Female	226 (28.6%)	109 (27.9%)	117 (29.3%)	
Level of education n (%)				
Low	178 (22.5%)	104 (26.6%)	74 (18.5%)	0.02
Middle	300 (37.9%)	137 (35.0%)	163 (40.8%)	
High	313 (39.6%)	150 (38.4%)	163 (40.8%)	
BMI (kg/m^2)	26.5±3.6	26.6±3.6	26.5±3.6	0.62
Physical activity (PAI score)	153±69	154±71	153±68	0.82
Current smoker n (%)	159 (20.1%)	81 (20.7%)	78 (19.5%)	0.74
Diabetes Mellitus n (%)	24 (3.0%)	10 (2.6%)	14 (3.5%)	0.57
Cardiovascular disease n (%)	93 (11.8%)	56 (14.3%)	37 (9.3%)	0.04
ApoE4 carriers n (%)	248 (30.9%)	124 (31.8%)	124 (31.2%)	0.93
Total homocysteine (µmol/L)	13.3±2.9	13.3±2.6	13.3±3.1	0.99
Vitamin B6 (nmol/L)	35.7±19.1	36.3±20.6	35.2±17.6	0.40
Folate (nmol/L)	12.5±4.5	12.4±4.3	12.6±4.6	0.50
Vitamin B12 (pmol/L)	315±105	317±114	313±96	0.53
DHA (%)*	0.60±0.21	0.60±0.22	0.59±0.20	0.85
EPA (%)*	1.11±0.69	1.14±0.76	1.08±0.61	0.26
Sum EPA and DHA (%)*	2.32±0.86	2.34±0.95	2.30±0.77	0.46
MMSE	28.6±1.5	28.6±1.3	28.5±1.7	0.32
Global cognition Z-score	0.00±0.66	0.02±0.67	-0.04±0.66	0.24
Episodic memory Z-score	0.00±0.94	0.02±0.67	-0.04±0.66	0.24
Information processing speed Z-score	0.00±0.84	0.03±0.80	-0.03±0.88	0.30
Executive functioning Z-score	0.00±0.84	0.04±0.73	-0.04±0.73	0.16

[1] FACIT subjects with available fatty acid and cognition data at both time points. Abbreviations: BMI: body mass index, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, MMSE: Mini Mental State Examination. Data are mean±SD or number (%).

* measured in cholesteryl esters

COGNITIVE PERFORMANCE

Global cognition

Global cognition improved over the three year study period in all groups. Folic acid treatment was more effective than placebo in improving global cognition in subjects in the low omega-3 fatty acid tertile (diff=0.155±0.042 (mean±SEM), p<0.01) (**table 2**). No difference in improvement in global cognition was observed between folic acid and placebo treatment in the middle and high omega-3 fatty acid tertile (respectively, diff=0.096±0.042, p=0.19; diff=-0.008±0.041, p=1.00). Furthermore, the interaction between folic acid treatment and omega-3 fatty acid tertile was significant (p=0.02). Compared to subjects in the high baseline omega-3 fatty acid tertile, the folic acid treatment effect was significantly larger in subjects within the low omega-3 fatty acid tertile (diff=0.163±0.059, p<0.01) (table 2, **figure 1-A**).

When focusing on EPA and DHA separately, we only observed a significant interaction between folic acid treatment and EPA tertile (p<0.01) (**supplementary table 2**). The folic acid treatment was significantly larger in subjects in the low and middle EPA tertile compared to subjects in the high EPA tertile (respectively, diff=0.182±0.059, p<0.01; diff=0.118±0.058, p=0.04). There was no significant overall interaction between folic acid treatment and DHA tertile (p=0.104).

Domain-specific cognition

Episodic memory

Despite the apparently larger difference in treatment effect in subjects in the low and middle omega-3 fatty acid tertile compared to subjects in the high tertile (respectively, diff=0.216±0.120, p=0.07; diff=0.190±0.120, p=0.11) (table 2, **figure 1-B**), there was no significant overall interaction between folic acid treatment and omega-3 fatty acid tertile (p=0.14).

Information processing speed

Information processing speed declined during the observation period in all groups, as indicated by the negative change in Z-score. The folic acid treatment was more effective than placebo in slowing the decline in information processing speed in subjects in the low omega-3 fatty acid tertile (diff=0.148±0.049, p=0.03), while no difference in treatment effectiveness was observed in subjects in the middle or high omega-3 fatty acid tertile (respectively, p=0.54; p=1.00) (table 2). The overall interaction between folic acid treatment and omega-3 fatty acid status was

significant (p=0.05). This was due to a larger treatment effect in the low compared to high omega-3 fatty acid tertile (diff= 0.167±0.068, p=0.01) (table 2, **figure 1-C**).

EXECUTIVE FUNCTIONING

Comparing folic acid and placebo treatment, no treatment effect was observed in any of the omega-3 fatty acid groups. Furthermore, there was no significant overall interaction between folic acid treatment and omega-3 fatty acid status (p=0.21) (table 2, **figure 1-D**).

APOE4 STATUS

The interaction between folic acid treatment and omega-3 fatty acid tertile on global cognition was not explained by ApoE4 status (p=0.14, data not shown). Similarly, ApoE4 status did not affect the interaction on episodic memory (p=0.17), information processing speed (p=0.58) and executive functioning (p=0.88).

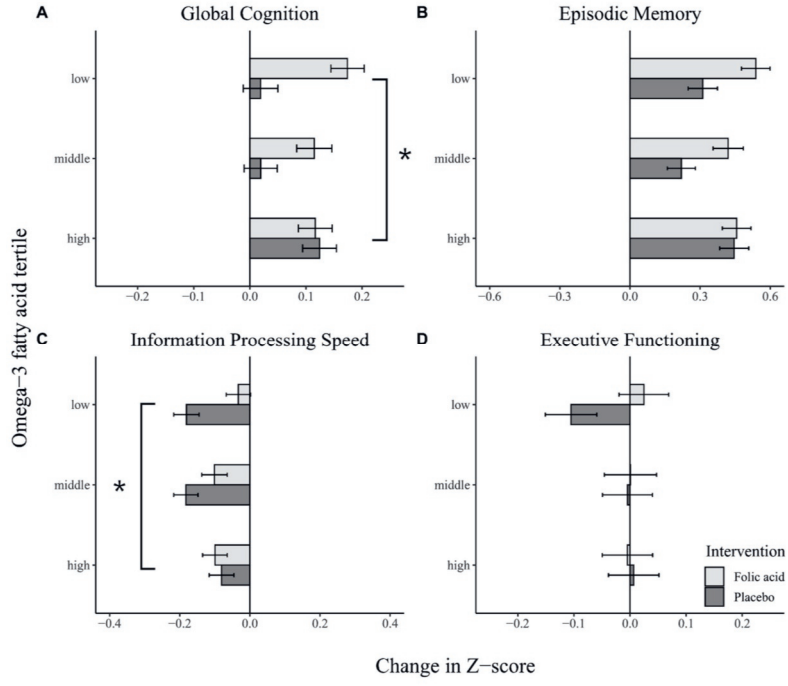

Figure 1: Changes in Z-scores (mean±SE) over the three year intervention period for global cognition (A) and the three cognitive domains (B-D) according to intervention group and omega-3 fatty acid status (n=791). * indicates a significant (p<0.05) difference in treatment effect between omega-3 fatty acid tertiles as analyzed by linear multiple regression.

Table 2: Changes in cognitive Z-scores following folic acid versus placebo supplementation according to omega-3 fatty acid status tertile.

	Treatment effect[1]			Overall interaction[2]	Tertiles pairwise comparison[3]		
	Crude	Adjusted	p-value	p-value	low vs middle	low vs high	middle vs high
Global cognition				0.02			
Low tertile	0.139±0.042	0.155±0.042	<0.01		diff=0.059±0.059 p=0.31	diff=0.163±0.059 p<0.01	diff=0.103±0.058 p=0.08
Middle tertile	0.107±0.043	0.096±0.042	0.19				
High tertile	-0.015±0.042	-0.008±0.041	1.00				
Episodic memory				0.14			
Low tertile	0.199±0.086	0.227±0.085	0.08		diff=0.026±0.120 p=0.83	diff=0.216±0.120 p=0.07	diff=0.190±0.120 p=0.11
Middle tertile	0.214±0.087	0.200±0.085	0.17				
High tertile	-0.007±0.086	0.011±0.085	1.00				
Information processing speed				0.05			
Low tertile	0.138±0.049	0.148±0.049	0.03		diff=0.066±0.068 p=0.33	diff=0.167±0.068 p=0.01	diff=0.101±0.069 p=0.14
Middle tertile	0.103±0.050	0.082±0.049	0.54				
High tertile	-0.021±0.050	-0.019±0.048	1.00				
Executive functioning				0.21			
Low tertile	0.108±0.062	0.130±0.062	0.29		diff=0.125±0.087 p=0.15	diff=0.141±0.087 p=0.11	diff=0.016±0.087 p=0.85
Middle tertile	0.013±0.063	0.005±0.062	1.00				
High tertile	-0.026±0.062	-0.011±0.062	1.00				

Data available for n=791 participants. Data is presented as mean±SEM. [1] Treatment effect is the difference in change in Z-score over time between the folic acid and placebo treatment groups within an omega-3 fatty acid tertile as analyzed using linear multiple regression, equal to Δ Z-score folic acid - Δ Z-score placebo. For example, in the low omega-3 tertile participants receiving folic acid had 0.155 units more improvement in the Z-score for global cognition over the three year period compared to participants receiving placebo. Crude model : adjusted for baseline cognitive Z-score; Adjusted model: adjusted for baseline cognitive Z-score, age, sex, level of education, ApoE4 status, baseline homocysteine concentration and baseline body mass index.

[2] The overall interaction indicates similarity of treatment effects in the low, middle and high omega-3 fatty acid tertiles.

[3] The pairwise comparison tests for differences in treatment effects between omega-3 fatty acid

DISCUSSION

This post-hoc analysis revealed that the efficacy of folic acid treatment on cognitive functioning in healthy older adults is dependent on omega-3 fatty acid status. With respect to global cognition, individuals in the low omega-3 fatty acid tertile benefited from the folic acid treatment, while individuals in the high omega-3 fatty acid tertile did not experience an advantage. The interaction is mainly driven by EPA. Regarding domain-specific performance, effectiveness of folic acid treatment was dependent on baseline omega-3 fatty acid levels for information processing speed, but not for episodic memory and executive functioning.

In contrast to our study, previous post-hoc analyses on the interaction between B-vitamins and omega-3 fatty acids were performed in persons with impaired cognitive functioning [13-15]. Remarkably, opposite effects were observed in the VITACOG trial [13, 14], a randomized controlled trial on the effect of two year daily supplementation with folic acid, vitamin B6 and B12 versus placebo in older adults (>70y) with MCI. This study showed that B-vitamin supplementation was beneficial in slowing down cognitive decline [13] and brain atrophy rates [14] in individuals with higher omega-3 fatty acid status, while individuals with lower omega-3 fatty acid status did not benefit from extra B-vitamins. Similarly, a post-hoc analysis from the OmegAD trial, in which AD patients were supplemented with EPA and DHA versus placebo for six months, showed that baseline homocysteine levels influence the effectiveness of omega-3 fatty acid supplementation on cognitive functioning, with only individuals with lower homocysteine levels benefitting from omega-3 fatty acid supplementation [15].

The difference in study population may explain the opposite findings. While our study included cognitively healthy older adults aged 50-70 years, the VITACOG and OmegAD trials focused on older participants (>70y) with MCI and AD. Older cognitively impaired persons may have higher needs for omega-3 fatty acids, due to changes in dietary intake and bioavailability [27]. Besides, membrane synthesis rate may be higher in response to increased neuronal tissue loss [27-29]. Hence, a higher neuronal turnover rate in MCI and AD patients may increase needs for omega-3 fatty acids. Phosphatidylcholine (PC) is one of the carriers involved in the transport of omega-3 fatty acids to the brain. It has been argued that PC levels can become a limiting factor resulting in insufficient transport of omega-3 fatty acids to the brain [14]. Interestingly, the formation of PC is dependent on B-vitamin levels. In the homocysteine-methionine cycle, vitamin B6, B12 and folic acid are crucial in the

conversion from homocysteine to methionine or cysteine. Insufficient levels of these B-vitamins result in accumulation of homocysteine and its precursor, S-adenosyl homocysteine (SAH) [30]. In turn, elevated SAH levels slow down phosphatidylethanolamine N-methyltransferase, an enzyme responsible for the conversion of phosphatidylethanolamine to PC [12]. This would imply that both B-vitamins and omega-3 fatty acid levels should be high in cognitively impaired older subjects: B-vitamins are needed to prevent accumulation of homocysteine and thereby stimulate the production of PC, and sufficient availability of omega-3 fatty acids are needed as these are an important constituent of neuronal membranes [30, 31]. As neuronal loss would be less in cognitively healthy older adults, it could be that lower levels of omega-3 fatty acids are sufficient to maintain synapses.

In addition to differences in omega-3 fatty acid requirements, study populations might differ with respect to inflammatory state. We hypothesize that participants from the VITACOG and OmegAD trials have a higher inflammatory state as higher age [32] and lower cognitive status [33] have been associated to higher inflammation levels. Inflammation is a key mechanism in the pathogenesis of age-related neurodegenerative diseases [34, 35]. As both B-vitamins and omega-3 fatty acids exhibit anti-inflammatory properties [36], and nutrients may have complementary anti-inflammatory effects [37, 38], it could be argued that this group has higher requirements for anti-inflammatory nutrients to counteract the harmful effect of elevated inflammation levels on cognition. To gain mechanistic insight in the role of inflammation in this interaction, future research could incorporate measurements of inflammation markers.

It is important to note that the opposing findings could also be attributed to differences in intervention or baseline nutrient status between study populations. Participants in our study were supplemented with folic acid only, while VITACOG participants received a combination of folic acid, vitamin B6 and B12. With respect to baseline nutrient status, unfortunately, omega-3 fatty acid levels cannot be compared directly between studies because of differences in fatty acid fractions analyzed, analytical methods and expressed measures. In our study population, the proportion of omega-3 fatty acids (EPA+DHA) in plasma cholesteryl esters was 2.32±0.86%. In the VITACOG trial, omega-3 fatty acid levels (EPA+DHA) were expressed in absolute amounts and measured in plasma in free, phospholipid, triglycerides and cholesteryl ester fractions with an average of 472 (95% CI: 439, 508) µmol/L [14]. In addition, homocysteine status, a possible factor in the mixed results

of B-vitamin supplementation trials [39], was approximately 2 µmol/L higher in our study compared to the previous trials. Vitamin B12 levels were comparable between study populations. This considerable role of the study population in the explanation of the opposite findings, highlights the importance of a personalized approach in the field of nutrition and cognitive ageing research.

We found that the effectiveness of folic acid treatment was dependent on EPA tertile, but not on DHA tertile group. Our findings are in contrast to the VITACOG trial on cognitive decline in which DHA appeared to be driving the interaction between B-vitamin treatment and omega-3 status [13]. With regard to brain atrophy, the interaction was driven by both EPA and DHA [14]. The above discussed differences between our and the VITACOG trials (study population, interventions, measures of fatty acids) could be responsible for the conflicting results.

A limitation of our study is that omega-3 fatty acid levels were only assessed at baseline. However, previous research on the FACIT trial showed that there was a positive correlation between dietary intake of fish at baseline and after three years [6] suggesting that participants did not change their fish intake limiting influence on omega-3 fatty acid plasma levels. Besides, it is important to emphasize that the current analysis is a subgroup analysis and therefore interpretation of results is limited. Strengths of our study include the long follow-up period of three years and the use of an extensive battery of cognitive tests, focusing on different domains.

Our analysis was limited to the interaction between folic acid and omega-3 fatty acids. Yet, other nutrients may be involved in the interaction. In a post-hoc analysis of the MAPT trial [40], Bowman and colleagues developed a blood-based nutritional risk index and investigated its predictive quality on cognitive decline. Score for the index ranged from zero to three, one point was given for each suboptimal level of erythrocyte omega-3 fatty acid, total plasma homocysteine and/or serum 25-hydroxyvitamin D. Participants with optimal levels for all three factors showed cognitive improvements, while participants with suboptimal levels for at least one of the factors declined over the study period, with each increase in score leading to faster rates of cognitive decline. This suggests that B-vitamins and omega-3 fatty acids may not capture the full complexity of the interaction, vitamin D potentially plays a role as well. Unfortunately, vitamin D status was not measured in the current study. Future research should consider to incorporate this measure as well as

incorporate extensive blood nutrient assessment to investigate the role of other dietary factors that have been linked to cognitive ageing.

In conclusion, this post-hoc analysis revealed that the effectiveness of folic acid treatment on cognitive functioning in healthy older adults is dependent on omega-3 fatty acid status. Healthy older adults with the lower omega-3 status benefited from folic acid supplementation, while individuals with the higher omega-3 fatty acid status did not show additional advantages of taking daily folic acid supplements. These results shed light on the presence of subgroups that benefit from B-vitamin supplementation, and emphasize the importance of a personalized approach. More research is needed to further disentangle the complex interaction between nutrition and cognitive aging, with a focus on investigating the role of other nutrients and the underlying mechanisms.

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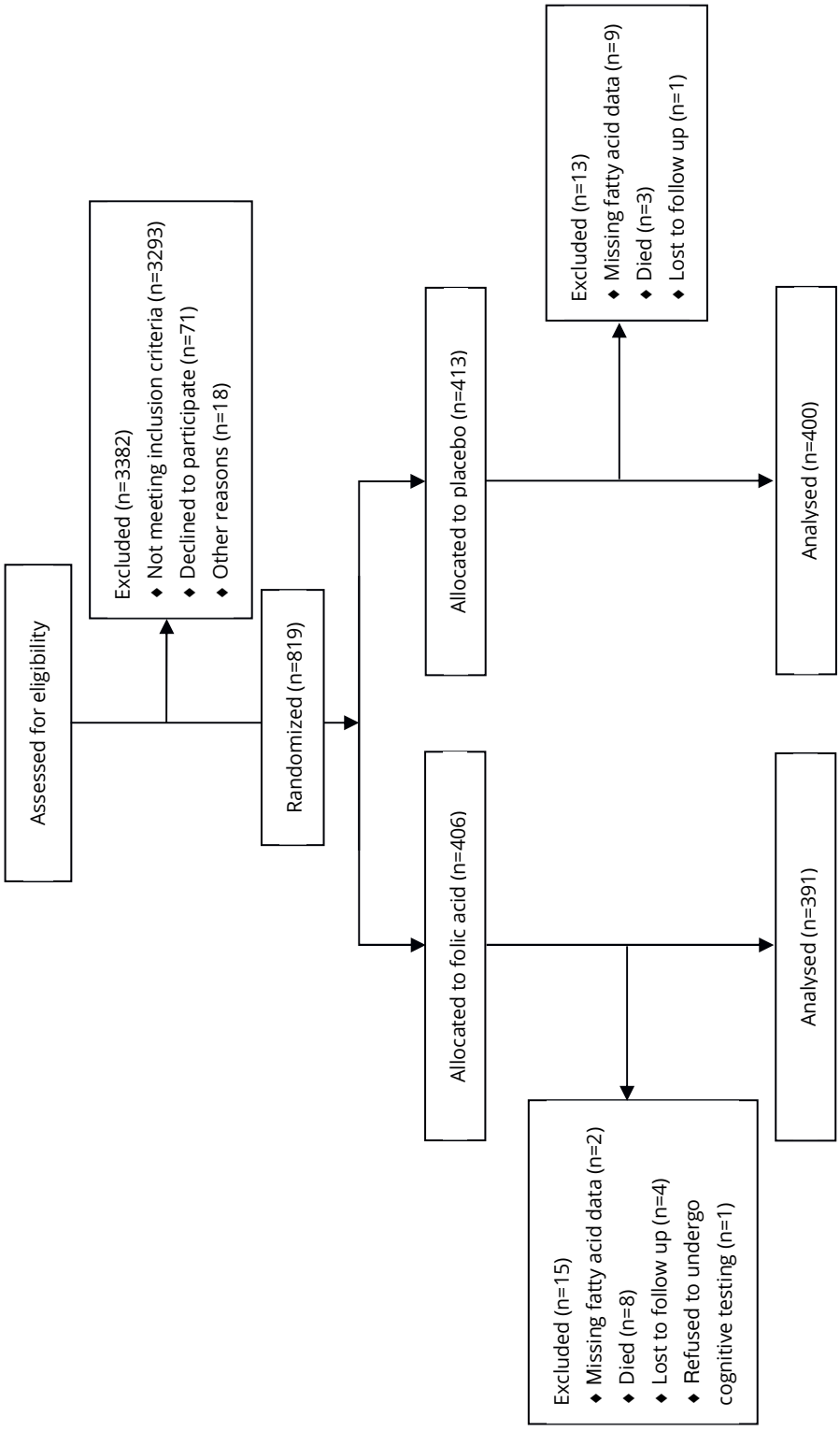
Supplementary materials

Supplementary table 1: Baseline characteristics per omega-3 fatty acid status group.

Characteristic	Overall (n=791)	Low (n=264)	Middle (n=264)	High (n=263)	p-value
Age (years)	60.2±5.6	60.0±5.9	59.8±5.4	60.7±5.5	0.16
Sex n(%)					0.10
Male	565 (71.4%)	200 (75.8%)	188 (71.2%)	177 (67.3%)	
Female	226 (28.6%)	64 (24.2%)	76 (28.9%)	86 (32.7%)	
Level of education n(%)					0.27
Low	178 (22.5%)	69 (26.1%)	58 (22.0%)	51 (19.4%)	
Middle	300 (37.9%)	94 (35.6%)	108 (40.9%)	98 (37.3%)	
High	313 (39.6%)	101 (38.3%)	98 (37.1%)	114 (43.3%)	
BMI (kg/m^2)	26.5±3.6	26.4±3.6	26.6±3.6	26.7±3.7	0.58
Physical activity (PAI score)	153±69	151±74	163±70	145±63	<0.01
Current smoker n(%)	159 (20.1%)	50 (18.9%)	54 (20.5%)	55 (20.9%)	0.84
Diabetes Mellitus n(%)	24 (3.0%)	10 (3.8%)	6 (2.3%)	8 (3.0%)	0.60
Cardiovascular disease n(%)	93 (11.8%)	32 (12.1%)	33 (12.5%)	28 (10.6%)	0.63
ApoE4 carrier n(%)	248 (30.9%)	89 (34.1%)	77 (29.2%)	82 (31.3%	0.48
Total homocysteine (µmol/L)	13.3±2.9	13.6±3.0	13.1±2.7	13.1±2.9	0.10
Vitamin B6 (nmol/L)	35.7±19.1	34.4±20.7	35.5±19.8	37.2±16.6	0.25
Folate (nmol/L)	12.5±4.5	12.5±4.4	12.5±4.4	12.5±4.6	0.99
Vitamin B12 (pmol/L)	315±105	305±104	310±99	331±111	0.01
DHA (%)*	0.60±0.21	0.43±0.10	0.57±0.12	0.80±0.19	<0.01
EPA (%)*	1.11±0.69	0.60±0.16	0.93±0.15	1.80±0.78	<0.01
Sum EPA and DHA (%)*	1.71±0.85	1.02±0.18	1.50±0.14	2.60±0.89	<0.01
MMSE	28.6±1.5	28.6±1.5	28.4±1.6	28.7±1.4	0.23
Global cognition Z-score	0.00±0.66	-0.03±0.63	-0.04±0.72	0.04±0.64	0.35
Episodic memory Z-score	0.00±0.94	-0.04±0.87	-0.03±0.98	0.07±0.95	0.34
Information processing speed Z-score	0.00±0.84	-0.01±0.87	-0.02±0.88	0.03±0.78	0.82
Executive functioning Z-score	0.00±0.73	-0.01±0.70	-0.03±0.78	0.04±0.71	0.51

Abbreviations: BMI: body mass index, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, MMSE: Mini Mental State Examination. Data are mean±SD or number (%).

* measured in cholesteryl esters.



Supplementary figure 1: Participant flow chart.

Supplementary table 2: Changes in global cognitive Z-score following folic acid versus placebo supplementation according to EPA and DHA tertiles separately.

	Treatment effect[1]			Overall interaction[2]	Tertiles pairwise comparison[3]		
	Crude	Adjusted	p-value	p-value	low vs middle	low vs high	middle vs high
EPA				<0.01			
Low tertile	0.155±0.042	0.163±0.042	<0.01		diff=0.063±0.059 p=0.28	diff=0.182±0.059 p<0.01	diff=0.118±0.058 p=0.04
Middle tertile	0.103±0.042	0.100±0.041	0.15				
High tertile	-0.025±0.042	-0.018±0.041	1.00				
DHA				0.104			
Low tertile	0.052±0.042	0.048±0.042	0.86		diff=0.106±0.058 p=0.07	diff=0.003±0.059 p=0.96	diff=0.110±0.059 p=0.06
Middle tertile	0.145±0.042	0.154±0.042	<0.01				
High tertile	0.037±0.043	0.045±0.042	0.89				

Data available for n=791 participants. Data is presented as mean±SEM.

[1] Treatment effect is the difference in change in Z-score over time between the folic acid and placebo treatment groups within an fatty acid tertile, equal to Δ Z-score folic acid - Δ Z-score placebo. For example, in the low EPA tertile participants receiving folic acid had 0.163 units more improvement in the Z-score for global cognition over the three year period compared to participants receiving placebo.

Crude model: adjusted for baseline cognitive Z-score.

Adjusted model: adjusted for baseline cognitive Z-score, age, sex, level of education, ApoE4 status, baseline homocysteine concentration and baseline body mass index.

[2] The overall interaction indicates similarity of treatment effects in the low, middle and high omega-3 fatty acid tertiles.

[3] The pairwise comparison tests for differences in treatment effects between omega-3 fatty acid tertiles.

CHAPTER 3

DHA status influences effects of B-vitamin supplementation on cognitive ageing

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ABSTRACT

Purpose: Trials aiming to lower homocysteine by B-vitamin supplementation have reported mixed results on slowing cognitive decline. We investigated if efficacy of B-vitamin supplementation is affected by baseline plasma omega-3 fatty acid levels.

Methods: This post-hoc analysis of the B-proof trial included 187 adults aged 65 years or older with baseline plasma total homocysteine ≥12μmol/L, randomly assigned to 400μg folic acid and 500μg vitamin B12 or placebo daily for two years. Global and domain-specific cognitive functioning were assessed at baseline and after two years. The effect of B-vitamin supplementation was analyzed according to tertiles of baseline plasma omega-3 fatty acids concentrations combined, and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) individually using multiple linear regression analyses.

Results: The mean±SD age of the participants was 71.6±5.9 years and median [IQR] Mini-Mental State Examination was 29 [28-30]. The treatment effect of B-vitamins on global cognition was larger in participants in the high compared to the middle DHA tertile (difference in z-score, mean±SE 0.22±0.10, p=0.03). There was no significant interaction between B-vitamin supplementation and combined omega-3 fatty acid (p=0.49) and EPA (p=0.99) tertiles. Similarly, the efficacy of B-vitamin treatment on domain-specific cognitive functioning did not link to omega-3 fatty acid, DHA, or EPA plasma levels.

Conclusion: This post-hoc analysis indicated that efficacy of B-vitamin supplementation in slowing cognitive decline relates to DHA status, with individuals with higher plasma DHA levels benefitting more from vitamin B12 and folic acid use. The results support earlier observations that positive effects of B-vitamins in cognitive ageing may be subgroup-specific.

Trial registration: registered at clinicaltrials.gov (NCT00696514) on June 12, 2008

Keywords: B-vitamins, omega-3 fatty acids, cognition, older adults, elderly, healthy ageing

INTRODUCTION

Age-related cognitive decline leading to dementia poses a societal challenge with major medical, social and economic impact. In the absence of curative treatment for dementia, the focus is on prevention by management of risk factors [1]. Epidemiological studies show that individuals with elevated homocysteine levels are at greater risk of cognitive decline and dementia, identifying homocysteine as a possible modifiable risk factor [2].

Elevated homocysteine levels may reflect impaired B-vitamin status [3]. B-vitamin supplementation to lower homocysteine levels and thereby slowing down cognitive decline would seem a straightforward solution, yet proof of clinical benefits is lacking. While clinical trials show that B-vitamin treatment, usually existing of vitamin B12, B6 and/or folic acid, is effective in lowering homocysteine levels, its effect on slowing down cognitive decline remains inconclusive [4].

It has been hypothesized that the efficacy of B-vitamin supplementation in slowing cognitive decline is dependent on omega-3 fatty acid status, with B-vitamin supplementation being only effective in individuals with higher omega-3 fatty acid plasma levels. Indeed, results from several post-hoc analyses of B-vitamin trials underline this hypothesis [5,6]. Surprisingly, opposite results have been demonstrated as well, with only individuals with lower omega-3 fatty acid status benefitting from B-vitamin supplementation [7]. This merits further research to disentangle the complex interaction between B-vitamins and omega-3 fatty acids in cognitive ageing.

Thus, the current study further investigates the interaction between B-vitamin supplementation and omega-3 fatty acids with respect to cognitive outcomes in healthy older adults without cognitive complaints. To this end, we investigated if the efficacy of B-vitamin supplementation was dependent on baseline omega-3 fatty acid plasma levels in cognitively healthy older adults in the B-proof trial (B-Vitamins for the Prevention of Osteoporotic Fractures). In the main study of the B-proof trial, no effects of B-vitamins on slowing cognitive decline were observed [8].

METHODS

STUDY DESIGN AND PARTICIPANTS

The present study was conducted as a post-hoc analysis within the B-proof trial, a randomized, double-blind placebo-controlled trial investigating the effect of folic acid and vitamin B12 supplementation on fracture incidence. Cognitive functioning

was measured as secondary outcome. Data was collected between October 2008 and March 2013 in three research centers in the Netherlands: Erasmus Medical Center (Rotterdam), VU University Medical Center (Amsterdam) and Wageningen University (Wageningen). This analysis is based on a subsample of the Wageningen participants for whom fatty acid data were available. The trial has been approved by the Medical Ethics committee from Wageningen University & Research and has been registered at clinicaltrials.gov (NCT00696514). All participants provided written informed consent.

Information on study design and participants has been described in detail previously [9]. In short, the intervention existed of daily administration of 400µg folic acid and 500 µg vitamin B12 tablets versus placebo tablets for a period of two years. Both intervention and placebo tablets contained 15µg vitamin D_3. Participants received tablets every 6 months and they were requested to return any remaining tablets, as a measure of compliance. Participants were men and women aged 65 years and older, with elevated plasma homocysteine levels (12-50µmol/L). Exclusion criteria were renal insufficiency (creatinine >150µmol/L), diagnosis of a malignancy in the past five years and current or recent (<4 months) use of supplements with very high dose of folic acid (>300µg) or intramuscular injections with vitamin B12. Fatty acid data was available for 205 participants. Our analysis included data of 191 participants. Data from 13 participants were excluded due to missing follow-up (n=3) or ApoE4 (n=10) data, and data from 1 participant was excluded due to a follow-up MMSE score of 19, indicating possible dementia.

COGNITIVE TESTING

Cognitive functioning was assessed at baseline and after two years of intervention with an extensive battery of cognitive tests administered by trained research assistants.

In the *Rey Auditory Verbal Learning Test* (RAVLT) [10], a list of 15 words was verbally presented to the participant at a rate of one word per two seconds. The participant was asked to recall the words in five trials immediately after presentation (subtest immediate), and after a 20-minute delay (subtest delayed). Subsequently, the participant was asked to identify the 15 words in a list of 30 verbally presented words (subtest recognition). The number of correctly recalled words in each subtest was recorded.

In the *Digit Span Task* [11], the participant was verbally presented with digit sequences and asked to recall the sequence in either forward or backward order. Starting at a sequence length of three digits in the forward and two digits in the backward task, the length increased each two trials until an error was made or the maximum length of nine digits in the forward and eight in the backward task was reached. The maximum sequence length for the forward and backward version was recorded.

In the *Trail Making Test* (TMT) [12], participants were presented with a paper containing 25 circles. In two subtests, participants were asked to connect 25 circles containing numbers in chronological order (part A), and to alternate connecting circles containing numbers and letters in chronological and alphabetical order (part B). Time to complete each part was recorded.

The *Stroop Color-Word test* [13] exists of three subtests, in which the participant was presented with color words written in black ink (part I), colored blocks (part II), or color words written in an incongruent color ink (part III). The participant is instructed to read aloud the words as fast as possible. The time needed to complete each part was documented.

In the *Symbol Digit Modalities Test* (SDMT) [14], symbols were paired with digits. The participant was presented with a sheet of symbols, and asked to match the symbols to the corresponding digit as fast as possible. The number of correctly matched pairs in 90 seconds was recorded.

In *Letter Fluency* [15], participants were given 60 seconds to name as many words as possible starting with the letter D, A and T (baseline) or K, O, and M (follow-up). The number of unique words was documented.

Parallel versions were used for RAVLT, TMT and Verbal Fluency to minimize learning effects. Individual cognitive test scores at baseline and follow-up were converted into Z-scores based on baseline mean and standard deviation, with higher scores indicating better cognitive functioning. The Z-scores for TMT and Stroop Color-Word test were reversed as lower scores indicate better cognitive functioning. Individual Z-scores were clustered into composite scores for global and domain specific cognitive functioning:

$$Global\ cognition = (Z_{RAVLTimmediate} + Z_{RAVLTdelayed} + Z_{RAVLTrecognition} + Z_{DigitSpan\ forward} + Z_{DigitSpan\ backward} + -Z_{Stroop\ mean\ I\ and\ II} + -Z_{TMTpartA} + Z_{SDMT} + -Z_{Stroop\ interference} + -Z_{TMTB/A} + Z_{Fluency})/\ 11$$

$$Episodic\ memory = (Z_{RAVLTimmediate} + Z_{RAVLTdelayed} + Z_{RAVLTrecognition})/3$$

$$Attention\ \&\ working\ memory = (Z_{DigitSpan\ forward} + Z_{DigitSpan\ backward})/2$$

$$Information\ processing\ speed = (-Z_{Stroop\ mean\ I\ and\ II} + -Z_{TMTpartA} + Z_{SDMT})/\ 3$$

$$Executive\ functioning = (-Z_{Stroop\ interference} + -Z_{TMTB/A} + Z_{Fluency})/\ 3$$

BIOCHEMICAL ASSAYS

Baseline omega-3 fatty acid concentrations were measured in the plasma phospholipid (PL) fractions from blood samples obtained after an overnight fast or a light breakfast. Samples had been collected by venipuncture using EDTA containing vacuum tubes. Plasma was obtained by centrifugation (10 min at 1200 g) and stored at -80°C. Studies have shown that polyunsaturated fatty acids remain stable for up to 12 years under these conditions [16]. Total lipids were extracted from plasma with isopropanol/hexane (2:3, v/v) and separated into cholesteryl and PL fractions by solid phase extraction using silica columns. Subsequently, fatty acids in the PL fractions were transesterified using boron trifluoride in methanol yielding their methyl esters. Analysis was performed by gas chromatography with flame-ionization detection. Peaks were identified based on comparison of retention times to known standards. Fatty acid concentrations are presented in relative concentrations of total fatty acids. The relative concentration of plasma omega-3 fatty acids was derived by adding the proportions of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A detailed description of the analytical procedure for fatty acids used in our lab has been published elsewhere [17].

Serum vitamin B12 and folate were determined using immune electrochemiluminescence assay (Elecsys, 2010, Roche). High-performance liquid chromatography was used to measure plasma total homocysteine [18]. DNA was isolated from buffy coats for genotyping. ApoE genotype was determined using TaqMan analysis.

DESCRIPTIVE CHARACTERISTICS

Trained research assistants measured height with a stadiometer to the nearest 0.1cm and weight with a calibrated scale to the nearest 0.5kg. Body mass index (BMI) was calculated as weight (kg) / (height (m))^2. Information on age, sex, education level

(low, middle, high), smoking status (never, former, current) and physical activity [19] was obtained via questionnaires. MMSE (0-30 points) [20] was assessed by trained research assistants following a standardized protocol.

STATISTICAL ANALYSIS

Data are expressed as n (%), mean (SD) or median (IQR) unless otherwise stated. Baseline characteristics between intervention and omega-3 fatty acid groups were compared using independent sample t-test, ANOVA or Kruskal-Wallis test for continuous variables and chi-square for categorical variables. Multiple linear regression was performed to investigate if the efficacy of B-vitamin supplementation was dependent on baseline omega-3 fatty acid levels. We modelled the change in cognition Z-score between baseline and post-intervention for global cognition and domain-specific cognition as a function of intervention group (B-vitamins, placebo), baseline omega-3 fatty acid group (low, middle, high) and their interaction. To investigate if DHA and/or EPA status modified B-vitamin supplementation efficacy separately, additional models were run replacing baseline omega-3 fatty acid levels (by groups) by either DHA or EPA concentrations (by groups). To create the omega-3 fatty acid status groups, baseline omega-3 fatty acid, DHA and EPA concentrations were divided into tertiles. The analyses were adjusted for baseline cognitive Z-score, age, sex, education, ApoE4 status, baseline homocysteine level, physical activity and smoking status, all measured at baseline. Tukey correction for multiple comparisons was applied when examining treatment effects within the omega-3 fatty acid tertiles. P-values <0.05 were considered statistically significant, for interaction terms the cutoff was set at $p<0.10$. All analyses were performed using RStudio Version 1.1.463 [21].

RESULTS

PARTICIPANT CHARACTERISTICS.

Table 1 presents baseline characteristics of the study population. The mean age of the participants was 71.5±5.8 years and 56% was male. The average BMI was 27.5±4.2 kg/m^2, with 76% being overweight (i.e. BMI≥25kg/m^2). Total homocysteine levels were elevated with a median of 13.7 [IQR 12.9-15.8] µmol/L. The study population was cognitively healthy, as indicated by a median MMSE score of 29 [IQR 28-30] at baseline. Five participants (2.6%) had MMSE scores equal to or lower than 24, indicating cognitive impairment. Participants in the B-vitamin group were younger (p=0.01) compared to participants in the placebo group. Furthermore, a larger proportion of participants in the middle omega-3 fatty acid tertile had never smoked compared to participants in the high omega-3 fatty acid tertile (p=0.02) (**supplementary table 1**). Mean baseline cognitive scores did not differ between either intervention or omega-3 fatty acid status groups. Compliance to treatment was high with an average of 97%. There was no difference in compliance between treatment and/or omega-3 fatty acid groups.

Comparing our subsample with the total Wageningen and B-proof study populations, our subsample was younger than the Wageningen (72.9±5.7, p<0.01) and B-proof (74.3±6.6y, p<0.01) study populations. Median Mini-Mental State Examination (MMSE) score was similar in our subsample and the total Wageningen study population (29 [28-30] for both, p=0.46). The total B-proof study population showed lower MMSE scores (28 [27-29], p<0.01).

COGNITIVE PERFORMANCE

GLOBAL COGNITIVE FUNCTIONING

The treatment effects of B-vitamins versus placebo on global cognition were numerically positive (i.e. larger than 0) in all omega-3 fatty acid (EPA and DHA combined) tertiles, indicating that the group that received B-vitamins improved more over time compared to the placebo group, irrespective of the omega-3 fatty acid blood levels (**table 2**, **figure 1**). Despite the larger treatment effect in participants in the high omega-3 fatty acid tertile (difference 0.16±0.07, p=0.25) compared to the middle and low tertiles (respectively, 0.08±0.07, p=0.75; 0.05±0.07, p=0.97), there was no significant overall interaction between B-vitamin supplementation and omega-3 fatty acid tertile (p=0.60), meaning that there is no difference in treatment effect of B-vitamins between the low, middle and high omega-3 fatty acid tertiles.

Table 1: Baseline characteristics per treatment group in the B-proof study[1]

Characteristic	Overall (n=191)	B-vitamin (n=94)	Placebo (n=97)	p-value
Age (years)	71.5±5.8	70.3±5.1	72.7±6.3	>0.01
Sex n (%)				
Male	107 (56%)	52 (55%)	55 (57%)	0.96
Female	84 (44%)	42 (45%)	42 (43%)	
Level of education n (%)				0.27
Low	76 (40%)	41 (43%)	35 (36%)	
Middle	46 (24%)	18 (19%)	28 (29%)	
High	69 (36%)	35 (37%)	34 (35%)	
BMI (kg/m^2)	27.5±4.2	27.5±4.4	27.5±4.0	0.95
Physical activity (kcal/d)	561 (358-863)	596 (386-879)	525 (326-810)	0.09
Smoking behavior n (%)				0.54
Current smoker	11 (6%)	7 (7%)	4 (4%)	
Former smoker	123 (64%)	61 (65%)	62 (64%)	
Never smoker	57 (30%)	26 (28%)	31 (32%)	
ApoE4 carriers n (%)	55 (29%)	28 (30%)	27 (28%)	0.89
Biochemical measures				
Total homocysteine (µmol/L)	13.7 (12.9-15.8)	13.7 (13.0-15.3)	13.7 (12.9-16.4)	0.65
Folate (nmol/L)	17.4 (14.1-23.5)	16.9 (13.9-22.4)	17.7 (14.3-24.7)	0.12
Vitamin B12 (pmol/L)	256 (201-334)	253 (203-308)	275 (196-366)	0.10
MMA (µmol/L)	0.22 (0.19-0.29)	0.22 (0.19-0.29)	0.22 (0.19-0.31)	0.80
holoTC (pmol/L)	62 (46-80)	63 (47-76)	62 (46-82)	0.73
25(OH)D (nmol/L)	60±23	61±24	60±22	0.84
Omega-3 status (sum DHA and EPA, %)*	5.7±1.9	5.5±1.8	5.9±2.1	0.20
DHA (%)*	4.3±1.2	4.2±1.2	4.5±1.3	0.11
EPA (%)*	1.4±0.9	1.3±0.8	1.4±1.0	0.54
MMSE score	29 (28-30)	29 (27-30)	29 (28-30)	0.85
Global cognition Z-score	0.00±0.52	0.01±0.54	-0.02±0.51	0.69
Episodic memory Z-score	0.00±0.70	0.03±0.70	-0.02±0.70	0.59
Attention & working memory Z-score	0.00±0.86	0.01±0.89	-0.00±0.85	0.94
Information processing speed Z-score	0.00±0.77	-0.02±0.79	0.02±0.76	0.73
Executive functioning Z-score	0.00±0.69	0.04±0.69	-0.05±0.69	0.37

[1] B-proof subjects with available fatty acid and cognition data at both time points. Abbreviations: BMI: body mass index, MMA: methylmalonic acid, holoTC: holotranscobalamin, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, MMSE: Mini Mental State Examination. Data are mean±SD, median (IQR) or number (%).

* measured in phospholipid fractions

Subsequently, we analysed the treatment effects related to EPA and DHA concentrations separately. The efficacy of B-vitamin supplementation related to plasma DHA levels. B-vitamin supplementation was more effective than placebo in maintaining global cognitive functioning in participants in the high DHA tertile (difference 0.24±0.07, p=0.01), while no difference in treatment effect was observed in participants in the middle or low DHA tertile (respectively, p=1.00 and p=0.95). The overall interaction between B-vitamin supplementation and DHA status was significant (p=0.06). Participants in the high DHA tertile benefited significantly more from B-vitamin supplementation compared to participants in the middle DHA tertile (difference 0.23±0.10, p=0.02). Furthermore, there was a trend towards a difference in treatment effect between the high and low DHA tertile (0.18±0.10 p=0.07).

Corresponding analyses for potential interaction with EPA, i.e. comparing B-vitamin and placebo supplementation for EPA status, revealed that no treatment effect was observed in any of the EPA groups. In addition, there was no significant overall interaction between B-vitamin supplementation and EPA status (p=0.97).

DOMAIN-SPECIFIC COGNITIVE FUNCTIONING

For none of the four cognitive domains separately; i.e. episodic memory, attention & working memory, information processing speed, and executive functioning, there was a difference in treatment effect in any of the combined omega-3 fatty acid groups (**supplementary table 2**). In addition, there was no significant overall interaction between B-vitamin supplementation and omega-3 fatty acid group. Similarly, when domain-specific performance was assessed in relation to tertiles of baseline concentrations of EPA and DHA individually, there were no significant treatment effects or interactions (**supplementary tables 3-4**).

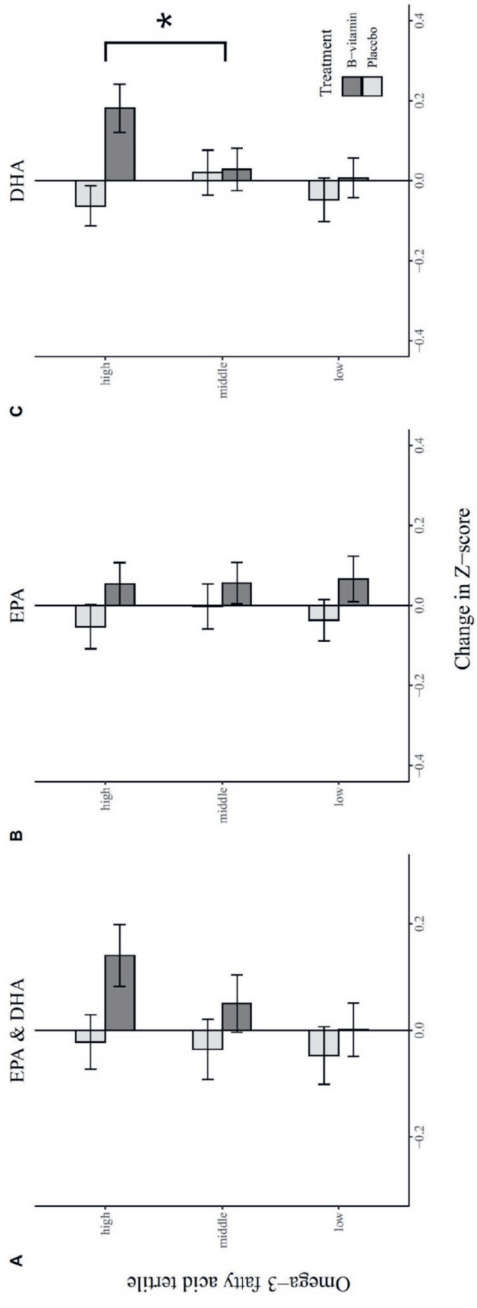

Figure 1: Changes in Z-scores (mean β±SE) in global cognition over the two year intervention period according to treatment group and (A) omega-3 fatty acid status, (B) eicosapentaenoic acid (EPA) status and (C) docosahexaenoic acid (DHA) status. * Significant (P<0.05) difference in treatment effect between omega-3 fatty acid tertiles, as analyzed by linear multiple regression.

Table 2: Changes in global cognition Z-scores following B-vitamin versus placebo supplementation according to omega-3 fatty acid, EPA and DHA status tertile.

	Treatment effect[1]			Overall interaction[2]	Tertiles pairwise comparison[3]		
	Crude	Adjusted	p-value	p-value	low vs middle	low vs high	middle vs high
Omega-3 fatty acid status				0.60			
Low tertile	0.10±0.07	0.05±0.07	0.97				
Middle tertile	0.13±0.07	0.08±0.07	0.75		diff=0.04±0.10 p=0.69	diff=0.10±0.10 p=0.32	diff=0.06±0.10 p=0.54
High tertile	0.16±0.07	0.16±0.07	0.25				
EPA status				0.97			
Low tertile	0.16±0.07	0.10±0.07	0.72				
Middle tertile	0.09±0.07	0.07±0.07	0.88		diff=0.02±0.10 p=0.82	diff=0.02±0.10 p=0.98	diff=0.02±0.10 p=0.84
High tertile	0.12±0.07	0.09±0.07	0.75				
DHA status				0.06			
Low tertile	0.12±0.07	0.06±0.07	0.95				
Middle tertile	0.04±0.07	0.01±0.07	1.00		diff=0.05±0.10 p=0.59	diff=0.18±0.10 p=0.07	diff=0.23±0.10 p=0.02
High tertile	0.24±0.07	0.24±0.07	0.01				

Data available for n=191 participants. Data is presented as mean β±SEM. Abbreviations: EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

[1] Treatment effect is the difference in change in Z-score over time between the B-vitamin and placebo treatment groups within an omega-3 fatty acid tertile as analyzed using linear multiple regression, equal to Δ Z-score B-vitamin - Δ Z-score placebo.

Crude model : adjusted for baseline cognitive Z-score; Adjusted model: adjusted for baseline cognitive Z-score, age, sex, level of education, ApoE4 status, baseline homocysteine concentration, baseline body mass index, physical activity, smoking status.

[2] The overall interaction indicates similarity of treatment effects in the low, middle and high omega-3 fatty acid tertiles.

[3] The pairwise comparison tests for differences in treatment effects between omega-3 fatty acid tertiles.

DISCUSSION

This post-hoc analysis of the B-proof trial showed that the efficacy of B-vitamin supplementation on global cognition may be related to plasma DHA levels, but not to plasma total omega-3 fatty acid or EPA levels. Individuals with higher DHA plasma levels benefitted from B-vitamin supplementation, while individuals with lower DHA plasma levels did not. With respect to domain-specific cognitive performance, plasma omega-3 fatty acid combined, DHA or EPA levels separately did not modify the treatment effect of B-vitamins on episodic memory, attention & working memory, information processing speed nor executive functioning.

To date, the interaction between B-vitamins and omega-3 fatty acids in relation to cognitive decline have been investigated in three post-hoc analyses and one clinical trial, with mostly similar [5,6,22,23] but also contrasting [7] findings. In line with our results, the VITACOG trial, in which older adults (>70y) with MCI were supplemented with B-vitamins (folic acid, vitamin B6 and B12) versus placebo for two years, showed that omega-3 fatty acid status influenced B-vitamin treatment efficacy. Only individuals with higher plasma omega-3 fatty acid levels showed slower rates of cognitive decline [5] and brain atrophy [6] following B-vitamin supplementation. Similarly, a post-hoc analysis of the OmegAD randomized controlled trial showed that adequate levels of both omega-3 fatty acids and B-vitamins are needed [22]. In the OmegAD trial on the effect of 6 month daily supplementation with EPA and DHA versus placebo in AD patients, only subjects with lower homocysteine status benefited from omega-3 fatty acid supplementation. Further proof comes from a recent randomized controlled trial with a factorial design, in which older adults with MCI were supplemented with placebo, 0.8mg folic acid, 0.8mg DHA or a combination of the two daily for 6 months. Combined intervention of folic acid and DHA was more effective in improving cognition compared to supplementation with only folic acid or DHA, adding proof for the interaction from a factorial clinical trial [23].

Contrary to our current results and previous studies, the post-hoc analysis of the FACIT trial [7], performed by our group, showed that either sufficient availability of omega-3 fatty acids or B-vitamins may be needed. In this randomized controlled trial on the effect of three year daily supplementation with folic acid in cognitively healthy middle-aged adults (50-70y) with elevated plasma homocysteine, folic acid supplementation was only beneficial in improving cognition in individuals with lower omega-3 fatty acid status, while individuals with higher omega-3 fatty acid status did not experience benefits.

The B-proof, VITACOG and OmegAD trials differed from the FACIT trial on various different aspects that could potentially explain the opposite findings. Importantly, B-proof, VITACOG and OmegAD participants were older, with an average age of over 70, versus an average age of 60 in the FACIT trial. In older individuals, needs for omega-3 fatty acids may be higher due to changes in dietary intake, bioavailability and increased membrane synthesis rates, as discussed previously [7]. Additionally, baseline omega-3 fatty acid status could be different between study populations, yet no direct comparison can be made due to differences in the fatty acid fractions analyzed, analytical methods and expressed measures. However, the omega-3 fatty acid distribution of our study population is similar to that of other study populations from European (non-Scandinavian) countries [24]. Vitamin B12 status also differed between study populations, as in the FACIT trial individuals with vitamin B12 deficiency were excluded. In our previous publication, we hypothesized that the contrasting findings of the FACIT trial could be attributed to differences in baseline homocysteine status and/or type of B-vitamin intervention. These factors now seem less probable, as homocysteine levels were both elevated in FACIT and B-proof trials and B-vitamin treatment included only folic acid in both the FACIT trial and in the clinical trial of Li and colleagues [7,23]. We strongly encourage researchers with access to data on both B-vitamin and omega-3 fatty acid status to perform post-hoc analyses to be able to better define populations that may benefit from a combination of B-vitamins and omega-3 fatty acids. These results can be the basis for the design of future clinical trials with a factorial design (comparing B-vitamin supplementation only, omega-3 fatty acid supplementation only, combined supplementation versus placebo).

A mechanistic explanation for the finding that B-vitamin supplementation was more effective in individuals with higher DHA status, may involve the interaction of B-vitamins with phospholipid metabolism [25]. Phosphatidylcholine (PC) plays a crucial role in the transport of omega-3 fatty acids, including DHA, to the brain. Interestingly, B-vitamins can influence the formation of PC [25]. In the one-carbon metabolism, the B-vitamins folic acid, B6 and B12 play an important role in regulating homocysteine levels. Inadequate B-vitamin status results in elevated levels of homocysteine and its precursor, S-adenosyl homocysteine (SAH) [26]. In turn, the accumulation of SAH slows down the enzyme phosphatidylethanolamine-N-methyltransferase, which converts phosphatidylethanolamine to PC [25]. In short, adequate B-vitamin status is needed to ensure sufficient PC production, and thus

transport of omega-3 fatty acids to the brain. To support this possible mechanistic explanation, for further research it would be interesting to measure the proportion of omega-3 fatty acids bound to PC.

Here we demonstrated that DHA status, but not EPA or total omega-3 fatty acid status, modified efficacy of B-vitamin supplementation. An explanation may again involve the regulatory role of B-vitamins for omega-3 fatty acid transport to the brain. EPA and DHA have different mechanisms to promote brain health. While EPA is particularly known for its anti-inflammatory effects and is only present in the brain in limited amounts, DHA is the most abundant fatty acid in the brain. This omega-3 fatty acid increases membrane fluidity which is critical for synaptic vesicles and transmission of signals, demonstrating the importance of adequate DHA levels in the brain for proper functioning of the neuronal membrane [27]. Alternatively, the differences in study populations (cognitively healthy versus MCI) and treatment (dose, combination of B-vitamins versus folic acid) between our study and previous studies, may be responsible for the lack of interaction with EPA in the current study.

The current analyses were limited to the interaction between vitamin B12/folic acid and omega-3 fatty acids, yet there are indications that also other nutrients may be involved. Bowman and colleagues [28] demonstrated a possible role for vitamin D, by showing that adequate vitamin D status further enhances the protective effect of sufficient homocysteine and omega-3 fatty acid levels in cognitive ageing. Additionally, omega-3 fatty acids may interact with antioxidants: a post-hoc analysis of an antioxidant supplementation trial demonstrated that the association of omega-3 fatty acid intake with cognitive functioning was modulated by a multi-nutrient antioxidant supplement containing ascorbic acid, vitamin E, beta-carotene, selenium and zinc [29], illustrating the importance of a multi-nutrient approach in slowing down cognitive ageing. For the current study, although we did have dietary and blood nutrient assessment data available, unfortunately we were limited by our sample size to further look into the role of other nutrients in the interaction. Further research with larger sample size should consider incorporating vitamin D status and/or antioxidant intake and status.

A major limitation of the current post-hoc analysis is that we performed exploratory analyses not designed and adequately powered to investigate the modifying potential of omega-3 fatty acid status on B-vitamin supplementation efficacy. The small sample size may be responsible for the lack of findings for domain-specific cognitive functioning, and for the lack of significant differences between the low and

high DHA tertiles. Additionally, omega-3 fatty acid status was only determined at baseline and in plasma phospholipids rather than red blood cells, which is a better proxy for long-term omega-3 fatty acid status. However, we assume that our measurements do represent longer-term status as dietary patterns (and thus omega-3 intake) in older adults are reasonably stable over time [30], and other factors that may influence variation (e.g. geographic and genetic reasons) also have remained stable. Though the two-year duration of the trial is a fairly short period of time to recognize cognitive deteriorations in healthy older individuals, it can still be considered a strength as an intervention period of two years is quite long in comparison with other nutrition intervention studies to slow cognitive decline. Another strength of the study is the use of an extensive cognitive test battery with a focus on domain-specific tests, instead of general tests such as the MMSE or Telephone Interview for Cognitive Status.

In conclusion, this post-hoc analysis demonstrated that B-vitamin supplementation effectiveness in cognitive ageing is related to plasma DHA levels, with older adults with higher plasma DHA levels benefitting more from B-vitamin supplementation. The results support earlier observations that positive effects of B-vitamins in cognitive ageing may be subgroup-specific. Further research is needed to optimize defining subgroups that may be susceptible for B-vitamin supplementation, and subsequently to confirm this finding in a clinical trial with a factorial design.

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Supplementary materials

Supplementary table 1: Baseline characteristics per omega-3 fatty acid tertile in the B-proof study[1]

Characteristic	Overall (n=191)	Low (n=64)	Middle (n=64)	High (n=63)	p-value
Age (years)	71.5±5.8	72.1±6.6	70.7±4.8	71.8±6.0	0.36
Sex n (%)					0.67
Male	107 (56%)	33 (52%)	37 (58%)	37 (59%)	
Female	84 (44%)	31 (48%)	27 (42%)	26 (41%)	
Level of education n (%)					0.84
Low	76 (40%)	28 (44%)	26 (41%)	22 (35%)	
Middle	46 (24%)	12 (19%)	17 (27%)	17 (27%)	
High	69 (36%)	24 (38%)	21 (33%)	24 (38%)	
BMI (kg/m^2)	27.5±4.2	26.6±4.0	28.1±4.5	27.9±3.9	0.08
Physical activity (kcal/d)	561 (358-863)	565 (356-943)	584 (361-864)	551 (374-815)	0.82
Smoking behavior n (%)					
Current smoker	11 (6%)	7 (11%)	1 (2%)	3 (5%)	0.02
Former smoker	123 (64%)	39 (61%)	37 (58%)	47 (75%)	
Never smoker	57 (30%)	18 (28%)	26 (41%)	13 (21%)	
ApoE4 carriers n(%)	55 (29%)	18 (28%)	13 (20%)	24 (38%)	0.09
Total homocysteine (µmol/L)	13.7 (12.9-15.8)	14.1 (13.3-15.9)	13.6 (12.8-16.0)	13.6 (12.9-15.3)	0.22
Folate (nmol/L)	17.4 (14.1-23.5)	16.7 (13.4-22.7)	18.4 (14.9-24.4)	17.6 (14.1-24.0)	0.32
Vitamin B12 (pmol/L)	256 (201-334)	254 (200-304)	257 (197-329)	280 (224-369)	0.18
MMA (µmol/L)	0.22 (0.19-0.29)	0.23 (0.19-0.32)	0.22 (0.18-0.27)	0.22 (0.19-0.28)	0.36
holoTC (pmol/L)	62 (46-80)	58 (46-71)	61 (46-77)	66 (48-96)	0.14
25(OH)D (nmol/L)	60±23	60±21	63±26	58±23	0.48
Omega-3 status (sum DHA+EPA, %)*	5.7±1.9	4.0±0.7	5.3±0.4	7.8±1.9	<.001
DHA (%)*	4.3±1.2	3.1±0.6	4.2±0.4	5.6±0.9	<.001
EPA (%)*	1.4±0.9	0.9±0.3	1.1±0.3	2.2±1.2	<.001
MMSE score	29 (28-30)	29 (27-29)	29 (28-30)	29 (27-30)	0.83
Global cognition Z-score	0.00±0.52	0.02±0.54	0.00±0.54	-0.03±0.50	0.90
Episodic memory Z-score	0.00±0.70	0.07±0.72	0.08±0.75	-0.13±0.62	0.19
Attention&working memory Z-score	0.00±0.86	-0.09±0.88	0.00±0.80	0.09±0.91	0.52
Information processing speed Z-score	0.00±0.77	-0.00±0.82	0.03±0.76	-0.03±0.75	0.89
Executive functioning Z-score	0.00±0.69	0.06±0.64	-0.09±0.71	0.03±0.71	0.41

[1]B-proof subjects with available fatty acid and cognition data at both time points. Abbreviations: BMI: body mass index, MMA: methylmalonic acid, holoTC: holotranscobalamin, DHA: docosahexaenoic acid, EPA: eicosapentaenoic acid, MMSE: Mini Mental State Examination. Data are mean±SD, median (IQR) or number (%). * measured in phospholipid fraction.

Supplementary table 2: Changes in domain-specific cognitive Z-scores following B-vitamin versus placebo supplementation according to omega-3 fatty acid status tertile.

	Treatment effect[1]			Overall interaction[2]	Tertiles pairwise comparison[3]		
	Crude	Adjusted	p-value	p-value	low vs middle	low vs high	middle vs high
Episodic memory				0.99			
Low tertile	0.17±0.15	0.10±0.15	0.98		diff=0.03±0.21 p=0.89	diff=0.00±0.21 p=0.99	diff=0.03±0.21 p=0.90
Middle tertile	0.19±0.14	0.13±0.15	0.95				
High tertile	0.07±0.15	0.11±0.15	0.98				
Attention & working memory				0.78			
Low tertile	0.00±0.15	0.03±0.16	1.00		diff=0.10±0.23 p=0.65	diff=0.05±0.23 p=0.82	diff=0.15±0.22 p=0.49
Middle tertile	-0.06±0.15	-0.07±0.16	0.99				
High tertile	0.08±0.15	0.08±0.16	1.00				
Information processing speed				0.61			
Low tertile	0.04±0.12	0.02±0.12	1.00		diff=0.15±0.17 p=0.38	diff=0.01±0.18 p=0.94	diff=0.14±0.17 p=0.41
Middle tertile	0.28±0.12	0.17±0.12	0.73				
High tertile	0.11±0.13	0.03±0.12	1.00				
Executive functioning				0.38			
Low tertile	0.17±0.13	0.10±0.13	0.97		diff=0.08±0.18 p=0.65	diff=0.16±0.18 p=0.38	diff=0.25±0.18 p=0.17
Middle tertile	0.06±0.13	0.02±0.13	1.00				
High tertile	0.29±0.13	0.26±0.13	0.34				

Data available for n=191 participants. Data is presented as mean β±SEM.

[1] Treatment effect is the difference in change in Z-score over time between the B-vitamin and placebo treatment groups within an omega-3 fatty acid tertile as analyzed using linear multiple regression, equal to Δ Z-score B-vitamin - Δ Z-score placebo.

Crude model : adjusted for baseline cognitive Z-score; Adjusted model: adjusted for baseline cognitive Z-score, age, sex, level of education, ApoE4 status, baseline homocysteine concentration, baseline body mass index, physical activity, smoking status.

[2] The overall interaction indicates similarity of treatment effects in the low, middle and high omega-3 fatty acid tertiles.

[3] The pairwise comparison tests for differences in treatment effects between omega-3 fatty acid tertiles.

CHAPTER 4

Concurrent nutrient deficiencies are associated with dementia incidence

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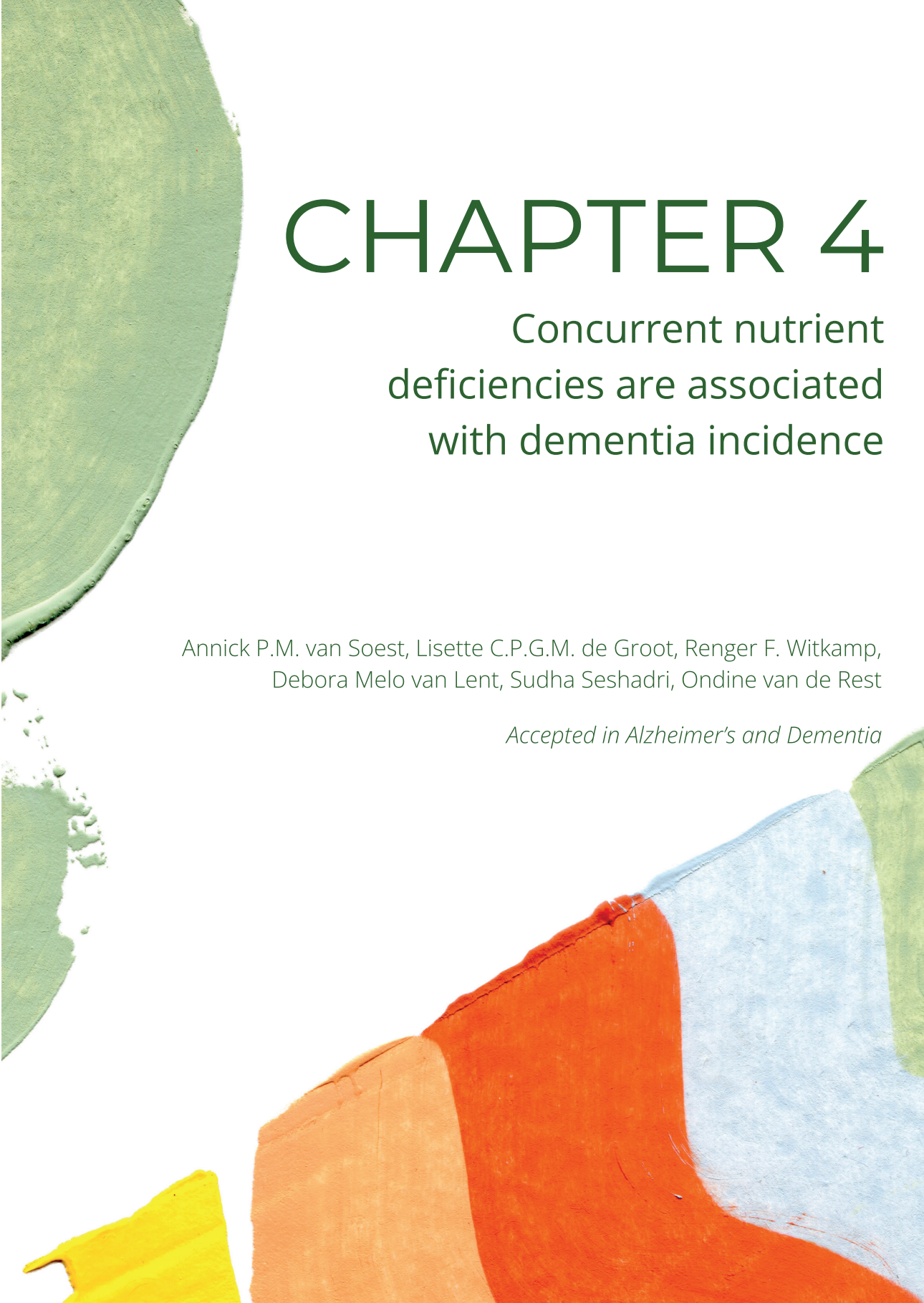

ABSTRACT

Introduction: While observational research suggests a protective role for nutrition in brain ageing, intervention studies remain inconclusive. This failing translation from observational to interventional research may result from overlooking nutrient interactions.

Methods: We developed a nutrient status index capturing the number of suboptimal statuses of omega-3 fatty acids, homocysteine and vitamin D (range 0-3). We associated this index with dementia incidence in a subsample (age≥50y) of the Framingham Heart Study Offspring cohort.

Results: Among 968 participants, 79 developed dementia over 15.5y (median follow-up). Each point increase in nutrient status index was associated with a 50% higher risk of dementia (Hazard ratio (HR) = 1.50; 95% CI = 1.16, 1.96). Participants with three high risk statuses had a 4-fold increased risk of dementia compared to participants without high risk statuses (HR = 4.68; 95% CI = 1.69, 12.94).

Discussion: Concurrent nutrient deficiencies are associated with the risk of dementia. The potential of optimizing nutritional status to lower dementia risk warrants further study.

Keywords: Nutrition, polyunsaturated fatty acids, B-vitamins, 25-hydroxyvitamin D, biomarkers, ApoE, Alzheimer's disease, ageing, older adults, elderly, prevention

INTRODUCTION

The rapidly increasing prevalence of dementia due to population ageing, in combination with the enormous social impact and economic costs of dementia, demonstrate the urgent need for action. In absence of curative treatment, the interest in preventive strategies is increasing [1].

Preclinical research has indicated that several nutritional factors have the potential to modulate brain ageing [2]. Nutrients of interest include omega-3 polyunsaturated fatty acids (n-3 PUFAs), B-vitamins, vitamin D, antioxidants, and polyphenols, which are mostly assumed to be effective due to their anti-oxidant, anti-inflammatory, and vascular health-promoting properties [2]. Epidemiological research into the association between nutrient intake or status of single nutrients and brain ageing generally confirms these preclinical findings [2]. However, clinical trials involving single nutrient supplementation, mainly demonstrate negative results [2]. This raises the question of which factors are responsible for the failure to translate findings from preclinical to clinical research.

A first explanation for the lack of effect of single nutrient supplementation in slowing down brain ageing is that nutrients are part of interacting processes with other nutrients quickly becoming limiting. Indeed, mechanisms underlying nutrition and brain ageing are considered multifactorial [3], and evidence for dietary patterns is stronger than for single nutrients [2]. A second explanation may be the lack of considering baseline nutrient status in the setup of clinical trials. In the majority of trials, participants are selected irrespective of their baseline nutrient status, while likely only individuals with a nutrient deficiency will benefit from nutrient supplementation [4]. This is supported by a secondary analysis of the VITACOG trial, in which the effect of B-vitamin supplementation on brain atrophy was dependent on baseline homocysteine levels [5].

A better understanding of the cumulative beneficial effects of nutrients and baseline nutrient status may advance the field. However, the literature on multiple nutritional deficiencies in relation to brain ageing is limited, with only two longitudinal studies having explored this topic. Here, it was demonstrated that a concurrent nutrient deficiency of n-3 PUFAs, B-vitamins and vitamin D was associated with steep rates of cognitive decline [6] and combined suboptimal statuses of n-3 PUFAs, carotenoids and vitamin D were strongly associated with an increased risk of dementia [7]. More longitudinal research is needed to reveal the complex interactions between multiple-nutrient suboptimal statuses and cognitive ageing. Specifically, the association

between a combined suboptimal status of B-vitamins, vitamin D and n-3 PUFAs with dementia incidence has not been investigated before. Therefore, we developed a nutrient status index including three nutrient biomarkers; homocysteine (as marker of vitamin B6, B12 and folate status), vitamin D, and n-3 PUFAs, and associated this index with dementia incidence in the Framingham Heart Study (FHS) Offspring cohort, a prospective community-based cohort.

METHODS

STUDY DESIGN AND POPULATION

The FHS is an ongoing prospective community-based cohort of residents of the city of Framingham, Massachusetts, USA. In 1948, the Original cohort was established to gain insight into the factors contributing to cardiovascular disease [8]. The Offspring cohort was established in 1971 as second-generation cohort, including children of the Original cohort and their spouses. A total of 5,124 participants have been enrolled in the Offspring cohort. To date, these participants have been studied over ten examination cycles, about once every four years [9]. The study was approved by the institutional review board of Boston University Medical Center and all participants have given written informed consent.

For the present study, we included data from participants aged ≥50y, free of dementia, with available blood biomarker data on homocysteine, 25-hydroxyvitamin D and n-3 PUFAs. We set the study baseline at exam 7, as biomarker data were measured at this time point. Among the 5,124 participants in the FHS Offspring cohort at exam 7, 1,525 participants had available data on all three biomarkers. All these participants were free of dementia at baseline. Data from 557 participants was excluded due to being younger than 50 years (n=130) or missing covariate data (n=427; of which n=169 education; n=25 ApoE carrier status; n=59 physical activity; n=8 smoking; n=107 alcohol intake; and n=59 depression). Thus our analysis included data of 968 participants.

LABORATORY MEASUREMENTS

Fasting serum, plasma and red blood cell samples had been collected and stored at -80°C until testing. In the samples collected at exam 7, plasma total homocysteine concentration was measured by high-performance liquid chromatography with fluorimetric detection [10] and serum 25-hydroxyvitamin D concentrations were determined by radioimmunoassay (DiaSorin, Stillwater, MN) [11]. In the samples collected at exam 8, the fatty acid composition of red blood cell membranes was

determined by gas chromatography according to the methods described by Tan and colleagues [12]. The omega-3 index was calculated using the sum of EPA and DHA and was expressed as weight percentage of total fatty acids.

While it would be preferred to have fatty acid composition data from exam 7, in line with the other biomarker data, we are confident that data from exam 8 are also valid. The fatty acids were measured in red blood cells which is preferred over measurement in serum or plasma, as red blood cell fatty acid composition is more biologically stable [13] and reflects dietary fatty acid intake over a longer time span (up to ~120d) [14]. Even though the time in between exam 7 and 8 is longer than this time interval, we assume that the red blood cell measurement provides a reliable and stable representation of the n-3 PUFA status, as dietary patterns (and thus n-3 PUFA intake) in elderly are reasonably stable over time [15] and other factors that may influence variation (e.g. geographic and genetic reasons) have remained stable.

ASCERTAINMENT OF INCIDENT DEMENTIA

Our outcome of interest was incidence of all-cause dementia, assessed through December 2018. Extensive explanation of the diagnostic procedures used has been published previously [16]. In short, participants were continuously screened for cognitive decline. They were flagged for being at risk when they experienced a decline in routinely administered Mini-Mental State Examination performance, when the participant, a family member or outside medical records reported subjective cognitive decline, or when they were referred for further screening by FHS staff or physicians. Subsequently, flagged participants underwent additional neuropsychological examination. A neurologist evaluated possible cognitive impairment or dementia, and referred for dementia review. Dementia diagnosis was made by consensus of at least one neurologist and one neuropsychologist, and was based on criteria from the Diagnostic and Statistical Manual of Mental Disorders, 4th edition [17]. If a participant passed away or was lost to follow-up, the review panel reviewed medical records up to the date of death/loss to follow-up to assess if the participant may have had cognitive decline.

COVARIATES

Data for all covariates were collected at study baseline (exam 7). Information on age, sex, education level (no high school degree, high school degree, some college, or college degree), smoking status (never, former or current) was obtained via medical questionnaires. ApoE genotype was determined as described previously [18], and classified into carriers and non-carriers of at least one ε4 allele. Physical activity was

self-reported and measured by the physical activity index [19]. Alcohol consumption was estimated from a food frequency questionnaire and classified as non-excessive or excessive (< or ≥21 units per week). Hypertension was defined as systolic blood pressure >140 mmHg, and/or use of antihypertensive medication, and diabetes was defined as random blood glucose ≥200 mg/dL or fasting blood glucose ≥126 mg/dL or on anti-diabetic medication. Finally, depression was defined as a score of ≥16 on the Center for Epidemiologic Studies Depression Scale (CES-D) [20].

CONSTRUCTION OF THE NUTRIENT STATUS INDEX

To construct the nutrient status index as used in our study, we combined the approaches of Bowman et al. [6] and Neuffer et al. [7]. This nutrient status index indicates the number of high-risk statuses for three nutrients: homocysteine (as marker of B-vitamin status), vitamin D, and n-3 PUFAs. These nutrient biomarkers were selected a priori on the basis of having plausible mechanism of action in preventing dementia, having proof from observational studies for beneficial associations between the nutrient biomarker and dementia risk [2], and being readily available in the FHS Offspring cohort. The cut-off for what level is high risk, was based on our own data a posteriori. We did this by visualizing the dose-response relationships between each nutrient and the risk of dementia, using penalized splines in a Cox proportional hazard model. Each model used age at exam 7 (delayed entry) and age at time of event or censoring (age as time scale), nutrient status winsorized at the 2.5th and 97.5th percentile, and the covariates sex, education and ApoE4 carrier status. After visualisation of the dose-response relationships, we set cut-offs based on graphical inspection of the curves where the splines crossed y=0.

Figure 1 shows the dose-response associations between the individual nutrient statuses with dementia. For homocysteine, the cut-off was set at 8 μmol/L. As homocysteine level was positively associated with dementia risk, participants with homocysteine status ≥8 μmol/L were classified in the 'high risk' category, and those with status <8 μmol/L were classified as 'low risk'. The cut-off for vitamin D (measured as 25-hydroxyvitamin D) was set at 15 ng/mL (37.5 nmol/L). For vitamin D levels between 5 and 25 ng/mL (12.5-62.5 nmol/L), there was an inverse association between vitamin D status and dementia incidence. To this end, participants with vitamin D levels ≤15 ng/mL were classified as 'high risk', and participant with a status >15 ng/mL as 'low risk'. Even though the line y=0 also crosses the spline at vitamin D level 28 ng/mL, we did not set another cut-off because only very few participants had vitamin D levels of ≥28 ng/mL, and this observation cannot

be explained from a physiological perspective. For omega-3 PUFAs, the cut-off was set at an omega-3 index of 5%. As omega-3 index was inversely associated with dementia incidence, participants with an omega-3 index ≤5% were classified as 'high risk' and participants with status >5% as 'low risk'. Again, while y=0 also crosses the spline at omega-3 index 8.5% we did not set a second cut-off because of the reasons explained before.

The nutrient status index captures the number of high risk statuses (range 0-3). In other words, we assigned score 0 if a participant fell into the 'low risk' category, and a score 1 if the participant had a 'high risk'. The ultimate nutrient status index sums the values for homocysteine, vitamin D and n-3 PUFA status.

STATISTICAL ANALYSIS

For the comparison of baseline characteristics, participants were grouped according to the number of high risk nutrient statuses. Baseline characteristics for these groups were compared using ANOVA or Kruskal-Wallis test for continuous variables and chi-square for categorical variables.

For the main analyses, we examined the longitudinal association between the nutrient status index and dementia incidence. We performed multivariate-adjusted Cox proportional hazard models and modelled delayed entry and age as time scale as a function of the nutrient status index (categorical and continuous). Model 1 was adjusted for the covariates age, sex, education, ApoE4 carrier status, and model 2 was additionally adjusted for physical activity, smoking, alcohol intake, hypertension, diabetes and depression. The proportional hazard assumption was met. Results are presented as adjusted hazard ratios accompanied by 95% confidence intervals. The hazard ratios represent the difference in dementia risk compared to participants with no high risk statuses (categorical), and the change in dementia risk by each unit increase in the nutrient status index (continuous).

As sensitivity analysis, we evaluated the robustness of the nutrient status index. We tested the effect of adjusting the cut-offs by 10%, by adopting same cut-offs as in a previous article [6], changing the definition of omega-3 PUFA status by including DPA, and by changing the age cut-off to ≥60y. The nutrient status indices thus obtained were again associated with dementia incidence similarly to the primary analysis.

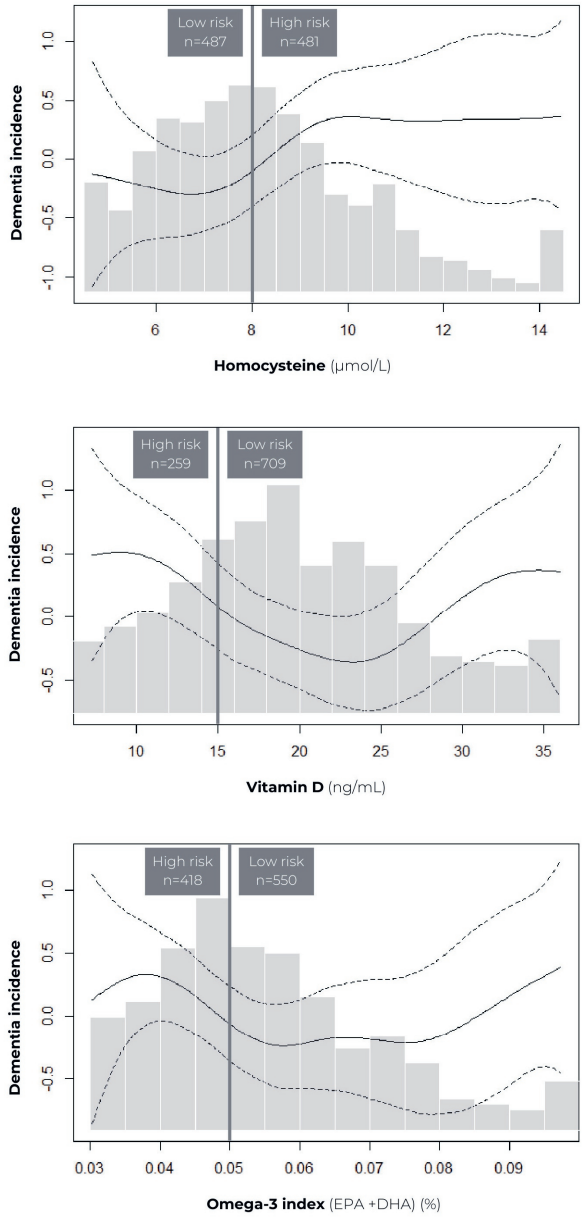

Figure 1: Dose-response relationships between nutrient statuses of homocysteine, vitamin D and omega-3 index and dementia risk, used to set cut-offs for optimal (low risk) and suboptimal (high risk) status to construct the nutrient status index. Abbreviations: EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid.

Additionally, to investigate if sex or ApoE4 carrier status modified the association, we tested for interactions with these two variables. A P-value <0.05 and <0.10 were considered statistically significant for the main analyses and for the tests for interactions, respectively. All analyses were performed using RStudio Version 1.1.463 [21].

RESULTS

PARTICIPANT CHARACTERISTICS

Baseline characteristics of the 968 participants are presented in **table 1**, and prevalence of high risk statuses in **supplementary table 1**. The participants were on average 61.4±7.6 years and 48% was male. A total of 22% of participants was carrier of at least 1 ApoE4 allele. The nutrient status index ranged from 0 (low risk for all nutrients) to 3 (high risk for all nutrients), with the majority of participants (40%) having 1 high risk nutrient status. Participants with a higher number of high risk statuses were more likely to be male, had on average a higher BMI, and were more often current or former smoker.

ASSOCIATION NUTRIENT STATUS INDEX WITH DEMENTIA INCIDENCE

Among the 968 participants, 79 developed dementia over a median follow-up of 15.5 [12.9, 19.0] years. In multivariable-adjusted models, the nutrient status index was associated with dementia incidence (**table 2**). Each point increase in nutrient status index was associated with a 50% higher risk of dementia (HR=1.50; 95% CI=1.16, 1.96). Moreover, participants with three high risk statuses had a 4-fold increased risk of dementia compared to participants without high risk statuses (HR=4.64; 95% CI=1.68, 12.83).

SENSITIVITY ANALYSES

We performed sensitivity analyses to assess the robustness of the association between the nutrient status index with dementia incidence. Overall, the association was robust to changes in nutrient status cut-offs (supplementary table 2). Varying the cut-offs by 10%, as well as adopting the same cut-offs as Bowman and colleagues[6], did not alter results. Similarly, results were robust to variations in study population and components. Changing the age cut-off to ≥60y or adapting the definition of the omega-3 index by including DPA in addition to EPA and DHA did not change results.

Subsequently, we tested for interactions to investigate if sex and ApoE4 carrier status modified the association between the nutrient status index and dementia

incidence (**supplementary table 3**). Interestingly, ApoE4 carrier status appeared to influence the association ($p_{interaction}$=0.01). The nutrient status index (continuous) was positively associated with dementia incidence in carriers ($HR_{per\ point\ increase}$=2.05, 95% CI=1.23, 2.44), but not in non-carriers ($HR_{per\ point\ increase}$=1.11, 95% CI=0.77, 1.59). There was no significant overall interaction between sex and nutrient status index (p=0.23).

Table 1: Characteristics of the Framingham Heart Study population per number of high risk statuses

	Overall (n=968)	Number of high-risk statuses				p-value
		0 (n=232)	1 (n=391)	2 (n=268)	3 (n=77)	
Dementia cases n (%)	79 (8%)	7 (3%)	38 (10%)	25 (9%)	9 (12%)	
Age (years)	61.4±7.6	60.8±7.1	61.4±7.8	62.1±7.7	61.1±7.8	0.33
Sex n (%)						<0.001
Male	461 (48%)	75 (32%)	185 (47%)	159 (59%)	42 (55%)	
Female	507 (52%)	157 (68%)	206 (53%)	109 (41%)	35 (54%)	
ApoE4 carrier n (%)	215 (22%)	50 (22%)	85 (22%)	60 (22%)	20 (26%)	0.85
Level of education n (%)						<0.001
High school no graduate	34 (4%)	4 (2%)	9 (2%)	18 (7%)	3 (4%)	
High school graduate	313 (32%)	66 (28%)	126 (32%)	94 (35%)	27 (35%)	
Some college	238 (25%)	70 (30%)	81 (21%)	67 (25%)	20 (26%)	
College graduate	383 (40%)	92 (40%)	175 (45%)	89 (33%)	27 (35%)	
BMI (kg/m^2)	28.1±5.1	26.4±4.3	28.3±5.1	28.9±5.1	29.8±5.4	<0.001
Physical activity (PAI)	38.2±6.4	38.7±5.9	37.7±6.3	38.6±7.1	37.9±5.9	0.18
Smoking behavior n (%)						0.02
Current smoker	30 (3%)	3 (1%)	9 (2%)	13 (5%)	5 (6%)	
Former smoker	563 (58%)	131 (56%)	229 (59%)	151 (56%)	53 (69%)	
Never smoker	374 (39%)	98 (42%)	153 (39%)	104 (39%)	19 (25%)	
Systolic blood pressure (mmHg)	126±17	124±17	127±17	127±17	127±16	0.07
Use of anti-hypertensives n (%)	337 (35%)	70 (30%)	148 (38%)	94 (35%)	25 (32%)	0.26
Depression (CES-D)	3 [0 – 6]	2 [1 – 7]	3 [0 – 6]	2 [0 – 6]	3 [1 – 7]	0.42
Cardiovascular disease n (%)	114 (12%)	20 (9%)	53 (14%)	33 (12%)	8 (10%)	0.30
Diabetes n (%)	84 (9%)	13 (6%)	31 (8%)	32 (12%)	8 (10%)	0.07
Omega-3 index (wt%)	5.6±1.7	6.8±1.5	5.7±1.7	4.7±1.2	4.0±0.7	<0.001
Plasma homocysteine (µmol/L)	8.4±3.8	6.4±1.1	8.1±2.3	9.5±2.5	11.7±9.9	<0.001
Serum 25-hydroxyvitamin D (ng/mL)	19.9±7.7	23.7±6.7	20.7±6.8	18.1±7.9	10.7±2.8	<0.001

Data are mean±SD, median [IQR] or number (%). ApoE: Apolipoprotein E; BMI: Body Mass Index; PAI: Physical Activity Index; CES-D: Center for Epidemiologic Studies Depression Scale.

Table 2: Risk of dementia by multi-nutrient status index

	Crude model	Model 1	Model 2
Number of high risk statuses			
0 (lowest risk)	REFERENCE	REFERENCE	REFERENCE
1	2.98 [1.33, 6.70]	2.79 [1.23, 6.33]	2.89 [1.27, 6.58]
	0.008	0.014	0.012
2	3.03 [1.31, 7.00]	3.09 [1.32, 7.24]	3.28 [1.38, 7.80]
	0.010	0.009	0.007
3 (highest risk)	4.30 [1.60, 11.56]	4.70 [1.74, 12.69]	4.68 [1.69, 12.94]
	0.004	0.002	0.003
Continuous	1.43 [1.11, 1.83]	1.48 [1.15, 1.93]	1.50 [1.16, 1.96]
	0.005	0.002	0.002

Data are hazard ratio [95% confidence interval] p-value. Model 1: adjusted for age, sex, education, and ApoE4 carrier status; Model 2: additionally adjusted for physical activity, smoking, alcohol consumption, diabetes, hypertension, and depression. In the continuous analysis, data are shown per point increment in nutrient status index

DISCUSSION

Using the nutrient status index developed for our study, we found that individuals with a higher index, i.e. having suboptimal statuses of homocysteine (as a marker of B-vitamins), vitamin D and n-3 PUFAs, had a higher risk of developing dementia compared to those with a lower index. Remarkably, a suboptimal status of all three nutrients was associated with a 4-fold increased risk of dementia compared to individuals without suboptimal statuses. In addition, ApoE4 carrier status appeared to influence the association between nutrient status index and dementia incidence, with the association only evident in ApoE4 carriers.

The effect size we observed was substantial: a 4-fold increased risk of developing dementia in individuals with combined suboptimal status of n-3 PUFAs, vitamin D and homocysteine. This effect size is large in comparison with other risk factors of dementia. In our sample, being current smoker or having diabetes doubled the risk, and being carrier of at least one ApoE4 allele tripled the risk of dementia.

Previous research complements our results and the large effect sizes. To our knowledge, the association between multiple-nutrient suboptimal statuses and brain ageing has been investigated in two other studies. In a secondary analysis of the Bordeaux Three-City (3C) study, Neuffer and colleagues developed a nutrient status index comprising of n-3 PUFAs (EPA+DHA+DPA), carotenoids and 25(OH)D. Similar to our approach, they set nutrient cut-offs based on their own data (n3-PUFA at 3 and 4.5%; carotenoids at 100 and 200 µg/mmol; vitamin D at 8 and 26 ng/mL). A higher nutrient status index (i.e. more nutrient suboptimal statuses) was

associated with a higher risk to develop dementia. The 13% of participants with highest nutrient status index, had a 4-fold increased chance of developing dementia compared to the 21% with lowest index scores [7]. Additionally, Bowman and colleagues investigated the role of combined deficiencies in n-3 PUFAs (EPA + DHA), 25(OH)D, and homocysteine on cognitive decline in a secondary analysis of the French Multi-domain Alzheimer's Prevention Trial (MAPT), with cut-offs for nutrient deficiencies set *a priori* (n-3 PUFA 4,82%; Hcy 14 µmol/L; vitamin D 20 ng/mL). Individuals without nutrient deficiencies of these nutrients showed cognitive improvements over three years, while each additional nutrient deficiency led to an incremental faster rate of cognitive decline [6].

While this previous research also demonstrates associations between multiple-nutrient suboptimal statuses and brain ageing, direct comparison between results is being complicated by differences in components and cut-offs.

Regarding components, in the 3C study data on carotenoid but not on homocysteine status were available. Carotenoids are also nutrients of prime interest in relation to the ageing brain. These nutrients reduce oxidative stress, a mechanism involved in the pathogenesis of dementia [22]. Additionally, higher carotenoid status has been associated with lower odds of dementia [23]. It is a limitation of the current study that that we did not have data available on carotenoid status, or on other anti-oxidant nutrient statuses like vitamin C or E. Further research on multi-nutrient suboptimal statuses should consider incorporating anti-oxidant nutrients, alongside n-3 PUFA's, homocysteine and vitamin D status.

With respect to cut-offs, our homocysteine cut-off (8 µmol/L) was lower compared to the French MAPT trial (14 µmol/L), which was anticipated because of folate fortification in the US. Our low cut-off is likely not applicable in countries where foods are not fortified. Vitamin D cut-offs among studies varied, ranging from 15 ng/mL in FHS, 20 ng/mL in MAPT and 8 and 26 ng/mL in 3C. Our cut-off is lower than the WHO guidelines of 20 ng/mL (50 nmol/L). However, this cut-off has been set for bone health rather than brain health. Our n-3 PUFA cut-off (5%) was slightly higher compared to the 3C study (3 and 4.5%) and MAPT trial (4.82%), but still low compared to the target range of 8-11% [24]. While increasing the cut-off to this target range could provide even stronger protective associations, the baseline omega-3 index of our study population was too low to obtain ensure sufficient contrast. All in all, optimal nutrient cut-offs may be population-specific and therefore it can be seen as

a limitation that we set cut-offs based on our own data. Nevertheless, the nutrient status index applied in our study was robust to variations in cut-offs as demonstrated in the sensitivity analyses, and this adds to the robustness of our findings. In addition, despite methodological difference between our and previous studies, results are remarkably consistent.

The observation that there is an association between multiple suboptimal nutrient statuses and dementia risk is biologically plausible. Preclinical studies underline that brain ageing depends on multiple, dynamically interacting mechanisms with nutrients playing distinctive roles. Vitamin D, among others, promotes healthy brain ageing by suppressing beta-amyloid deposition, regulating calcium homeostasis and reducing oxidative stress and inflammation [25]. Omega-3 fatty acids also possess anti-inflammatory and anti-oxidant properties, as well as vascular health-promoting effects. In addition, these fatty acids serve as building blocks for neuronal tissue [26]. Homocysteine has been shown to negatively impact the ageing brain through impairing vascular functioning, by increasing tau phosphorylation, and via inhibition of methylation reactions [27]. Considering the multi-factorial nature of dementia, it is conceivable that these single effects of the nutrients targeting different mechanisms of action have additive effects.

As well as these additive effects, it is possible that nutrients act in a synergistic manner. This has been hypothesized for homocysteine and n-3 PUFAs, as a consequence of the regulatory role of homocysteine in the transport of n-3 PUFAs to the brain [28]. DHA is transported with the help of phosphatidylcholine (PC), the formation of which is dependent on homocysteine levels. Elevated homocysteine levels decrease the activity of phosphatidylethanolamine N-methyltransferase, the enzyme responsible for the conversion of phosphatidylethanolamine to PC. Consequently, this results in low transport of n-3 PUFAs to the brain [28]. Indeed, the synergistic effects between homocysteine and n-3 PUFAs have been confirmed in secondary analyses of B-vitamin [29-31] and n-3 PUFA [32] supplementation trials.

For further research, we strongly encourage researchers with access to data on multiple nutrient statuses and brain ageing outcomes to further investigate the potential of multi-nutrient suboptimal statuses. This will give more insight in the optimal nutrient status cut-offs. Additionally, these results can be the basis for the design of clinical trials, in which the nutrient status index can be used to select participants at nutritional risk for dementia [4]. Participants then may undergo nutrient supplementation to correct suboptimal status. Instead of nutrient

supplementation, participants could undergo a diet intervention targeted at improving general dietary intake, as suboptimal status of the three nutrients investigated in this research could also be a proxy for general suboptimal nutritional status.

Another topic that deserves further investigation is the interaction with ApoE4 genotype. We observed an interaction with ApoE4 genotype, with strong associations between multi-nutrient suboptimal statuses and dementia in carriers, but not in non-carriers of the ApoE4 allele. This interaction has not been investigated in the two previous articles on multi-nutrient suboptimal statuses [6,7]. However, a large body of literature is available on the interaction between ApoE genotype and n-3 PUFAs in relation to brain ageing. According to this literature, ApoE4 carriers seem more susceptible to the benefits of omega-3 PUFAs in preclinical stages, while benefits in the clinical stages are limited to non-carriers [33]. The mechanistic rationale why ApoE4 carriers may need more n-3 PUFAs during the preclinical stage is that they experience accelerated DHA catabolism and less efficient transport of DHA both across the blood brain barrier and within the brain. These processes occur before the onset of neurodegeneration [33]. At baseline, our study population was likely in the preclinical stage of dementia, considering the relatively young age (≥50y). Literature on vitamin D and homocysteine in relation to ApoE genotype is limited, but a similar pattern as for n-3 PUFAs has been demonstrated for other preventive strategies to lower dementia risk, with ApoE4 carriers benefitting more in preclinical stages [34]. However, it is important to emphasize that these results come from a subgroup analysis and thus interpretation is limited. Further research is required to confirm the interaction with ApoE4 genotype.

In conclusion, in our community-based sample, concurrent suboptimal status of n-3 PUFAs, homocysteine and vitamin D is associated with the risk of dementia. The results support earlier observations that multiple-nutrient suboptimal statuses are highly detrimental for brain ageing, suggesting that nutrition is a key modifiable risk factor for dementia. Further research is needed to optimize nutrient status cut-offs and to study the potential of optimizing nutritional status to lower dementia risk.

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Supplementary materials

Supplementary table 1: Overview of the prevalence of high risk nutrient statuses of homocysteine, vitamin D and omega-3 polyunsaturated fatty acids

Nutrient(s) with high risk status	Sample size, n (%)
0 risk statuses (lowest risk)	232 (24%)
1 risk status	391 (40%)
Hcy	186 (19%)
Vit D	67 (7%)
n-3 PUFA	138 (14%)
2 risk statuses	268 (28%)
Hcy & vit D	65 (7%)
Hcy & n-3 PUFA	153 (16%)
Vit D & n-3 PUFA	50 (5%)
3 risk statuses (highest risk)	77 (8%)

Abbreviations: Hcy: homocysteine; n-3 PUFA: omega-3 polyunsaturated fatty acid; vit D: vitamin D

Supplementary table 2: Association between nutrient status index and dementia incidence following changes in definitions exposures and cut-offs

	Effect size			
	Crude	Model 1	Model 2	p-value
Adapting data-based cut-offs				
Homocysteine				
Cut-off 10% lower	1.40 [1.07, 1.84]	1.45 [1.10, 1.92]	1.44 [1.08, 1.93]	0.01
Cut-off 10% higher	1.40 [1.10, 1.79]	1.42 [1.10, 1.83	1.43 [1.10, 1.85]	0.008
Vitamin D				
Cut-off 10% lower	1.53 [1.18, 1.97]	1.61 [1.23, 2.10]	1.64 [1.25, 2.16]	<0.001
Cut-off 10% higher	1.37 [1.08, 1.74]	1.43 [1.11, 1.83]	1.45 [1.12, 1.87]	0.004
Omega-3 index				
Cut-off 10% lower	1.46 [1.13, 1.88]	1.53 [1.18, 1.98]	1.55 [1.19, 2.02]	0.001
Cut-off 10% higher	1.41 [1.10, 1.81]	1.46 [1.14, 1.88]	1.44 [1.11, 1.86]	0.006
Adapting literature-based cut-offs				
Cut-offs according to Bowman 2019[1]	1.49 [1.10, 2.01]	1.45 [1.06, 1.98]	1.41 [1.03, 1.94]	0.03
Changing definition omega-3 index				
Including DPA	1.42 [1.10, 1.83]	1.49 [1.15, 1.94]	1.48 [1.13, 1.93]	0.005
Changing age cut-off				
≥60y	1.42 [1.09, 1.83]	1.48 [1.13, 1.93]	1.48 [1.13, 1.94]	0.004

Data are HR [95% CI] per point increase in nutrient status index. Model 1: adjusted for age, sex, education, and ApoE4 carrier status; Model 2: additionally adjusted for physical activity, smoking, alcohol consumption, diabetes, hypertension, and depression.

Supplementary table 3: Association between nutrient status index and dementia incidence, stratified by sex and ApoE4 carrier status

	Effect size				Overall interaction
	Crude	Model 1	Model 2	p-value	p-value
ApoE4 carrier status					
Carrier (n=215, of which 33 dementia cases)	2.10 [1.42, 3.10]	2.14 [1.42, 3.22]	2.05 [1.23, 2.44]	0.001	0.01
Non-carrier (n=753, of which 46 dementia cases)	1.13 [0.81, 1.57]	1.10 [0.79, 1.55]	1.11 [0.77, 1.59]	0.57	
Sex					
Female (n=507, of which 40 dementia cases)	1.55 [1.11, 2.15]	1.62 [1.17, 2.25]	1.74 [1.23, 2.44]	0.002	0.23
Male (n=461, of which 39 dementia cases)	1.23 [0.83, 1.82]	1.28 [0.85, 1.93]	1.27 [0.82, 1.94]	0.28	

Data are HR [95% CI] per point increase in nutrient status index. Model 1: adjusted for age, education, and ApoE4 carrier status or sex; Model 2: additionally adjusted for physical activity, smoking, alcohol consumption, diabetes, hypertension, and depression.

CHAPTER 5

Associations between pro- and anti-inflammatory gastro-intestinal microbiota, diet and cognitive functioning

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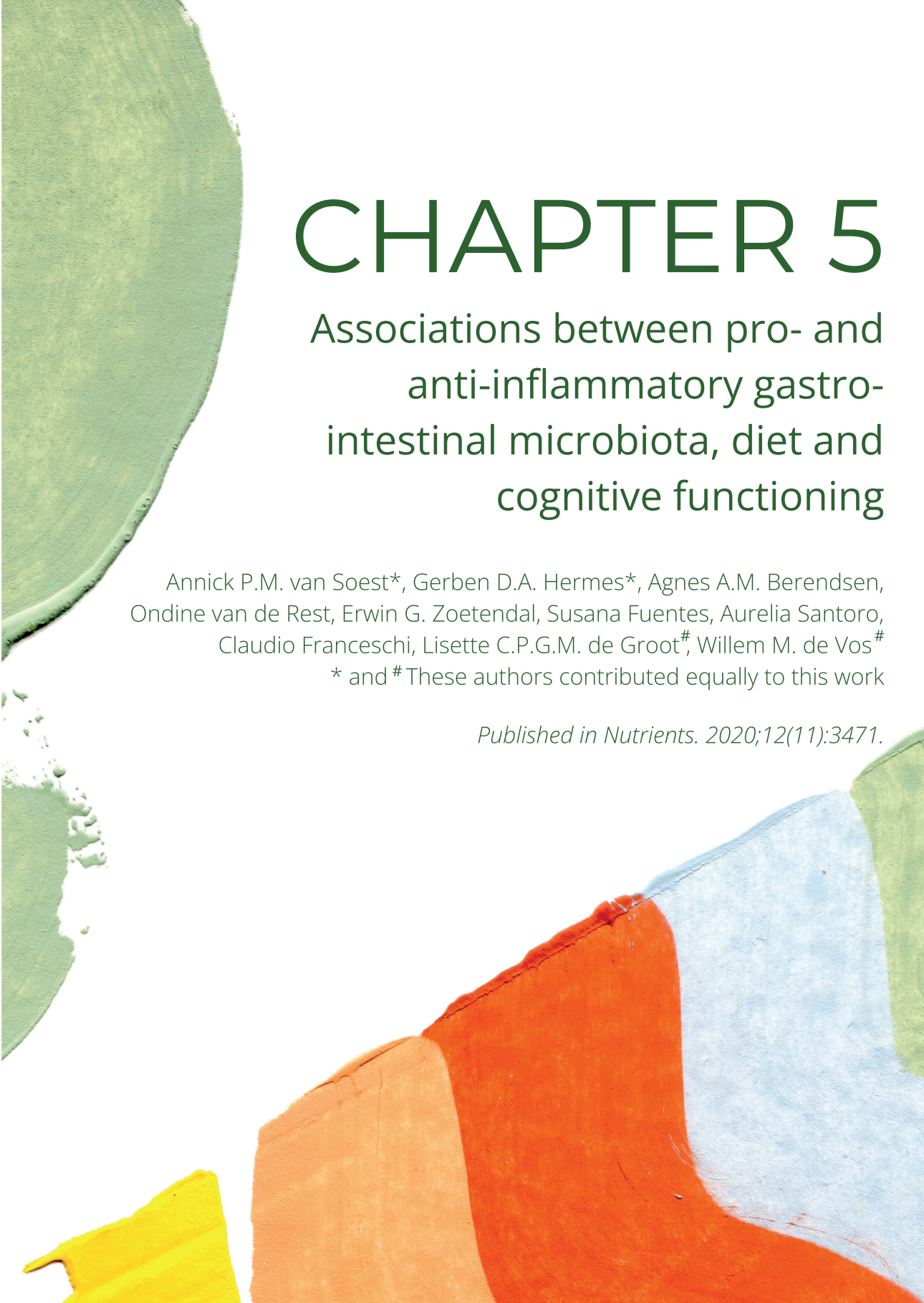

ABSTRACT

Dietary modulation of the gastro-intestinal microbiota is a potential target in improving healthy ageing and age-related functional outcomes, including cognitive decline. We explored the association between diet, gastro-intestinal microbiota and cognition in Dutch healthy older adults of the NU-AGE study. The microbiota profile of 452 fecal samples from 226 subjects was determined using a 16S ribosomal RNA gene-targeted microarray. Dietary intake was assessed by 7-day food records. Cognitive functioning was measured with an extensive cognitive test battery. We observed a dietary and microbial pro- to anti-inflammatory gradient associated with diets richer in animal- or plant-based foods. Fresh fruits, nuts, seeds and peanuts, red and processed meat and grain products were most strongly associated to microbiota composition. Plant-rich diets containing fresh fruits, nuts, seeds and peanuts were positively correlated with alpha-diversity, various taxa from the Bacteroidetes phylum and anti-inflammatory species, including those related to *Faecalibacterium prausnitzii* and *Eubacterium rectale* and *E. biforme*. Animal product-rich diets associated with pro-inflammatory species, including those related to *Ruminococcus gnavus* and *Collinsella spp.*. Cognition was neither associated with microbiota composition nor alpha-diversity. In conclusion, diets richer in animal- and plant-based foods were related to a pro- and anti-inflammatory microbial profile, while cognition was associated with neither.

Keywords: Gut microbiota; Dietary intake; Cognitive decline; Elderly; Healthy ageing; Inflammation

INTRODUCTION

The ageing population is growing rapidly. Worldwide, the number of people aged 65 years or over is currently estimated at 703 million. Due to a steep rise in life expectancy, this number is expected to double to 1.5 billion in 2050 [1]. Unfortunately, as the longer lifespan is not accompanied by improvements of health outcomes [2], the increase in life expectancy poses serious challenges to the health care system, economy and society [3]. Therefore, there is an urgent need for strategies to improve healthy ageing.

The gastro-intestinal (GI) microbiota has been implicated as a potential target to enhance healthy ageing [4]. Ageing is accompanied by several physiological and lifestyle changes, including altered GI tract function, elevated inflammation levels and dietary changes, that affect the GI microbiota [5, 6]. Compared to younger adults, the GI microbiota in older adults has been shown to exhibit larger inter-individual and temporal variation. It was also strongly correlated to diet, which was linked to residence location in the community [7, 8]. Despite the larger variation, several universal changes in the GI microbiota that occur with ageing have been identified. Generally, the relative abundance of *Bifidobacterium* spp. Was found to be lower in older adults with concomitant higher levels of Enterobacteriaceae and other pathobionts [5, 6].

Changes in GI microbiota composition may influence age-related functional outcomes, such as cognitive decline. In the past decade, the link between altered GI microbiota composition and cognition has been demonstrated in various rodent models, including germ-free animals and several microbiota modulation strategies, such as antibiotics, pre- or probiotics, and fecal microbiota transplants [9]. For example, rodents with disrupted GI microbial homeostasis, due to infection or treatment with antibiotics, perform worse on cognitive tests compared to animals with an undisturbed GI microbiota. Restoring this homeostasis by administration of probiotics or via fecal microbiota transplantation positively influenced cognitive performance of rodents [9]. In humans, administration of *Bifidobacterium* and *Lactobacillus* species for 12 weeks has shown to positively affect cognitive functioning in older adults [10, 11], providing preliminary evidence for a relation between GI microbiota and cognition in humans, thus proposing the GI microbiota as a target to prevent or delay age-related cognitive decline.

Modification of diet has been suggested as a strategy to both maintain cognition and GI homeostasis. There is special interest in the Mediterranean diet (MedDiet), which

is characterized by a high intake of vegetables, fruits, legumes and olive oil and moderate to low intake of animal-based food products [12]. Greater adherence to the MedDiet has been associated to slower rates of age-related cognitive decline [13, 14] and beneficial changes in GI microbiota composition [15, 16].

To our knowledge, to date only one human study has investigated the relation between diet, cognition and GI microbiota. Data from all European partners of the NU-AGE study, a one-year Mediterranean-like dietary intervention, showed that individuals with better adherence to this diet had higher relative abundances of several microbial groups, including *Faecalibacterium prausnitzii, Anaerostipes* and *Roseburia* [16], which have previously been linked to beneficial health effects. For instance, these species exhibit anti-inflammatory properties, are able to produce the short chain fatty acid (SCFA) butyrate and have been inversely associated with diabetes mellitus type 2 and colorectal cancer [17-19]. In turn, higher relative abundances of these beneficial species were weakly, but positively, associated with cognitive function measured by BabCock Memory and Constructional Praxis performance [16].

These results provide preliminary evidence for the potential of the MedDiet to prevent age-related cognitive decline by modulating GI microbiota. However, it remains unclear which specific food groups of the MedDiet are responsible for the potentially beneficial effects on cognition and GI microbiota composition. Moreover, in the previous study, cognitive function was measured by means of single tests [16], whereas the assessment of multiple cognitive tests representing all cognitive domains and combining these tests into composite cognitive scores is a more robust measure of cognitive functioning [20]. Therefore, the current study aims to explore the relation between diet, GI microbiota composition and cognitive function in healthy older adults (65-79 years).

MATERIALS AND METHODS

STUDY DESIGN AND PARTICIPANTS

We used data from the Dutch cohort of the NU-AGE study, a parallel randomized one-year study investigating the effect of a dietary intervention on inflammation in European older adults [21]. Cognitive functioning and microbiota composition were determined as secondary outcomes. Information on participants, recruitment and the dietary intervention has previously been described in detail [22, 23]. In short, 252 healthy Dutch older adults aged 65-79 years were randomized to the intervention or

control group. Participants in the intervention group received individually tailored dietary advice to follow a Mediterranean-like diet. The control group received no specific dietary advice except for a leaflet describing the national guidelines for a healthy diet. Analyses showed that the intervention did not affect GI microbiota. Therefore, the current study has a cross-sectional design, in which data from both pre and post intervention are combined. Participants were non-frail (Fried frailty ≤1 [24]) and free of major diseases including cancer, dementia, diabetes mellitus type I and II and organ failure, and did not use antibiotics in the three months prior to inclusion. Dietary intake, GI microbiota composition and cognitive functioning were assessed at baseline and post intervention. Data from 26 participants were excluded due to missing GI microbiota assessments at either pre or post intervention. The NU-AGE study has been registered at clinicaltrials.gov (identifier: NCT01754012). This study was conducted according to the Declaration of Helsinki and written informed consent was obtained from all participants. The study protocol was approved by the Medical Ethics Committee of Wageningen University & Research (ABR 37818.081.11).

DIETARY ASSESSMENT

At baseline and post intervention, dietary intake was assessed by a 7-day food record. Participants were instructed to record all consumed foods and their amounts based on household measures. All food records were reviewed by a trained research dietician during an interview. Consumed food products were coded according to standardized coding procedures. Nutrient intake data was calculated by use of the Dutch food composition table (NEVO 2011). Consumed food products with similar composition were grouped into food groups according to the EPIC-Soft Classification [25] with some local modifications. Additional groups were created for ready-to-eat meals and savory bread spreads as products in these groups were not included in the current EPIC-Soft list. Separate groups were created for low fat, and salt and sugar options within the dairy food groups based on the Dutch dietary guidelines [26]. Products containing artificial sweeteners were placed in a separate group as sweeteners have been shown to influence GI microbiota composition [27]. In addition, a separate group was made for legume-based ready to eat soups due to the relatively high fiber content. Finally, the food group meat was divided into red meat, processed meat, poultry and meat replacers instead of groups based on animal origin to limit the number of food groups.

MICROBIOTA COMPOSITION PROFILING

At baseline and post intervention, participants were instructed to collect a fecal sample at home with the help of a stool collection kit and store them immediately at -20°C. Samples were transported in coolers and then stored at -20°C and later at -80°C before being processed. DNA extraction from fecal samples has been described in detail elsewhere [28]. In brief, DNA was extracted using a combination of column purification and Repeated-Bead-Beating. Purity and concentration of DNA were assessed with a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). The composition analysis was then performed utilizing a previously benchmarked custom made, phylogenetic microarray, the Human Intestinal Tract Chip (HITChip) [29, 30]. The HITChip contains a duplicated set of 3,631 probes, which target the V1 and V6 hypervariable regions of the 16S rRNA gene of 1140 intestinal bacterial phylotypes. After extraction of DNA, the full-length 16S rRNA gene was amplified by PCR using primers T7prom-Bact-27-for and Uni-1492-rev [30]. This was followed by in vitro transcription and labelling of the resulting RNA with Cy3/Cy5 before hybridization to the array. The signal intensity data from the microarray hybridizations were collected from the Agilent G2505C scanner (Agilent Technologies) using the Agilent Feature Extraction software, version 10.7.3.1 and pre-processed using an in-house MySQL database and custom R scripts. Each scanner channel from the array was separately spatially normalized using polynomial regression, followed by outlier detection and filtering in each set of probes with a $\chi 2$ test. Each sample was hybridized at least twice to ensure reproducibility. Duplicate hybridizations with a Pearson correlation <.98 were not considered for further analysis. Microbiota profiles were summarized to genus-like 16S rRNA gene sequence groups with a sequence similarity >90% referred to as species and relatives ('et rel.'). Measurements of probes that belong to the same phylotype were normalized with Robust Probabilistic Averaging [31, 32]. Log10-transformed hybridization signals were used as a proxy for bacterial abundance.

COGNITIVE FUNCTIONING

Cognitive functioning was assessed at baseline and post intervention with an extensive battery of cognitive tests which were administered by trained research assistants. The battery included cognitive tests from the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) test battery [33] plus five additional tests. In the *Verbal fluency category test* [34], participants were asked to name as many animals as possible within 60 seconds. The number of uniquely named animals was

recorded. Participants were presented with four figures in the *Constructional praxis test* [35], and asked to copy these figures on blank paper immediately after presentation (subtest immediate) and after a few minutes (subtest recall). Scoring was based on the number of correct responses. In the *Word List Memory test* [33], participants were visually presented with ten random words. The number of correctly recalled words directly after presentation in three trials (subtest immediate) and after five minutes in one trial (subtest delayed) was recorded. Finally, the participant was asked to identify the ten words from a verbally presented list of twenty words (subtest recognition). Next, participants were read a brief story in the *Babcock story recall test* [36] and asked to retell the story immediately (subtest immediate) and after 20 minutes (subtest delayed). Scoring was based on the correctly recalled parts of the story. In the *Trail Making Test* [37], participants were instructed to connect 25 numbers in chronological order (part A) and to connect numbers and letters in chronological and alphabetical order alternately (part B). Time to complete each task was recorded. In the *Number cancellation test* [38], participants were presented with a list of random numbers. The number of correctly crossed out 4s in 30 seconds was documented. In the *Pattern comparison test* [39], participants were asked to indicate if two patterns were similar or different. Scoring was based on the number of correct responses.

Scores for each of the cognitive tests were converted into Z-scores with baseline mean and standard deviation of the whole population. The Z-score for the Trail Making Test was reversed as lower scores represent better cognitive functioning. The individual Z-scores for the cognitive tests were clustered into four cognitive domains:

$$Episodic\ memory = (zWordList_{immediate} + zWordList_{delayed} + zWordList_{recognition} + zBabcockStoryRecall_{immediate} + zBabcockStoryRecall_{delayed})/5$$

$$Executive\ functioning = (zVerbal\ Fluency + -zTrailMakingTest_{B/A})/2$$

$$Information\ processing\ speed = (-zTrailMakingTest_{A} + zNumberCancellation + zPatternComparison)/3$$

$$Visuospatial\ ability = (-zConstructionalPraxis_{immediate} + -zConstructionalPraxis_{recall})/2$$

ASSESSMENT OF PHENOTYPICAL CHARACTERISTICS

Body weight and height were measured by trained research assistants. Weight was determined while wearing light clothing to the nearest 0.1 kg using a calibrated scale. Height was measured using a stadiometer to the nearest 0.1 cm. Body mass index

(BMI) was calculated as weight/height^2. Data on age, sex, education (number of years) and smoking status (never, former or current) were collected using questionnaires. Frailty status (non-frail/pre-frail) [24] and Mini-Mental State Examination (MMSE) [40] were assessed by trained research assistants following standardized procedures. MMSE scores from 24 to 30 are considered within the normal range [40]. Physical activity was measured using the Physical Activity Scale for Elderly (PASE). For individuals aged 70 to 75, average values for PASE are 89.1 for women and 102.4 for men [41].

STATISTICAL ANALYSES

All microbiota analyses were performed in R version 3.4.0 [42]. Redundancy analysis (RDA) was performed to determine the multivariate effects of the explanatory variables on microbiota composition using the *rda* function from the vegan package [43]. RDA is a technique summarizing the linear relationships between a set of variables i.e., GI microbiota composition explained by a set of explanatory variables i.e., dietary and host variables. The effect of an explanatory variable is defined as R^2, which is the percentage of variation explained from the total amount of microbiota variation. All numerical environmental variables (food groups, nutrients, phenotype and cognition) were normalized to ensure that the input variables had similar scales before performing the RDA. We first determined the simple effects of all explanatory variables on microbiota composition to help understand what was driving the interactions. Because the dietary intervention had no significant effect on microbiota composition, we performed a cross-sectional analysis with both pre and post intervention samples to increase power. To determine which set of food groups resulted in the most parsimonious model (i.e. explaining microbiota variation), we performed forward and reverse automatic stepwise model selection for constrained ordination methods using permutation tests with the *ordistep* function from the vegan package, which bases the term choice on Akaike's information criterion and p-value. This ordination configuration was used to test which other explanatory variables (nutrients, phenotype and cognition) significantly correlated with microbiota composition by post-hoc fitting these as vectors using the *envfit* function from vegan. $P<0.05$ was considered significant. Richness, Inverse Simpson and Shannon diversity were calculated to define microbial alpha-diversity using the microbiome package [44]. In ecology, alpha-diversity is defined as the species diversity within a sample. We used to commonly applied methods to determine diversity, *viz* Shannon diversity and Inverse Simpson diversity. Diversity of the

microbiota was based on non-logarithmic oligo-level signals and probes were counted in each sample to measure richness, by using an 80% quantile threshold for detection. To correlate microbial alpha-diversity with the significant explanatory variables we used Pearson correlations and visualized these using heatmaps with the *psych* package [45]. P-values were corrected for multiple testing using the Benjamini-Hochberg procedure [46] and q<0.05 was considered significant.

RESULTS

PARTICIPANT CHARACTERISTICS

At baseline, the mean age of participants was 70.9±4.1 years and 44.4% of the study population was male (**table 1**). The average body mass index (BMI) at baseline was 25.9±3.6 kg/m^2 and mean score on the mini-mental state examination (MMSE) was 27.7±1.8 points., indicating that our study population was cognitively healthy. The mean PASE score was 137±54, indicating that the physical activity level was slightly higher than normal compared to a study population with similar age [41].

Table 1. Baseline characteristics of 226 healthy Dutch older adults

Characteristic	n=226
Age, years	70.9±4.1
Sex, male n (%)	100 (44.2%)
Education, years	12.3±3.7
BMI, kg/m^2	25.9±3.6
Smoking status, n(%)	
Never	117 (51.8%)
Former	103 (45.6%)
Current	6 (2.7%)
MMSE (score 0-30)	27.7±1.8
Physical activity (PASE score)	137±54
Frailty, n (%)	
Non-frail	178 (78.8%)
Pre-frail	48 (21.2%)

Abbreviations: BMI: body mass index; MMSE: Mini Mental State Examination; PASE: Physical Activity Scale for Elderly. Data are presented as mean ±SD or number (%).

VARIABLES AFFECTING GI MICROBIOTA COMPOSITION

To determine how the different environmental variables impact the microbiota, we first calculated their simple effects (i.e. the effect of the environmental variable on the microbiota without any other covariates). As previously described in the methods, the dietary intervention had no significant effect on microbiota composition (p=1.0, R^2=0.08%). A total of 41 variables, existing of phenotypical

characteristics, food groups and nutrients, significantly correlated to GI microbiota composition as shown in **figure 1**. The largest proportion of GI microbiota variation was explained by individuals (R^2=40.0%) (**supplementary figure 1**). The phenotypical characteristics BMI (R^2=0.73%) and sex (R^2=0.22%) were both correlated with microbiota composition. BMI explained the largest proportion of microbiota variation out of all microbiota covariates. With respect to the dietary variables, 29 nutrients and 10 food groups were significantly correlated with GI microbiota composition. Concerning the food groups, fresh fruits explained the highest proportion of variation in GI microbiota composition (R^2=0.51%). Further zooming in on the fresh fruits showed that berries and grapes were the fruits most contributing to this observation. Other significant food groups were nuts, seeds and peanuts (R^2=0.45%), grain products (R^2=0.39%) and both processed and red meat (R^2=0.36% and R^2=0.25% respectively). Among the nutrients, total protein (R^2=0.46%) and protein from animal (R^2=0.62%) and plant (R^2=0.42%) sources explained the largest proportion of variation. In addition, various forms of carbohydrates, water-soluble vitamins, minerals and omega-3 fatty acids were significantly associated to GI microbiota composition, while other fatty acids and fat-soluble vitamins did not. None of the cognitive functioning domains was significantly correlated with GI microbiota composition.

To visualize the relations between dietary factors and phenotypical characteristics with microbiota composition, their conditional effects (the impact on the microbiota with the effect of other variables in the model) were calculated and plotted in two RDA bi-plots (**figure 2**). We observed a gradient of participants with higher intakes of plant-based foods and participants consuming higher amounts of animal-based foods. Higher intakes of these animal-based foods, animal protein, cholesterol, vitamin B12, low fat cheese, and red and processed meat, were correlated with a higher BMI. The participants with lower intake of animal-based foods and higher intake of plant-based foods could be further divided into two groups; those consuming higher amounts of fresh fruits, nuts, seeds and peanuts and vitamin C, and those with higher intakes of grain products and digestible carbohydrates.

Consumption of animal-based foods and BMI was positively associated with species related to *Ruminococcus gnavus, Streptococcus* spp. (*S. mitis and bovis*) and *Collinsella*. Conversely, animal-based foods were inversely associated with *Akkermansia muciniphila,* uncultured Clostridiales I and II and species related to *Sporobacter termitidis.* Consumption of fresh fruits, its associated nutrient vitamin

C, and nuts, seeds and peanuts were associated with several genera from the Bacteroidetes phylum, including *Bacteroides* spp., *Parabacteroides*, *Alistipes* and *Prevotella,* and Firmicutes such as species related to *Faecalibacterium prausnitzii, Oscillospira guillermondii* and *Eubacterium rectale* and *E. biforme.* Grain products and carbohydrates were positively associated with *Dialister* and species related to *Clostridium difficile* (recently renamed to *Clostridioides difficile*). Although this group is named after *C. difficile*, the observed differences do likely not relate to this potential pathogen but probes targeting *C. bifermentans*, *C. bartlettii* and *C. glycolicum.*

VARIABLES ASSOCIATED WITH MICROBIAL ALPHA-DIVERSITY

The relations between the significant variables in the RDA (phenotypical characteristics, nutrients, food groups) and indices that contribute to microbial alpha-diversity were calculated and visualized in **figure 3A**. BMI was negatively correlated with alpha-diversity. With respect to the food groups, only fresh fruits and nuts, seeds and peanuts were positively correlated with alpha-diversity, with correlation coefficients ranging from 0.1 to 0.17. Among the fresh fruits, alpha diversity positively correlated with berries and grapes, citrus fruits and stone fruits in **supplementary table 1**. Nutrients that were positively correlated to alpha-diversity included vitamin C, various minerals, forms of carbohydrate and plant protein, with correlation coefficients between 0.09 and 0.14. None of the nutrients was negatively associated with alpha-diversity.

With correlation coefficients ranging from -0.04 to 0.05, none of the cognitive domains was significantly correlated to any of the diversity indices (**figure 3B**).

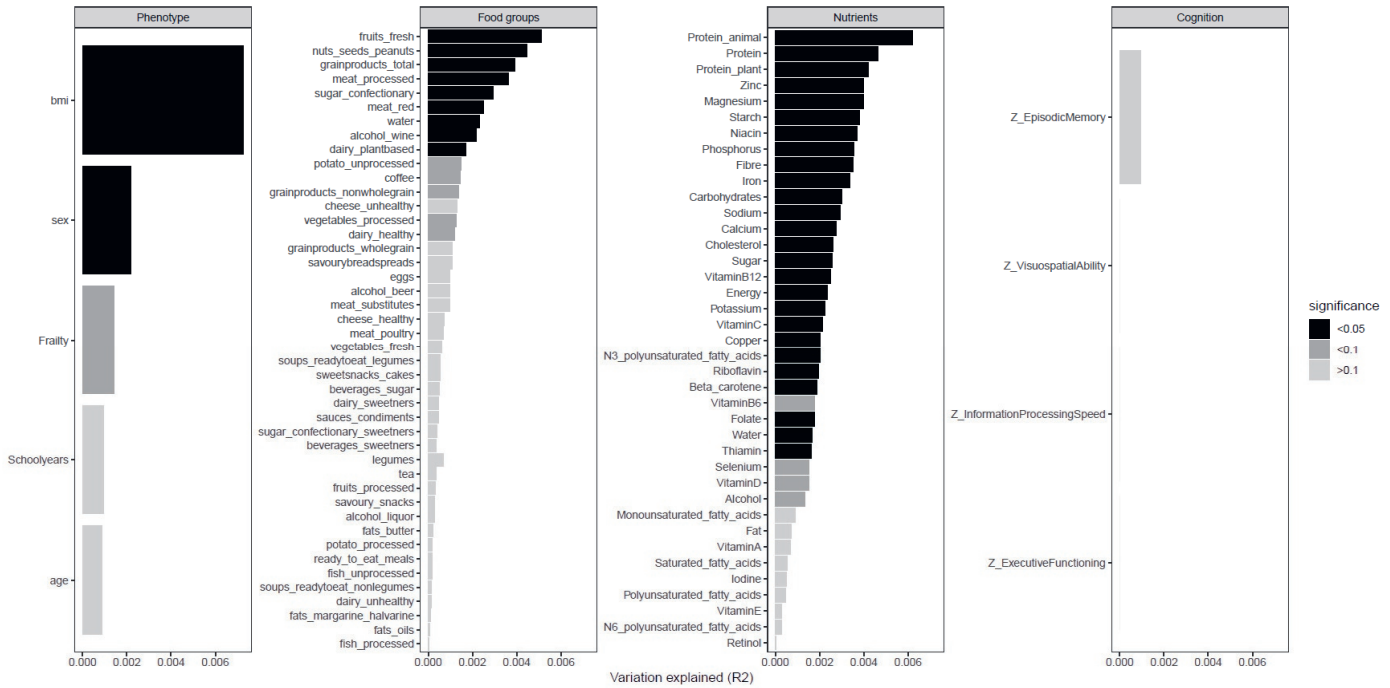

Figure 1. Microbiota covariates. Impact of all measured variables on microbiota composition defined as percentage variation explained (R^2) out of all the total microbiota variation. A higher R^2 implies a stronger effect size.

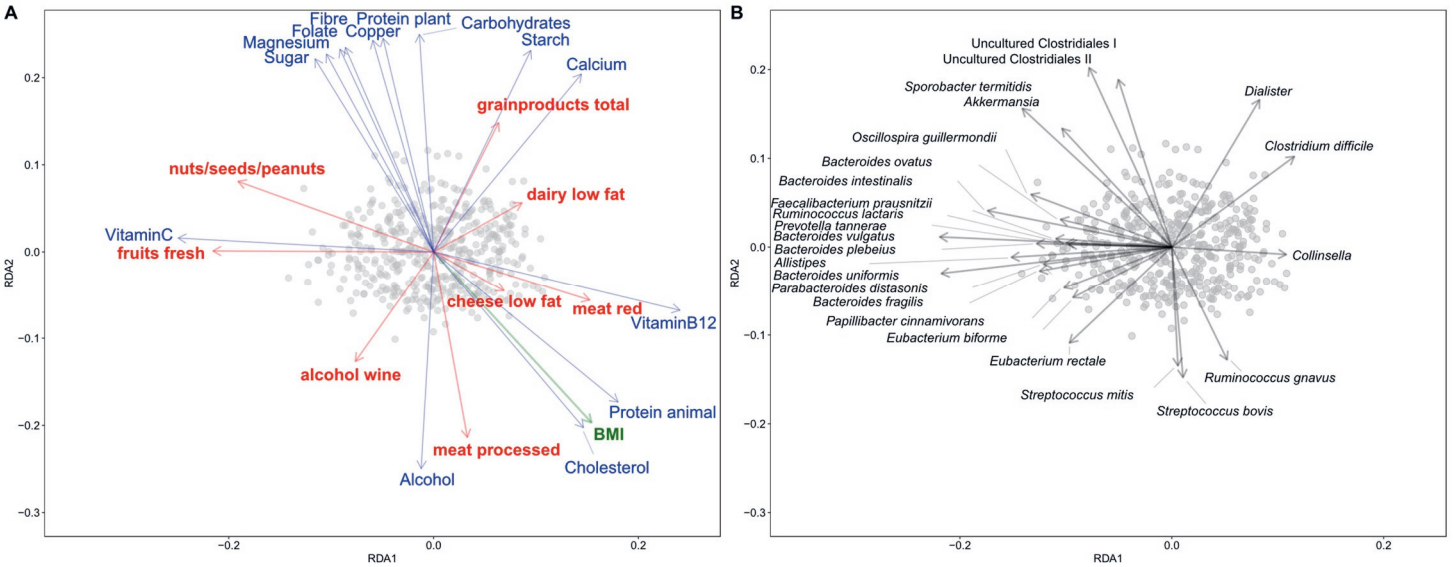

Figure 2. Association of microbiota with food groups, nutrients and BMI. Samples are plotted as grey circles. A) Redundancy analysis (RDA) bi-plot of microbiota with explanatory variables; food groups (red), nutrients (blue) and phenotypical characteristics (green). B) RDA bi-plot of samples with the associated microbial taxa (indicated as genera or species-level groups) The direction of the arrows depicts the abundance of microbial taxa. Length of the arrows is a measure of fit. The variable arrows approximate the correlation between species and explanatory variables. Samples near the coordinate origin (zero point) suggest near zero correlation. The further a sample falls in the direction indicated by the arrow, the higher the correlation.

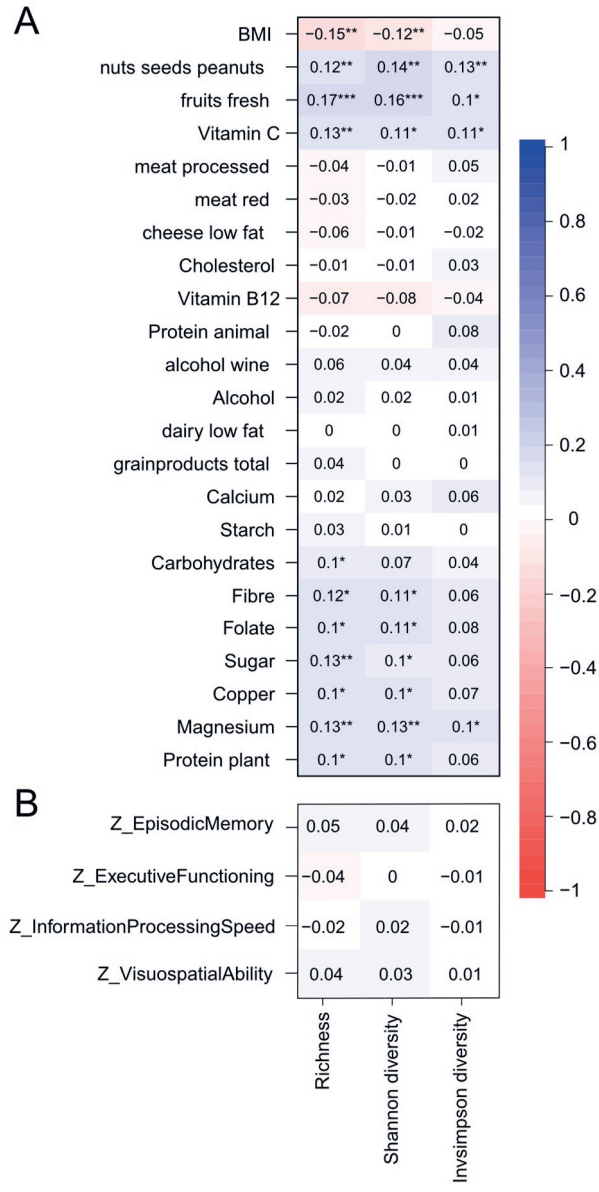

Figure 3. Correlation of alpha-diversity with microbiota covariates (A) and cognition variables (B). Pearson correlation of significant microbiota covariates were calculated. P values are corrected for multiple testing using the Benjamini-Hochberg procedure. *** q<0.001, ** q<0.01 * q<0.05

DISCUSSION

By exploring associations between diet, GI microbiota and cognition in healthy Dutch older adults using food groups as the primary input, we showed that fresh fruits, nuts, seeds and peanuts, red and processed meat, grain products, low fat dairy and cheese and wine are important dietary factors in GI microbiota composition. Of these food groups, fresh fruits (berries and grapes in particular), and nuts, seeds and peanuts positively correlated with alpha-diversity. Overall, fresh fruits and nut seeds and peanuts correlated with various taxa from the Bacteroidetes phylum and species related to *Faecalibacterium prausnitzii*, grain products correlated with *Dialister,* while higher intake of animal-based foods was associated with a higher abundance of *Collinsella* and *Streptococcus* spp. as well as species related to *Ruminococcus gnavus*. Cognitive functioning was neither associated with GI microbiota composition nor alpha-diversity.

Our study is the first to investigate which food groups are related to whole GI microbiota composition and alpha-diversity in older adults. In younger adults, several studies have investigated this association before. In a large cross-sectional study with GI microbiota data from 1135 Dutch adults, 78 dietary factors, including fruit, frequency of nut consumption, red and processed meat and protein, were important dietary factors in explaining GI microbiota variation [47]. The associations of fruit and meat with GI microbiota composition were confirmed in a large cross-sectional Belgian study with adults (n=1106) [48] and the French Milieu Intérieur study (n=862) [49] showed that fruit influenced the GI microbiota. With respect to alpha-diversity, our finding that individuals with higher intakes of fresh fruit and nuts had a more diverse GI microbiota was confirmed by the studies of Dutch and French adults [47, 49] and the association between nuts, seeds and peanuts and alpha-diversity was also observed Dutch adults [47].

Despite the fact that the dietary intervention did not have a significant impact on the GI microbiota in our cohort, we could clearly identify associations between dietary variables and microbiota composition. We observed a gradient between participants consuming a diet richer in foods from animal origin and a diet richer in foods from plant origin, from now on referred to as animal- and plant-rich diets. The animal-rich diet was characterized by higher intakes of processed and red meat, low fat cheese and dairy, vitamin B12 and cholesterol. The plant-rich diet was higher in vitamin C, fresh fruits and nuts, seeds and peanuts. In addition to the classification based on origin of the food products and nutrients, these diets can also be classified as pro-

and anti-inflammatory according to the dietary inflammatory index, in which various dietary factors have been scored based on their inflammatory potential [50]. Vitamin B12 and cholesterol, both associated with the animal-rich diet, were considered pro-inflammatory. With respect to the plant-rich diet, nutrients present in fresh fruits (vitamin C, flavonoids, fiber) and nuts, seeds and peanuts (polyphenols, omega-3 fatty acids, fiber) were all classified as anti-inflammatory.

Interestingly, classification of the GI microbiota based on inflammatory potential showed a similar pattern. The consumption of the pro-inflammatory diet rich in animal foods positively correlated with *Collinsella* and *Streptococcus* spp. as well as species related to *R. gnavus*. Overall, these bacteria have been classified as pro-inflammatory. Increased abundance of *Collinsella* has been observed in several inflammatory diseases, including type 2 diabetes mellitus [51, 52], atherosclerosis [53] and rheumatoid arthritis [54]. Even though *Streptococcus* is a normal inhabitant of the upper GI tract, increased abundance in the colon has been associated with pro-inflammatory nutrients of animal origin [15]. Finally, higher abundance of *R. gnavus* has been linked to several inflammatory diseases as well, such as spondyloarthritis [55], eczema in infants [56] and inflammatory bowel disease, especially during active disease episodes [57]. In addition to the connection with inflammatory diseases, it has recently been shown that *R. gnavus* synthesizes an inflammatory polysaccharide that induces secretion of the inflammatory cytokine tumor necrosis factor-alpha by dendritic cells [58].

The anti-inflammatory plant-rich diet was associated with species related to *F. prausnitzii*, *E. rectale* and *E. biforme*. These species can be classified as anti-inflammatory due to their ability to produce butyrate. Butyrate has been shown to exhibit anti-inflammatory effects through their regulation of leukocyte function via inhibition of histone deacetylase and activation of G-protein coupled receptors [59]. These anti-inflammatory effects of butyrate have been demonstrated *in vivo*, in both animal models [60] and human clinical trials [61]. *F. prausnitzii* specifically has been shown to exhibit anti-inflammatory effects *in vitro* and *in vivo*. In peripheral blood mononuclear cells, *F. prausnitzii* led to higher levels of the anti-inflammatory cytokine IL-10 and lower production of the pro-inflammatory cytokines IL-12 and IFN-γ. In a mouse model with induced acute colitis, administration of living *F. prausnitzii* decreased colitis [62]. Moreover, in humans lower abundance of these species has been observed in several inflammatory diseases. A meta-analysis in inflammatory bowel disease patients showed that patients suffering from an active

disease episode had lower abundance of *F. prausnitzii* compared to patients in remission [63] and *E. rectale* were reduced in Crohn's disease patients compared to healthy controls [64]. The plant-rich diet also positively correlated to the mucin degrading species *A. muciniphila*. Similarly, lower abundance of *A. muciniphila* has been observed in inflammatory conditions including obesity and type 2 diabetes [65-67]. Moreover, a recent human intervention trial showed that daily administration of *A. muciniphila* cells for three months increased barrier function, by decreasing the levels of pro-inflammatory lipopolysaccharides in prediabetic human subjects [68]. Overall, the links between these bacteria and inflammatory diseases and compounds, indicate that the consumption of an animal-rich diet might correlate with a more pro-inflammatory GI microbiota profile, while the plant-rich diet correlates to a more anti-inflammatory GI microbiota profile.

Moreover, several species associated to the plant-rich diet, including *F. prausnitzii* and *E. rectale*, have been previously associated with a high adherence to the MedDiet in various European countries [16]. This might imply that certain food groups that were part of the plant-rich diet, i.e. nuts, seeds and peanuts and fresh fruits, are important dietary factors in the MedDiet with respect to GI microbiota modulation. The beneficial associations of these food groups could be due to the fiber present in fruits and nuts. Fermentation of fibers in the gut leads to the production of SCFA, which have beneficial effects on health as previously discussed [69]. An additional factor underlying the beneficial associations might be the presence of polyphenols in fruit and nuts. These plant metabolites are poorly absorbed in the small intestine and reach the colon where they can interact with microbiota. Polyphenols have been shown to have prebiotic-like effects. Various types of polyphenols enhanced growth of lactobacilli and bifidobacteria as well as *Akkermansia*, in both *in vitro* and *in vivo* (animal and human) studies [70, 71].

In addition to the association between the plant-rich diet and the anti-inflammatory species, the diet rich in plant foods also positively correlated with several genera from the Bacteroidetes phylum such as *Parabacteroides*, *Alistipes*, and mostly *Bacteroides* and *Prevotella* spp. Members of the latter two maintain a complex and generally beneficial relationship with the host. Bacteroidetes are abundantly present in the human gut and many genera within this phylum respond to changes in diet. Generally, diets rich in fiber are linked with increased abundance of *Prevotella* spp. [72], while higher abundance of *Bacteroides* spp. is associated to diets rich in fat and protein from animal origin [73]. However, the latter group has also been linked to

plant-based complex carbohydrates and inversely associated with dietary fat and protein [15], in line with our results. It is well known that microorganisms have context-dependent functions and a changing metabolism, depending on environmental conditions and the presence and function of other microbes. For instance, *Bacteroides* spp. contain a large repertoire of enzymes to break down complex plant carbohydrates [74], which likely underlies their association in the current study. However, several *Bacteroides* spp are also bile resistant [75] and could thus be more prevalent in individuals consuming high fat diets with little complex carbohydrates. Additionally, different species or strains within the *Bacteroides* and *Prevotella* genera have been shown to be genetically diverse and associated with different dietary components, such as plant-based diets while some are associated with animal-based nutrients [76, 77]. Another factor in the ambiguity of the health associations of *Bacteroides* spp. is their status as a pathogen, as several species (notably *B. fragilis*) can cause significant pathology, including bacteremia and abscess formation in multiple body sites [75]. Similarly, several *Prevotella* spp. have been associated with chronic inflammatory conditions [78]. In contrast, *Bacteroides* spp. have also been linked to beneficial effects on health. This apparent duality was exemplified by the observation of a cohort specific positive or negative association with markers of insulin resistance in overweight insulin resistant males [79]. For example, *Bacteroides* spp. can contribute to the formation the SCFA propionate via the succinate pathway [80]. Propionate has been linked to several health benefits, including regulation of appetite and lipid synthesis in *in vivo* animal studies, and anti-colorectal cancer effects in *in vitro* models [81].

Specific food groups, such as berries and nuts, seeds and peanuts, were correlated with several anti-inflammatory microbial species. In addition, these food groups have been associated with slower rates of cognitive decline [82, 83]. Although inflammation is a major mechanism underlying cognitive decline [84], we did not find associations between cognitive functioning and the GI microbiota composition or alpha-diversity. To our knowledge, the association between diet, gastro-intestinal microbiota and cognitive functioning in humans has only been investigated in a single other study [16]. Here, the authors showed that European individuals with high adherence to a Mediterranean-like diet had high relative abundance of several beneficial, anti-inflammatory, butyrate producing microbial groups, including *Faecalibacterium prausnitzii, Anaerostipes* and *Roseburia.* Increased relative abundance of these species was associated with improved cognitive function

measured by single tests. Our approach augments this paper, but also differed in two aspects. First, we used diet as a combination of different food groups, while in the previous paper diet was only considered as adherence to the Mediterranean diet in general. Hence, it was not clear which specific food groups of the Mediterranean diet were responsible for the beneficial effect on cognition and gastro-intestinal microbiota composition. Second, we incorporated cognitive functioning outcomes using a robust measure of cognitive functioning by calculating mean scores per cognitive domain (composite cognitive scores). The previous research only considered scores of single cognitive tests. Aside from the use of a more robust measure of cognitive functioning, there are several other explanations for the apparent differing results with regard to the association of microbiota with cognitive function.

From animal studies, there is strong evidence for a relation between the gut and the brain, which has been shown with (germ-free) rodent studies, using microbiota modulating strategies such as antibiotics and fecal microbiota transplants [9]. However, there are many differences between rodents and humans, such as differences in GI tract anatomy and physiology and microbiota composition [85], severely limiting translation from rodents to humans. In addition, rodent models allow for more extreme interventions, have a very homogeneous genetic background and there is a high level of control over external factors, which allow for the demonstration of subtle effects. In contrast, we investigated cross-sectional relations in a healthy population of older adults in which diets and microbiota were relatively homogeneous. There were no extreme variations in intake of food components between participants and the dietary intervention that half of the participants underwent, resulted in small changes in dietary intake (e.g. increase of one slice of whole-wheat bread, one third of an apple, and half a serving spoon of vegetables extra per day) [23]. This may have limited the demonstration of associations between cognitive functioning and GI microbiota.

Moreover, our study population consisted of cognitively healthy older adults as shown by the mean MMSE score of 27.7 points out of 30, as scores from 24 to 30 are considered within the normal range [40]. Cognitively healthy indicates that these participants were no mild cognitive impairment or dementia patients. It is important to emphasize that cognitively healthy older individuals can benefit from the effects of diet on cognition. Cognitive health is not static, but rather a progressive phenomenon. The process of age-related cognitive decline starts from the late 20's

and continuous throughout the lifespan [86]. The rate of decline can be influenced by several lifestyle factors, including nutrition. Previous research has already demonstrated that several dietary patterns can slow down cognitive decline with ageing. For example, this has been shown for the Mediterranean, Dietary Approaches to Stop Hypertension (DASH) and Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diets [87].

Nevertheless, gastro-intestinal microbiota targeted interventions to slow down cognitive decline may be more effective in cognitively impaired individuals, i.e. mild cognitive impairment or Alzheimer's disease patients. Mild cognitive impairment and Alzheimer's disease patients have shown decreased microbial diversity and similar changes in GI microbiota compared to healthy older adults [88]. In line with this, the effectiveness of probiotic supplementation on cognition in humans likely depends on the degree of cognitive impairment. In human intervention studies, the effect of probiotic supplementation on cognitive functioning is mainly effective in cognitively impaired individuals (i.e. mild cognitive impairment or Alzheimer's disease patients), [10, 89, 90] while the effectiveness in relatively healthy older adults has been inconsistent [11, 91, 92]. Similarly, the efficacy of other dietary interventions to slow down cognitive decline has been shown to be dependent on the extent of cognitive impairment as well [93]. Therefore, our study population might have been too healthy to demonstrate the link between cognition and GI microbiota. Indeed, changes in GI microbiota in older adults seem to be more strongly associated with health status rather than with chronological age [5, 94].

The study population is an important limitation of this study. We did not demonstrate associations between cognitive functioning and GI microbiota, possibly due to relatively small differences in diet and microbiota between subjects and the high cognitive health status of our study population. Further research on the association between diet, GI microbiota and cognitive ageing in humans would benefit from focusing on cognitively impaired study populations and study populations that are more heterogeneous with respect to dietary intake.

CONCLUSIONS

This cross-sectional investigation into the association between diet, GI microbiota and cognition showed that the anti-inflammatory potential of a plant-rich diet high in fresh fruits and nuts, seeds and peanuts was linked to a GI microbiota profile with a higher anti-inflammatory potential. Conversely, a pro-inflammatory animal-rich

diet was associated with a more pro-inflammatory GI microbiota profile. Despite the prominent role of inflammation in cognitive decline, we did not demonstrate associations between cognitive functioning and GI microbiota.

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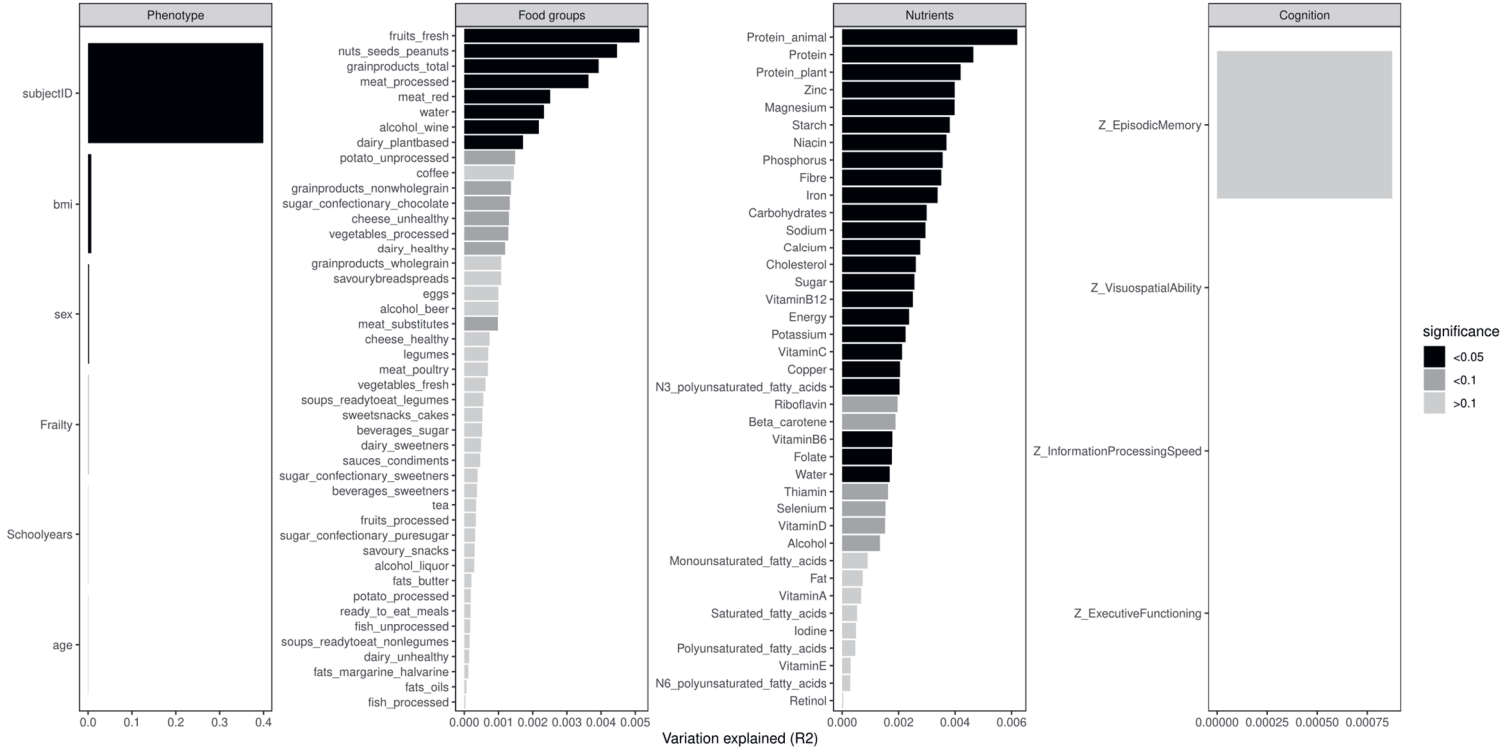
Supplementary materials



Supplementary figure 1: **Microbiota covariates.** Impact of the individual and all measured variables and on microbiota composition defined as percentage variation explained (R^2) out of the total microbiota variation. A higher R^2 implies a stronger effect size.

Supplementary table 1. Pearson correlation of alpha-diversity with fresh fruit categories. P-values are corrected for multiple testing using the Benjamini-Hochberg procedure.

	Richness	Corrected p-value	Shannon diversity	Corrected p-value	Inverse Simpson diversity	Corrected p-value
Total fresh fruits	0.170	<0.001	0.157	<0.001	0.105	0.026
Citrus fruits	0.133	0.005	0.105	0.025	0.086	0.067
Apples and pears	0.098	0.037	0.071	0.129	0.022	0.640
Berries and grapes	0.130	0.006	0.115	0.015	0.102	0.030
Banana	-0.065	0.169	-0.048	0.309	-0.092	0.051
Stone fruits	0.062	0.192	0.085	0.071	0.095	0.044
Melon	-0.010	0.827	-0.008	0.867	-0.019	0.692
Tropical fruits	0.086	0.067	0.087	0.063	0.055	0.246

CHAPTER 6

The association between adherence to a plant-based diet and cognitive ageing

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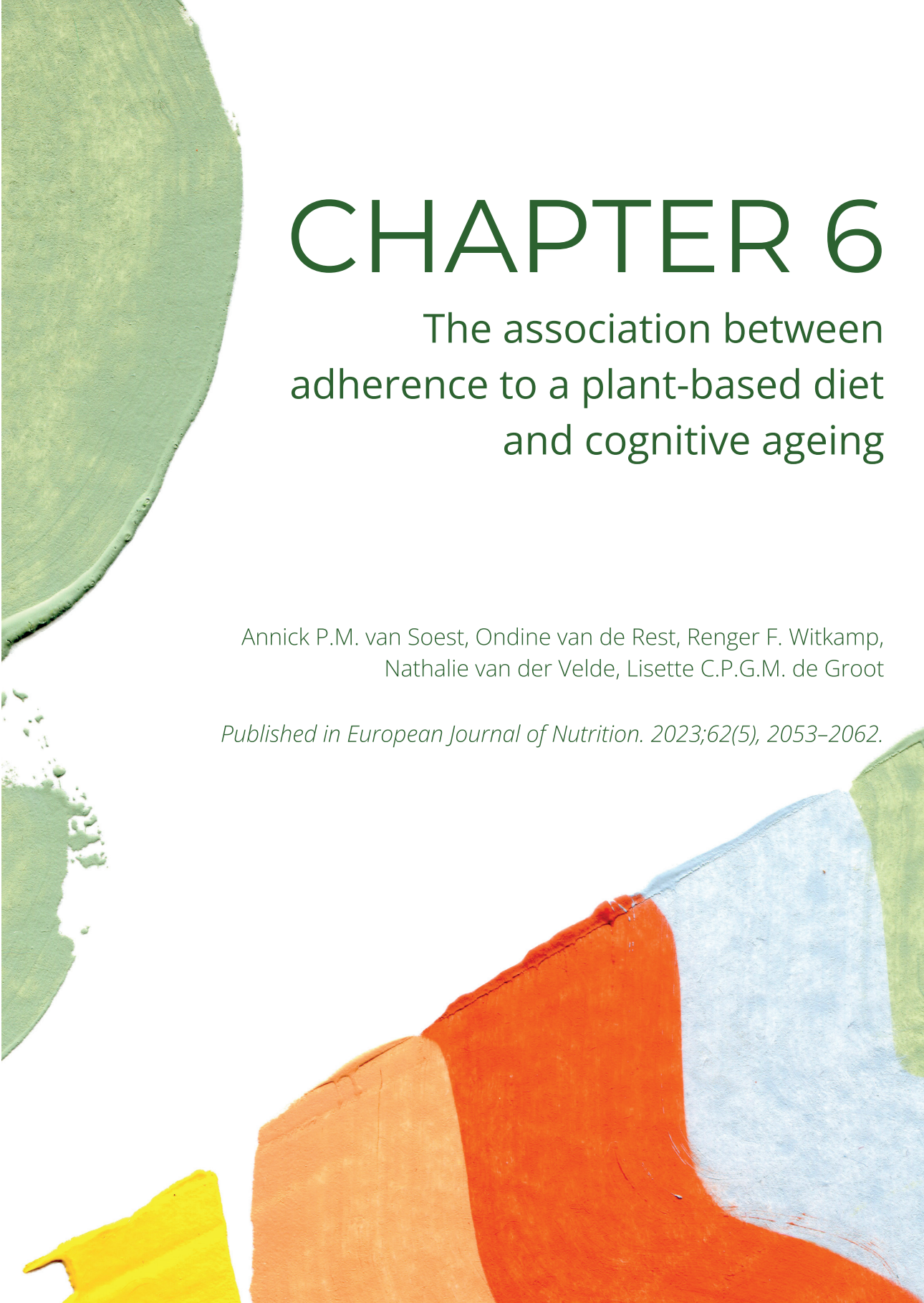

ABSTRACT

Purpose: While the benefits of adopting a more plant-based diet for sustainability and animal welfare are clear, its long-term health impacts, including the impact on cognitive ageing, are limited studied. Therefore, we investigated the associations between plant-based diet adherence and cognitive ageing.

Methods: Data from a previous intervention study involving community-dwelling adults aged ≥65 years were analysed at baseline (n=658) and after 2-year follow-up (n=314). Global and domain-specific cognitive functioning were assessed at both timepoints. Overall, healthful and unhealthful plant-based dietary indices were calculated from a 190-item food frequency questionnaire. Multivariate-adjusted linear regression models were applied to test for associations.

Results: After full-adjustment, higher overall adherence to a plant-based diet was not associated with global cognitive function (difference in Z-score, tertile 1 versus 3 [95%CI]: 0.04 [-0.05,0.13] p=0.40) or cognitive change (-0.04 [-0.11,0.04], p=0.35). Similarly, healthful and unhealthful plant-based diet indices were not associated with cognitive functioning (respectively p=0.48; p=0.87) or change (respectively p=0.21, p=0.33). Interestingly, we observed fish consumption to influence the association between plant-based diet adherence and cognitive functioning (p-interaction=0.01), with only individuals with a fish consumption of ≥0.93 portion/week benefitting from better overall plant-based diet adherence (β per 10-point increment [95%CI]: 0.12 [0.03,0.21] p=0.01).

Conclusion: We did not demonstrate associations of a more plant-based diet with cognitive ageing. However, possibly such association exists in a subpopulation with higher fish intake. This would be in line with earlier observations that diets rich in plant foods and fish, such as the Mediterranean diet, may be beneficial for cognitive ageing.

Trial registration: registered at clinicaltrials.gov (NCT00696514) on June 12, 2008.

Keywords: Plant-based diet, omega-3 fatty acids, cognition, older adults, elderly, healthy ageing

INTRODUCTION

Consumers are increasingly opting for more plant-based diets, for various reasons related to sustainability, animal welfare and presumed health benefits. Nevertheless, the evidence supporting health benefits of shifting to a plant-based diet remains limited. While protective associations have been demonstrated for cardiovascular disease [1], cancer [2], and diabetes [3], little is known about the long-term effects of a shift towards a more plant-based diet on healthy ageing, including the effect on cognitive abilities.

At the same time, there is emerging evidence for beneficial effects of individual plant-derived components and foods on cognitive ageing. For example, higher consumption of polyphenols, vitamins C and E, carotenoids and unsaturated fatty acids, and plant foods rich in these plant-derived components, including vegetables, berries, nuts, olive oil, tea and coffee, have been associated to favourable brain ageing outcomes [4]. Similarly, higher adherence to the Mediterranean diet (MedDiet) and the Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) diet has been associated with better cognitive performance and slower rates of cognitive decline [5]. These dietary patterns, though not exclusively plant-based, are plant-centred and rich in brain-health promoting plant-derived components and foods.

Whereas these plant-derived components, foods and plant-centred dietary patterns have been shown to contribute to healthy cognitive ageing, there is no direct evidence to support the benefits of higher adherence to a plant-based dietary pattern. The few preliminary studies on the role of a more plant-based or vegetarian diet show promising positive associations with cognitive ageing outcomes [6-9], though not all studies support these findings [10,11]. This merits further research on the role of a more plant-based diet on cognitive ageing.

To this end, the current study aims to investigate the association between plant-based diet adherence and cognitive functioning and 2-year cognitive decline in cognitively healthy older adults. To categorize plant-based diet adherence, we used the approach proposed by Satija et al [12] based on an overall, healthful and unhealthful plant-based dietary index.

METHODS

PARTICIPANTS AND STUDY DESIGN

The present study made use of data from the B-vitamins for the Prevention of Osteoporotic Fractures (B-proof) trial [13], a randomized double-blind placebo-controlled trial on the effect of 2 year supplementation with B-vitamins in community-dwelling adults aged ≥65y with elevated homocysteine levels (12–50 μmol/L) on fracture incidence. Cognition was measured pre- and post-intervention as secondary outcome. The intervention existed of B-vitamin supplementation (400μg folic acid and 500μg vitamin B12) versus placebo. Participants did not suffer from renal insufficiency (creatinine > 150 μmol/L) and did not have a diagnosis of a malignancy in the past 5 years. For the present study, we used baseline data to perform a cross-sectional analysis. Next to this we performed a longitudinal analysis using follow-up data from the control group only, in order to eliminate any influence of the B-vitamin intervention, as previous research has demonstrated that this intervention may have slowed down cognitive decline in a subpopulation [14]. Data were collected between October 2008 and March 2013 in three research centres in the Netherlands: Erasmus Medical Center (Rotterdam), VU University Medical Center (Amsterdam) and Wageningen University (Wageningen). The current analysis is based on the Wageningen participants, as only this subpopulation underwent extensive cognitive testing (n=856) and completed a food frequency questionnaire (FFQ) (n=664). Of these 664 participants, data from 6 participants were excluded due to unreliable energy intake data (for men <800 kcal or >4200 kcal, women <500kcal or >3500 kcal, n=2) or due to missing baseline cognition data (n=4). The final study sample comprised 658 participants. The study was approved by the Medical Ethics committee of Wageningen University & Research and has been registered at clinicaltrials.gov as NCT00696514. All participants had given written informed consent.

DIETARY ASSESSMENT

Habitual dietary intake was assessed at baseline by a 190-item FFQ, of which validity has been reported previously [15,16]. Participants were asked how often they had consumed a food item in the past month. Portion sizes were estimated using standard portion sizes and commonly used household measures. Average daily nutrient intakes were calculated based on the Dutch food composition database [17].

As a measure of plant-based diet adherence, we calculated the overall, healthful and unhealthful plant-based diet index (PDI, hPDI, uPDI, respectively) [12]. These indices included a total of 18 food groups, of which 7 are designated as healthy plant-based groups (whole grains, fruits, vegetables, nuts, legumes, vegetable oils, and tea & coffee), 5 as unhealthy plant-based groups (fruit juices, refined grains, potatoes, sugar-sweetened beverages, and sweets) and 6 classified as animal-based food groups (animal fat, dairy, fish, meat, eggs, and miscellaneous animal-based) (**supplementary table 1**). For scoring of the indices, intake of each food group was ranked into cohort-specific quintiles and each quintile was assigned a score ranging from 1 to 5. In the PDI (overall), both healthy and unhealthy plant-based groups were scored positively (i.e. higher intakes received higher scores). For the hPDI, healthy plant food groups were given positive scores and unhealthy plant-foods received reversed scores. For the uPDI, healthy plant-foods received reversed scores and unhealthy plant foods received positive scores. Animal-based food groups were scored reversely in all three indices. The 18 food group quintile scores were summed to obtain the index scores. Alcohol and margarine intake were not included in the indices but adjusted for in the analysis, in line with previous research [12]. Furthermore, the diet indices were adjusted for energy intake using the residual method [18].

COGNITIVE TESTING

Cognitive functioning was assessed by trained research assistants with an extensive battery of cognitive tests at baseline and after 2 years. This battery included the Rey Auditory Verbal Learning Test (RAVLT) (subtests immediate, delayed and recognition) [19], the Digit Span task [20], the Trail Making Test (TMT) (part A and B) [21], the Stroop Colour-Word test (part I, II and III) [22], the Symbol Digit Modalities Test (SDMT) [23], and Letter Fluency [24] (**supplementary table 2**). Parallel versions were used for RAVLT, TMT and letter fluency to reduce learning effects.

To limit the number of cognition outcomes, cognitive composite scores were created. Individual cognitive test scores at baseline and after 2 y were converted into Z-scores based on population mean and standard deviation at baseline. The Z-scores for the TMT and Stroop Colour-Word test were reversed as lower scores represent better cognitive functioning. Individual Z-scores per test were clustered into composite scores for global and domain-specific cognitive functioning.

$$Global\ cognition = \left(Z_{Episodic\ memory} + Z_{Attention\ \&\ working\ memory} + Z_{Information\ processing\ speed} + Z_{Executive\ functioning}\right)/4$$

$$Episodic\ memory = \left(Z_{RAVLT\ immediate} + Z_{RAVLT\ delayed} + Z_{RAVLT\ recognition}\right)/3$$

$$Attention\ \&\ working\ memory = \left(Z_{Digit\ span\ forward} + Z_{Digit\ span\ backward}\right)/2$$

$$Information\ processing\ speed = \left(-Z_{Stroop\ mean\ I\ and\ II} + -Z_{TMT\ part\ A} + Z_{SDMT}\right)/3$$

$$Executive\ functioning = \left(-Z_{Stroop\ interference} + -Z_{TMT\ part\ B/A} + Z_{Fluency}\right)/3$$

Finally, Mini-Mental State Examination [25] was administered following standardized procedures. This score was measured for descriptive purposes (as an indicator of the cognitive state of the participants) rather than as outcome variable.

COVARIATES

Information on age, gender, education level (low, middle, high), smoking status (never, former, current) was collected via questionnaires. Body weight and height were measured by trained research assistants. Body mass index (BMI) was calculated as weight (kg)/(height (m))^2. Physical activity was assessed using the LASA physical activity questionnaire [26], and expressed in metabolic equivalent hours per week (MET h/w) covering activities of walking, cycling, sports, gardening and housework. Alcohol and margarine intake were derived from the FFQ.

STATISTICAL ANALYSIS

Data are expressed as n (%), mean (SD) or median (IQR) unless otherwise stated. Participant characteristics between PDI tertiles were compared using ANOVA or Kruskal-Wallis test for continuous variables, and chi-square test for categorical variables. Multiple linear regression analyses were performed to investigate the association between plant-based diet adherence and cognition. For the cross-sectional analysis, we modelled cognitive function at baseline as a function of plant-based diet adherence (PDI, hPDI and uPDI, in tertiles and continuous), using data of the total study population. For the longitudinal analysis, the change in cognition Z-score between baseline and after 2y was modelled as a function of plant-based diet adherence (PDI, hPDI and uPDI, in tertiles and continuous). Here, only data from the control group were used, to eliminate interference of the B-vitamin intervention. All analyses were adjusted for age (in years), gender, education level (low, middle, high), BMI (in kg/m^2), physical activity (in MET h/w), smoking (never, current, former), alcohol intake (light, moderate, excessive), and margarine intake (portions/d). The longitudinal analysis was additionally adjusted for baseline cognition Z-scores. To investigate if consumption of specific animal food groups modified the association, stratified analyses by fish, meat, egg and dairy consumption (median split) were

performed. P-values smaller than 0.05 were considered significant. All analyses were performed using RStudio Version 1.4.0 [27].

RESULTS

BASELINE CHARACTERISTICS

Participant characteristics are shown in **table 1**. The mean age at baseline was 72.1 ± 5.4 years, and 59% was male. On average, participants were overweight with a mean BMI of 27.2 ± 3.9 kg/m^2 and cognitively healthy as indicated by a median MMSE score of 29 [28-30]. Participants who fell into the lowest tertile with respect to their plant-based diet adherence were on average more often male (p=0.02), had a higher BMI (p<0.01) and consumed more alcohol (p<0.01) compared to individuals classified in the tertile with highest adherence. Nutrient intake differed between plant-based diet adherence tertiles (**supplementary table 3**). Participants with higher adherence to a plant-based diet had higher intakes of carbohydrates, sugar, fibre, and folic acid, while their intakes of protein, EPA, DHA and vitamin B12 were lower.

CROSS-SECTIONAL ANALYSIS

In the fully adjusted models, a higher overall adherence to a plant-based diet, as well as higher adherence to either a healthful or an unhealthful plant-based diet were not associated with global cognitive functioning (difference tertile 1 vs 3 [95% CI]: PDI 0.04 [-0.05, 0.13] p=0.40; hPDI -0.03 [-0.13, 0.06] p=0.48; uPDI -0.01 [-0.10, 0.09] p=0.87) (**table 2**). With respect to domain-specific cognitive functioning, individuals with a higher overall adherence to a plant-based diet showed better episodic memory compared to individuals with lower overall plant-based diet adherence (difference tertile 1 vs 3: 0.16 [0.03, 0.28], p=0.01) (**supplementary table 4**). However, this finding was not confirmed in the continuous analysis (p-trend=0.08). For the remaining three cognitive domains, no associations were found between overall, healthful or unhealthful plant-based diet adherence and attention & working memory, information processing speed, or executive functioning.

Table 1: Participant characteristics according to overall plant-based diet index tertiles

Characteristic	Overall (n=658)	Tertile 1 (n=226)	Tertile 2 (n=202)	Tertile 3 (n=230)	p-value
PDI score	54.0 ± 6.3	47.2 ± 3.4	54.0 ± 1.4	60.7 ± 3.1	<0.001
Age (years)	72.1 ± 5.4	71.8 ± 5.2	72.3 ± 5.6	72.3 ± 5.3	0.59
Sex n (%)					0.02
Male	391 (59%)	151 (67%)	110 (54%)	130 (57%)	
Female	267 (41%)	75 (33%)	92 (46%)	100 (43%)	
Level of education n (%)					0.42
Low	280 (43%)	103 (46%)	50 (39%)	99 (43%)	
Middle	157 (24%)	50 (22%)	47 (23%)	60 (26%)	
High	221 (34%)	73 (32%)	77 (38%)	71 (31%)	
Ethnicity n (%)					
White	624 (95%)	217 (96%)	192 (95%)	215 (93%)	0.06
Asian	25 (4%)	3 (1%)	10 (5%)	12 (5%)	
Unknown	9 (1%)	6 (3%)	0 (0%)	3 (1%)	
BMI (kg/m^2)	27.2 ± 3.9	28.1 ± 3.6	27.2 ± 4.5	26.3 ± 3.4	<0.001
Physical activity (MET h/w)	53.4 [33.4-79.8]	51.5 [31.5-76.4]	53.1 [34.1-79.4]	56.7 [35.4-85.5]	0.15
Smoking behavior n (%)					0.19
Never smoker	200 (30%)	56 (25%)	70 (35%)	74 (32%)	
Current smoker	70 (11%)	27 (12%)	22 (11%)	21 (9%)	
Former smoker	388 (59%)	143 (63%)	110 (54%)	135 (59%)	
Alcohol consumption					<0.001
Light	424 (64%)	115 (51%)	135 (67%)	174 (76%)	
Moderate	213 (32%)	101 (45%)	59 (29%)	53 (23%)	
(Very) excessive	21 (3%)	10 (4%)	8 (4%)	3 (1%)	
Margarine intake (portion/d)	15.4 [5.1-27.9]	14.9 [5.3-28.2]	12.8 [3.3-24.6]	18.8 [6.0-31.1]	0.01
MMSE score	29 [28-30]	29 [27-30]	29 [28-30]	29 [28-30]	0.29

Abbreviations: PDI: plant-based diet index, BMI: body mass index, MMSE: Mini Mental State Examination. Data are mean ± SD, median (IQR) or number (%).

LONGITUDINAL ANALYSIS

Higher adherence to an overall or healthful plant-based diet was not associated with the rate of cognitive decline over 2 years (difference tertile 1 vs 3 [95% CI]: PDI -0.04 [-0.11, 0.04], p=0.35; hPDI 0.05 [-0.03, 0.12] p=0.21) (**table 2**). Individuals with the highest adherence to an unhealthful plant-based diet did not show steeper rates of cognitive decline compared to those with lowest adherence (difference T1 vs T3: -0.04 [-0.11, 0.04], p=0.33), though the continuous analysis indicated a significant trend (β per 10-point increment: -0.05 [-0.09, 0.00], p=0.04).

With respect to domain-specific cognitive functioning, attention & working memory was influenced by the degree of adherence to a plant-based diet (**supplementary table 5**). Better adherence to a healthful plant-based diet was associated with slower rates of cognitive decline in attention & working memory (difference tertile 1 vs 3: 0.23 [0.05, 0.41], p=0.01, p-trend=0.01), while higher unhealthful plant-based diet adherence was associated with faster rates of decline (difference tertile 1 vs 3: -0.18 [-0.36, -0.01], p=0.04, p-trend<0.01). Overall adherence to a plant-based diet was not associated with a decline in attention & working memory (p-trend=0.29).

We did not find associations between the plant-based dietary indices and episodic memory, information processing speed, or executive functioning.

SENSITIVITY ANALYSIS

To investigate if consumption of specific animal food groups modified the association between adherence to a plant-based diet and cognitive ageing, we performed stratified analyses by fish, meat, egg and dairy consumption based on a median split.

For the sensitivity analysis stratified by fish intake, participants were divided into two groups, those with lower and higher fish intake than the median fish intake of 0.93 portion per week. Interestingly, fish consumption appeared to influence the association between adherence to a plant-based diet and cognition (**table 3**). Cross-sectionally, higher overall plant-based diet adherence was associated with better global cognitive functioning in individuals with higher fish consumption (β per 10-point increment 0.12 [0.03, 0.21], p=0.01), while in individuals with lower fish consumption no association was observed (β per 10-point increment -0.03 [-0.12, 0.06], p=0.52; p-interaction=0.01). Longitudinally, the association between the rate of cognitive change with healthful plant-based diet adherence appeared to be modified by fish consumption in a similar manner (p-interaction <0.01): higher

healthful plant-based diet adherence was associated with slower rates of cognitive decline in individuals with higher fish consumption (0.07 [0.00, 0.14], p=0.04), but not in those with lower fish consumption (-0.02 [-0.08, 0.04], p=0.56). The association between overall plant-based adherence and cognitive decline appeared to be influenced by fish consumption as well (p-interaction <0.01), but in the opposite direction. We did not find an association between overall adherence to a plant-based diet and cognitive decline in individuals with higher fish consumption (0.05 [-0.04, 0.13], p=0.27), while a negative association became apparent in individuals with lower fish consumption (-0.10 [-0.16, -0.03], p<0.01). This interaction in opposite direction was solely driven by the lower episodic memory performance of the individuals with lower fish intake, and was not observed for the other cognitive domains (data not shown).

We did not find proof for modification by the other animal food groups, i.e. meat, egg, or dairy (data not shown).

Table 2: Regression output energy-adjusted overall, healthful and unhealthful plant based diet index and global cognitive functioning (cross-sectional) and change (longitudinal)

	PDI			hPDI			uPDI		
	Crude model	Model 1	Model 2	Crude model	Model 1	Model 2	Crude model	Model 1	Model 2
Cross-sectional									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	0.11	0.07	0.09	0.06	0.03	0.03	-0.03	0.00	0.00
	[0.01, 0.22]	[-0.03, 0.16]	[-0.01, 0.18]	[-0.04, 0.16]	[-0.06, 0.12]	[-0.06, 0.12]	[-0.14, 0.07]	[-0.09, 0.09]	[-0.09, 0.09]
	0.03	0.16	0.07	0.26	0.52	0.52	0.55	0.98	0.98
Tertile 3	0.04	0.03	0.04	0.07	-0.01	-0.03	-0.14	-0.02	-0.01
	[-0.06, 0.15]	[-0.06, 0.12]	[-0.05, 0.13]	[-0.03, 0.18]	[-0.10, 0.08]	[-0.13, 0.06]	[-0.24, -0.04]	[-0.12, 0.07]	[-0.10, 0.09]
	0.40	0.56	0.40	0.16	0.81	0.48	0.01	0.65	0.87
Continuous	0.05	0.03	0.04	0.06	0.00	-0.02	-0.08	-0.02	-0.01
	[-0.02, 0.12]	[-0.03, 0.09]	[-0.02, 0.10]	[-0.01, 0.12]	[-0.06, 0.05]	[-0.08, 0.03]	[-0.15, -0.02]	[-0.07, 0.04]	[-0.06, 0.05]
	0.14	0.31	0.22	0.08	0.87	0.46	0.01	0.55	0.83
Longitudinal									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	-0.09	-0.08	-0.08	0.00	0.00	0.00	-0.03	-0.03	-0.03
	[-0.16, -0.01]	[-0.15, 0.00]	[-0.16,-0.01]	[-0.08, 0.07]	[-0.07, 0.07]	[-0.07, 0.07]	[-0.10, 0.04]	[-0.11, 0.04]	[-0.10, 0.05]
	0.02	0.04	0.03	0.89	0.99	0.92	0.42	0.35	0.47
Tertile 3	-0.02	-0.01	-0.04	0.06	0.06	0.05	-0.03	-0.03	-0.04
	[-0.09, 0.05]	[-0.08, 0.06]	[-0.11, 0.04]	[-0.01, 0.14]	[-0.01, 0.13]	[-0.03, 0.12]	[-0.11, 0.04]	[-0.11, 0.04]	[-0.11, 0.04]
	0.65	0.73	0.35	0.10	0.11	0.21	0.42	0.41	0.33
Continuous	-0.01	-0.01	-0.03	0.04	0.04	0.03	-0.04	-0.04	-0.05
	[-0.06, 0.04]	[-0.06, 0.04]	[-0.08, 0.02]	[0.00, 0.08]	[0.00, 0.09]	[-0.01, 0.08]	[-0.08, 0.01]	[-0.09, 0.00]	[-0.09, 0.00]
	0.73	0.70	0.30	0.08	0.06	0.17	0.09	0.06	0.04

Abbreviations: PDI; plant-based diet index, hPDI; healthful plant-based diet index, uPDI; unhealthful plant-based diet index.
Model 1: adjusted for age, gender and education. Model 2: additionally adjusted for BMI, physical activity, smoking, alcohol consumption and margarine consumption. Longitudinal analysis was additionally adjusted for baseline cognition score. Data are β [95% CI] p-value. In the continuous analysis, β is shown per 10 points increment in plant-based diet index.

Table 3: Association between overall, healthful and unhealthful plant-based diet index and cognitive functioning and change, stratified by fish consumption

	PDI				hPDI				uPDI			
	Effect size			Overall inter-action	Effect size			Overall inter-action	Effect size			Overall inter-action
	Crude	Adjusted	p-value	p-value	Crude	Adjusted	p-value	p-value	Crude	Adjusted	p-value	p-value
Cross-sectional												
Fish intake < 0.93 portion/w	-0.02 [-0.12, 0.07]	-0.03 [-0.12, 0.06]	0.52	0.01	0.03 [-0.06, 0.12]	-0.04 [-0.12, 0.05]	0.38	0.44	-0.09 [-0.18, 0.00]	-0.02 [-0.11, 0.06]	0.57	0.58
Fish intake ≥ 0.93 portion/w	0.13 [0.03, 0.23]	0.12 [0.03, 0.21]	0.01		0.08 [0.00, 0.16]	0.01 [-0.07, 0.09]	0.77		-0.09 [-0.18, 0.01]	0.00 [-0.09, 0.09]	0.97	
Longitudinal												
Fish intake < 0.93 portion/w	-0.08 [-0.13, -0.02]	-0.10 [-0.16, -0.03]	<0.01	<0.01	-0.03 [-0.08, 0.03]	-0.02 [-0.08, 0.04]	0.56	<0.01	-0.01 [-0.06, 0.05]	-0.02 [-0.08, 0.04]	0.52	0.34
Fish intake ≥ 0.93 portion/w	0.06 [-0.02, 0.15]	0.05 [-0.04, 0.13]	0.27		0.10 [0.03, 0.17]	0.07 [0.00, 0.14]	0.04		-0.07 [-0.15, 0.00]	-0.07 [-0.15, 0.01]	0.08	

Abbreviations: PDI; plant-based diet index, hPDI; healthful plant-based diet index, uPDI; unhealthful plant-based diet index.

Adjusted model was adjusted for age, gender and education, BMI, physical activity, smoking, alcohol consumption and margarine consumption. Longitudinal analysis was additionally adjusted for baseline cognition score. Data are β [95% CI] and β is shown per 10 points increment in plant-based diet index.

DISCUSSION

In this cohort of Dutch cognitively healthy older adults, there was no evidence for a beneficial association between adherence to a plant-based diet and cognitive ageing. While individuals who adhered better to a plant-based diet consumed more fibre and less cholesterol and saturated fatty acids, their intakes of vitamin B12, EPA and DHA were lower. Interestingly, a higher consumption of fish, rich in the latter nutrients, appeared to partly influence the association between adherence to a plant-based diet and cognitive ageing.

To our knowledge, the association between the degree of adherence to a plant-based or vegetarian diet with cognitive ageing has been investigated in six other studies, with mixed results. Three studies demonstrated positive associations: a more plant-based dietary pattern as derived from principle component analysis was associated with better cognitive functioning in older adults [7], and higher scores on the overall and healthful plant-based diet index were associated with a lower risk of cognitive impairment in two Asian cohorts [6,9]. At the same time, mixed results were observed in an American study [8]. Here, higher adherence to a healthful plant-based diet was associated with slower rates of decline in different cognitive domains in older African Americans, but no association was observed for White Americans in this same cohort. A null-finding comes from a small sample of non-demented community dwelling older adults, in which vegetarians did not perform better on cognitive tests or had lower odds of mild cognitive impairment compared to omnivores [11]. In addition, a higher pro-vegetarian score was not associated with 6 year change in Telephone Interview of Cognitive Status (TICS) scores in middle-aged to older adults [10]. The reason for the inconsistency in findings is hard to pinpoint, as comparability is limited due to differences in study population, duration of follow-up, exposure variable and outcome measures. Importantly, even studies that make use of the plant-based diet index as exposure variable cannot be compared directly, as this index makes use of population-specific cut-offs. An important limitation of our analysis that could be responsible for our null-finding is the duration of follow-up. Two years is relatively short to detect cognitive decline in cognitively healthy older individuals. Nevertheless, we used an extensive cognitive test battery to be able to capture subtle cognitive deteriorations rather than the more general MMSE or TICS. Furthermore, the degree of adherence to a plant-based diet was only determined at baseline. However, we do assume that our measurement represents long-term intake as dietary patterns in the elderly are fairly stable over time [28].

Whatever the true association between plant-based diet adherence and cognitive ageing may be, the lack of beneficial association in our analysis can be explained from a nutrient perspective. A diet rich in plant foods contains many nutrients that are beneficial for healthy brain ageing, including vitamin C, vitamin E, polyphenols, carotenoids and unsaturated fatty acids. These nutrients have demonstrated anti-oxidant and anti-inflammatory properties, via which they could slow down cognitive decline during ageing [4]. However, a diet predominantly containing plant foods may be lacking some crucial nutrients for optimal brain functioning, including vitamin B12, EPA and DHA. Vitamin B12, in conjunction with vitamin B6 and folic acid, plays an important role in regulating homocysteine levels, an important risk factor for cognitive decline and dementia [29]. EPA and DHA are involved in different mechanisms shown to be important to maintain brain health. For example, these long chain omega-3 polyunsaturated fatty acids are important building blocks of brain tissue, and have anti-inflammatory, anti-oxidative and vascular health promoting effects [30].

In our sensitivity analysis, we found that fish consumption modified the association between adherence to a plant-based diet and cognition, with only individuals with a higher fish consumption seeming to benefit from adhering to a plant-based diet. While fish, rich in vitamin B12, EPA and DHA, has been shown to slow cognitive decline on its own [31], combining fish with a diet rich in plant foods may have additional benefits. A multi-nutrient approach seems crucial for healthy brain ageing, as the mechanisms underlying nutrition and brain ageing are multifactorial [32]. This is also evidenced by the stronger evidence for dietary patterns versus single nutrients or foods [4] and the synergistic effect of omega-3 fatty acids and anti-oxidants [33]. Alternatively, the modification by fish may be explained by a shift in of animal-based product consumption, i.e. from meat to fish. Meat is an important source of saturated fatty acids, which have been associated to worse cognitive functioning and higher risks of mild cognitive impairment and dementia [4]. In addition, various dietary patterns low in meat have been associated with favourable brain ageing outcomes [5]. However, observational studies on the association between meat intake and cognitive ageing mostly demonstrate no associations [34], thus direct evidence for a possible negative effect of meat is lacking.

Observational studies can confirm the combined beneficial associations of a diet rich in plant foods and fish in cognitive ageing. The MedDiet, a diet rich in vegetables, fruits, whole grains, nuts and fish, has been associated with better cognitive

functioning, slower rates of cognitive decline and lower chance of dementia [5]. Similar benefits have been demonstrated for the MIND diet, which composition is based on the MedDiet but with emphasis on the specific brain foods such as berries and leafy greens [5]. In addition, a study into the association between the plant-based diet index and cognitive ageing showed that plant-based dietary patterns including fish were more protective against risk of cognitive impairment compared to plant-based dietary patterns without fish [9].

While the modification by fish intake can be explained from a nutrient perspective and observational studies support this finding, it needs to be mentioned that our findings result from a subgroup analysis which limits the interpretability of these observations. Possibly, this is also an explanation for the inconsistency in findings, as we only demonstrated the modification by fish intake for PDI in the cross-sectional analysis, and hPDI in the longitudinal analysis. These results should be interpreted as preliminary and the analyses have to be replicated in other datasets before definite conclusions can be drawn.

Finally, a remark should be made with regard to the protein content of plant-based diets. Even though protein is not considered a nutrient of prime interest for the ageing brain, adequate consumption of high-quality protein is crucial for the ageing muscle and the prevention of sarcopenia [35]. Consuming a plant-based diet increases the risk of inadequate protein intake, due to the lower protein density and suboptimal essential amino acid content of plant foods [36]. Therefore, caution is warranted before advising older adults to reduce their intake of animal-based products.

In conclusion, we did not demonstrate a beneficial association of better adherence to a plant-based diet with cognitive ageing, which could be due to the lower intakes of vitamin B12, DHA and EPA in individuals with higher plant-based diet adherence. Possibly, such association between plant-based diet adherence and cognition exists in a subpopulation of fish-consumers with a fish intake of at least one portion per week. This would be in line with earlier findings that plant-centred diets that include regular fish consumption, such as the MedDiet and MIND diet, may offer benefits for the ageing brain.

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Supplementary materials

Supplementary table 1: Overview of food items constituting the 18 food groups.

Plant food groups	
Healthy	
Whole grains	Whole grain breakfast cereal, cooked oatmeal, wheat porridge, whole grain rusk, whole grain crispbread, rye bread, whole grain bread, whole grain pasta, brown rice, bulgur, millet, couscous
Fruits	Apple, banana, orange, strawberry, other fruits
Vegetables	Cauliflower, broccoli and other cabbages, spinach, beets, endive, green beans, other cooked vegetables, lettuce, raw endive, other raw vegetables
Nuts	Peanut butter, peanuts, cocktail nuts, walnuts, mixed nuts, other nuts and seeds
Legumes	Legumes, soy products
Vegetable oils	Olive oil, dressing based on oil, other oils
Tea & coffee	Tea, coffee
Less healthy	
Fruit juices	Orange juice, other juices
Refined grains	Cornflakes, white rusk, rice waffles, cream crackers, croissants, white bread, raisin bread, gingerbread, white pasta, white rice
Potatoes	Fries, chips, cooked and baked potatoes, mashed potatoes
Sugar sweetened beverages	Regular soft drinks, light soft drinks
Sweets and desserts	Sweet bread toppings, sugar, cookies, cake, chocolate, candy bars, candy, water ice
Animal food groups	
Animal fat	Butter, lard
Dairy	Milk, buttermilk, chocolate milk, yoghurt, custard, drink breakfast, cheese, cream, ice cream,
Egg	Eggs
Fish and seafood	Shellfish, mussels, flounder, trout, herring, salmon, other types of fish
Meat	Liver, ham, bacon, chicken, turkey, minced meat, beef, pork, organ meats, smoked sausage, fried meat snacks
Misc. animal-based food	Pizza, pancakes, creamy salad dressings, mayonnaise, fried spring roll, meat/fish salads

Supplementary table 2: Description of cognitive tests

Domain	Test	Description	Scoring
Episodic memory	RAVLT immediate	Recall of 15 words in five trials	0-45
	RAVLT delayed	Delayed recall of the 15 words after 20 minutes	0-15
	RAVLT recognition	Recognition of the 15 words in a list of 30 words	0-30
Attention & Working memory	Digit span forward	Recall of digit sequences with increasing length in forward order	0-9
	Digit span backward	Recall of digit sequences with increasing length in backward order	0-8
Information processing speed	Stroop part I and II	Naming colour words written in black ink (part I) and coloured blocks (part II) as fast as possible. Outcome is mean part I and II	0 – ∞ s
	Trail making test part A	Draw lines connecting numbers in chronological order as fast as possible	0 - 300 s
	SDMT	Match symbols with digits within 90 s as fast as possible.	0 - 110
Executive functioning	Stroop interference	Naming colour words written in black ink (part I), coloured blocks (part II) and colour words written in an incongruent colour ink (part III) Outcome is part III corrected by parts I and II	0 – ∞
	Trail making test part B/A	Draw lines connecting numbers in chronological order (part A) or numbers and letters alternating in chronological and alphabetical order (part B). Outcome is ratio part B/A	0-300 s
	Letter fluency	Name as many words as possible starting with a specific letter in 60 s	0 - ∞

Abbreviations: Rey Auditory Verbal Learning Test (RAVLT); Symbol Digit Modalities Test (SDMT)

Supplementary table 3: Nutrient intake according to overall plant-based diet index tertile

Nutrient	Total (n=658)	Tertile 1 (n=226)	Tertile 2 (n=202)	Tertile 3 (n=230)	p-value
Energy (kCal)	1945 ± 508	1981 ± 505	1863 ± 530	1980 ± 483	0.02
Protein (g)	73 ± 18	77 ± 18	71 ± 19	71 ± 16	<0.01
Protein, animal origin (g)	45 ± 13	51 ± 14	44 ± 13	41 ± 11	<0.001
Protein, plant origin (g)	28 ± 8	26 ± 8	27 ± 8	31 ± 8	<0.001
Carbohydrates (g)	214 ± 62	202 ± 61	204 ± 62	234 ± 59	<0.001
Sugar (g)	111 ± 40	105 ± 42	105 ± 38	122 ± 38	<0.001
Starch (g)	102 ± 32	97 ± 31	98 ± 32	111 ± 29	<0.001
Fibre (g)	23 ± 7	21 ± 6	23 ± 7	26 ± 7	<0.001
Fat (g)	78 ± 27	83 ± 28	74 ± 29	76 ± 25	<0.01
Cholesterol (mg)	202 ± 80	246 ± 90	194 ± 71	167 ± 53	<0.001
SFA (g)	28 ± 11	32 ± 14	27 ± 10	26 ± 8	<0.001
MUFA (g)	26 ± 10	28 ± 9	25 ± 10	27 ± 10	0.03
PUFA (g)	16 ± 8	16 ± 7	15 ± 8	16 ± 7	0.09
Alcohol (g)	14 ± 14	17 ± 16	14 ± 13	10 ± 11	<0.001
linoleic acid (g)	13 ± 7	13 ± 6	12 ± 7	14 ± 7	0.05
α-linolenic acid (g)	1.3 ± 0.7	1.3 ± 0.7	1.2 ± 0.8	1.4 ± 0.7	0.11
EPA (g)	0.07 ± 0.08	0.08 ± 0.08	0.07 ± 0.07	0.06 ± 0.08	0.01
DHA (g)	0.11 ± 0.11	0.13 ± 0.12	0.11 ± 0.10	0.10 ± 0.12	0.01
Vitamin B12 (mg)	4.1 ± 2.0	4.8 ± 2.3	4.0 ± 1.9	3.5 ± 1.4	<0.001
Folic acid (mcg)	187 ± 55	180 ± 52	185 ± 60	197 ± 52	<0.01

Abbreviations: SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid. Data are mean ±SD.

Supplementary table 4: Regression output energy-adjusted overall, healthful and unhealthful plant based diet index and domain specific cognitive functioning (cross-sectional)

	PDI			hPDI			uPDI		
	Crude model	Model 1	Model 2	Crude model	Model 1	Model 2	Crude model	Model 1	Model 2
Episodic memory									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	0.09	0.03	0.06	0.00	-0.04	-0.03	-0.07	-0.02	-0.02
	[-0.05, 0.22]	[-0.09, 0.16]	[-0.06, 0.19]	[-0.13, 0.13]	[-0.16, 0.08]	[-0.16, 0.09]	[-0.20, 0.06]	[-0.15, 0.10]	[-0.14, 0.11]
	0.20	0.61	0.33	0.95	0.54	0.58	0.32	0.70	0.80
Tertile 3	0.16	0.12	0.16	0.09	0.03	0.01	-0.16	-0.01	0.03
	[0.03, 0.29]	[0.00, 0.24]	[0.03, 0.28]	[-0.04, 0.22]	[-0.10, 0.15]	[-0.12, 0.13]	[-0.29, -0.03]	[-0.14, 0.12]	[-0.10, 0.16]
	0.02	0.06	0.01	0.17	0.67	0.91	0.02	0.90	0.67
Continuous	0.08	0.05	0.07	0.07	0.01	0.00	-0.09	0.00	0.03
	[-0.01, 0.16]	[-0.03, 0.13]	[-0.01, 0.16]	[-0.01, 0.14]	[-0.06, 0.09]	[-0.08, 0.07]	[-0.17, -0.01]	[-0.08, 0.08]	[-0.05, 0.11]
	0.08	0.19	0.08	0.10	0.76	0.91	0.03	1.00	0.46
Attention & working memory									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	0.14	0.08	0.09	0.08	0.06	0.05	-0.01	0.05	0.03
	[-0.02, 0.30]	[-0.07, 0.23]	[-0.06, 0.25]	[-0.08, 0.24]	[-0.09, 0.21]	[-0.10, 0.20]	[-0.18, 0.15]	[-0.11, 0.20]	[-0.12, 0.19]
	0.09	0.30	0.24	0.31	0.45	0.48	0.86	0.56	0.69
Tertile 3	-0.11	-0.12	-0.11	0.00	-0.09	-0.10	-0.09	0.03	0.03
	[-0.27, 0.05]	[-0.27, 0.03]	[-0.26, 0.05]	[-0.16, 0.16]	[-0.25, 0.06]	[-0.25, 0.06]	[-0.26, 0.07]	[-0.12, 0.19]	[-0.14, 0.19]
	0.18	0.11	0.17	0.99	0.22	0.22	0.27	0.68	0.74
Continuous	-0.02	-0.03	-0.02	0.01	-0.06	-0.06	-0.06	0.01	0.01
	[-0.12, 0.09]	[-0.13, 0.07]	[-0.12, 0.08]	[-0.09, 0.11]	[-0.15, 0.03]	[-0.15, 0.03]	[-0.16, 0.04]	[-0.09, 0.10]	[-0.09, 0.10]
	0.75	0.51	0.69	0.84	0.20	0.22	0.22	0.87	0.91

Supplementary table 4 (*continued*)

	PDI			hPDI			uPDI		
	Crude model	**Model 1**	**Model 2**	**Crude model**	**Model 1**	**Model 2**	**Crude model**	**Model 1**	**Model 2**
Information processing speed									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	0.08	0.06	0.08	0.07	0.05	0.06	-0.07	-0.06	-0.05
	[-0.07, 0.23]	[-0.08, 0.20]	[-0.06, 0.22]	[-0.08, 0.22]	[-0.09, 0.18]	[-0.08, 0.19]	[-0.23, 0.08]	[-0.20, 0.08]	[-0.19, 0.09]
	0.32	0.40	0.28	0.37	0.49	0.41	0.34	0.39	0.48
Tertile 3	0.05	0.06	0.05	0.08	-0.01	-0.04	-0.15	-0.05	-0.02
	[-0.10, 0.20]	[-0.08, 0.19]	[-0.09, 0.19]	[-0.07, 0.23]	[-0.15, 0.13]	[-0.18, 0.10]	[-0.30, 0.00]	[-0.19, 0.09]	[-0.16, 0.13]
	0.50	0.42	0.46	0.31	0.91	0.56	0.06	0.50	0.80
Continuous	0.06	0.05	0.04	0.05	0.00	-0.03	-0.07	-0.02	0.00
	[-0.04, 0.16]	[-0.04, 0.14]	[-0.05, 0.13]	[-0.04, 0.14]	[-0.08, 0.08]	[-0.11, 0.06]	[-0.16, 0.02]	[-0.10, 0.07]	[-0.09, 0.09]
	0.22	0.32	0.39	0.26	0.97	0.51	0.12	0.67	0.97
Executive functioning									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	0.15	0.09	0.11	0.08	0.04	0.04	0.02	0.05	0.04
	[0.01, 0.28]	[-0.03, 0.22]	[-0.02, 0.24]	[-0.06, 0.21]	[-0.08, 0.17]	[-0.09, 0.16]	[-0.12, 0.15]	[-0.08, 0.18]	[-0.08, 0.17]
	0.04	0.15	0.09	0.26	0.51	0.53	0.79	0.44	0.50
Tertile 3	0.07	0.06	0.06	0.12	0.02	-0.01	-0.13	-0.02	-0.02
	[-0.06, 0.21]	[-0.07, 0.18]	[-0.07, 0.19]	[-0.01, 0.25]	[-0.10, 0.15]	[-0.13, 0.12]	[-0.27, 0.00]	[-0.15, 0.11]	[-0.16, 0.11]
	0.27	0.37	0.34	0.08	0.72	0.93	0.06	0.75	0.76
Continuous	0.08	0.06	0.06	0.09	0.03	0.01	-0.10	-0.03	-0.03
	[-0.01, 0.17]	[-0.02, 0.14]	[-0.02, 0.15]	[0.01, 0.17]	[-0.05, 0.10]	[-0.07, 0.08]	[-0.18, -0.02]	[-0.11, 0.04]	[-0.12, 0.05]
	0.09	0.15	0.15	0.03	0.49	0.90	0.02	0.38	0.40

Abbreviations: PDI; plant-based diet index, hPDI; healthful plant-based diet index, uPDI; unhealthful plant-based diet index. Model 1: adjusted for age, gender and education. Model 2: additionally adjusted for BMI, physical activity, smoking, alcohol consumption and margarine consumption. Longitudinal analysis was additionally adjusted for baseline cognition score. Data are β [95% CI] p-value. In the continuous analysis, β is shown per 10 points increment in plant-based diet index.

Supplementary table 5: Regression output energy-adjusted overall, healthful and unhealthful plant based diet index and domain specific cognitive change (longitudinal)

	PDI			hPDI			uPDI		
	Crude model	**Model 1**	**Model 2**	**Crude model**	**Model 1**	**Model 2**	**Crude model**	**Model 1**	**Model 2**
Episodic memory									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	-0.05	-0.06	-0.07	0.02	0.01	0.01	-0.08	-0.07	-0.05
	[-0.21, 0.11]	[-0.21, 0.10]	[-0.23, 0.10]	[-0.13, 0.17]	[-0.14, 0.17]	[-0.15, 0.16]	[-0.23, 0.08]	[-0.22, 0.09]	[-0.21, 0.11]
	0.53	0.48	0.42	0.80	0.86	0.92	0.32	0.39	0.54
Tertile 3	-0.04	-0.03	-0.06	-0.02	-0.02	-0.04	-0.07	-0.03	-0.03
	[-0.19, 0.11]	[-0.18, 0.13]	[-0.22, 0.10]	[-0.17, 0.14]	[-0.18, 0.13]	[-0.20, 0.12]	[-0.22, 0.09]	[-0.19, 0.13]	[-0.19, 0.14]
	0.61	0.72	0.49	0.84	0.76	0.64	0.41	0.71	0.76
Continuous	-0.04	-0.04	-0.06	0.02	0.01	0.00	-0.06	-0.04	-0.04
	[-0.14, 0.06]	[-0.14, 0.07]	[-0.17, 0.05]	[-0.08, 0.11]	[-0.09, 0.11]	[-0.10, 0.10]	[-0.16, 0.03]	[-0.14, 0.05]	[-0.14, 0.06]
	0.44	0.47	0.27	0.75	0.83	0.97	0.21	0.39	0.47
Attention & working memory									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	-0.17	-0.18	-0.20	0.00	-0.01	0.00	0.00	0.00	0.01
	[-0.34,-0.01]	[-0.36,-0.01]	[-0.37,-0.02]	[-0.16, 0.17]	[-0.18, 0.15]	[-0.17, 0.16]	[-0.17, 0.17]	[-0.17, 0.17]	[-0.16, 0.18]
	0.04	0.03	0.03	0.97	0.89	0.96	0.99	0.98	0.92
Tertile 3	0.08	0.06	0.05	0.25	0.22	0.23	-0.21	-0.19	-0.18
	[-0.09, 0.24]	[-0.10, 0.23]	[-0.13, 0.22]	[0.08, 0.42]	[0.05, 0.39]	[0.05, 0.41]	[-0.38, -0.04]	[-0.36,-0.01]	[-0.36, -0.01]
	0.35	0.47	0.59	<0.01	0.01	0.01	0.02	0.04	0.04
Continuous	0.06	0.05	0.04	0.15	0.14	0.14	-0.17	-0.16	-0.16
	[-0.05, 0.17]	[-0.06, 0.16]	[-0.08, 0.16]	[0.05, 0.26]	[0.04, 0.24]	[0.03, 0.25]	[-0.27, -0.06]	[-0.27,-0.06]	[-0.27, -0.05]
	0.29	0.36	0.48	<0.01	0.01	0.01	<0.01	<0.01	<0.01

Supplementary table 5 (*continued*)

	PDI			hPDI			uPDI		
	Crude model	**Model 1**	**Model 2**	**Crude model**	**Model 1**	**Model 2**	**Crude model**	**Model 1**	**Model 2**
Information processing speed									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	-0.15	-0.13	-0.13	0.03	0.04	0.04	-0.07	-0.07	-0.08
	[-0.28,-0.01]	[-0.26, 0.00]	[-0.26, 0.01]	[-0.10, 0.16]	[-0.09, 0.17]	[-0.08, 0.17]	[-0.21, 0.06]	[-0.20, 0.06]	[-0.22, 0.05]
	0.04	0.05	0.06	0.65	0.55	0.50	0.29	0.29	0.21
Tertile 3	-0.08	-0.08	-0.11	0.06	0.04	0.00	-0.06	-0.04	-0.07
	[-0.21, 0.05]	[-0.21, 0.05]	[-0.25, 0.02]	[-0.07, 0.20]	[-0.10, 0.17]	[-0.13, 0.14]	[-0.20, 0.07]	[-0.17, 0.10]	[-0.21, 0.06]
	0.21	0.21	0.09	0.35	0.60	0.99	0.36	0.58	0.30
Continuous	-0.04	-0.05	-0.07	0.05	0.03	0.01	-0.05	-0.05	-0.07
	[-0.13, 0.05]	[-0.14, 0.03]	[-0.16, 0.01]	[-0.03, 0.13]	[-0.05, 0.12]	[-0.07, 0.09]	[-0.13, 0.03]	[-0.13, 0.03]	[-0.16, 0.01]
	0.36	0.23	0.10	0.25	0.40	0.80	0.21	0.24	0.09
Executive functioning									
Tertile 1	REF	REF	REF	REF	REF	REF	REF	REF	REF
Tertile 2	0.09	0.09	0.09	-0.01	-0.02	-0.01	0.02	0.02	0.02
	[-0.04, 0.22]	[-0.03, 0.22]	[-0.04, 0.22]	[-0.13, 0.12]	[-0.14, 0.11]	[-0.14, 0.11]	[-0.11, 0.14]	[-0.10, 0.15]	[-0.10, 0.15]
	0.17	0.15	0.15	0.90	0.80	0.81	0.78	0.74	0.72
Tertile 3	0.03	0.04	0.01	0.07	0.04	0.01	0.05	0.08	0.06
	[-0.09, 0.16]	[-0.08, 0.16]	[-0.11, 0.14]	[-0.06, 0.20]	[-0.08, 0.17]	[-0.12, 0.14]	[-0.08, 0.18]	[-0.05, 0.21]	[-0.07, 0.20]
	0.60	0.56	0.82	0.28	0.50	0.84	0.44	0.21	0.34
Continuous	0.05	0.04	0.03	0.03	0.01	-0.01	0.03	0.04	0.03
	[-0.03, 0.13]	[-0.04, 0.12]	[-0.06, 0.11]	[-0.05, 0.11]	[-0.06, 0.09]	[-0.09, 0.07]	[-0.05, 0.11]	[-0.04, 0.12]	[-0.05, 0.12]
	0.26	0.30	0.52	0.47	0.70	0.84	0.47	0.28	0.40

Abbreviations: PDI; plant-based diet index, hPDI; healthful plant-based diet index, uPDI; unhealthful plant-based diet index.

Model 1: adjusted for age, gender and education. Model 2: additionally adjusted for BMI, physical activity, smoking, alcohol consumption and margarine consumption. Longitudinal analysis was additionally adjusted for baseline cognition score. Data are β [95% CI] p-value. In the continuous analysis, β is shown per 10 points increment in plant-based diet index.

CHAPTER 7

The association between adherence to the EAT-Lancet diet and cognitive ageing

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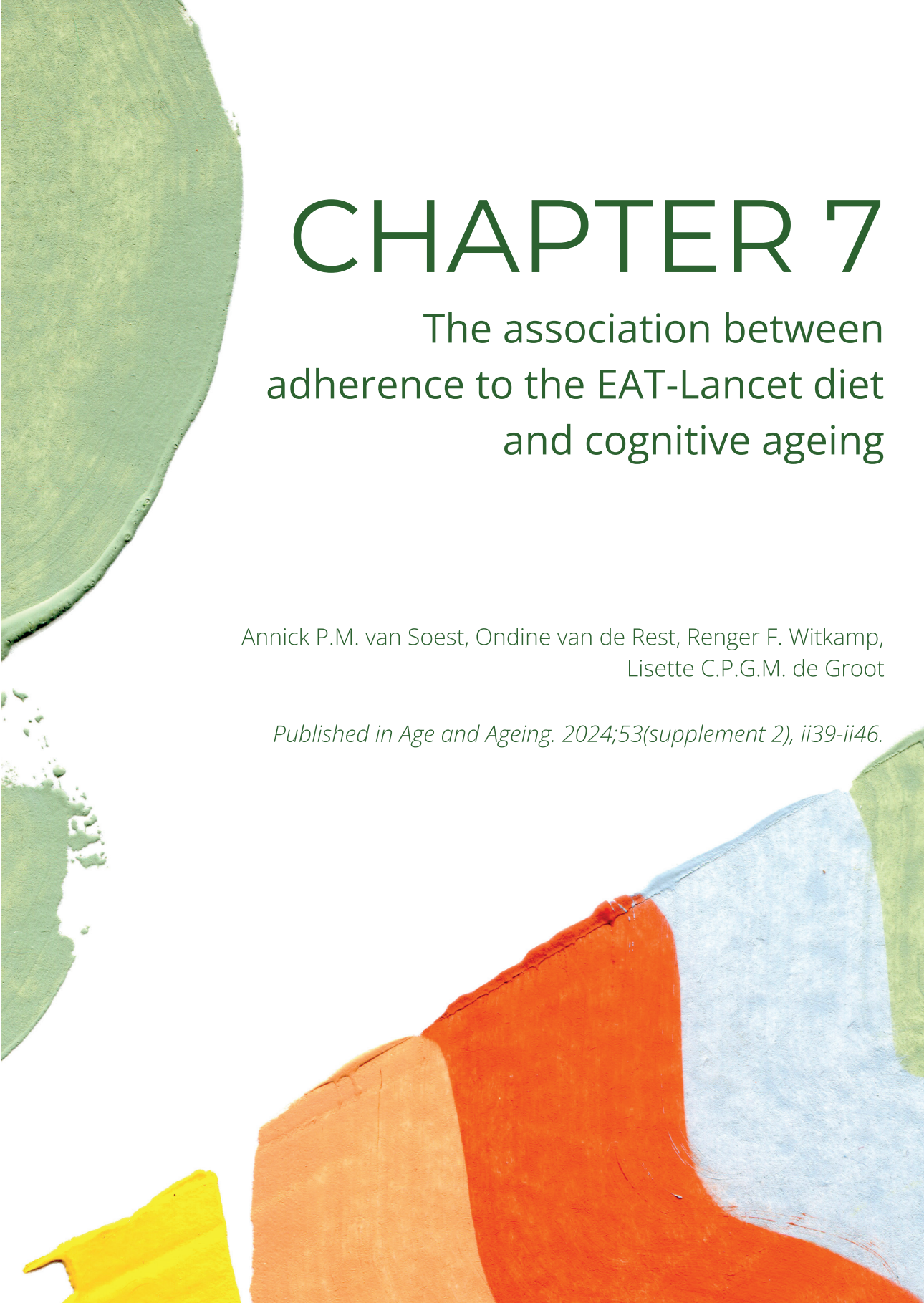

ABSTRACT

Background: The EAT-Lancet commission has proposed a dietary pattern that is both sustainable and healthy. However, the impact of this diet on cognition in older adults remains unexplored. Therefore, we examined the association between adherence to the EAT-Lancet diet and cognitive ageing.

Methods: We used data from a previous intervention study involving cognitively healthy community-dwelling adults aged ≥65 years. Adherence to the EAT-Lancet diet was calculated using a recently published index and a 190-item food frequency questionnaire. Global and domain-specific cognitive functioning were assessed at baseline and after two years using a neuropsychological test battery. Multivariate-adjusted linear regression was conducted to examine associations between EAT-Lancet diet adherence and cognitive functioning (n=630) and 2-year change (n=302).

Results: Greater adherence to the EAT-Lancet diet was associated with better global cognitive functioning (β per SD=3.7 points [95% CI]: 0.04 [0.00, 0.08]) and slower rate of decline (β per SD [95% CI]: 0.05 [0.02, 0.08]). With respect to domain-specific functioning, beneficial associations were observed cross-sectionally for executive functioning ($p<0.01$), and longitudinally for change in executive functioning ($p<0.01$) and attention and working memory ($p<0.01$). The degree of adherence to the EAT-Lancet was not associated with (changes in) information processing speed or episodic memory.

Conclusion: We demonstrated that greater adherence to the EAT-Lancet diet is associated with better global cognitive functioning and slower cognitive decline among cognitively healthy older adults. Further research is needed to confirm these findings and assess the potential benefits of the EAT-Lancet diet for the ageing population in a broader context.

Keywords: planetary health diet; plant-based diet; nutrition; brain ageing; healthy ageing; older people.

Key points

- This study explores the association between the EAT-Lancet diet, a healthy and sustainable dietary pattern, and cognitive ageing
- EAT-Lancet diet adherence was positively associated with global cognition and slower decline in cognitively healthy older adults
- Replication of these findings and extension to other outcomes would be required to verify the relevance to the ageing population

INTRODUCTION

The diet we consume has a major impact on both human and environmental health. Poor quality diets, such as the Western diet, are associated with an increased risk of chronic diseases [1]. Simultaneously, food production is among the most important drivers of global environmental change, depletion of water and biodiversity loss [2]. Consequently, there is an urgent need for diets that promote both human and environmental health.

The EAT-Lancet commission has proposed the EAT-Lancet diet, a healthy reference diet that fits within planetary boundaries [3]. This diet was developed based on extensive literature review and consists of a large variety in plant foods with few foods from animal sources. Specifically, intakes of vegetables, fruits, legumes, whole grains, nuts, and fish are emphasized. Eggs, poultry, and dairy are considered optional foods that should be consumed in moderation, and intake of red meat and starchy vegetables should be minimized [3].

While a substantial body of literature supports the health benefits of individual components of the EAT-Lancet diet, the diet as a whole has only been studied for a limited number of health domains. For example, studies have demonstrated that better adherence to the EAT-Lancet diet is associated with lower risk of mortality [4] and type 2 diabetes [5-7], as well as favourable cardiovascular [8-10] and anthropometric [11, 12] outcomes. Yet, the probable benefits of the EAT-Lancet diet on healthy ageing, including the effect on cognitive abilities, have not been studied.

Therefore, the current study aims to investigate the association between adherence to the EAT-Lancet diet and cognitive functioning and two year cognitive decline in healthy older adults without cognitive complaints.

METHODS

PARTICIPANTS AND STUDY DESIGN

We made use from data of the B-proof study, a randomized double-blind controlled trial on the effect of B-vitamin supplementation (400µg folic acid and 500µg vitamin B12) versus placebo for 2 years on fracture incidence. Cognitive functioning was measured as secondary outcome. Detailed information on study design and participants has been described previously [13]. In short, participants were community dwelling older adults aged 65 years and older with elevated homocysteine levels (12–50 µmol/L). None of the participants had a renal

insufficiency (creatinine >150 µmol/L) or a cancer diagnosis in the past five years. In the present study, we performed a cross-sectional analysis using baseline data and a longitudinal analysis spanning 2 years, using data from the control group only. Follow-up data from the intervention group was excluded to eliminate potential interference from the B-vitamin supplementation. While the B-vitamin intervention was not effective in slowing cognitive decline in the complete B-proof study population [14], a post-hoc analysis indicated that the B-vitamins may have slowed cognitive ageing in a subgroup [15]. Data were collected between October 2008 and March 2013 at three locations in the Netherlands: Erasmus Medical Center, VU University Medical Center and Wageningen University & Research. The current analysis is based on the Wageningen subgroup only, as extensive dietary and cognitive assessment was only done for Wageningen participants. Of the n=664 participants with available dietary and cognition data, data from 6 participants were excluded due to unreliable energy intake (men<800 kcal or >4200 kcal, women<500 kcal or >3500 kcal, n=2), missing baseline cognition (n=4) or missing ApoE genotype (n=28) data. The final study sample comprised n=630 participants. The B-proof trial was approved by the Medical Ethics committee from Wageningen University & Research and was registered under NCT00696514 at clinicaltrials.gov. All participants provided informed consent.

DIETARY ASSESSMENT

Dietary intake was assessed at baseline by a validated 190-item food frequency questionnaire (FFQ) [16, 17], that covered consumption frequencies in the past month. Standard portion sizes and commonly used household measures were used to estimate portion sizes. Average daily nutrient intakes were computed using data from the Dutch food composition database [18] and an added sugar composition database [19].

As a measure of EAT-Lancet diet adherence, we calculated the EAT-Lancet index based on the methods proposed by Stubbendorff and colleagues [4]. This index is based on 14 food components, divided into 7 'emphasized' food components (vegetables, fruits, unsaturated oils, legumes, nuts, whole grains and fish) and 7 'limited' intake food components (beef and lamb, pork, poultry, eggs, dairy, potatoes and added sugar) (**supplementary table 1**). To calculate the index score, first an energy-adjustment was applied according to the nutrient density approach [20]. The intake of a food component in grams was converted to intake in grams per 2500 kcal, as the EAT-Lancet diet is based on an intake of 2500 kcal/day. Subsequently,

each energy-adjusted food component was assigned a score ranging from 0 to 3 depending on the level of intake according to the cut-offs set by Stubbendorff et al. [4]. For 'emphasized' food components, higher intakes received higher scores whereas 'limited' food components received lower scores for higher intakes. The scores for the 14 food components were totalled to calculate the EAT-Lancet index, with score 0 representing poorest adherence to score 42 for perfect adherence.

COGNITIVE TESTING

Cognitive functioning was assessed by trained research assistants at baseline and after 2 years. This was done with a comprehensive battery of cognitive tests including six different tests: Rey Auditory Verbal Learning Test (RAVLT) (subtests immediate, delayed and recognition) [21], Digit Span task [22], Trail Making Test (TMT) (part A and B) [23], Stroop Colour-Word test (part I, II and III) [24], Symbol Digit Modalities Test (SDMT) [25], and Letter Fluency [26] (**supplementary table 2**). To limit learning effects, we used parallel versions at year 2 assessment for RAVLT, TMT and letter fluency.

To reduce the number of cognition outcomes, raw scores from each test were converted into cognitive composite Z-scores using population baseline mean and standard deviation. We reversed the Z-scores for the TMT and Stroop Colour-Word test as smaller values indicate better cognition. The individual Z-scores per test were combined into composite scores for global and domain-specific cognitive functioning according to the formulas below.

$$Global\ cognition = \left(Z_{Episodic\ memory} + Z_{Attention\ and\ working\ memory} + Z_{Information\ processing\ speed} + Z_{Executive\ functioning}\right)/4$$

$$Episodic\ memory = \left(Z_{RAVLT\ immediate} + Z_{RAVLT\ delayed} + Z_{RAVLT\ recognition}\right)/3$$

$$Attention\ and\ working\ memory = \left(Z_{Digit\ span\ forward} + Z_{Digit\ span\ backward}\right)/2$$

$$Information\ processing\ speed = \left(-Z_{Stroop\ mean\ I\ and\ II} + -Z_{TMT\ part\ A} + Z_{SDMT}\right)/3$$

$$Executive\ functioning = \left(-Z_{Stroop\ interference} + -Z_{TMT\ part\ B/A} + Z_{Fluency}\right)/3$$

Additionally to the cognitive test battery, Mini-Mental State Examination (MMSE) [27] was administered following standardized protocol. This test was assessed for descriptive purposes and serves as an indicator of the participants' cognitive state.

COVARIATES

Trained research assistants collected data on age, sex, education level, smoking status and physical activity [28] via questionnaires. Height and weight were measured following standardized protocol and were used to calculate body mass index (BMI) using the formula weight (kg)/(height (m))^2. ApoE genotype was determined using TaqMan analysis.

STATISTICAL ANALYSIS

Data are reported as n (%), mean (SE) or median (IQR). Differences in baseline characteristics across tertiles were compared using ANOVA for continuous variables and chi-square test for categorical variables. We performed multiple linear regression analysis to examine the association between adherence to the EAT-Lancet diet and global and domain-specific cognitive functioning and decline. For the cross-sectional analysis, we modelled the cognitive composite Z-score as a function of EAT-Lancet index score (categorical and continuous) using data from the complete study population. In the longitudinal analysis, the change in cognition Z-score between baseline and after 2 years of follow-up was modelled as a function of the EAT-Lancet index score (categorical and continuous) in the control group only. We excluded participants in the B-vitamin intervention group to eliminate influence of the supplementation. We adjusted the final model for the covariates age (years), sex, education (low, middle, high), ApoE4 carrier status, BMI (in kg/m^2), physical activity (METh/w), smoking (never, former, current) and alcohol intake (light, moderate, excessive). Additionally, the longitudinal analysis was adjusted for baseline cognitive composite Z-score. A p-value smaller than 0.05 was considered significant. All analyses were performed using RStudio version 1.4.0 [29].

RESULTS

BASELINE CHARACTERISTICS

The average age was 72.1±5.3 years and 60% of the participants were male (**table 1**). The mean BMI was 27.2±3.9 kg/m^2, indicating that on average participants were overweight. Additionally, participants were cognitively healthy as demonstrated by a median MMSE of 29 [28-30]. The EAT-Lancet index score ranged from 8 to 33 in the total study population, with scores ranging from 8-18 in the low, 19-21 in the middle, and 22-33 in the high tertiles. Participants classified in the lowest tertile of EAT-Lancet diet adherence had on average a higher BMI, had a lower level of education, were more often current smoker and scored lower on MMSE compared to individuals who fell into the tertile with highest adherence.

Table 1: Baseline characteristics of the B-proof study population per tertile of the EAT-Lancet index

Characteristic	Overall (n=630)	Low (n=241)	Middle (n=203)	High (n=186)	p-value
EAT-Lancet index (range)	8-33	8-18	19-21	22-33	<0.01
Age (years)	72.1±5.3	72.2±5.3	72.5±5.2	71.4±5.4	0.11
Sex n (%)					0.18
Male	378 (60%)	144 (60%)	131 (65%)	103 (55%)	
Female	252 (40%)	97 (40%)	72 (35%)	83 (45%)	
Level of education n (%)					<0.01
Low	268 (43%)	127 (53%)	72 (35%)	69 (37%)	
Middle	151 (24%)	54 (22%)	55 (27%)	42 (23%)	
High	211 (34%)	60 (25%)	76 (37%)	75 (40%)	
ApoE4 carrier	192 (31%)	60 (25%)	67 (33%)	65 (35%)	0.05
BMI (kg/m^2)	27.2±3.9	27.7±3.8	27.0±4.2	26.7±3.7	0.03
Physical activity (METh/w)	53 (33-80)	51 (31-77)	56 (34-84)	54 (35-77)	0.32
Smoking behavior n (%)					0.01
Current smoker	67 (11%)	35 (15%)	20 (10%)	12 (6%)	
Former smoker	372 (59%)	130 (54%)	133 (66%)	109 (59%)	
Never smoker	191 (30%)	76 (32%)	50 (25%)	65 (35%)	
Alcohol intake n (%)					0.99
Light	405 (64%)	157 (65%)	131 (65%)	117 (63%)	
Moderate	204 (32%)	76 (32%)	65 (32%)	63 (34%)	
Excessive	21 (3%)	8 (3%)	7 (3%)	6 (3%)	
MMSE score	29 (28-30)	29 (27-29)	29 (28-30)	29 (28-30)	0.01

Abbreviations: BMI: body mass index, MMSE: Mini Mental State Examination. Data are mean±SD, median (IQR) or number (%).

Additionally, nutrient intake differed largely between tertiles (**supplementary table 3**). Participants with higher adherence to the EAT-Lancet diet consumed more plant protein, polyunsaturated fatty acids in general, omega-3 fatty acids and fibres, while their intakes of animal protein, cholesterol and saturated fat were lower.

The EAT-Lancet diet differs from other dietary patterns with demonstrated benefits for the ageing brain (MIND diet, Mediterranean diet) (**supplementary table 4**). To obtain a maximum adherence score, the EAT-Lancet diet allows fewer portions of animal-based foods, and requires more portions of plant-based foods.

CROSS-SECTIONAL ANALYSIS

Higher adherence to the EAT-Lancet diet was associated with better global cognitive functioning (**table 2**). For each SD (3.7 points) increase in EAT-Lancet diet score, the global cognition composite increased with 0.04 units (95% CI 0.00, 0.08; p=0.03). However, this finding was not confirmed in the categorical analysis (difference T1 vs T3 [95% CI]: 0.07 [-0.03, 0.16], p=0.17).

With respect to domain-specific cognitive functioning, higher adherence to the EAT-Lancet diet was associated with better executive functioning performance (β per SD [95% CI]: 0.07 [0.01, 0.12], p=0.02), but again this finding was not replicated in the categorical analysis (p=0.27). There was a trend towards an association between information processing speed performance and the level of adherence to the EAT-Lancet diet (β per SD [95% CI]: 0.06 [0.00, 0.12], p=0.05). For episodic memory and attention and working memory, no associations were found between EAT-Lancet diet adherence in both continuous and categorical analyses.

LONGITUDINAL ANALYSIS

The level of adherence to the EAT-Lancet diet was associated with cognitive decline, with a 0.05 unit (95% CI 0.02, 0.08; p<0.01) slower rate in global cognitive decline per SD (3.7 point) increase in EAT-Lancet diet (**table 3**). This equals being 4.5 years younger in age per SD increase in EAT-Lancet diet score. Similarly, comparing tertiles of adherence, individuals with highest adherence to the EAT-Lancet diet showed slower cognitive decline compared to individuals with lowest adherence (difference T1 vs T3 [95% CI]: 0.12 [0.05, 0.20], p<0.01).

A similar pattern was observed with respect to the cognitive domains attention and working memory and executive functioning. Higher adherence to the EAT-Lancet diet was associated with slower rates of cognitive decline in attention and working memory (β per SD [95% CI]: 0.11 [0.04, 0.18], p<0.01) and executive functioning (β per SD [95% CI]: 0.07 [0.02, 0.12], p=0.01), equalling to being 9.7 and 4.4 years younger in age, respectively. For both cognitive domains, findings were confirmed in the categorical analysis. However, we did not find associations between the level of adherence to the EAT-Lancet diet with change in episodic memory and information processing speed.

Table 2: Regression output association EAT-Lancet index and cognitive functioning (cross-sectional).

	Crude model	Model 1	Model 2
Global cognition			
Tertile 1	REF	REF	REF
Tertile 2	0.11 [0.01, 0.21]	0.06 [-0.04, 0.15]	0.04 [-0.05, 0.13]
	0.02	0.23	0.37
Tertile 3	0.19 [0.09, 0.29]	0.08 [-0.01, 0.18]	0.07 [-0.03, 0.16]
	<0.01	0.09	0.17
Continuous	0.10 [0.06, 0.14]	0.05 [0.01, 0.09]	0.04 [0.00, 0.08]
	<0.01	0.01	0.03
Episodic memory			
Tertile 1	REF	REF	REF
Tertile 2	-0.06 [-0.18, 0.07]	-0.07 [-0.19, 0.05]	-0.09 [-0.21, 0.04]
	0.39	0.27	0.17
Tertile 3	0.07 [-0.06, 0.20]	0.00 [-0.13, 0.12]	-0.03 [-0.16, 0.09]
	0.27	0.94	0.60
Continuous	0.05 [-0.01, 0.10]	0.01 [-0.05, 0.06]	0.00 [-0.06, 0.05]
	0.08	0.81	0.88
Attention and working memory			
Tertile 1	REF	REF	REF
Tertile 2	0.20 [0.04, 0.36]	0.10 [-0.05, 0.25]	0.10 [-0.05, 0.26]
	0.01	0.20	0.19
Tertile 3	0.21 [0.05, 0.37]	0.07 [-0.09, 0.23]	0.08 [-0.08, 0.24]
	0.01	0.37	0.34
Continuous	0.10 [0.04, 0.17]	0.03 [-0.03, 0.10]	0.04 [-0.03, 0.11]
	<0.01	0.30	0.23
Information processing speed			
Tertile 1	REF	REF	REF
Tertile 2	0.15 [0.00, 0.29]	0.11 [-0.03, 0.25]	0.08 [-0.06, 0.22]
	0.05	0.12	0.24
Tertile 3	0.25 [0.10, 0.40]	0.15 [0.00, 0.29]	0.12 [-0.02, 0.26]
	<0.01	0.04	0.10
Continuous	0.11 [0.05, 0.18]	0.07 [0.01, 0.13]	0.06 [0.00, 0.12]
	<0.01	0.02	0.05
Executive functioning			
Tertile 1	REF	REF	REF
Tertile 2	0.17 [0.04, 0.30]	0.07 [-0.05, 0.20]	0.06 [-0.07, 0.19]
	0.01	0.24	0.35
Tertile 3	0.20 [0.07, 0.34]	0.09 [-0.04, 0.22]	0.07 [-0.06, 0.20]
	<0.01	0.18	0.27
Continuous	0.12 [0.07, 0.18]	0.07 [0.02, 0.12]	0.07 [0.01, 0.12]
	<0.01	0.01	0.02

Model 1: adjusted for age, gender, education and ApoE4 carrier status; Model 2: additionally adjusted for BMI, physical activity, smoking and alcohol consumption. Data are β [95% CI] p-value. In the continuous analysis, β is shown per SD=3.7 points increment in EAT-Lancet index.

Table 3: Regression output association EAT-Lancet index and cognitive change (longitudinal).

	Crude model	Model 1	Model 2
Global cognition			
Tertile 1	REF	REF	REF
Tertile 2	0.06 [-0.01, 0.14] 0.08	0.07 [-0.01, 0.14] 0.07	0.06 [-0.02, 0.13] 0.14
Tertile 3	0.13 [0.05, 0.20] <0.01	0.13 [0.06, 0.21] <0.01	0.12 [0.05, 0.20] <0.01
Continuous	0.05 [0.02, 0.08] <0.01	0.05 [0.02, 0.08] <0.01	0.05 [0.02, 0.08] <0.01
Episodic memory			
Tertile 1	REF	REF	REF
Tertile 2	0.01 [-0.15, 0.16] 0.94	0.01 [-0.15, 0.18] 0.87	-0.01 [-0.17, 0.16] 0.93
Tertile 3	0.10 [-0.06, 0.25] 0.21	0.10 [-0.06, 0.26] 0.21	0.08 [-0.08, 0.24] 0.32
Continuous	0.03 [-0.03, 0.09] 0.35	0.03 [-0.04, 0.09] 0.42	0.02 [-0.05, 0.08] 0.58
Attention and working memory			
Tertile 1	REF	REF	REF
Tertile 2	0.05 [-0.12, 0.21] 0.57	0.01 [-0.17, 0.18] 0.94	-0.01 [-0.18, 0.17] 0.94
Tertile 3	0.32 [0.15, 0.48] <0.01	0.28 [0.11, 0.46] <0.01	0.27 [0.10, 0.45] <0.01
Continuous	0.12 [0.05, 0.19] <0.01	0.11 [0.04, 0.18] <0.01	0.11 [0.04, 0.18] <0.01
Information processing speed			
Tertile 1	REF	REF	REF
Tertile 2	0.10 [-0.03, 0.24] 0.13	0.11 [-0.02, 0.25] 0.11	0.10 [-0.04, 0.24] 0.15
Tertile 3	0.08 [-0.05, 0.22] 0.21	0.08 [-0.05, 0.22] 0.24	0.06 [-0.07, 0.20] 0.35
Continuous	0.03 [-0.02, 0.09] 0.21	0.03 [-0.03, 0.08] 0.30	0.02 [-0.03, 0.08] 0.46
Executive functioning			
Tertile 1	REF	REF	REF
Tertile 2	0.17 [0.05, 0.30] 0.01	0.17 [0.04, 0.30] 0.01	0.16 [0.02, 0.29] 0.02
Tertile 3	0.17 [0.05, 0.29] 0.01	0.15 [0.02, 0.27] 0.03	0.13 [0.00, 0.26] 0.06
Continuous	0.09 [0.04, 0.13] <0.01	0.08 [0.02, 0.13] <0.01	0.07 [0.02, 0.12] 0.01

Crude model: adjusted for baseline cognition score; Model 1: additionally adjusted for age, gender, education and ApoE4 carrier status; Model 2: additionally adjusted for BMI, physical activity, smoking and alcohol consumption. Data are β [95% CI] p-value. In the continuous analysis, β is shown per SD=3.7 points increment in EAT-Lancet index.

DISCUSSION

In this cohort of cognitively healthy older adults, higher adherence to the EAT-Lancet diet was found to have a beneficial impact on cognitive ageing, manifested by associations with better global cognitive function and a slower rate of global cognitive decline. With respect to domain-specific cognitive functioning, individuals with a higher adherence to the EAT-Lancet diet showed better executive functioning and slower decline in this domain, as well as slower decline in attention and working memory. No associations were observed for episodic memory and information processing speed.

To the best of our knowledge, the association between the EAT-Lancet diet and cognitive ageing has previously been described in only one article [30]. In line with our findings, this article demonstrated that better adherence to the EAT-Lancet diet in midlife was associated with lower odds of poor cognitive function among Asian adults in later life. These findings are interesting, considering that the EAT-Lancet diet is more plant-based and thus more sustainable compared to other dietary patterns with demonstrated benefits for the ageing brain [31]. Further support that a shift towards more plant-based diets might positively impact cognition comes from research on the healthy plant-based diet index [32-34]. Interestingly, the addition of fish to a plant-rich diet was superior over a plant-rich dietary pattern without fish [32, 35], in line with the composition of the EAT-Lancet diet.

Previous research on the EAT-Lancet diet in relation to other health outcomes with shared aetiology as cognitive ageing further substantiates our findings. Higher adherence to the EAT-Lancet diet has been associated with lower risk of type 2 diabetes in three European cohorts [5-7], though not in a cohort of Mexican women [36]. Additionally, the EAT-Lancet diet seems beneficial for cardiovascular health as higher adherence to the diet has been associated with lower risk of coronary events [8], cardiovascular disease [10], subarachnoid stroke [37], and improved cardiometabolic markers including blood pressure and cholesterol [9]. However, two studies showed a null-association with cardiovascular outcomes [12, 38]. Finally, higher scores on the EAT-Lancet diet were associated with favourable obesity outcomes, as measured by BMI [12] and waist circumference [11].

From the perspective of nutrient composition, a positive link between the EAT-Lancet diet and cognitive ageing is conceivable. The EAT-Lancet diet is plant-focused and delivers various plant nutrients including carotenoids, polyphenols, vitamin E and

some B-vitamins. These nutrients have anti-inflammatory and anti-oxidant properties, via which they can support healthy brain ageing [39]. Importantly, the EAT-Lancet diet also recommends consumption of fish and does not completely restrict consumption of other animal foods, thus providing the brain with omega-3 fatty acids and vitamin B12. Omega-3 fatty acids contribute to healthy brain ageing due to their anti-inflammatory and vascular health promoting properties. Additionally, they are an important structural component of the brain [40]. Vitamin B12, together with folic acid and vitamin B6, prevent homocysteine accumulation and thereby help lower the risk of dementia [41].

However, while the EAT-Lancet diet does contain all nutrients required for healthy brain ageing, concerns have been raised if the diet supplies some of these nutrients in adequate amounts.

The first nutrient of concern is vitamin B12. According to the original EAT-Lancet diet publication, adopting the EAT-Lancet diet would improve intake of all nutrients but vitamin B12 [3]. Similarly, a nutrient adequacy analysis confirmed that the EAT-Lancet diet does not provide enough vitamin B12, with estimated intake being 93% of recommended nutrient intake [42]. Vitamin B12 is not only of great importance for brain health, but also for bone health [43]. The lack of vitamin B12 is especially of concern for the ageing population, as vitamin B12 absorption decreases with age and can be compromised with use of certain medications [44].

Secondly, the calcium content of the EAT-Lancet diet may be inadequate, with the estimated intake being 86% of the recommended nutrient intake [42]. Even though calcium is not considered a nutrient of prime interest for the ageing brain, this nutrient is essential for the bones and in the prevention of osteoporosis and fractures [45]. Adequate intake of calcium is especially relevant in the context of a plant-based diet high in phytates, as this anti-nutrient may decrease the absorption of calcium. Fortunately, this is particularly the case in non-balanced diets low in minerals but high in phytate, and may be less relevant when adhering to a balanced diet such as the EAT-Lancet diet [46].

Finally, the protein and amino acid content of the diet may be a point of concern, especially in the light of the increase in protein requirements with ageing [47]. The EAT-Lancet diet recommends largely replacing animal by plant protein, despite the lower protein quality (e.g. suboptimal essential amino acid content) of plant foods compared to animal foods [47]. While it is possible to consume a plant-based diet

high in protein with a complete amino acid profile, it requires knowledge on protein content of plant foods and how to combine these protein sources. The possible deficit of protein is particularly of relevance for the muscle, as adequate consumption of high-quality protein is crucial to prevent age-related decline in muscle mass and strength [47].

Further research is needed to investigate if the EAT-Lancet diet sufficiently supplies the ageing population with these nutrients of concern, and to find out how adherence to the EAT-Lancet diet relates to other age-related outcomes. For now, caution is warranted before advising older adults to adopt the EAT-Lancet diet. Intake and/or status of nutrients at risk should be monitored, and possibly supplementation or consumption of fortified foods may be required. Furthermore, it is crucial that older adults are educated on how to create balanced high protein plant-based meals that cover amino acid needs.

Finally, each study has limitations that should be considered. An important limitation of the current study is that the variation in dietary intake between participants was small. This is evidenced by the narrow range of EAT-Lancet diet scores in the middle tertile of adherence, and happened despite the extensive dietary assessment. The low variation in scores on the EAT-Lancet diet may explain why some result were only significant in the continuous, but not in the categorical analyses. Regardless, continuous results should be prioritized over categorical results. Another limitation includes the two year duration of follow-up, which is a relatively short period to capture cognitive deteriorations in older adults without cognitive complaints. Nonetheless, the use of an extensive cognitive test battery as a sensitive method to pick up cognitive differences allowed us to demonstrate associations.

In conclusion, we demonstrated a beneficial association between better adherence to the EAT-Lancet diet with cognitive ageing in cognitively healthy older adults. It is too early, however, to recommend the ageing population to adopt the EAT-Lancet diet, as the diet may provide inadequate amounts of several nutrients of major importance to healthy ageing. Further research focussing on other age-related conditions would be required to verify its relevance to the ageing population.

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Supplementary materials

Supplementary table 1: Overview of food items constituting the 14 EAT-Lancet diet food groups.

Emphasized food components	
Vegetables	Cauliflower, broccoli and other cabbages, spinach, beets, endive, green beans, other cooked vegetables, lettuce, raw endive, other raw vegetables
Fruits	Apple, banana, orange, strawberry, other fruits
Unsaturated oils	Olive oil, dressing based on oil, other oils
Legumes	Legumes, soy products
Nuts	Peanut butter, peanuts, cocktail nuts, walnuts, mixed nuts, other nuts and seeds
Whole grains	Whole grain breakfast cereal, cooked oatmeal, wheat porridge, whole grain rusk, whole grain crispbread, rye bread, whole grain bread, whole grain pasta, brown rice, bulgur, millet, couscous
Fish	Shellfish, mussels, flounder, trout, herring, salmon, other types of fish
Limited intake food components	
Beef and lamb*	Beef, lamb, beef/lamb liver, beef minced meat, organ meats, beef fried meat snacks
Pork*	Pork, pork liver, bacon, pork minced meat, smoked sausage, pork fried meat snacks
Poultry*	Chicken, turkey, chicken fried meat snacks
Eggs	Eggs
Dairy	Milk, buttermilk, chocolate milk, yoghurt, custard, drink breakfast, cheese, cream, ice cream
Potatoes	Fries, cooked and baked potatoes, mashed potatoes
Added sugar	Calculated based on added sugar composition database by Sluik and colleagues

* Some meat products could not be classified into one component. To this end, we included meat products from other animal origin (horse, hare) for 50% in the beef and lamb component, and for 50% in the pork component. The same approach was taken for meat products with unknown origin and meat products containing both beef and pork in equal amounts (i.e. half-half minced meat). Finally, meat products containing both beef and pork but consisting primarily (≥75%) out of either beef or pork, have been assigned to the respective group.

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Supplementary table 2: Description of cognitive tests.

Domain	Test	Description	Scoring
Episodic memory	RAVLT immediate	Recall of 15 words in five trials	0-45
	RAVLT delayed	Delayed recall of the 15 words after 20 minutes	0-15
	RAVLT recognition	Recognition of the 15 words in a list of 30 words	0-30
Attention and working memory	Digit span forward	Recall of digit sequences with increasing length in forward order	0-9
	Digit span backward	Recall of digit sequences with increasing length in backward order	0-8
Information processing speed	Stroop part I and II	Naming colour words written in black ink (part I) and coloured blocks (part II) as fast as possible. Outcome is mean part I and II	0 – ∞ s
	Trail making test part A	Draw lines connecting numbers in chronological order as fast as possible	0 - 300 s
	SDMT	Match symbols with digits within 90 s as fast as possible.	0 - 110
Executive functioning	Stroop interference	Naming colour words written in black ink (part I), coloured blocks (part II) and colour words written in an incongruent colour ink (part III) Outcome is part III corrected by parts I and II	0 – ∞
	Trail making test part B/A	Draw lines connecting numbers in chronological order (part A) or numbers and letters alternating in chronological and alphabetical order (part B). Outcome is ratio part B/A	0-300 s
	Letter fluency	Name as many words as possible starting with a specific letter in 60 s	0 - ∞

Abbreviations: Rey Auditory Verbal Learning Test (RAVLT); Symbol Digit Modalities Test (SDMT)

Table is reused from supplementary information from van Soest et al. (2023).

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van Soest APM, van de Rest O, Witkamp RF, et al.; The association between adherence to a plant-based diet and cognitive ageing. *European Journal of Nutrition* 2023;**62**(5):2053-2062. doi: 10.1007/s00394-023-03130-y.

Supplementary table 3: Nutrient intake of the B-proof study population according to EAT-Lancet index tertile.

Nutrient	Overall (n=630)	Low (n=241)	Middle (n=203)	High (n=186)	p-value
Energy (kCal)	1948 ± 508	1932 ± 506	2010 ± 527	1899 ± 487	0.09
Protein (g)	73 ± 18	73 ± 16	76 ± 18	71 ± 18	<0.01
Protein, animal origin (g)	45 ± 13	48 ± 12	47 ± 13	40 ± 13	< 0.001
Protein, plant origin (g)	28 ± 8	25 ± 7	29 ± 9	31 ± 8	< 0.001
Carbohydrates (g)	214 ± 63	210 ± 62	224 ± 68	208 ± 56	0.02
Sugar (g)	111 ± 40	110 ± 42	117 ± 43	106 ± 35	0.02
Starch (g)	103 ± 32	100 ± 32	107 ± 34	101 ± 29	0.08
Fibre (g)	23 ± 7	21 ± 6	25 ± 7	25 ± 7	< 0.001
Fat (g)	78 ± 27	79 ± 28	79 ± 27	76 ± 27	0.42
Cholesterol (mg)	202 ± 79	230 ± 89	199 ± 71	168 ± 58	< 0.001
SFA (g)	28 ± 11	31 ± 13	28 ± 10	25 ± 8	< 0.001
MUFA (g)	26 ± 10	26 ± 10	27 ± 10	26 ± 11	0.87
PUFA (g)	16 ± 8	14 ± 7	16 ± 8	17 ± 9	< 0.001
Alcohol (g)	14 ± 14	13 ± 14	14 ± 12	15 ± 15	0.34
linoleic acid (g)	13 ± 7	12 ± 6	14 ± 7	14 ± 8	< 0.001
α-linolenic acid (g)	1.3 ± 0.8	1.2 ± 0.6	1.3 ± 0.8	1.4 ± 0.9	< 0.001
EPA (g)	0.07 ± 0.08	0.05 ± 0.05	0.08 ± 0.09	0.10 ± 0.10	< 0.001
DHA (g)	0.11 ± 0.11	0.08 ± 0.07	0.12 ± 0.12	0.14 ± 0.14	< 0.001
Vitamin B12 (mg)	4.1 ± 2.0	4.0 ± 1.8	4.4 ± 2.3	3.8 ± 1.9	0.01
Folic acid (mcg)	188 ± 56	173 ± 51	200 ± 59	193 ± 54	<0.001

Data are mean ± SD. Abbreviations: SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid.

Supplementary table 4: Overview food components and portion sizes for the EAT-Lancet, MIND and Mediterranean diet.

EAT-Lancet diet[1]			MIND diet[2]		Mediterranean diet[3]	
Components	**Portion size (grams)**	**Portion size (portion)[4]**	**Components**	**Portion size (portion)**	**Components**	**Portion size (portion)**
Vegetables	>300 g/d	>6/d	Green leafy vegetables	≥ 6/w	Vegetables	>4/d
			Other vegetables	≥ 1/d		
Fruits	>200 g/d	>2/d	Berries	≥2/w	Fruits	>3/d
Unsaturated oils	>40 g/d	>4/d	Olive oil	Primary oil	Olive oil	≥1/d
			Butter, margarine	< T/d		
Legumes	>75 g/d	>1.5/d	Beans	>3/w	Legumes, nuts & beans	>6/w
Nuts	>50 g/d	>2/d	Nuts	≥5/w		
Whole grains	>232 g/d	>5/d	Whole grains	≥ 3/d	Non-refined grains	>4/d
Fish	>28 g/d	>2/w	Fish	>1/w	Fish	>6/w
Beef and lamb	<7 g/d	<0.5/w	Red meat and products	<4/w	Red meat and products	≤1/w
Pork	<7 g/d	<0.5/w				
Poultry	<29 g/d	<2/w	Poultry	≥2/w	Poultry	≤3/w
Eggs	<13 g/d	<2/w				
Dairy	<250 g/d	<2/d	Cheese	<1/w	Full-fat diary	≤10/w
Potatoes	<50 g/d	<0.5/d			Potatoes	>2/d
Added sugar	<31 g/d	n/a	Pastries, sweets	< 5/w		
			Fast/fried foods	<1/w		
			Wine	1/d	Alcohol	<300mL/d but >0

Green colour indicates emphasized food components, orange colour indicates limited intake food components.

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[1] Based on Stubbendorff, A., Sonestedt, E., Ramne, S., Drake, I., Hallström, E., & Ericson, U. (2022). Development of an EAT-Lancet index and its relation to mortality in a Swedish population. *The American journal of clinical nutrition*, *115*(3), 705-716.

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CHAPTER 8

The Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) Diet for the ageing brain: a systematic review

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ABSTRACT

The Mediterranean-Dietary Approaches to Systolic Hypertension diet intervention for neurodegenerative delay (MIND) diet seems a promising approach to preserve brain function during ageing. Previous systematic reviews have demonstrated benefits of the MIND diet for cognition and dementia, though an update is needed. Additionally, other outcomes relevant to brain ageing have not been summarized. Therefore, this systematic review aims to give an up-to-date and complete overview on human studies that examined the MIND diet in relation to brain ageing outcomes in adults aged ≥40y. Ovid Medline, Web of Science core collection, and Scopus were searched up to July 25, 2023. Study quality was assessed using the Newcastle Ottawa Scale and the Cochrane Risk-of-Bias tool. We included 40 articles, of which 32 unique cohorts. Higher MIND diet adherence was protective of dementia in 7 of 10 cohorts. Additionally, positive associations were demonstrated in 3 of 4 cohorts for global cognition, and 4 of 6 cohorts for episodic memory. The protective effects of the MIND diet on cognitive decline are less apparent, with only 2 of 7 longitudinal cohorts demonstrating positive associations for global decline, and 1 of 6 for episodic memory decline. For other brain outcomes (domain-specific cognition, cognitive impairments, Parkinson's disease, brain volume, and pathology) results were mixed or only few studies had been performed. Many of the cohorts demonstrating protective associations were of North-American origin, raising the question if the most favourable diet for healthy brain ageing is population-dependent. In conclusion, this systematic review provides observational evidence for protective associations between the MIND diet with global cognition and dementia risk, but evidence for other brain outcomes remains mixed and/or limited. The MIND diet may be the preferred diet for healthy brain ageing in North-American populations, though evidence for other populations seems less conclusive.

Keywords: MIND diet; dietary pattern; nutrition; diet; cognitive function; Alzheimer's disease; healthy ageing; older adults; elderly.

INTRODUCTION

With increasing age, the functioning of the brain gradually declines. Processing speed, executive function and episodic memory performance start impairing during midlife, and further decline into older age [1]. This decline in cognitive performance is accompanied by changes in the brain. For example, the volume of the brain shrinks and abnormal proteins accumulate. In case of accelerated ageing, these and other changes may eventually lead to age-related brain diseases, including various types of dementia and Parkinson's disease (PD) [2].

As it is not possible to completely stop brain ageing nor to cure age-related brain diseases, there is increasing interest in preventive strategies to ensure optimal brain ageing. Nutrition is considered an important lifestyle factor that can influence the brain ageing trajectory. Over recent decades, the research field has shifted from studying single nutrients and foods towards dietary patterns [3]. Studying dietary patterns is thought to be a more powerful approach to unravel the role of nutrition in brain ageing, as it allows to capture synergistic beneficial effects of nutrients. Indeed, evidence for dietary patterns is stronger than that for single nutrients and foods [3].

A dietary pattern that seems promising is the Mediterranean-Dietary Approaches to Systolic Hypertension (DASH) diet intervention for neurodegenerative delay (MIND) diet, which is specifically developed to preserve brain function during ageing. The MIND diet is a hybrid of the Mediterranean and DASH diets and further emphasizes intake of food groups with neuroprotective properties, including berries and leafy green vegetables. According to the developers of the MIND diet, the diet is more protective against cognitive decline [4] and Alzheimer's disease [5] as compared to the Mediterranean and DASH diets.

The possible beneficial role of the MIND diet in healthy brain ageing has been summarized systematically in five reviews and two meta-analyses [6-12]. However, these previously published papers are either in need of an update and/or only focussed on cognitive functioning and/or dementia rather than taking a broader perspective on the ageing brain. To this end, we aim to 1) give an updated overview on the MIND diet in relation to cognitive functioning, cognitive decline and dementia risk, and 2) to extend this overview to other brain ageing outcomes, including neuroimaging and pathology outcomes and incidence of other age-related neurodegenerative diseases.

METHODS

PROTOCOL REGISTRATION

We conducted this systematic review in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [13]. The study protocol was registered in PROSPERO (CRD42022254625).

INFORMATION SOURCES AND SEARCH STRATEGY

A systematic search was performed in three databases: Ovid Medline, Web of Science core collection and Scopus. No date restrictions were applied. An initial search was conducted on October 12, 2022. After this date, an automatic alert was set up within these databases to identify new articles published until July 25, 2023. The searches were conducted using predefined terms related to the MIND diet and the ageing brain (full search strategy in **supplementary tables S1A-C**). Search terms were determined in consultation with a librarian.

STUDY SELECTION AND ELIGIBILITY CRITERIA

The web tool CADIMA was used to organize the systematic review [14]. Duplicates were automatically removed by the web tool.

Two researchers (AvS and SB) independently reviewed the title and abstract of all obtained literature and subsequently full-text for eligibility.

For eligibility the following criteria were applied: 1) The study was a human observational or interventional study. Meta-analyses, reviews, commentaries, editorials, abstracts, unpublished studies, letters, news, or newspaper articles were excluded; 2) The study population comprised middle-aged and older individuals, all aged ≥40y. In case only mean age was stated, the mean age minus two times the standard deviation had to be ≥40y. This age cut-off was chosen since brain ageing is already present during midlife [15]; 3) The exposure variable was a measure of MIND diet adherence (observational studies) or a MIND diet intervention (interventional studies); 4) The comparator was lower adherence to the MIND diet (observational studies) or no MIND diet intervention (interventional studies); 5) The outcome measure was related to brain ageing, including cognitive performance, cognitive decline, incidence of any type of dementia or PD, or brain volume and pathology outcomes. Outcome measures related to depression, brain tumours and/or multiple sclerosis were excluded; 6) An effect size was given for the association between

MIND diet exposure and brain ageing outcome; 7) The article was published in English in a peer-reviewed journal.

Two researchers (AvS and SB) resolved disagreements by discussion. Remaining disagreements were discussed among all contributing authors until consensus was reached.

DATA EXTRACTION

Data extraction was independently performed by two researchers (AvS and SB). The following variables were extracted from eligible studies: first author, year of publication, country, name of study, study design, study duration (duration of follow-up or intervention), sample size, description of the study population, description of the exposure variable, outcome measure(s), results including effect size, and covariates. In case various models were run with different covariates, we collected the results of the most extensively adjusted model. Studies were organized based on outcome variable, with the exception of the randomized controlled trials (RCTs) that were tabled together. Outcome variables are categorized as cognitive function, cognitive decline, dementia, cognitive impairments, PD, and brain volume and pathology.

QUALITY ASSESSMENT

Two independent researchers (AvS and SB) assessed the quality of the included studies. The instruments used for quality assessment were based on the Cochrane handbook for Systematic Reviews for interventions [16]. The Newcastle Ottawa Scale (NOS) was used to rate the quality of observational cohort and case-control studies (**supplementary table S2 and S3**), and an adapted version of the NOS for quality of cross-sectional studies [17, 18] (**supplementary table S4**). Cohort and case-control studies were scored on the domains 'selection', 'comparability' and 'outcome/exposure', with maximum scores for the individual domains being four, two and four, respectively. The maximum score for cross-sectional studies was seven, of which maximum three, two, and two points could be retrieved from the domains selection, comparability, and outcome, respectively. Quality was categorized as either good, fair or poor. Threshold scores for categorizing the study quality are shown in **supplementary tables S2-4**.

In addition, the risk of bias of randomized controlled trials was assessed using the Cochrane Risk-of-bias tool for randomized trials (ROB2) [19]. ROB2 is structured into five domains of bias; randomization process, deviations from intended

interventions, missing outcomes, measurement of the outcome, and selection of reported result. Within each domain a series of signalling questions could be answered as 'yes', 'no', 'do not know or unclear', or 'not applicable'. These answers led to the judgement of 'low risk of bias', 'some concerns', or 'high risk of bias'.

Disagreements were resolved by discussion between two researchers (AvS and SB). Remaining disagreements were discussed among all contributing authors until consensus was reached.

RESULTS

IDENTIFICATION AND SELECTION

Out of the 321 studies identified in the database searches, a total of 40 articles met the inclusion criteria (**figure 1**).

STUDY CHARACTERISTICS

The characteristics of the 40 articles are presented in **tables 1-7**, and quality assessments are presented in **supplementary tables 5-8**. Two of the included articles were RCTs, and 38 articles had an observational design. Among the included articles, some cohorts have been used multiple times. The Rush Memory and Ageing Project (MAP) (n=8) [4, 5, 20-25], Health and Retirement Study (n=3) [11, 26, 27], Framingham Heart Study (n=2) [11, 28], UK Biobank (n=2) [29, 30], and the Women's Health Initiative (n=2) [20, 31] cohorts were used by multiple articles. This results in the inclusion of 32 unique cohorts in this systematic review.

The majority of included cohorts were conducted in North America (n=12), followed by Europe (n=11). The remaining studies were performed in Asia (n=6), Australia (n=2), and in South America (n=1).

In the articles with an observational design, MIND diet adherence was assessed as continuous measure, as quantiles, tertiles, and/or as low/high adherence. Adherence to the MIND diet was mostly assessed by Food Frequency Questionnaires (FFQ's), five cohorts used 24h recalls [12, 29, 30, 32, 33], two cohorts used a short MIND adherence questionnaire [34, 35], one cohort used a dietician interview [36], and one cohort used the combination of a FFQ and a 24h recall [37]. In addition, interpretation and scoring of MIND diet components varied largely (**supplementary table 9**). Sample sizes ranged from n=37 [38] up to n=114,684 [30]. The majority of included cohorts involved participants aged ≥ 60y (n=27) and participants free of dementia (n=23).

COGNITIVE FUNCTION

A total of 14 articles with 13 unique cohorts assessed the cross-sectional association between adherence to the MIND diet with cognitive function. Cognitive function was either reported as global cognition composite (n=5), domain-specific cognition (n=7) or generic screening test outcome, such as Mini-Mental State Examination (MMSE) score or Telephone Interview for Cognitive Status (TICS) score (n=5) (table 1).

Among the five studies that assessed global cognition [26-28, 39, 40], there were four unique cohorts, all originating from North-America. Three of the four unique cohorts demonstrated a positive association between MIND adherence and global cognitive function. In two cohorts of middle-aged to older adults, a one-point increase in MIND diet score was associated with β±SE 0.03±0.01 (p=0.004) [28] and β=0.027 (95% CI: 0.008, 0.046) [40] point increase in global cognition (z-score). In addition, another cohort demonstrated that individuals in the lowest tertile of adherence to the MIND diet scored significantly worse on a global cognition composite compared to individuals with highest adherence (mean±SE; T1 14.9±0.10; T3 15.6±0.09; p for trend: <0.001) [26]. The study by Berendsen and colleagues [39] was the only cohort that did not demonstrate an association. This cohort differed with respect to study population, as it was performed in female nurses rather than in a general older population of males and females. In addition, quality of this study was rated as fair, in contrast to the good quality of the other cohorts assessing global cognition.

Seven cohorts assessed domain-specific cognition [12, 28, 33, 34, 39, 41, 42], among which three North-American cohorts. Domain-specific cognition either involved composite scores that combined multiple tests into a domain [12, 33, 39, 41, 42] or single tests as a proxy for domain-specific cognition [28, 34]. Episodic memory was positively associated with MIND diet adherence in four [12, 28, 39, 41] out of six articles [33, 42]. Higher MIND diet score was associated with better episodic memory composite (z-score) in a Chinese ($\beta_{\text{per 3points}}$=0.102, 95% CI: 0.051, 0.153) [12], German ($\beta_{\text{per 1 point}}$=0.045, 95% CI: 0.003, 0.087) [41] and North-American (Mean difference$_{\text{Q1 vs Q5}}$=0.04, 95% CI: 0.01, 0.07) [39] cohort. In addition, each point increase in MIND diet score was associated with improved visual reproductions delayed recall (β±SE=0.03±0.01, p=0.01) and logical memory delayed recall (β±SE=0.03±0.01, p=0.02) in another a North-American cohort [28]. Two cohorts [33, 42] did not find associations with episodic memory, though these study had small sample sizes (n=132 and n=141, respectively). Evidence for the other cognitive domains is largely lacking. Positive associations were demonstrated for executive functioning in two

[28, 33] out of five cohorts [34, 41, 42], for processing speed in one [28] out of two cohorts [33], for working memory one [28] out of four cohorts [33, 34, 41] and for visuospatial memory one [28] out of three cohorts [34, 41]. None of two cohorts found a beneficial association between better adherence to the MIND diet and semantic memory [41, 42]. Among the seven cohorts assessing domain-specific cognition, four cohorts were rated as good quality [12, 28, 33, 41], one as fair [39] and two as poor [34, 42]. The cohort rated as fair showed a positive association with episodic memory, and both cohorts with poor quality all showed null-associations.

The generic tests were assessed in six cohorts [12, 36, 39, 43-45]. Only two of these cohorts demonstrated a positive dose-response association between the level of adherence to the MIND diet and cognition [12, 36]. A Greek cohort showed better MMSE performance in participants with better adherence to an adapted 9-point MIND score (β (r) = 0.24 (0.32), p<0.001; 95% CI/SD/SE not shown) [36]. In a Chinese cohort, higher adherence to a Chinese adapted MIND diet was associated with better cognition as measured with the TICS-m (β=0.110, 95% CI: 0.060, 0.159) [12]. Two studies also showed differences between tertiles of MIND adherence [43, 45], but only the lowest and middle tertile of MIND diet adherence differed significantly rather than the lower and highest tertile. Finally, two cohorts did not find proof for an association between MIND diet adherence and cognition as measured with generic tests [39, 44]. Overall, quality was low with only two articles scoring good [12, 44], two fair [39, 45] and two poor [36, 43].

COGNITIVE DECLINE

Thirteen articles using data from ten unique cohorts assessed the association between the adherence to the MIND diet with change in cognition. Change in cognition was reported as global cognition composite (n=9), domain-specific cognition (n=8) or a generic test score (n=5) (table 2).

Of the nine studies that studied global cognition [4, 20, 22, 24, 28, 39, 40, 46, 47], data from seven unique cohorts were used. Five cohorts did not find associations between adherence to the MIND diet and change in global cognition [20, 28, 39, 46, 47], while two cohorts (presented in five articles) did demonstrate a positive association [4, 20, 22, 24, 40]. For each point increase in MIND diet score, global cognition increased with β=0.0213 (95% CI: 0.008, 0.034) in a cohort of Puerto Ricans living in USA [40], and with 0.0106 ± 0.0023 (β ± SE, p<0.001) in the MAP cohort of American older adults [4]. The MIND diet was also protective of cognitive decline in

a subpopulation of the MAP cohort with stroke [22]. Overall quality was good, with seven articles scoring good [4, 20, 22, 28, 40, 46, 47], one scoring fair [39], and one scoring poor [24]. Of these lower-quality articles, one demonstrated a positive association [24], and one a null-association [39].

With respect to change in domain-specific cognitive function, seven unique cohorts were identified among the eight articles that assessed this outcome [4, 12, 22, 28, 35, 39, 46, 47]. Only the two articles using data from the American MAP cohort [4, 22] and an Israelian study [47] demonstrated positive associations with change of domain-specific cognitive function in at least one domain. In the MAP cohort, Morris et al. (2015) demonstrated that one point increase in MIND diet score was associated with an increase in episodic memory (β±SE, 0.0090±0.0028, p=0.001), working memory (β±SE, 0.0060±0.0024, p=0.01), semantic memory (β±SE, 0.0113±0.0027, p<0.0001), visuospatial ability (β±SE, 0.0077±0.0025, p=0.002), and perceptual speed (β±SE,0.0097±0.0023, p<0.0001). The Israelian study showed a positive association with each point increase in MIND diet score with executive functioning (β±SE, 0.00978±0.00446, p=0.028), but not with episodic memory, attention, or language [47]. The other five cohorts, originating from North-America, Europe and Asia, did not show an association between adherence to the MIND diet with change in any cognitive domain [12, 28, 35, 39, 46]. The majority of articles were scored as good quality [4, 22, 28, 46, 47], with exception of three articles [12, 35, 39]. These three studies all showed null-associations.

Among the five studies that assessed change in cognition using generic tests [12, 39, 46, 48, 49], two demonstrated beneficial associations with better MIND adherence [48, 49]. In two European cohorts of cognitively healthy older adults, MMSE increased by β=0.006 (95% CI: 0.003, 0.009) per 1 point increase in MIND diet score [49] and STICS-m increased by β=0.27 (95% CI: 0.05, 0.48) per 1.5 point increase in MIND diet score [48]. However, these two cohorts had been rated as having poor [49] and fair [48] quality.

DEMENTIA

Eight articles using data from ten unique cohorts studied the association between MIND diet adherence with risk of all-cause dementia and/or Alzheimer's disease (AD). In addition, two case-control studies assessed odds of dementia and early onset dementia (table 3).

All-cause dementia was assessed in seven articles including ten cohorts [11, 20, 29, 30, 37, 50, 51], of which seven out of ten cohorts [11, 20, 37, 50, 51] showed that better adherence to the MIND diet was associated with a lower risk of all-cause dementia. Each point increase on a French-adapted MIND diet score was associated with a 10% lower risk of all-cause dementia (HR=0.90, 95% CI: 0.83, 0.96) [37]. Positive associations were also observed in an Australian cohort (OR=0.72, 95% CI: 0.54, 0.95) [51], and four American cohorts (HR=0.95, 95% CI: 0.92, 0.97; HR=0.91, 95% CI: 0.83, 1.00; HR=0.82, 95% CI: 0.68,0.99; HR=0.76, 95% CI: 0.57,1.00) [11, 20]. Both positive and null associations have been demonstrated in the same cohort from the Netherlands [50]: in one sample of participants better MIND diet adherence decreased the risk of all-cause dementia over an average of 15.6 years (HR=0.79, 95% CI 0.70, 0.91), while another largely non-overlapping sample that was followed for a mean of 5.9 years did not demonstrate an association (HR=0.99, 95% CI: 0.94, 1.05). Finally, in two UK cohorts [11, 29, 30] and a biracial American [20] cohort no association with all-cause dementia was demonstrated. The majority of studies scored good on study quality [11, 20, 30, 37, 50] with two studies scoring fair [29, 51]. The studies with fair quality demonstrated a positive association [51] and a null-association [29].

Among the three studies that studied risk of Alzheimer's disease [5, 29, 37], two showed beneficial associations [5, 37]. The study of Morris and colleagues (2015) showed the largest effect size: individuals in the American MAP cohort in the highest versus lowest tertile of MIND diet adherence had 52% lower risk to develop Alzheimer's disease (T1 vs T3: HR=0.48, 95% CI 0.29, 0.79) [5]. These findings were confirmed in a sample of French older adults, with a French-adapted MIND diet score (HR=0.89, 95% CI: 0.81, 0.97) [37]. Both these studies scored good on quality. No association was demonstrated in an UK sample of older adults [29], that was rated as fair quality.

The two case-control studies on MIND adherence and dementia showed lower odds of dementia (OR=0.43, 95% CI: 0.29, 0.63) [36], early onset dementia (OR=0.66, 95% CI: 0.47, 0.91) and early onset AD (OR=0.97, 95% CI: 0.46, 0.98) [52], but not for early onset frontotemporal dementia [52]. The study quality was rated as poor for both case-control studies.

COGNITIVE IMPAIRMENT

An overview of all articles on cognitive impairment outcomes is shown in table 4.

Mild cognitive impairment (MCI) was assessed in three cohorts [45, 51, 53]. Two cohorts demonstrated protective associations: higher MIND diet adherence was cross-sectionally associated with lower odds of MCI in a Chinese a sample of older adults (T1 vs T3 OR=0.60, 95% CI: 0.51, 0.72) [45] and, longitudinally with lower odds of MCI in Australian older adults after 12 years of follow-up (T1 vs T3 OR=0.47, 95% CI: 0.24, 0.91) [51]. The third cohort did not find a cross-sectional association between MIND diet adherence and odds of cognitive impairment in British PD patients (β=-0.23, 95% CI/SD/SE not shown, p=0.070) [53]. The study quality was rated as fair [45], good [51], and poor [53].

The only study that assessed risk of subjective memory complaints was of good quality and demonstrated that better adherence to the MIND diet was associated with lower risk of memory complaints in older adults aged ≥70y (HR=0.87, 95% CI: 0.78, 0.98), but not in older adults aged 60-69y (HR=1.00, 95% CI: 0.95, 1.05) [32].

One study assessed the longitudinal association between cognitive resilience and adherence to the MIND diet. This study showed that higher MIND diet adherence was associated with higher cognitive resilience, based on change in global cognition adjusted for neuropathologies (mean difference=0.07, 95% CI: 0.02, 012) [25]. The quality of the study was rated as good.

PARKINSON'S DISEASE (PD)

PD outcomes were assessed in one cross-sectional [54] and one longitudinal study [21] (table 5). Cross-sectionally, Canadian PD patients adhering better to the MIND diet developed the disease at a later age (β=2.2, 95% CI/SD/SE not shown, p=0.002) [54]. Longitudinally, each point increase in MIND diet adherence was associated with a lower risk of incident PD (HR=0.89, 95% CI: 0.83, 0.96) and a smaller change in PD progression (β±SE=0.008±0.0037, p=0.04) in the American MAP cohort [21]. The study quality of both studies was rated as poor.

BRAIN VOLUMES

Brain volume outcomes were assessed in three cross-sectional [28, 30, 42] and one longitudinal study [31] (table 6). With respect to total brain volume, cross-sectional associations with MIND diet adherence were demonstrated in one ($\beta_{per\ 1\ point}$±SE=0.02±0.01, p=0.02) [28] out of two cohorts [30]. Longitudinally, MIND diet adherence was not associated with the change in total brain volume over 7-10y [31]. Furthermore, no cross-sectional or longitudinal associations were demonstrated with grey matter (region), white matter (region), and subcortical areas [28, 31, 42].

Two studies were rated as good quality [28, 31], and two as fair quality [30, 42]. The studies with fair quality did not demonstrate any associations with brain volumes.

BRAIN PATHOLOGY

A total of four studies assessed neuropathological markers, focusing on global AD pathology (n=2), beta-amyloid load (n=2), tangles (n=2), brain infarcts (n=2), atherosclerosis (n=1), and measures from cerebrospinal fluids (n=1) [23, 24, 28, 35] (table 6).

Two studies made use of data of the American MAP cohort, resulting in three unique cohorts. Surprisingly, the two studies using data from the MAP study showed different results: while Agarwal and colleagues (2023) demonstrated an association of MIND diet adherence with lower global AD pathology ($\beta_{continuous}$±SE=-0.24±0.011, p=0.025) and beta-amyloid load (β_{T1vsT3}±SE=-0.246±0.123, p=0.047; $\beta_{continuous}$±SE=-0.062±0.034, p=0.071) using a n=581 sample from the MAP cohort [23], Dhana et al. (2021) did not confirm this using data from n=596 older individuals from the same cohort (global AD pathology: $\beta_{continuous}$±SE =-0.013±0.024, p=0.578, beta-amyloid: $\beta_{continuous}$±SE =-0.03±0.049, p=0.395) [24]. Both MAP cohort studies did not demonstrate an association between MIND diet adherence and tau-tangles. Furthermore, null-associations between MIND diet adherence with brain infarcts [24, 28], cerebral atherosclerosis [24] and with cerebrospinal fluid biomarkers [35] were demonstrated. Quality was rated as good in three studies [23, 24, 28] and poor in one study [35]. The study of poor quality demonstrated a null-association with cerebrospinal fluid biomarkers.

RANDOMIZED CONTROLLED TRIALS

The effect of a MIND diet intervention on cognitive change and brain volume was reported in two articles [38, 55] (table 7). In both articles, a calorie-restricted MIND diet was compared to a calorie-restricted control diet.

An American trial (n=564) did not demonstrate an effect of a 3-year MIND diet intervention in older adults with overweight on change in global cognition (z-score) (mean change=0.035, 95% CI: −0.022, 0.092), domain-specific cognition, and brain volumes [55]. This trial was rated as good quality, thus low risk of bias. A small Iranian trial (n=37) in middle-aged females with obesity did demonstrate short-term beneficial effects of a MIND diet intervention. After a 3-month intervention, the MIND diet group improved their cognitive functioning more compared to the control group on six of eight cognitive tests, covering working memory, verbal memory and

attention domains. This article also included brain volume outcomes, though as no effect sizes were reported this data is no part of this systematic review [38]. The study quality of the Iranian article was rated as with 'some concerns of bias'.

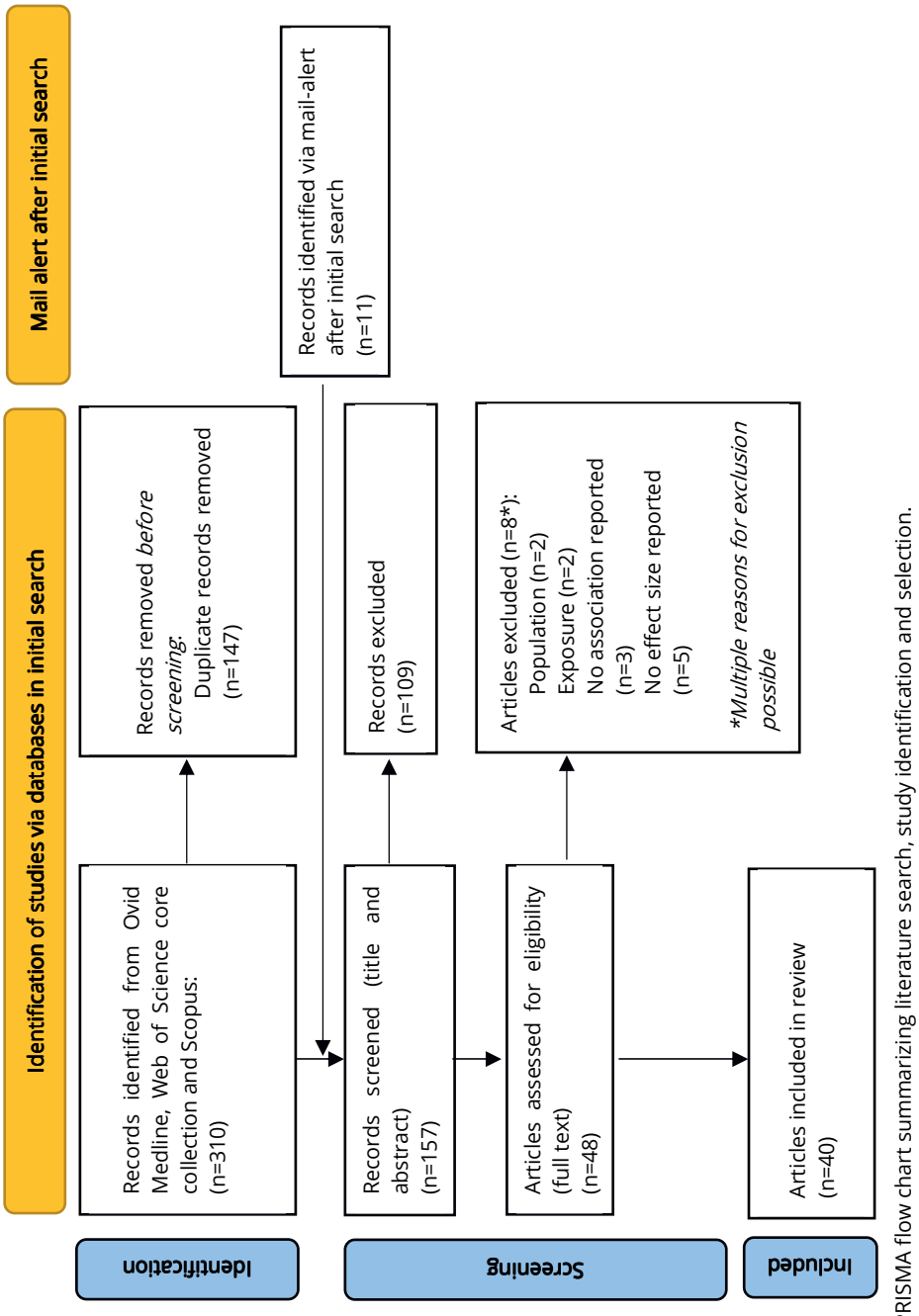

Figure 1. PRISMA flow chart summarizing literature search, study identification and selection.

Table 1. Description of included cross-sectional studies describing the association between MIND diet and cognitive function.

Author (year)	Study (country)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
McEvoy (2017) [26]	Health and Retirement Study (USA)	5907	Older adults (mean age 67.8 ±10.8y), without history of stroke or dementia	15-MIND diet adherence (tertiles) based on 163-item sFFQ	Cognition measured by (1) Global cognition (range 0-27, based on immediate, delayed word list, backward counting, and serial 7 subtraction)	Mean (SE) T1: 14.9 (0.10) T2: 15.2 (0.09) T3: 15.6 (0.09) p for trend: **<0.001**	Sex, age, race, low education, current smoking, total wealth, obesity, hypertension, diabetes mellitus, physical inactivity, depression, total energy intake	Good
					(2) Impaired cognition (defined by more than 1SD (=4.3 points) below mean global cognition score)	OR (95% CI) $T1_{vs}T2$: 0.85 (0.70, 1.03), p=0.10 $T1_{vs}T3$: **0.70 (0.56, 0.86), p=0.001**		
Ahn (2022) [27]	Health and Retirement Study (USA)	3463	Older adults (≥50y), without history of stroke or dementia	15-MIND diet adherers (score ≥7.5) vs non-adherers (<7.5), based on 163-item sFFQ	Cognition measured by (1) Global cognition (range 0-27, based on immediate and delayed recall word list, serial seven subtraction, backward counting);	Mean difference (95% CI) Physically inactive persons: **0.81 (0.50, 1.11), p<0.001** Regular physically active persons: **0.60 (0.08, 1.12), p<0.001**	Age, sex, race, education, annual income, smoking history, hypertension, diabetes mellitus, depression, obesity	Good
					(2) Impaired cognition (defined by more than 1SD (=4.5 points) below mean global cognition score)	OR (95% CI) Physically inactive persons: **0.68 (0.54, 0.86), p<0.01**; Regular physically active persons: 0.73 (0.48, 1.11), p>0.05		
Van Lent (2021) [28]	Framingham Heart Study, offspring cohort (USA)	2092	Older adults (mean age 61 ± 9y), free of dementia	15-MIND diet adherence (continuous) based on 126-item sFFQ	Cognition measured by (1) Visual Reproductions Delayed Recall;	β±SE **0.03±0.01, p=0.01**	Age, age^2, sex, ApoE4 status, total energy intake, education, BMI, physical activity, smoking, diabetes, CVD, depressive symptoms, anti-hypertensive medication, systolic blood pressure, total cholesterol:HDL, time interval between FFQ and outcome measure.	Good
					(2) Logical Memory Delayed Recall;	**0.03±0.01, p=0.02**		
					(3) TMT A;	**0.03±0.01, p=0.01**		
					(4) TMT B/A;	0.01±0.01, p=0.30		
					(5) Hooper Visual Organization Test;	0.01±0.01, p=0.28		
					(6) Similarities;	**0.03±0.01, p=0.02**		
					(7) Global cognition (comp. of tests above)	**0.03±0.01, p=0.004**		

Table 1. (*continued*)

Author (year)	Study (country)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Berendsen (2018) [39]	Nurses' Health Study (USA)	16058	Older women (≥70y), free of stroke, free of dementia	15-MIND diet adherence (quantiles) based on 116-item sFFQ	Cognition measured by (1) TICS;	Mean difference (95% CI) $Q1_{vs}Q2$: -0.05 (-0.18, 0.07) $Q1_{vs}Q3$: 0.00 (-0.13, 0.12) $Q1_{vs}Q4$: -0.02 (-0.14, 0.11) $Q1_{vs}Q5$:-0.09 (-0.21, 0.04)	Age, education, physical activity, calorie intake, alcohol intake, smoking status, multivitamin use, BMI, depression, history of high blood pressure, hypercholesterolemia, myocardial infarction, diabetes mellitus.	Fair
					(2) Verbal (episodic) memory (comp. of immediate and delayed recalls of the East Boston Memory Test and delayed recall of the TICS);	$Q1_{vs}Q2$: 0.01 (-0.02, 0.05) $Q1_{vs}Q3$: 0.02 (-0.01, 0.05) $Q1_{vs}Q4$: 0.02 (-0.01, 0.06) $Q1_{vs}Q5$: **0.04 (0.01, 0.07)**		
					(3) Global cognition (comp. of aforementioned tests and category fluency digit span backward)	$Q1_{vs}Q2$: 0.00 (-0.02, 0.03) $Q1_{vs}Q3$: 0.00 (-0.03, 0.03) $Q1_{vs}Q4$: 0.00 (-0.03, 0.03) $Q1_{vs}Q5$: 0.00 (-0.03, 0.03)		
Boumenna (2022) [40]	The Boston Puerto Rican Health Study (USA)	1081	Middle-aged to older adults (mean age 52.7±7.9)	15-MIND diet adherence (quantiles and continuous) assessed by FFQ	Global cognition (comp. of MMSE, 16 word list learning, digit span forward and backward, stroop test, clock drawing and figure copying, verbal fluency)	β (95% CI) $Q1_{vs}Q2$: -0.065 (-0.162, 0.033) $Q1_{vs}Q3$: -0.005 (-0.085, 0.075) $Q1_{vs}Q4$: 0.047 (-0.035, 0.129) $Q1_{vs}Q5$: **0.092 (0.002, 0.182)** Continuous: **0.027 (0.008, 0.046), p=0.0062**	Age, sex, BMI, physical activity score, diabetes, hypertension, educational level, smoking, alcohol use, APOE4, energy intake, job complexity score, poverty index	Good
Huang (2023) [12]	China Health and Nutrition Survey (China)	4066	Older adults (≥55y), free of dementia	12-MIND diet adherence (tertiles and continuous) based on 3 24h dietary recalls	Cognition measured by (1) Global cognition, comp. of items TICS-m;	β (95% CI) (1) Global cognition/TICS-m $T1_{vs}T2$: 0.017 (-0.027, 0.061) $T1_{vs}T3$: **0.071 (0.026, 0.116)** Continuous (per 3 points): **0.110 (0.060, 0.159)**	Age, age square, sex, education, residence, region, income, smoking status, drinking status, BMI, total energy, physical activities, hypertension, diabetes, myocardial infarction	Good
					(2) Verbal memory, comp. of immediate and delayed recall	$T1_{vs}T2$: 0.003 (-0.042, 0.049) $T1_{vs}T3$: **0.068 (0.021, 0.115)** Continuous (per 3 points): **0.102 (0.051, 0.153)**		

Table 1. (*continued*)

Author (year)	Study (country)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Wesselman (2021) [41]	The DZNE-Longitudinal Cognitive Impairment and Dementia Study (Germany)	383	Older adults (mean age 69.3 ±5.6y), free of dementia	15-MIND diet adherence (continuous) based on 148-item sFFQ	Cognition measured by (1) Memory (comp. of cognitive subscale word list, delayed recall and cognition, free and cued selective reminding test, free recall and cue efficiency, Wechsler Memory Scale, logic memory, figure savings, SDMT, incidental learning, Face Name Test);	β (95% CI) **0.045 (0.003, 0.087)**	Age, sex, education, APOe4- status, total daily energy intake, BMI, smoking status, physical activity	Good
					(2) Language (comp. of verbal fluency; groceries and animals, Boston naming test, FCSRT naming);	0.039 (-0.002, 0.079)		
					(3) Executive functioning (comp. of TMT A+B, number cancelation, SDMT, Flanker Task);	0.014 (-0.029, 0.057)		
					(4) Working memory (comp. of Digit Span Forward + Backward, FCSRT: interference task);	0.031 (-0.014, 0.076)		
					(5) Visuospatial functioning (comp. of Clock copying + drawing, CERAD figure copying).	0.014 (-0.024, 0.052)		
Escher (2022) [42]	UCSF Memory and Aging Center's Longitudinal Brain Aging Program (USA)	132	Older adults (mean age 71.7±19y), free of dementia	15-MIND diet adherence (continuous) based on FFQ	Cognition measured by (1) Episodic memory (California Verbal Learning Test - long delay);	β (95% CI) 0.03 (-0.01, 0.08)	Age, sex, education, vascular burden score, PASE, MIND*PASE	Poor
					(2) Executive function (Stroop interference, Digit Span Backwards, phonemic fluency, D-KEFS design fluency, modified TMT);	0.15 (-0.03,0.33)		
					(3) Language (Boston naming test, category fluency)	0.18 (-0.001, 0.04)		

Table 1. (*continued*)

Author (year)	Study (country)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Gauci (2022) [33]	Memory and Attention Supplement Trial cohort (Australia)	141	Middle-aged adults (40-65y), free of dementia	15-MIND diet adherence (continuous) based on multiple (2 to 4) 24h recalls	Cognition measured by computer-based tests (1) Reaction and decision speed (comp. of simple reaction time, choice reaction time);	β (95% CI/SD/SE not shown) -0.06, n.s.	Age, sex, education, energy intake	Good
					(2) Visual processing (comp. of immediate recognition, delayed recognition, contextual memory task);	-0.12, n.s.		
					(3) Stroop processing (comp. of difference incongruent and congruent stroop tasks);	**0.19, p<0.035**		
					(4) Spatial working memory (comp. of 14 spatial working memory trials)	-0.13, n.s.		
Zare (2023) [34]	No name (Iran)	60	Older adults (≥60y) with T2DM, free of dementia	14-MIND diet adherence (continuous) based on a MIND dietary scoring questionnaire	Cognition measured by (1) Stroop task 1 (time)	r (p-value) -0.217 (n.s.)	None	Poor
					(2) Stroop task 1 (errors)	-0.164 (n.s.)		
					(3) Stroop task 2 (time)	0.025 (n.s.)		
					(4) Stroop task 2 (errors)	-0.092 (n.s.)		
					(5) TMT	-0.165 (n.s.)		
					(6) Forward digit span	0.194 (n.s.)		
					(7) Letter digit modality task (total)	0.247 (0.057)		
					(8) Letter digit modality task (true responses)	0.245 (0.060)		
Huang (2022) [45]	Chinese Longitudinal Healthy Longevity Study (China)	11245	Older adults (mean age 84 ± 11y) without stroke or dementia	12-MIND diet adherence (tertiles and continuous) based on simplified FFQ	Cognition measured by MMSE	β (95% CI) $T1_{vs}T2$: **0.60 (0.37, 0.82)** $T1_{vs}T3$: 1.01 (0.76, 1.26)	Sex, age, region, education, BMI, smoking, drinking, exercise, social engagement, hypertension, diabetes, depression, hearing impairment.	Fair

Table 1. (*continued*)

Author (year)	Study (country)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Vassilo-poulou (2022) [36]	No name (Greece)	167 (115 dementia; 52 cognitively healthy)	Older adults (mean age 72.6±8.1 (dementia); 70.2±4.6 (healthy))	9-MIND diet adherence (continuous) based on dietitian interview	Cognition measured by MMSE	β (r) (95% CI/SD/SE not shown): **0.24 (0.32), p<0.001**	Sex, age, BMI, DASS-21	Poor
Calil (2018) [43]	No name (Brazil)	96 (36 cognitively healthy, 30 MCI, 30 AD)	Older adults (≥60y) from neurology outpatient clinics	15-MIND diet adherence (tertiles) based on 98-item FFQ	Cognition measured by (1) MMSE;	β (95% CI) in cognitively healthy participants $T1_{vs}T2$: **3.21 (0.95, 5.48), p= 0.007** $T1_{vs}T3$: 1.51 (-0.78, 3.79), p=0.188	(1) Age, education, partner, MedDiet score (2) Age, partner, MedDiet score	Poor
					(2) Learning score of Brief Cognitive Screening Battery	$T1_{vs}T2$: 0.46 (-0.66, 1.60), p= 0.404 $T1_{vs}T3$: **1.39 (0.30, 2.49), p=0.014** *No association in MCI and AD patients (data not shown)*		
Yeung (2022) [44]	MrOs and MsOs study (Hong Kong/China)	3730	Older adults (≥65y)	9-MIND diet adherence (continuous) based on 280-item FFQ	Cognition (low/high performance based on median split of 4 items of MMSE; orientation to date, orientation to address, registration of three objects, and attention and calculation)	OR (95% CI) In men: 0.98 (0.88, 1.10), p=0.743 In women: 1.00 (0.89, 1.14), p=0.946	Age, BMI, education level, subjective social status, PASE score, daily energy intake, current smoker status, current alcohol use, number of chronic diseases.	Good

Abbreviations: AD: Alzheimer's disease, BMI: Body mass index, CERAD: Consortium to Establish a Registry for Alzheimer's disease, CI: Confidence Interval, comp.: composition score, CVD: cardiovascular disease, DASS-21: Depression Anxiety Stress Scale, D-KEFS: Delis-Kaplan Executive Function System, FCSRT: Free and Cued Selective Reminding Test, MCI: mild cognitive impairment, MedDiet: Mediterranean diet, MIND: Mediterranean-Dietary Approaches to Systolic Hypertension (DASH) diet Intervention for Neurodegenerative Delay, MMSE: Mini-Mental-State Examination, OR: odds ratio, PASE: Physical Activity Scale for the Elderly, SDMT: Symbol Digit Modalities Test, SE: standard error, sFFQ: simplified Food Frequency Questionnaire, TICS: Telephone Interview for Cognitive Status, TMT: Trail Making Test, T2D: Type 2 diabetes.

[1] Study quality was assessed with the Newcastle Ottawa Scale.

Table 2. Description of included longitudinal studies describing the association between MIND diet and cognitive decline

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Vu (2022) [20]	Chicago Health and Aging Project (USA)	Not shown	2449 (946 white, 1503 black)	Older adults (≥65y), free of dementia, either white or African (black) Americans	15-MIND diet adherence (tertiles, continuous) based on sFFQ	Change in global cognition (comp. of east Boston story immediate and delayed, symbol digit modalities test, MMSE)	β (95% CI) White participants: $T1_{vs}T2$: 0.0001 (-0.01, 0.01), p=0.99 $T1_{vs}T3$: -0.0008 (-0.01, 0.01), p=0.89 Continuous: -0.004 (-0.003, 0.002), p=0.78 Black participants: $T1_{vs}T2$: 0.0003 (-0.01, 0.01), p=0.95 $T1_{vs}T3$: -0.003 (-0.01, 0.01), p=0.51 Continuous: -0.00002 (-0.003, 0.003), p=0.99	Age, sex, study centre, education, income, global cognition score, late life cognitive activity, history of diabetes, hypertension, stroke, heart disease, smoking, calorie intake, BMI, depressive symptoms, physical activity	Good
Vu (2022) [20]	Rush Memory and Aging Project (USA)	Not shown	725	Older adults (mean age 82y), free of dementia	15-MIND diet adherence (tertiles, continuous) based on sFFQ	Change in global cognition (comp of word list memory, recall and recognition, east Boston story immediate and delayed, logical memory IIa immediate and delayed, Boston naming test, verbal fluency, reading test, digit span forward and backward, digit ordering, symbol digit modalities test, number comparison, stroop word reading and colour naming, judgement of line orientation, standard progressive matrices)	β (95% CI) $T1_{vs}T2$: 0.006 (-0.01, 0.02), p=0.50 $T1_{vs}T3$: **0.03 (0.01, 0.05), p=0.001** Continuous: **0.006 (0.003, 0.01), p=0.002**	Age, sex, study centre, education, income, global cognition score, late life cognitive activity, history of diabetes, hypertension, stroke, heart disease, smoking, calorie intake, BMI, depressive symptoms, physical activity	Good

Table 2. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Cherian (2019) [22]	Rush Memory and Aging Project (USA)	5.9 (mean follow-up)	106	Older adults (mean age 82.8y) with a clinical history of stroke and no dementia	15-MIND diet adherence (tertiles) based on 144-item sFFQ	Change in cognition measured by (1) Global cognition (comp. of all domains)	β (95% CI) $T1_{vs}T2$: 0.058 (-0.011, 0.128) $T1_{vs}T3$: **0.083 (0.007, 0.158)**	Age, sex, education, APOE4, caloric intake, smoking, participation in cognitive and physical activity	Good
						(2) Episodic memory (comp. of word list immediate, recall & recognition, East Boston immediate & delayed recall, logic memory immediate & delayed)	$T1_{vs}T2$: 0.025 (-0.048, 0.098) $T1_{vs}T3$: 0.041 (-0.038, 0.121)		
						(3) Semantic memory (comp. of Boston naming test, category fluency, reading test)	$T1_{vs}T2$: 0.030 (-0.033, 0.093) $T1_{vs}T3$: **0.070 (0.001, 0.138)**		
						(4) Working memory (comp. of digits forward, digits backwards, digit ordering)	$T1_{vs}T2$: 0.023 (-0.041, 0.087) $T1_{vs}T3$: 0.033 (-0.037, 0.102)		
						(5) Visuospatial memory/ perceptual orientation (comp. of line orientation, progressive matrices)	$T1_{vs}T2$: 0.062 (-0.001, 0.126) $T1_{vs}T3$: 0.061 (-0.008, 0.130)		
						(6) Perceptual speed (comp. of symbol digits modality, number comparison, stroop colour naming, stroop word reading)	$T1_{vs}T2$: 0.047 (-0.019, 0.113) $T1_{vs}T3$: **0.071 (0.000, 0.142)**		
Dhana (2021) [24]	Memory and Aging Project (USA)	Not shown	569	Older adults (mean age at death 90.8 ± 6.1y), some were diagnosed with AD	15-MIND diet adherence (continuous, per 1SD=1.42 point) based on 144-item sFFQ	Change in global cognition proximate to death (comp. of East Boston Story immediate/delayed recall, Story A from Logical Memory, Word List Memory, Word List Recall/recognition, Boston Naming Test, Verbal Fluency, Word reading test, Digit Span Forward/Backward, Digit Ordering)	β ± SE **0.119±0.040, p=0.003**	Age at death, sex, education, APOE4, late-life cognitive activities, total energy intake.	Poor

Table 2. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Morris (2015) [4]	Rush Memory and Aging Project (USA)	4.7 (mean follow-up)	835 - 860 (depends on outcome)	Older adults (mean age 81.4 ±7.2y), free of dementia	15-MIND diet adherence (continuous) based on 144-item sFFQ	Change in cognition measured by (1) Global cognition (comp. of all domains)	β ± SE **0.0106 ± 0.0023, p<0.0001**	Age at first cognitive assessment, sex, education, participation in cognitive activities, APOE4, smoking history, physical activity hours per week, total energy intake, time, history of stroke, myocardial infarction, diabetes, hypertension, interaction terms between time and each model covariate, MIND diet score	Good
						(2) Episodic memory (comp. of word list memory, recall and recognition, East Boston story immediate and delayed recall, story A from logical memory of Wechsler memory scale-revised)	**0.0090 ±0.0028, p=0.001**		
						(3) Working memory (comp. of digit span forward and backward, digit ordering)	**0.0060 ±0.0024, p=0.01**		
						(4) Semantic memory (comp. of Boston naming test, verbal fluency, national adult reading test)	**0.0113 ±0.0027, p<0.0001**		
						(5) Visuospatial ability (comp. of judgement of line orientation, standard progressive matrices)	**0.0077 ±0.0025, p=0.002**		
						(6) Perceptual speed (comp. of symbol digit modalities test, number comparison, stroop test)	**0.0097 ±0.0023, p<0.0001**		
Dong (2023) [35]	Wisconsin Registry for Alzheimer's Prevention (USA)	Not shown	1078	Older adults (mean age 63.5±6.7y), free of dementia	15-MIND diet adherence (continuous) based on 15-item self-reported diet questionnaire	Change in cognition measured by (1) Preclinical Alzheimer cognitive composite (PACC)	β (p-value) 0.0087 (0.388).	None	Poor
						(2) Immediate learning (Rey auditory verbal learning test total trials 1–5, Wechsler memory scale–revised logical memory subtest immediate recall, and brief visuospatial memory test immediate recall)	-0.0038 (0.770. *Data on delayed recall and executive function were also available but no effect sizes were given*		

Table 2. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Lotan (2022) [47]	Israel Diabetes and Cognitive Decline study (Israel)	4.1±2.1 (mean follow-up)	960	Older adults (≥65y) with T2DM, free of dementia	15-item MIND diet adherence (continuous) based on FFQ	Change in cognition measured by (1) Global cognition (comp. of all domains)	β±SE 0.00604±0.00354, p=0.087	Age, sex, education, daily calories, duration of T2D at baseline, baseline cholesterol, creatinine, HbA1c, triglycerides, systolic blood pressure, diastolic blood pressure, BMI, diabetic medication, physical activity	Good
						(2) Episodic memory (comp. of word list immediate, delayed and recognition)	0.00219±0.00584, p=0.707		
						(3) Attention/working memory (comp. of shape cancellation, digit span forward and backward)	0.00030±0.0054, p=0.954		
						(4) Language/semantic categorization (comp. of similarities, animal fluency and 15-item boston naming test)	0.00559±0.00374, p=0.135		
						(5) Executive function (comp. of TMT A and B, praxis, and digit symbol substitution test)	**0.00978±0.00446, p=0.028**		
Shakersain (2018) [49]	The Swedish National Study on Aging and Care in Kungsholmen (Sweden)	6	2223	older adults (≥ 60y), free of dementia	14-MIND diet adherence (continuous and tertiles) adapted to range 0-66, based on 98-item sFFQ	(1) Change in cognition measured by MMSE	β (95% CI) $T1_{vs}T2$: **0.075 (0.012, 0.138), p=0.019** $T1_{vs}T3$: **0.126 (0.064, 0.188), p<0.001** Continuous: **0.006 (0.003, 0.009), p<0.001**	Total calorie intake, age, sex, education, civil status, physical activity, smoking, body mass index, vitamin/mineral supplement intake, vascular disorders, diabetes, cancer, ApoE4, dietary components other than main exposures	Poor
						(2) Risk of cognitive decline, defined as MMSE score of ≤24 after 6y	HR (95% CI) $T1_{vs}T2$: 0.781 (0.494, 1.235) p=0.289 $T1_{vs}T3$: **0.468 (0.261, 0.840) p=0.011** Continuous: **0.965 (0.941, 0.989) p=0.005**		

Table 2. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Berendsen (2018) [39]	Nurses' Health Study (USA)	6	16058	Older women (≥70y), free of stroke and dementia	15-MIND diet adherence (quantiles) based on 116-item sFFQ	Change in cognition measured by (1) TICS	Mean difference (95% CI) $Q1_{vs}Q2$: 0.14 (-0.018, 0.045) $Q1_{vs}Q3$: 0.003 (-0.030, 0.035) $Q1_{vs}Q4$: -0.011 (-0.043, 0.020) $Q1_{vs}Q5$: 0.004 (-0.028, 0.036)	Age, education, physical activity, calorie intake, alcohol intake, smoking status, multivitamin use, BMI, depression, history of high blood pressure, hypercholesterolemia, myocardial infarction, diabetes mellitus.	Fair
						(2) Verbal (episodic) memory (comp. of East Boston Memory Test immediate and delayed TICS delayed recall)	$Q1_{vs}Q2$: 0.000 (-0.009, 0.009) $Q1_{vs}Q3$: -0.007 (-0.017, 0.002) $Q1_{vs}Q4$: -0.003 (-0.013, 0.006) $Q1_{vs}Q5$: 0.002 (-0.008, 0.011)		
						(3) Global cognition (comp. of aforementioned tests and category fluency digit span backward)	$Q1_{vs}Q2$: 0.001 (-0.0007, 0.009) $Q1_{vs}Q3$: -0.004 (-0.011, 0.004) $Q1_{vs}Q4$: -0.002 (-0.010, 0.006) $Q1_{vs}Q5$: 0.001 (-0.007, 0.009)		
Munoz-Garcia (2020) [48]	Seguimiento Universidad de Navarra Project (Spain)	6	806	Older adults (>55y), free of dementia	15-MIND diet adherence (tertiles and continuous) based on 136-item sFFQ	Change in cognition measured by STICS-m	β (95% CI) $T1_{vs}T2$: 0.17 (-0.28, 0.62) $T1_{vs}T3$: 0.47 (-0.07,1.02) Continuous (per 1SD/1.5 points): **0.27 (0.05. 0.48)**	Age, sex, follow-up time until baselines STICS-m, years of university education, APOE4, smoking status, package-years, total energy intake, physical activity, BMI, alcohol intake, hypertension, high cholesterol, low HDL, and prevalent disease at recruitment (depression, cardiovascular disease, diabetes).	Fair

Table 2. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Nishi (2021) [46]	PREvención con DIeta MEDiterráne a-Plus trial (Spain)	2	5714	Older adults (55-75y) with overweight or obesity and metabolic syndrome	15-MIND diet adherence (tertiles) based on 143-item sFFQ	Change in cognition measured by (1) Global cognition (comp. of test below)	β (95% CI) $T1_{vs}T2$: -0.020 (-0.057, 0.016) $T1_{vs}T3$: 0.023 (-0.017, 0.063)	Age, sex, intervention group, centre size, corrected for clusters, respective cognitive test score at baseline, baseline education level, civil status, smoking habits, BMI, hypertension, hypercholesterolemia, diabetes, and depressive symptomology, baseline physical activity, and total energy intake.	Good
						(2) MMSE	$T1_{vs}T2$: 0.044 (-0.007, 0.095) $T1_{vs}T3$: 0.039 (-0.014, 0.092)		
						(3) Clock drawing test	$T1_{vs}T2$: 0.002 (-0.056, 0.060) $T1_{vs}T3$: 0.030 (-0.030, 0.090)		
						(4) Verbal fluency semantical	$T1_{vs}T2$: -0.003 (-0.051, 0.045) $T1_{vs}T3$: -0.036 (-0.086, 0.014)		
						(5) Verbal fluency phonological	$T1_{vs}T2$: -0.030 (-0.077, 0.018) $T1_{vs}T3$: 0.015 (-0.035, 0.064)		
						(6) TMT A	$T1_{vs}T2$: 0.023 (-0.031, 0.076) $T1_{vs}T3$: -0.017 (-0.077, 0.044)		
						(7) TMT B	$T1_{vs}T2$: 0.045 (-0.003, 0.094) $T1_{vs}T3$: 0.022 (-0.031, 0.075)		
						(8) Digit span forward	$T1_{vs}T2$: -0.043 (-0.095, 0.009) $T1_{vs}T3$: -0.007 (-0.065, 0.051)		
						(9) Digit span backward	$T1_{vs}T2$: 0.006 (-0.045, 0.057) $T1_{vs}T3$: 0.055 (-0.001, 0.112)		
Huang (2023) [12]	China Health and Nutritio n Survey (China)	3 (median follow-up)	4066	Older adults (≥55y), free of dementia	12-MIND diet adherence (tertiles and continuous) based on 3 24h dietary recalls	Change in cognition measured by (1) Global cognition (comp. of items of TICS-m)	β (95% CI) $T1_{vs}T2$: **0.016 (0.004, 0.029)** $T1_{vs}T3$: 0.010 (-0.003, 0.023) Continuous (per 3 points): 0.006 (-0.009, 0.020)	Age, age square, sex, education, residence, region, income, smoking status, drinking status, BMI, total energy, physical activities, hypertension, diabetes, myocardial infarction	Fair
						(2) Verbal memory (comp. of immediate and delayed recall)	$T1_{vs}T2$: 0.012 (-0.001, 0.025) $T1_{vs}T3$: 0.007 (-0.006, 0.021) Continuous (per 3 points): 0.004 (-0.011, 0.019)		

Table 2. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Van Lent (2021) [28]	Framingham Heart Offspring Study (USA)	6.6 ± 1.1 (mean follow-up)	2092	Older adults (mean age 61 ± 9y), free of dementia	15-MIND diet adherence (continuous) based on 126-item sFFQ	Change in cognition measured by (1) Visual reproductions delayed recall	β ± SE -0.01±0.02, p=0.58	Age, age[2], sex, ApoE4 status, total energy intake, education, BMI, physical activity, smoking, diabetes, CVD, depressive symptoms, anti-hypertensive medication, systolic blood pressure, total cholesterol to HDL ratio, time interval between FFQ and outcome measure.	Good
						(2) Logical memory delayed recall	-0.02±0.02, p=0.32		
						(3) TMT A	-0.004±0.02, p=0.79		
						(4) TMT B/A	-0.02±0.02, p=0.28		
						(5) Hooper visual organization test	-0.02±0.02, p=0.31		
						(6) Similarities	0.03±0.02, p=0.05		
						(7) Global cognition (comp. of tests above)	-0.002±0.02, p=0.87		
Boumenna (2022) [40]	The Boston Puerto Rican Health Study (USA)	8	573	Middle to older-aged adults (45-75y)	15-MIND diet adherence (quantiles and continuous) based on FFQ	Change in global cognition (comp. of MMSE, 16 word list learning, digit span forward and backward, stroop test, clock drawing and figure copying, verbal fluency)	β (95% CI) $Q1_{vs}Q2$: 0.005 (-0.053, 0.064) $Q1_{vs}Q3$: 0.006 (-0.043, 0.055) $Q1_{vs}Q4$: 0.047 (-0.006, 0.099) $Q1_{vs}Q5$: **0.093 (0.035, 0.152)** Continuous: **0.0213 (0.008, 0.034), p=0.0013**	Age, sex, BMI, physical activity score, diabetes, hypertension, education level, smoking, alcohol use, ApoE4 carrier, energy intake, job complexity score, poverty index	Good

Abbreviations: AD: Alzheimer's disease, BMI: Body mass index, CI: Confidence Interval, comp.: composition score, CVD: cardiovascular disease, HR: Hazard Ratio, MIND: Mediterranean-Dietary Approaches to Systolic Hypertension (DASH) diet Intervention for Neurodegenerative Delay, MMSE: Mini-Mental-State Examination, PACC: Preclinical Alzheimer cognitive composite, SE: standard error, sFFQ: simplified Food Frequency Questionnaire, TICS: Telephone Interview for Cognitive Status, TMT: Trail Making Test, T2D: Type 2 diabetes. [1] Study quality was assessed with the Newcastle Ottawa Scale.

Table 3. Description of included studies describing the association between MIND diet adherence and dementia.

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Vassilo-poulou (2022) [36]	No name (Greece)	N.A.; case-control	167 (115 dementia; 52 cognitively healthy controls)	Older adults; either dementia (mean age 72.6±8.1) or cognitively healthy (mean age 70.2±4.6)	9-MIND diet adherence (continuous) based on dietitian interview	Odds of dementia	OR (95% CI) **0.43 (0.29, 0.63)**	Sex, age, BMI, DASS-21, MMSE	Poor
Filippini (2020) [52]	No name (Italy)	N.A.; case-control	108 (n=54 cases)	Early onset dementia patients (cases) and caregivers (controls) (mean age 65y)	15-MIND diet adherence (continuous, tertiles) based on 188-item sFFQ	Odds of (1) Early onset dementia	OR (95% CI) $T1_{vs}T2$: **0.32 (0.12, 0.83)** $T1_{vs}T3$: **0.31 (0.11, 0.90)** Continuous: **0.66 (0.47, 0.91)**	Sex, age, educational attainment, total energy intake	Poor
						(2) Early onset AD	$T1_{vs}T2$: 0.39 (0.13, 1.15) $T1_{vs}T3$: 0.32 (0.09, 1.13) Continuous: **0.67 (0.46, 0.98)**		
						(3) Early onset frontotemporal dementia spectrum	$T1_{vs}T2$: 0.31 (0.07, 1.28) $T1_{vs}T3$: 0.45 (0.10, 2.00) Continuous: 0.66 (0.41, 1.08)		
Thomas (2022) [37]	The Three-City Bordeaux study (France)	9.7	1412	Older adults (mean age 75.8±4.8), free of dementia	15-MIND diet adherence (tertiles and continuous) based on 148-item FFQ and one 24h recall	Incident (1) All-cause dementia	HR (95% CI) $T1_{vs}T2$: 0.93 (0.73, 1.17) $T1_{vs}T3$: **0.73 (0.55, 0.97)** Continuous: **0.90 (0.83, 0.96), p=0.003**	Sex, APOE4 status, educational level, total energy intake, BMI, tobacco consumption, practice of regular physical activity, diabetes, history of cerebral and cardiovascular disease, hypertension, hypercholesterolemia, depressive symptoms (age as time scale)	Good
						(2) AD	$T1_{vs}T2$: 0.96 (0.72, 1.27) $T1_{vs}T3$: 0.70 (0.49, 1.00) Continuous: **0.89 (0.81, 0.97), p=0.008**		

Table 3. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Morris (2015) [5]	Rush Memory and Aging Project (USA)	4.5	789	Older adults (58-98y), free of AD	15-MIND diet adherence (tertiles) based on 144-item sFFQ	Incident AD	HR (95% CI) $T1_{vs}T2$: **0.64 (0.42, 0.97)** $T1_{vs}T3$: **0.48 (0.29, 0.79)** p for trend = **0.003**	Age, sex, education, APOE4, participation in cognitively stimulating activities, physical activity, total energy intake, cardiovascular conditions (hypertension, myocardial infarction, diabetes, stroke)	Good
Vu (2022) [20]	Chicago Health and Aging Project (USA)	not shown	2449 (946 white, 1503 black)	Older adults (≥65y), either white or African (black) Americans, free of dementia	15-MIND diet adherence (tertiles, continuous) based on sFFQ	Incident all-cause dementia	OR (95% CI) In white participants $T1_{vs}T2$: 0.87 (0.30, 2.54), p=0.80 $T1_{vs}T3$: 1.23 (0.47, 3.18), p=0.68 Continuous: 1.00 (0.81, 1.25), p=0.97 In black participants $T1_{vs}T2$: 0.86 (0.36, 2.05), p=0.74 $T1_{vs}T3$: 1.48 (0.51, 4.27), p=0.47 Continuous: 1.08 (0.79, 1.48), p=0.61	Age, sex, study centre, education, income, global cognition score, late life cognitive activity, history of diabetes, hypertension, stroke, heart disease, smoking, calorie intake, BMI, depressive symptoms, physical activity	Good
Vu (2022) [20]	Rush Memory and Aging Project (USA)	not shown	725	Older adults (mean age 82y), free of dementia	15-MIND diet adherence (tertiles and continuous) based on sFFQ	Incident all-cause dementia	HR (95% CI) $T1_{vs}T2$: 0.85 (0.62, 1.16), p=0.31 $T1_{vs}T3$: **0.63 (0.42, 0.92), p=0.02** Continuous: 0.91 (0.83, 1.00), p=0.06	Age, sex, study centre, education, income, global cognition score, late life cognitive activity, history of diabetes, hypertension, stroke, heart disease, smoking, calorie intake, BMI, depressive symptoms, physical activity	Good
Vu (2022) [20]	Women's Health Initiative Memory Study (USA)	not shown	5308	Older female (≥65y), free of dementia	15-MIND diet adherence (tertiles and continuous) based on sFFQ	Incident all-cause dementia	HR (95% CI) $T1_{vs}T2$: **0.87 (0.79, 0.97), p=0.008** $T1_{vs}T3$: **0.80 (0.72, 0.89), p<0.0001** Continuous: **0.95 (0.92, 0.97), p<0.0001**	Age, study centre, randomization status, education, income, global cognition score, history of diabetes, hypertension, stroke, heart disease, smoking, calorie intake, BMI, depressive symptoms, physical activity	Good

Table 3. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
de Crom (2022) [50]	Rotterdam Study (Netherlands)	15.6 (baseline 1) 5.9 (baseline 2)	5375 (baseline 1) 2861 (baseline 2)	Older adults (≥55y), free of dementia	15-MIND diet adherence (continuous) based on 170-item (baseline 1) or 389-item FFQ (baseline2)	Incident all-cause dementia	HR (95% CI) baseline 1: 0.99 (0.94, 1.05) baseline 2: **0.79 (0.70, 0.91)**	Sex, age, age^2, educational attainment, smoking status, physical activity, daily energy intake, BMI, diabetes, hypercholesterolemia, hypertension.	Good
Hosking (2019) [51]	The 60's cohort of the Personality and Total Health Through Life (Australia)	12	961	Older adults (60-64y), free of dementia	13-MIND diet adherence (continuous) based on 183-item sFFQ	Incident dementia	OR (95% CI) **0.72 (0.54, 0.95)**	Age, sex, energy intake	Fair
Cornelis (2023) [29]	UK Biobank (UK)	10.5±1.8 (mean follow-up)	77398	Older adults ≥55y, free of dementia	15-MIND diet adherence (tertiles and continuous) based on 1 to 4 Oxford webQs (web-based 24h dietary assessment tool)	Incident (1) All-cause dementia	HR (95% CI) $T1_{vs}T2$: 1.06 (0.90, 1.24) $T1_{vs}T3$: 0.90 (0.74, 1.09) Continuous 0.99 (0.95, 1.03)	Age, sex, self-reported race/ethnicity, education, Townsend deprivation index, income, employment status, global cognition score, family history of dementia; history of hypertension, diabetes, heart disease, stroke and depression; self-reported health, smoking, physical activity, BMI, fast meal consumption,energy intake	Fair
						(2) AD	$T1_{vs}T2$: 1.00 (0.78, 1.30) $T1_{vs}T3$: 0.96 (0.72, 1.28) Continuous: 1.01 (0.95, 1.07)		
Zhang (2023) [30]	UK Biobank (UK)	9.4	114684	Middle-aged to older adults (40-69y), free of dementia	14-MIND diet adherence (tertiles and continuous) based on 2-4 Oxford web-based 24h dietary assessment tool, scored according to quintiles of intake	Incident dementia	HR (95% CI) $T1_{vs}T2$: 0.91 (0.73, 1.14) $T1_{vs}T3$: 0.89 (0.71, 1.12)	Age, sex, educational level, Townsend deprivation index, BMI, smoking status, alcohol consumption, regular physical activity, sleep duration, time on watching TV, family history of AD, APOE genotypes, cancer, CVD, diabetes	Good

Table 3. (*continued*)

Author (year)	Study (country)	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Chen (2023) [11]	Whitehall II study (UK)	12.9	8358	Older adults (≥45y), free of dementia	14-MIND diet adherence (tertiles and continuous), rescaled to 15-points, based on FFQ	Incident dementia	HR (95% CI) $T1_{vs}T2$: 1.03 (0.73, 1.45) $T1_{vs}T3$: 0.96 (0.66, 1.38) Continuous (per 3 points): 0.97 (0.72, 1.30)	Age, sex, education level, occupational class, vigorous physical activity, smoking status, energy intake, BMI, depressive symptoms, hypertension, hypercholesterolemia, diabetes, stroke, cardiovascular diseases	Good
Chen (2023) [11]	Health and Retirement Study (USA)	5.0	6758	Older adults (≥45y), free of dementia	15-MIND diet adherence (tertiles and continuous) based on FFQ	Incident dementia	HR (95% CI) $T1_{vs}T2$: 0.95 (0.73, 1.25) $T1_{vs}T3$: 0.83 (0.63, 1.09) Continuous (per 3 points): **0.82 (0.68-0.99)**	Age, sex, education level, household income, vigorous physical activity, smoking status, energy intake, BMI, depressive symptoms, hypertension, diabetes, stroke, cardiovascular diseases	Good
Chen (2023) [11]	Framingham Heart Study, Offspring cohort (USA)	10.7	3020	Older adults (≥45y), free of dementia	15-MIND diet adherence (tertiles and continuous) based on FFQ	Incident dementia	HR (95% CI) $T1_{vs}T2$: 0.96 (0.70, 1.33) $T1_{vs}T3$: **0.69 (0.48, 0.99)** Continuous (per 3 points): 0.76 (0.57, 1.00)	Age, sex, education level, household income, vigorous physical activity, smoking status, energy intake, BMI, depressive symptoms, hypertension, hypercholesterolemia, diabetes, stroke, cardiovascular diseases	Good

Abbreviations: AD: Alzheimer's disease, BMI: Body mass index, CI: Confidence Interval, CVD: cardiovascular disease, DASS-21: Depression Anxiety Stress Scale, EO-AD: Early onset Alzheimer's disease, EOD: Early onset dementia, EO-FTP: Early onset frontotemporal dementia spectrum HR: Hazard Ratio, MIND: Mediterranean-Dietary Approaches to Systolic Hypertension (DASH) diet Intervention for Neurodegenerative Delay, MMSE: Mini-Mental-State Examination, OR: Odds Ratio, sFFQ: simplified Food Frequency Questionnaire. [1] Study quality was assessed with the Newcastle Ottawa Scale.

Table 4. Description of included studies describing the association between MIND diet adherence and cognitive impairment, subjective memory complaints, and cognitive resilience.

Author (year)	Study (country)	Design	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Lawrie (2022) [53]	Oxford Parkinson's Disease Discovery Cohort (UK)	Cross-sectional	N.A.	131	Older adults (67±9y) with Parkinson's disease, free of dementia	15-MIND diet adherence (continuous) based on FFQ	Odds of mild cognitive impairment (MoCA, adjusted for education)	β (95% CI/SD/SE not shown) -0.23, p=0.070	Age, sex, kcal, disease duration, physical activity level, education, smoking status	Poor
Huang (2022) [45]	Chinese Longitudinal Healthy Longevity Study (China)	Cross-sectional	N.A.	11245	Older adults (84±11y) without stroke or dementia	Chinese-adapted 12-MIND diet adherence (tertiles and continuous) based on sFFQ	Odds of cognitive impairment (MMSE, adjusted for education)	OR (95% CI) $T1_{vs}T2$: **0.81 (0.71, 0.92)** $T1_{vs}T3$: **0.60 (0.51, 0.72)** Continuous: **0.86 (0.82, 0.89)**	Sex, age, region, education, BMI, smoking, drinking, exercise, social engagement, hypertension, diabetes, depression, hearing impairment.	Fair
Adjibade (2019) [32]	NutriNet-Santé cohort (France)	Longi-tudinal	6	6011	Older adults (≥60y), free of dementia	15-MIND diet adherence (tertiles and continuous), based on 3 non-consecutive 24h dietary records	Subjective memory complaints (SMC) (cognitive difficulty scale (CDS), cut-off score of 43)	HR (95% CI) total population $T1_{vs}T2$: 0.97 (0.84, 1.12) $T1_{vs}T3$: 0.94 (0.79, 1.11) Continuous: 0.98 (0.93, 1.02), p=0.32 HR (95% CI) 60-69y $T1_{vs}T2$: 1.00 (0.85, 1.18) $T1_{vs}T3$: 0.97 (0.80, 1.17) Continuous: 1.00 (0.95, 1.05), p=0.96 HR (95% CI) ≥70y $T1_{vs}T2$: 0.84 (0.60, 1.17) $T1_{vs}T3$: 0.81 (0.55, 1.20) Continuous: **0.87 (0.78, 0.98), p=0.02**	Age, sex, material status, educational level, occupational category, household income per consumption unit, energy intake without alcohol, number of recording days, inclusion moth, smoking status, physical activity, BMI, comorbid conditions during follow-up, depressive symptoms at the end of the follow-up, baseline CDS score.	Good

Table 4. (*continued*)

Author (year)	Study (country)	Design	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Hosking (2019) [51]	The 60's cohort of the Personality and Total Health Through Life (Australia)	Longi-tudinal	12	961	Older adults (60-64y), free of dementia	13-MIND diet adherence (tertiles and continuous) based on 183-item sFFQ	Incident MCI (Winbald criteria)	OR (95% CI) $T1_{vs}T2$: 0.94 (0.57, 1.56) $T1_{vs}T3$: **0.47 (0.24, 0.91)** p for trend: **0.026**	Energy intake, age, sex, APOE4 status, education, mental activity, physical activity, smoking status, depression, diabetes, BMI, hypertension, heart disease, stroke	Good
Wagner (2023) [25]	Memory and Ageing Project (USA)	Longi-tudinal	9±4	578	Older adults (mean age diet assessment 84.1±5.8; death 91.4±6.1), free of dementia	15-MIND diet adherence (tertiles and continuous) based on 144-item sFFQ	(1) Cognitive resilience mean level, based on change in global cognition (comp. of 17 tests) adjusted for neuropathologies	Mean difference (95% CI) $T1_{vs}T2$: 0.23 **(0.04, 0.41) p=0.02** $T1_{vs}T3$: **0.34 (0.14, 0.55) p=0.001** Continuous: **0.07 (0.02, 0.12) p=0.01**	Sex, education, age at first dietary assessment, total energy intake, smoking status, number of depressive symptoms, number of medical conditions, physical activity, frequency of participation in cognitively stimulating activities	Good
							(2) Cognitive resilience slope, based on the slope of global cognitive decline given a specific profile of neuropathologies	$T1_{vs}T2$: **0.20 (0.01, 0.39) p=0.04** $T1_{vs}T3$: **0.27 (0.05, 0.48) p=0.01** Continuous: 0.05 (-0.003, 0.10) p=0.06		

Abbreviations: BMI: Body mass index, CDC: cognitive difficulty score, CI: Confidence Interval, comp.: composition score, HR: Hazard Ratio, MIND: Mediterranean-Dietary Approaches to Systolic Hypertension (DASH) diet Intervention for Neurodegenerative Delay, MMSE: Mini-Mental-State Examination, MoCA: Montreal Cognitive Assessment, OR: Odds Ratio, SD: standard deviation, SE: standard error, sFFQ: simplified Food Frequency Questionnaire, SMC: Subjective memory complaints.[1] Study quality was assessed with the Newcastle Ottawa Scale.

Table 5. Description of included studies describing the association between MIND diet adherence and Parkinson's disease

Author (year)	Study (country)	Design	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Metcalfe-Roach (2021) [54]	no name (Canada)	Cross-sectional	N.A.	n=121	Older adults (mean age 65y) with diagnosis of PD	15-MIND diet adherence (continuous) based on FFQ, MIND score adjusted to 0-10 scale	Age of PD onset	Beta (95% CI/SE/SD not shown) **2.2, p=0.002**	Disease duration, kcal, sex, smoking, years of education, exercise	Poor
Agarwal (2018) [21]	Rush Memory and Aging Project (USA)	Longi-tudinal	4.6y (mean follow-up)	n=706	Older adults (59-97y), free of PD and dementia	15-MIND diet adherence (tertiles, continuous) based on 114-item FFQ	(1) Incident PD	HR (95% CI) $T1_{vs}T2$ **0.70 (CI not shown) p=0.008** $T1_{vs}T3$ **0.58 (CI not shown) p=0.0003** Continuous **0.89 (0.83-0.96) p<0.05**	Age, sex, smoking, total energy intake, BMI, depressive symptoms	Poor
							(2) Change in PD progression	β (SE) Continuous **-0.008 (0.0037), p= 0.04**		

Abbreviations: BMI: Body Mass index, CI: Confidence Interval, FFQ: Food Frequency Questionnaire, HR: Hazard ratio, kcal: kilocalories, MIND: Mediterranean-Dietary Approaches to Systolic Hypertension (DASH) diet Intervention for Neurodegenerative Delay, PD: Parkinson's disease, SD: Standard deviation, SE: Standard error. [1] Study quality was assessed with the Newcastle Ottawa Scale.

Table 6. Description of included studies describing the association between MIND diet adherence and brain volume and pathology outcomes.

Author (year)	Study (country)	Design	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
van Lent (2021) [28]	Framingham Heart Study, Offspring cohort (USA)	Cross-sectional	N.A.	1904	Older adults (61±9y), free of dementia	15-MIND diet adherence (continuous) based on 126-item sFFQ	Brain volume (% of intracranial volume) measured by	β (SE)	Age, age[2], sex, ApoE4 status, total energy intake, education, BMI, physical activity, smoking, diabetes, CVD, depressive symptoms, anti-hypertensive medication, systolic blood pressure, total cholesterol to HDL ratio, time interval between FFQ and outcome measure.	Good
							(1) Total brain	**0.02 (0.01), p=0.02**		
							(2) Lateral ventricular	-0.007 (0.01), p=0.59		
							(3) Hippocampal	0.02 (0.01), p=0.20		
							(4) White matter hyperintensity	-0.02 (0.01), p=0.15		
							(5) Odds of silent brain infarcts	OR (95% CI) 0.99 (0.91, 1.09), p=0.89		
Dhana (2021) [24]	Rush Memory and Aging Project (USA)	Longi-tudinal	Not shown	569	Older adults (≥65y; mean age at death 90.8±6.1y), some were diagnosed with AD	15-MIND diet adherence (continuous, per 1SD=1.42 point) based on 144-item sFFQ	Brain pathology measured by	β (SE)	Age at death, sex, education, APOE4, late-life cognitive activities, total energy intake.	Good
							(1) Global AD pathology (comp. of neurotic, diffuse plaques, and neurofibrillary tangles)	-0.013 (0.024), p=0.578		
							(2) β-amyloid	-0.03 (0.049), p=0.395		
							(3) Tangles	0.058 (0.332), p=0.862		
							(4) Macroinfarcts	0.038 (0.091), p=0.680		
							(5) Microinfarcts	0.132 (0.095), p=0.163		
							(6) Arteriolosclerosis	0.087 (0.098), p=0.378		
							(7) Cerebral atherosclerosis	0.033 (0.104), p=0.754		

Table 6. (*continued*)

Author (year)	Study (country)	Design	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Agarwal (2023) [23]	Memory and Ageing Project (USA)	Longi-tudinal	6.8±3.9y (mean follow-up)	581	Older adults (mean age diet assessment 84.2±5.8; age death 91.3±6.1)	15-MIND diet adherence (tertiles and continuous) based on 144-item sFFQ	Brain pathology measured by (1) Global AD pathology	β (SE) $T1_{vs}T2$: -0.027 (0.037) p=0.461 $T1_{vs}T3$: -0.077 (0.038) p=0.044 Continuous: -0.024 (0.011) p=0.025	Age at death, sex, education, ApoE4 status, total calories, time between last dietary assessment and death	Good
							(2) Beta-amyloid load	$T1_{vs}T2$: -0.099 (0.118) p=0.402 $T1_{vs}T3$: -0.246 (0.123) p=0.047 Continuous: -0.062 (0.034) p=0.071		
							(3) Phosphorylated tau-tangle	$T1_{vs}T2$: -0.139 (0.130) p=0.285 $T1_{vs}T3$: -0.108 (0.134) p=0.422 Continuous: -0.024 (0.037) p=0.528		
Dong (2023) [35]	Wisconsin Registry for Alzheimer's Prevention (USA)	Longi-tudinal	Unknown	924	Older adults (mean age 63.5±6.7y)	15-MIND diet adherence (continuous) based on 15-item diet questionnaire	Cerebrospinal fluid biomarkers (1) P-tau	β (p-value) -0.1842 (0.37)	None	Poor
							(2) T-tau	-2.244 (0.31)		
Escher (2022) [42]	UCSF Memory and Aging Center's Longitudinal Brain Aging Program (USA)	Cross-sectional	N.A.	77	Older adults (≥50y)	15-MIND diet adherence (continuous) based on FFQ	Total intracranial volume of (1) Grey matter	β (95% CI) 0.01 (0.00, 0.01)	Age, sex, education, vascular burden score, PASE, MIND*PASE	Fair
							(2) White matter	0.001 (-0.005, 0.01)		

Table 6. (*continued*)

Author (year)	Study (country)	Design	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Zhang (2023) [30]	UK Biobank (UK)	Cross-sectional	N.A.	18214	Middle-aged to older adults (40-69y), free of dementia	14-MIND diet adherence (continuous) based on 2 to 4 Oxford webQs (web-based 24h dietary assessment tool), scored according to quintiles of intake	Brain volume (mm^3) measured by	β±SD (p-value, significance set at $p<6.6*10^{-4}$; multiple testing correction)	Age, sex, educational level, APOE, BMI, smoking status, alcohol consumption, regular physical activity, time on watching TV, sleep duration, Towsend deprivation index, family history of dementia, cancer, cardiovascular disease, diabetes	Fair
							(1) Total brain	14.40±469.36 (0.976)		
							(2) Grey matter	-144.44±276.68 (0.602)		
							(3) White matter	158.83±304.02 (0.601)		
							(4) Superior frontal gyrus	2.11±22.41 (0.925)		
							(5) Inferior frontal gyrus	-13.71±10.81 (0.205)		
							(6) Middle frontal gyrus	-37.09±22.86 (0.105)		
							(7) Supplementary motor cortex	-12.74±7.88 (0.106)		
							(8) Precentral gyrus	-7.92±22.45 (0.724)		
							(9) Postcentral gurus	21.24±18.89 (0.261)		
							(10) Precuneus	-6.28±19.12 (0.743)		
							(11) Superior parietal lobe	22.46±11.42 (0.049)		
							(12) Parahippocampal gyrus	13.60±6.94 (0.050)		
							(13) Middle temporal gyrus	13.22±19.88 (0.506)		
							(14) Inferior temporal gyrus	13.73±16.33 (0.400)		
							(15) Hippocampus	12.40±5.77 (0.032)		
							(16) Putamen	4.12±6.52 (0.527)		
							(17) Thalamus	17.03±9.73 (0.080)		
							(18) Caudate	7.53±6.10 (0.217)		
							(19) Amygdala	4.77±3.23 (0.140)		

Table 6. (*continued*)

Author (year)	Study (country)	Design	Duration (years)	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Chen (2021) [31]	Women's Health Initiative Hormone Replacement Therapy trial (USA)	Longi-tudinal	7-10	1302	Older woman (65-79y), free of dementia	15-MIND diet adherence (continuous, per 0.5 point) based on 122-item sFFQ	Brain volume (mm^3) measured by (1) Total brain	β (95% CI), adjusted p-value 0.10 (-0.17, 0.38), 0.90	Intracranial volume, age, race, U.S. regions, education level, employment, smoking status, alcohol consumption, BMI, physical activity, history of hypertension, diabetes, hypercholesterol-emia, cardiovascular disease	Good
							(2) Normal brain (excluding areas with evidence of small vessel ischemic disease)	0.23 (-0.15, 0.61), 0.90		
							(3) Total white matter	0.74 (0.001, 1.48), 0.33		
							(4) Frontal lobe white matter	0.33 (-0.01, 0.67), 0.33		
							(5) Parietal lobe white matter	0.18 (-0.03, 0.39), 0.43		
							(6) Temporal lobe white matter	0.19 (0.002, 0.37), 0.33		
							(7) Corpus callosum white matter	0.001 (-0.02, 0.02), 0.90		
							(8) Hippocampus	0.0007 (-0.02, 0.02), 0.90		

Abbreviations: AD: Alzheimer's disease, BMI: Body Mass index, CVD: Cardiovascular Disease, HDL: High-Density-Lipoprotein, MIND: Mediterranean-Dietary Approaches to Systolic Hypertension (DASH) diet Intervention for Neurodegenerative Delay, PASE: Physical Activity Scale for the Elderly, SD: Standard deviation, SE: Standard error, sFFQ: simplified Food Frequency Questionnaire. [1] Study quality was assessed with the Newcastle Ottawa Scale.

Table 7. Description of included randomized controlled trials describing the effect of the MIND diet on cognitive decline and brain volume

Author (year)	Study (country)	Duration	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Arjmand (2022) [38]	MIND Diet Intervention and Cognitive Performance trial (Iran)	3 months	37	Obese middle-aged women (48 ± 5.38y), without any metabolic complication and free of dementia	14-MIND diet intervention with caloric restriction vs control diet with caloric restriction	Change in cognition measured by (1) Letter number sequencing task (LNST)	Mean difference (95% CI) *(calculated based on given numbers)* 1.31 (0.79, 1.95), **p≤0.001**	None	Some concerns
						(2) Auditory verbal learning test	1.54 (3.30, 6.40), **p≤0.001**		
						(3) SDMT	3.75 (2.43, 5.07), **p≤0.001**		
						(4) Digit span forward	1.75 (1.15, 2.35), **p≤0.001**		
						(5) Digit span backward	0.44 (0.01, 0.86), **p=0.041**		
						(6) TMT A	-5.86 (-9.16, -2.22), **p=0.002**		
						(7) TMT B	-2.63 (-6.34, 1.09), p=0.161		
						(8) Stroop task	-10.24 (-23.6, 3.09), p=0.128		
Barnes (2023) [55]	Trial of the MIND diet (USA)	3y	519-564 (n=268-275 intervention group; depending on outcome)	Overweight older adults (≥65y), free of dementia	14-MIND diet intervention with mild caloric restriction vs control diet with mild caloric restriction	Change in cognition measured by (1) Global cognition (comp. of all tests below)	Mean change between groups (95% CI) 0.035 (−0.022, 0.092)	None	Low bias
						(2) Episodic memory (comp. of word list memory, recall & recognition, East Boston story immediate & delayed recall)	0.045 (-0.046, 0.137)		
						(3) Semantic memory (comp. of category fluency and multilingual naming test)	-0.043 (-0.144, 0.057)		
						(4) Executive functioning (comp. of TMT B and flanker inhibitory control and attention test)	0.070 (-0.033, 0.173)		
						(5) Perceptual speed (comp. of oral symbol digit modality test, pattern comparison test, and TMT A)	(5) Perceptual speed: 0.008 (-0.078, 0.094)		

Table 7. (*continued*)

Author (year)	Study (country)	Duration	Sample size (n)	Population	Exposure	Outcome	Results	Covariates	Study quality[1]
Barnes (2023) [55]	Trial of the MIND diet (USA)	3y	193-200 (97-101 intervention group; depending on outcome)	Overweight older adults (≥65y), free of dementia	14-MIND diet intervention with mild caloric restriction vs control diet with mild caloric restriction	Brain volume measured by	Mean change between groups (95% CI)	Clinical site	Low bias
						(1) Grey and white matter	0.001(−0.003, 0.005)		
						(2) Hippocampal volume	0.005 (−0.016, 0.026)		
						(3) White-matter hyperintense lesions	−0.019 (−0.046, 0.008)		

Abbreviations: CI: Confidence Interval, MIND: Mediterranean-Dietary Approaches to Systolic Hypertension (DASH) diet Intervention for Neurodegenerative Delay, SDMT: Symbol digit modality task, TMT: Trail making test.[1] Study quality was assessed with the Cochrane risk-of-bias tool for randomized trials

DISCUSSION

In this review, we summarized the evidence on the MIND diet in relation to brain ageing. The only intervention study with good quality did not demonstrate beneficial effects of a MIND diet intervention on cognition or brain volumes. With respect to observational research, the majority of studies indicated that the MIND diet reduces the risk of all-cause dementia and Alzheimer's disease. The evidence for the protective associations of the MIND diet with cognition, however, is more mixed. While there are studies supporting cross-sectional associations with global cognition and episodic memory, these protective associations primarily originate from North-American populations. In addition, longitudinal evidence as well as evidence for other cognitive domains is limited. Neuroimaging, pathology and PD outcomes have only been addressed in few studies, that so far do not hint towards benefits. Overall study quality was adequate and excluding articles poor or fair quality did not change findings. Interestingly, the MIND diet works especially well for the Rush Memory and Ageing Project (MAP), being the only cohort where associations with brain pathology and cognitive decline in multiple domains has been demonstrated.

From a mechanistic point of view, protective associations could be expected as the MIND diet is rich in all nutrients considered relevant for healthy brain ageing. Polyphenols and anti-oxidants from berries and vegetables, and vitamin E from nuts and olive oil have anti-inflammatory, anti-oxidant and/or vascular-health promoting properties [3]. Omega-3 fatty acids from fish also possess these properties, and act as building block for neurons [56]. Finally, B-vitamins coming from leafy greens, whole grains and poultry, maintain homocysteine levels [57]. These multiple nutrients targeting different mechanisms are crucial, as the mechanisms underlying nutrition and brain ageing are multifactorial [58]. This is further substantiated by the findings that evidence for dietary patterns is stronger than that for single nutrients and foods [3] and that nutrients have synergistic properties [59, 60].

Our findings, however, do not conclusively prove the benefits of the MIND diet for brain ageing. The only randomized controlled trial with good quality did not show protective effects. Regarding observational studies, while we did find evidence for global cognitive functioning and dementia, the benefits of the MIND diet for global cognitive decline were only demonstrated in two out of seven cohorts.

A possible explanation why the MIND diet trial showed null-results is the choice of the control diet. In this trial, the effect of the MIND diet with mild caloric restriction

was compared to a control diet with also mild caloric restriction. Over the three years of follow-up, both arms lost a similar amount of weight. Weight loss in itself may be responsible for improved cognition, i.e. via lowering inflammation or improving insulin sensitivity, which may have overruled the benefits of the MIND diet intervention. Alternatively, selection bias could have occurred. The participants in the MIND diet trial were on average more highly educated, had a healthier medical history and a higher baseline MIND-diet score compared to participants of the MAP cohort in which the MIND diet was shown to be beneficial [4, 5].

With respect to the observational evidence, a first hypothesis why the MIND diet works for some but not all cohorts is that the preferred diet for brain ageing may be population-specific. This population-dependency has already been demonstrated for Mediterranean and Nordic dietary patterns [49, 61]. Better adherence to the Mediterranean diet was associated with a risk of all-cause mortality in both Mediterranean and non-Mediterranean countries, although effect sizes were larger in Mediterranean countries [61]. Similarly, in the context of brain ageing, a Nordic dietary pattern was more strongly protectively associated with cognitive decline than the MIND diet in a Swedish population [49].

This hypothesis that the preferred diet for brain ageing may be population-specific can be substantiated by differences in cultural practices between populations, which is an important factor influencing dietary behaviour [62]. For example, a traditional Dutch way to consume leafy green vegetables is by eating 'stamppot', a dish that combines cooked leafy greens with mashed potatoes and meat. This is different from the way green leafy vegetables are likely being consumed in other countries, i.e. raw as a salad. In addition, MIND diet specific foods, such as berries, are not considered part of all cultures [63]. As a consequence, the MIND diet scoring system might capture different dietary patterns in different populations, depending on cultural practices.

The MIND diet may be the most preferred diet for brain ageing in North-America. This is supported by our findings, as cross-sectional protective associations were primarily observed in North-American populations. The MIND diet was also especially protective for participants in the MAP cohort, the first cohort in which the MIND diet has been tested [4, 5]. Furthermore, some of the studies originating outside North-America showing beneficial associations had adapted the MIND diet to their local eating habits. For example, a French study changed scoring thresholds to French guidelines and replaced berry intake by total polyphenol intake [37], and

a Chinese study replaced wine by tea consumption [45]. Further research is required to discover if traditional eating habits with components of the MIND diet are more protective of brain ageing than the original MIND diet.

Another possible explanation for the mixed findings is that study populations were not adequately selected. Preferably, there is a large variation in exposure and outcome between participants to allow easier detection of associations. In terms of exposure this means a wide range of variation in dietary intake, i.e. in MIND diet score. More variation in outcome can be achieved by selecting participants at risk of brain ageing as opposed to the general population, as an at-risk population is more likely to decline. This can be exemplified by comparing the MAP cohort with the Nurses' Health Study cohort, of which the MAP cohort did demonstrate beneficial associations [4], and the Nurses' Health Study cohort did not [39]. Overall, there was more variation in MIND score in the MAP cohort compared to the Nurses' Health Study cohort (2.5-12.5 vs 2.6-11.0) and participants in the MAP cohort were at higher risk of cognitive decline compared to Nurses' Health Study participants, as evidenced by a larger proportion of smokers and individuals with cardiovascular complaints [4, 39].

Alternatively, it could be that focussing on diet only is a too simplistic view, as we know that many other factors can influence the association between the MIND diet and brain ageing. For example, ApoE4 genotype may be an effect modifier, as reported by studies on other dietary patterns and brain ageing [64-66]. Among our included studies, the interaction between ApoE4 genotype and the MIND diet has been demonstrated as well. Findings are inconsistent, however, with some studies reporting improved MIND-diet related brain ageing among carriers [20, 28, 50], others among non-carriers [20], and the majority demonstrating no interaction [23, 29, 37, 39, 51].

In addition to genotype, other potential effect modifiers include income, physical activity and exposure to fine particulate matter. Only individuals with higher income [67] or lower levels of physical activity [27, 42] benefitted from better adherence to the MIND diet. In addition, exposure to fine particulate matter was only harmful for brain ageing in females not adhering well to the MIND diet [31]. These studies illustrate that the association between the MIND diet with brain ageing is an interplay between many different factors.

The importance of interactions between various factors is now largely recognized and implemented in multi-domain interventions. A well-known example is the FINGER trial, the first randomized controlled trial evidencing that a multidomain lifestyle intervention can slow cognitive decline in older adults at risk of dementia. Further building on this trial, the world-wide FINGERS network has been set up. This network of multi-domain interventions for dementia prevention aims to extend the findings of FINGER to multiple populations and settings around the world. In several of these interventions, the MIND diet has been chosen as a basis for the nutrition component of the multi-domain lifestyle (i.e. US Pointer, clinicaltrials.gov NCT03688126; FINGER-NL, clinicaltrials.gov NCT05256199; LatAm-FINGERS [68]). These trials will give insight in the interplay between the MIND diet and other lifestyle factors in healthy brain ageing.

Finally, our results should be interpreted with care because of several methodological limitations. There was a large variation in exposure assessment, with differences in dietary assessment methods (FFQ, food dairy), timing of assessment and interpretation and scoring of MIND components that limits comparability between studies. In addition, measurement of outcomes varied largely. Without consensus on the optimal neuropsychological test battery to capture cognitive changes, especially in the pre-clinical phase, and no rules on how to construct cognitive domains, it is hard to draw firm conclusions [69]. Because of this heterogeneity in outcomes, we chose to not perform a meta-analysis. Also, as the majority of included studies had an observational design, there is a risk of reverse causation, residual confounding and over-adjustment. Another limitation is that many articles made use of data from the MAP cohort, which may give a limited perspective on the state of evidence. Finally, we assessed quality of individual articles using NOS and ROB2, but we did not assess overall quality of evidence using e.g. the GRADE approach.

To conclude, this systematic review shows observational evidence for a beneficial association between the MIND diet with global cognitive function and dementia risk, but evidence for cognitive decline, cognitive impairment, brain volume, pathology and PD remains mixed and/or limited. The preferred diet for brain ageing may be population-specific, with the MIND diet being the favoured diet for North-American populations.

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Supplementary materials

Table S1A: Search strategy Ovid Medline.

	***Ovid Medline*: 12-10-2022**	**Hits**
1	MIND diet*.mp	105
2	Mediterranean-DASH.mp	97
3	1 or 2	134
4	cognit*.mp	545719
5	Exp Dementia/	195928
6	dementia.mp	151270
7	Alzheimer*.mp	190802
8	parkinson*.mp	150303
9	brain.mp	1566911
10	4 or 5 or 6 or 7 or 8 or 9	2186540
11	3 and 10	91

Table S1B: Search strategy Web of Science core collection.

	***Web of Science core collection*: 12-10-2022**	**Hits**
1	ALL=("MIND diet*")	112
2	ALL=("Mediterranean-DASH")	88
3	#1 or #2	136
4	ALL=(cognit*)	984686
5	ALL=(dementia)	207765
6	ALL=(Alzheimer*)	298393
7	ALL=(Parkinson*)	233128
8	ALL=(brain)	1865420
9	4 or 5 or 6 or 7 or 8	2842640
10	#3 and #9	108

Table S1C: Search strategy Scopus.

	***Scopus*: 12-10-2022**	**Hits**
1	TITLE-ABS-KEY("MIND diet*")	143
2	TITLE-ABS-KEY({Mediterranean-DASH})	84
3	#1 OR #2	157
4	TITLE-ABS-KEY(cognit*)	1080735
5	TITLE-ABS-KEY (dementia)	221084
6	TITLE-ABS-KEY(alzheimer*)	259343
7	TITLE-ABS-KEY(parkinson*)	209188
8	TITLE-ABS-KEY(brain)	2437047
9	4 OR 5 OR 6 OR 7 OR 8	3535872
10	#3 AND #9	111

Table S2. Newcastle-Ottawa Quality Assessment Scale (NOS) Cohort studies.

Selection (max. 4)
1) Representativeness of the exposed cohort
a) truly representative of the average in target population in the community *
b) somewhat representative of the average in target population in the community *
c) selected group of users e.**g.** nurses, volunteers
d) no description of the derivation of the cohort
2) Selection of the non-exposed cohort
a) drawn from the same community as the exposed cohort *
b) drawn from a different source
c) no description of the derivation of the non-exposed cohort
3) Ascertainment of exposure (MIND diet)
a) FFQ/ ≥3x 24h recall/ food diary AND MIND total score ≥13 points *
b) structured (dietician) interview AND MIND total score ≥13 points *
c) written self-report OR MIND total score <13 points
d) no description
4) Demonstration that outcome of interest was not present at start of study
a) yes *
b) no
Comparability (max 2)
1) Comparability of cohorts on the basis of the design or analysis
a) study controls for age, sex, and education *
b) study controls for any additional lifestyle or genetic factor (e.g. smoking, alcohol, physical activity, BMI, APOE4) *
Outcome (max 4)
1) Assessment of outcome
a) independent or blind assessment *
b) record linkage *
c) self-report
d) no description
2) Was follow-up long enough for outcomes to occur
a) yes (≥2 years for cognitive decline, MRI data and brain pathology, ≥5 years for dementia/MCI incidence and cognitive screeners (e.g. MMSE, MoCA, TICs) *
b) no
3) Adequacy of follow up of cohorts
a) complete follow up - all subjects accounted for *
b) subjects lost to follow up unlikely to introduce bias - small number lost - > 70% follow up, or description provided of those lost *
c) follow up rate < 70% and no description of those lost
d) no statement
4) Statistical test
a) the statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented including confidence intervals and the probability level (p value) *
b) the statistical test is not appropriate, not described or incomplete

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for the comparability question.
Scoring: Good quality: ≥3 stars in selection domain AND 2 stars in comparability domain AND ≥2 stars in outcome domain. Fair quality: ≥2 stars in selection domain AND ≥1 stars in comparability domain AND ≥2 stars in outcome domain. Poor quality: <2 stars in selection domain OR 0 stars in comparability domain OR <2 stars in outcome domain.

Table S3. Newcastle-Ottawa Quality Assessment Scale (NOS) Case-control studies.

Selection (max. 4)
1) Is the case definition adequate?
a) yes, with independent validation (e.g. >1 person/record/time/process to extract information, or reference to primary record source such as x-rays or medical/hospital records)*
b) yes, eg record linkage*
c) based on self-reports
d) no description
2) Representativeness of the cases
a) consecutive or obviously representative series of cases (All eligible cases with outcome of interest over a defined period of time, all cases in a defined catchment area, all cases in a defined hospital or clinic, group of hospitals, health maintenance organisation, or an appropriate sample of those cases (e.g. random sample) *
b) potential for selection biases or not stated
3) Selection of Controls
a) community controls (same community as cases) *
b) hospital controls (within same community as cases, but derived from hospitalised population)
c) no description
4) Definition of Controls
a) no history of disease (endpoint) *
b) no description of source
Comparability (max 2)
1) Comparability of cases and controls on the basis of the design or analysis
a) study controls for age, sex, and education *
b) study controls for any additional lifestyle or genetic factor (e.g. smoking, alcohol, physical activity, BMI, APOE4) *
Exposure (max 4)
1) Ascertainment of exposure (MIND diet)
a) FFQ/ ≥3x 24h recall/ food diary AND MIND total score ≥13 points *
b) structured (dietician) interview AND MIND total score ≥13 points *
c) written self-report OR MIND total score <13 points
d) no description
2) Same method of ascertainment for cases and controls
a) yes *
b) no
3) Non-Response rate (drop-outs)
a) same rate for both groups *
b) non respondents described
c) rate different and no designation
4) Statistical test
a) The statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented including confidence intervals and the probability level (p value) *
b) The statistical test is not appropriate, not described or incomplete

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for the comparability question. Scoring: Good quality: ≥3 stars in selection domain AND 2 stars in comparability domain AND ≥3 stars in exposure domain. Fair quality: ≥2 stars in selection domain AND ≥1 stars in comparability domain AND ≥3 stars in exposure domain. Poor quality: <2 stars in selection domain OR 0 stars in comparability domain OR <3 stars in exposure domain.

Table S4. Newcastle-Ottawa Quality Assessment Scale (NOS) adopted for cross-sectional studies.

Selection (max. 3)
1) Representative of the sample
a) truly representative of the average in target population in the target population (random sample or whole population) *
b) somewhat representative of the average in target population in the target population (non-random sample) *
c) selected group/convenience sample
d) no description of the sampling strategy
2) Non-respondents
a) comparability between respondents and non-respondents characteristics is established, or the response rate is satisfactory (>70%)*
b) the response rate is unsatisfactory, and the comparability between respondents and non-respondents is unsatisfactory
c) no description of the response rate or the characteristics of the responders and non-responders
3) Ascertainment of the exposure (MIND diet)
a) FFQ/ ≥3x 24h recall/ food diary AND MIND total score ≥13 points *
b) structured (dietician) interview AND MIND total score ≥13 points *
c) written self-report OR MIND total score <13 points
d) no description
Comparability (max. 2)
4) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled for
a) study controls for age, sex, and education *
b) study controls for any additional lifestyle or genetic factor (e.g. smoking, alcohol, physical activity, BMI, APOE4) *
Outcome (max. 2)
5) Assessment of the outcome (brain health)
a) independent or blind assessment *
b) record linkage *
c) self-report
d) no description
6) Statistical test
a) the statistical test used to analyse the data is clearly described and appropriate, and the measurement of the association is presented including confidence intervals and the probability level (p value) *
b) the statistical test is not appropriate, not described or incomplete

Note: This scale was a modified version of the NOS scale, as used in several other review studies. A maximum of two point can be given for Comparability. Scoring: Good quality: ≥2 stars in selection domain AND ≥2 stars in comparability domain AND ≥1 stars in outcome domain. Fair quality: ≥1 stars in selection domain AND ≥1 stars in comparability domain AND ≥1 stars in outcome domain. Poor quality: 0 stars in selection domain OR 0 stars in comparability domain OR 0 stars in outcome domain.

Table S5. The Newcastle-Ottawa Scale (NOS) scores for cohort studies included in the review.

Study (authors)	Outcome category	Representative of exposed sample	Selection non-exposed	Ascertain-ment exposure	Not present at start	Design or analysis	Assess-ment of outcome	Follow -up	Adequacy follow-up	Statistics	Study quality
Van Lent (2021)	Cognitive decline	1	1	1	0	2	0	1	1	1	Good
Dhana (2021)	Cognitive decline	1	1	1	0	1	0	0	0	1	Poor
Cherian (2019)	Cognitive decline	1	1	1	0	2	1	1	0	1	Good
Morris (2015)	Cognitive decline	1	1	1	0	2	1	1	1	1	Good
Berendsen (2018)	Cognitive decline	0	1	1	0	2	1	1	1	1	Fair
Nishi (2021)	Cognitive decline	1	1	1	1	2	0	1	1	1	Good
Vu (2022) - CHAP	Cognitive decline	1	1	1	0	2	1	0	0	1	Good
Vu (2022) - MAP	Cognitive decline	1	1	1	0	2	1	0	0	1	Good
Boumenna (2022)	Cognitive decline	1	1	1	0	2	1	1	1	1	Good
Munoz-Garcia (2020)	Cognitive decline	0	1	1	0	2	0	1	1	1	Fair
Shakersain (2018)	Cognitive decline	0	1	0	0	2	0	1	0	1	Poor
Lotan (2022)	Cognitive decline	1	1	1	0	2	1	1	1	1	Good
Huang (2023)	Cognitive decline	1	1	0	0	2	0	0	1	1	Fair

Table S5. (*continued*)

Study (authors)	Outcome category	Representative of exposed sample	Selection non-exposed	Ascertain-ment exposure	Not present at start	Design or analysis	Assess-ment of outcome	Follow -up	Adequacy follow-up	Statistics	Study quality
Adjibade (2019)	SMC	1	1	1	1	2	1	1	1	1	Good
Dong (2023)	Cognitive decline	1	1	0	0	0	0	0	0	0	Poor
Hosking (2019	MCI	1	1	1	1	2	1	1	1	1	Good
Thomas (2022)	Dementia	1	1	1	1	2	1	1	0	1	Good
Morris (2015)	Dementia	1	1	1	1	2	1	0	0	1	Good
Vu (2022) - CHAP	Dementia	1	1	1	1	2	1	0	0	1	Good
Vu (2022) - MAP	Dementia	1	1	1	1	2	1	0	0	1	Good
Vu (2022) - WHIMS	Dementia	1	1	1	1	2	1	0	1	1	Good
de Crom (2022)	Dementia	1	1	1	1	2	1	1	0	1	Good
Hosking (2019)	Dementia	1	1	1	1	1	1	1	1	1	Fair
Cornelis (2023)	Dementia	1	1	0	0	2	1	1	1	1	Fair
Zhang (2023)	Dementia	1	1	0	1	2	0	1	0	1	Good
Chen (2023), Whitehall II study	Dementia	0	1	1	1	2	1	1	1	1	Good

Table S5. (*continued*)

Study (authors)	Outcome category	Representative of exposed sample	Selection non-exposed	Ascertain-ment exposure	Not present at start	Design or analysis	Assess-ment of outcome	Follow -up	Adequacy follow-up	Statistics	Study quality
Chen (2023), Health and Retirement Study	Dementia	1	1	1	1	2	1	0	1	1	Good
Chen (2023), Framingham Heart Study	Dementia	1	1	1	1	2	1	1	0	1	Good
Agarwal (2018)	Parkinson 's disease	1	1	1	1	1	1	0	0	0	Poor
Dhana (2021)	Brain pathology	1	1	1	0	2	1	0	0	1	Good
Chen (2021)	Brain volumes	1	1	1	0	2	1	1	1	1	Good
Agarwal (2023)	Brain pathology	1	1	1	0	2	1	1	1	1	Good
Dong (2023)	Brain pathology	1	1	0	0	0	1	0	0	0	Poor
Wagner (2023)	Cognitive resilience	1	1	1	0	2	1	1	0	1	Good

Abbreviations: MCI: Mild cognitive impairment; SMC: subjective memory complaints

Table S6. The Newcastle-Ottawa Scale (NOS) scores for case-control studies included in the review.

Author (year)	Outcome category	Case definition adequate	Represent-ativeness cases	Selection controls	Definition controls	Design or analysis	Assess-ment of exposure	Same method	Non-response rate	Statistics	Study quality
Vassilo-poulou (2022)	Dementia	1	0	0	1	1	0	1	0	1	Poor
Filippini (2020)	Dementia	0	0	1	0	2	1	1	1	1	Poor

Table S7. The Newcastle-Ottawa Scale (NOS) scores for cross-sectional studies included in the review.

Author (year)	Outcome category	Representative of exposed sample	Non-respondents	Ascertainment exposure	Design or analysis	Assessment of outcome	Statistics	Study Quality
Van Lent (2021)	Cognitive function	1	1	1	2	0	1	Good
Vassilopoulou (2022)	Cognitive function	0	0	0	1	1	0	Poor
Berendsen (2018)	Cognitive function	0	0	1	2	1	1	Fair
Calil (2018)	Cognitive function	0	0	1	0	1	1	Poor
McEvoy (2017)	Cognitive function	1	1	1	2	0	1	Good
Gauci (2022)	Cognitive function	1	1	0	2	1	0	Good
Huang (2022)	Cognitive function	1	0	0	2	0	1	Fair
Ahn (2022)	Cognitive function	1	0	1	2	0	1	Good
Boumenna (2022)	Cognitive function	1	1	1	2	1	1	Good
Yeung (2022)	Cognitive function	1	1	0	2	0	1	Good
Wesselman (2021)	Cognitive function	1	1	1	2	0	1	Good
Escher (2022)	Cognitive function	0	0	1	2	0	0	Poor
Huang (2023)	Cognitive function	1	1	0	2	0	1	Good
Zare (2023)	Cognitive function	1	0	0	0	1	0	Poor

Table S7. (*continued*)

Author (year)	Outcome category	Representative of exposed sample	Non-respondents	Ascertainment exposure	Design or analysis	Assessment of outcome	Statistics	Study Quality
Lawrie (2022)	Mild Cognitive Impairment	1	0	1	2	0	0	Poor
Huang (2022)	Mild Cognitive Impairment	1	0	0	2	0	1	Fair
Metcalfe-Roach (2021)	Parkinson's disease	1	0	1	2	0	0	Poor
van Lent (2021)	Brain Volumes	1	0	1	2	1	1	Good
Escher (2022)	Brain Volumes	0	0	1	2	1	1	Fair
Zhang (2023)	Brain Volumes	1	1	1	2	1	1	Fair

Table S8. The Cochrane Risk of Bias tool in Randomized Controlled Trials (Rob2) scoring of studies included in the review.

Study (authors)	Outcome category	Randomization Process	Deviation from intended interventions	Missing outcome data	Measurement of the outcome	Selection of the reported result	Overall Bias
Arjmand (2022)	Cognition	0	+	+	+	0	0
Barnes (2023)	Cognition	+	+	+	+	+	+
Barnes (2023)	Brain Volumes	+	+	+	+	+	+

Key: +: Low risk of bias; 0: some concerns; -: high concerns; n/a: not assessed.

Table S9. Overview MIND diet scoring methodology per included article.

	Components			Scoring		
Author (year)	# of items	Similar to original MIND diet	Explanation	Type of scoring	Similar to original MIND diet	Explanation
McEvoy (2017)	15	No	Several cabbages considered green leafy vegetable; peas considered beans	Based on set serving size	Yes	n/a
Ahn (2022)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Van Lent (2021)	15	Yes	n/a	Based on set serving size	Yes	n/a
Berendsen (2018)	15	Unknown	No detailed food classification provided	Based on set serving size	Yes	n/a
Boumenna (2022)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Huang (2023)	12	No	Excluded: olive oil, butter/margarine, and cheese. Red wine replaced with tea	Based on cut-points	No	Based on tertiles
Wesselman (2021)	15	Yes	n/a	Based on set serving size	Yes	n/a
Escher (2022)	15	Unknown	No detailed food classification provided	Based on set serving size	Yes	n/a
Gauci (2022)	15	Unknown	No detailed food classification provided	Based on set serving size	Yes	n/a
Zare (2023)	14	No	Excluded: wine	Based on set serving size	Yes	n/a

Table S9. (*continued*)

	Components			Scoring		
Author (year)	# of items	Similar to original MIND diet	Explanation	Type of scoring	Similar to original MIND diet	Explanation
Huang (2022)	12	No	Replaced: whole grains to type of staple food, berries to fresh fruit, beans to soybeans, olive oil to vegetable oil, wine to tea, green leafy vegetable and other vegetables to fresh vegetables and mushroom/ algae. Removed: Poultry, butter/ margarine, cheese, red meat and products, fast fried foods. Added: Garlic	Based on set serving size	No	Scoring differs for all components but fish and nuts
Vassilopoulou (2022)	9	No	Excluded: other vegetables, beans, poultry, cheese, olive oil, red meat	Based on set serving size	Unknown	Referred to original article, but additional explanation inadequate to make a proper comparison
Calil (2018)	15	Yes	n/a	Based on set serving size	No	Scoring differs for vegetables and whole cereals
Yeung (2022)	9	No	Excluded: olive oil, beans, fish, poultry, fried/fast foods, and red meat and products	Based on set serving size	Unknown	Not reported
Vu (2022)	15	Unknown	Supplementary table cannot be opened	Based on set serving size	Unknown	Supplementary table cannot be opened
Cherian (2019)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Morris (2015a)	15	Yes	Original article	Based on set serving size	Yes	Original article

Table S9. (*continued*)

	Components			Scoring		
Author (year)	# of items	Similar to original MIND diet	Explanation	Type of scoring	Similar to original MIND diet	Explanation
Dhana (2021)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Not reported
Nishi (2021)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Lotan (2022)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Dong (2023)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Munoz-Garcia (2020)	15	Unknown	No detailed food classification provided	Based on set serving size	Yes	n/a
Shakersain (2018)	14	No	Replaced: olive oil to vegetable oil. Excluded: nuts	Based on cut-points	No	Based on sex-specific population median. For brain healthy foods, intake below the median was scored 0 and scores 1 to 5 were assigned to quintiles of intakes above the median. For brain unhealthy foods, scoring was reversed.
Filippini (2020)	15	Unknown	No detailed food classification provided	Unknown	Unknown	Referred to original article, but no additional explanation given

Table S9. (*continued*)

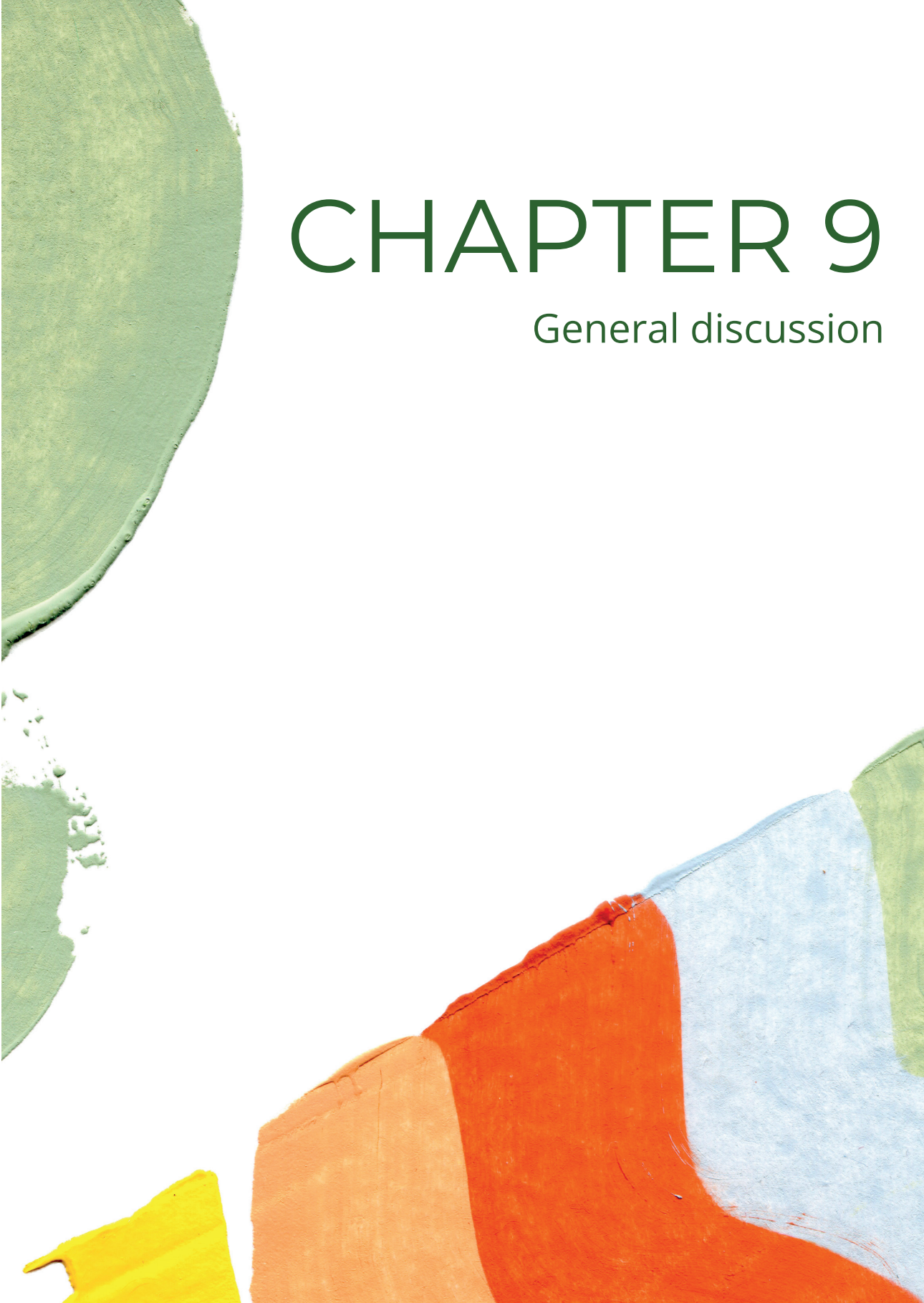
	Components			Scoring		
Author (year)	# of items	Similar to original MIND diet	Explanation	Type of scoring	Similar to original MIND diet	Explanation
Thomas (2022)	15	No	Replaced: berry intake to total polyphenol intake	Based on set serving size	No	Scoring adapted to French dietary habits and guidelines for fish, other vegetables, green leafy vegetables, nuts, whole grain
Morris (2015b)	15	Yes	Original article	Based on set serving size	Yes	Original article
de Crom (2022)	15	No	Cabbage included as green leafy vegetable, flax seeds included as nuts, mussels included as fish	Unknown	Unknown	Referred to original article, but no additional explanation given
Hosking (2019)	13	No	Excluded: butter/margarine and olive oil	Unknown	Unknown	Referred to original article, but no additional explanation given
Cornelis (2023)	15	No	Seeds included as nuts, shellfish included as fish	Based on set serving size	No	For butter/margarine scoring is reported to be opposite to original (might be error in reporting). Scoring differs for wine
Zhang (2023)	14	No	Excluded: olive oil	Based on cut-points	No	Based on quintiles
Chen (2023) - WII cohort	14	No	Excluded: olive oil	Based on set serving size	No	Scoring differs for berries, nuts and whole grains (whole grains might be error in reporting)
Chen (2023) - HRS & FHS - offspring	15	Unknown	No detailed food classification provided	Based on set serving size	No	Scoring differs for berries, nuts and whole grains (whole grains might be error in reporting)

Table S9. (*continued*)

	Components			Scoring		
Author (year)	# of items	Similar to original MIND diet	Explanation	Type of scoring	Similar to original MIND diet	Explanation
Lawrie (2022)	15	Unknown	Supplementary table cannot be opened	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Adjibade (2019)	15	Unknown	No detailed food classification provided	Based on set serving size	Yes	n/a
Wagner (2023)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Metcalfe-Roach (2021)	15	Unknown	No detailed food classification provided	Based on set serving size	Yes	n/a
Agarwal (2018)	15	Unknown	No detailed food classification provided	Based on set serving size	Unknown	Referred to original article, but no additional explanation given
Agarwal (2023)	15	Yes	n/a	Based on serving size	Unknown	Referred to original article, but no additional explanation given
Chen (2021)	15	No	Canola included as olive oil. Kiwi included as berry. Seeds included as nuts.	Based on serving size	No	Scoring differs for whole grains, butter/margarine
Arjmand (2022)	15	No	Replaced: wine to grape juice	Based on serving size	Yes	n/a
Barnes (2023)	14	No	Excluded: wine	Unknown	Unknown	Not reported

CHAPTER 9

General discussion



AIM AND MAIN FINDINGS

The central question addressed in this thesis was to what extent combinations of specific nutrients were beneficial for brain ageing. In the first part (**chapters 2-4**), this question was approached by studying the effect of interactions between specific nutrients on brain ageing. The second part (**chapters 5-8**) focussed on dietary patterns that provide different nutrients that are considered relevant to maintain brain function during ageing.

The main findings of these chapters are summarized in **table 1**. First, we demonstrated that the efficacy of folic acid supplementation on cognitive functioning was dependent on omega-3 fatty acid status. Only individuals with a lower omega-3 fatty acid status benefited from supplementation (**chapter 2**). In **chapter 3**, we included a second B-vitamin, specifically B12, and studied the interaction between supplementation of vitamin B12 and folic acid with omega-3 fatty acid status. Surprisingly, in this study only individuals with *higher* DHA status benefitted from supplementation with vitamin B12 and folic acid. In **chapter 4** we further demonstrated the importance of considering interactions between nutrients by showing a considerable association between a combined suboptimal status of omega-3 fatty acids, vitamin D and homocysteine with dementia risk. With respect to the dietary patterns, we demonstrated in **chapter 5** that whilst the consumption of a plant-rich diet was related to a gastro-intestinal microbiota profile with anti-inflammatory potential, it was not associated with better cognition. Similarly, better adherence to a more plant-based diet was not associated with better cognitive function or slower cognitive decline. At the same time, greater adherence to a more plant-based diet with fish (**chapter 6**) and the EAT-Lancet diet (**chapter 7**) were associated with healthier cognitive ageing. Finally, in our systematic literature review we presented observational evidence for a beneficial association between the MIND diet with global cognition and dementia risk, but not for other brain ageing outcomes (**chapter 8**). Overall, these findings support our hypothesis that we should shift our focus from single nutrients to combinations and these findings feature the importance of certain B-vitamins, omega-3 fatty acids, vitamin D, antioxidants and polyphenols, all of which are more abundant in plant-rich diets and fatty fish.

Table 1: Summary of main findings of this thesis.

Ch.	Type of study	Exposure	Study population	Outcome	Results
2	Subgroup analysis of intervention study	Folic acid supplementation by omega-3 status	791 cognitively healthy adults aged 50-70y with elevated homocysteine levels from the FACIT trial	Cognitive decline	Efficacy of folic acid supplementation was related to omega-3 fatty acid status, with individuals with lower status benefiting more from folic acid supplementation compared to those with higher status (mean difference in treatment effect: 0.16±0.06 SU).
3	Subgroup analysis of intervention study	Vitamin B12 and folic acid supplementation by omega-3 status	187 cognitively healthy adults aged ≥65y with elevated homocysteine levels from the B-proof trial	Cognitive decline	Efficacy of B-vitamin supplementation was related to plasma DHA levels, but not to total plasma omega-3 or plasma EPA levels. Individuals with higher plasma DHA benefitted from B-vitamin supplementation, with a mean difference in treatment effect of 0.24±0.06 SU.
4	Longitudinal	Nutrient status index	968 non-demented adults aged ≥50y from the Framingham Heart Study, Offspring cohort.	Dementia incidence	Individuals with concurrent deficiencies of omega-3 fatty acids, homocysteine and vitamin D showed a 4-fold higher dementia risk compared to individuals with optimal levels for all nutrients.
5	Cross-sectional	Food groups and microbiota composition	226 cognitively healthy adults aged ≥65y from the NU-AGE trial	Cognition	Plant-rich diets high in fresh fruits and nuts, seeds and peanuts were related to a more anti-inflammatory microbial profile, but not to cognitive functioning.
6	Cross-sectional and longitudinal	Plant-based diet	658 cognitively healthy adults aged ≥65y with elevated homocysteine levels from the B-proof trial	Cognition & cognitive decline	Better adherence to a plant-based diet was not associated with cognitive ageing. Possibly, such association exists in a subpopulation of fish-consumers. Benefits translate to being 2.5 years younger in cognitive age for each standard deviation increase in plant-based diet adherence score.
7	Cross-sectional and longitudinal	EAT-Lancet diet	630 cognitively healthy adults aged ≥65y with elevated homocysteine levels from the B-proof trial	Cognition & cognitive decline	Better adherence to the EAT-Lancet diet was associated with better cognition and slower cognitive decline, equivalent to a cognitive age 4.5 years younger for each standard deviation increase in EAT-Lancet diet adherence score.
8	Systematic review of the literature	MIND diet	40 studies in adults aged ≥40y	Variety of outcomes related to brain ageing	Available evidence indicates positive associations between MIND diet adherence with global cognition and dementia, especially in North-American cohorts. Evidence was mixed for cognitive decline, and limited for brain volume, brain neuropathology and Parkinson's disease.

Abbreviations: EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; MIND: Mediterranean-Dietary Approaches to stop Hypertension Intervention for Neurodegenerative Delay.

In the present chapter, we will discuss the proposed mechanisms of action of combinations of nutrients, as well as how our findings fit with literature. Subsequently, we will reflect on methodological considerations in the field of nutrition and brain ageing, including exposures and interventions, outcome measures and populations to study. Finally, suggestions for further research are discussed.

BIOLOGICAL MECHANISMS OF ACTION

Brain ageing is a complex and multifactorial process, influenced by a combination of factors rather than being determined by a single cause. Indeed, as described in **chapter 1**, several biological changes take place in the brain during ageing. These include, but are not limited to, a reduction in brain size, a decline in the number of neurons and synapses, and the accumulation of the proteins β-amyloid and tau. Key age-related processes such as inflammation, oxidative stress and vascular dysfunction contribute to these biological changes.

Because brain ageing is a multifactorial process, interventions that target multiple aspects simultaneously are likely to provide the best outcomes to enhance brain health during ageing. The various nutrients investigated in this thesis act upon different mechanisms, as summarized in **figure 1**. Each single nutrient may be able to influence one or more of the key biological changes that occur during brain ageing. Via additive effects, it is likely that nutrient combinations may provide a better result than a nutrient in isolation. Similarly, if one of the key nutrients is limiting, adding other nutrients may have little effect.

In addition, it is possible that nutrients act in a synergistic manner. Possible mechanisms of action for synergistic effects between omega-3 fatty acids and B-vitamins, and omega-3 fatty acids and antioxidants have been proposed. As described in **chapter 2** and **3**, B-vitamins have a regulatory role in the transport of omega-3 fatty acids to the brain, via the regulation of one-carbon metabolism [1]. The mechanistic hypothesis for effectiveness of the combination of omega-3 fatty acids and antioxidants, as found in plant-rich diets with fish (**chapters 6-8**), involves the ability of anti-oxidants to decrease peroxidation of omega-3 fatty acids in neuronal membranes [2, 3]. To this end, the main finding of this thesis, that a combination of specific nutrients is beneficial for slowing brain ageing, is biologically plausible. Presumably, this is achieved via a combination of additive and synergistic mechanisms of action.

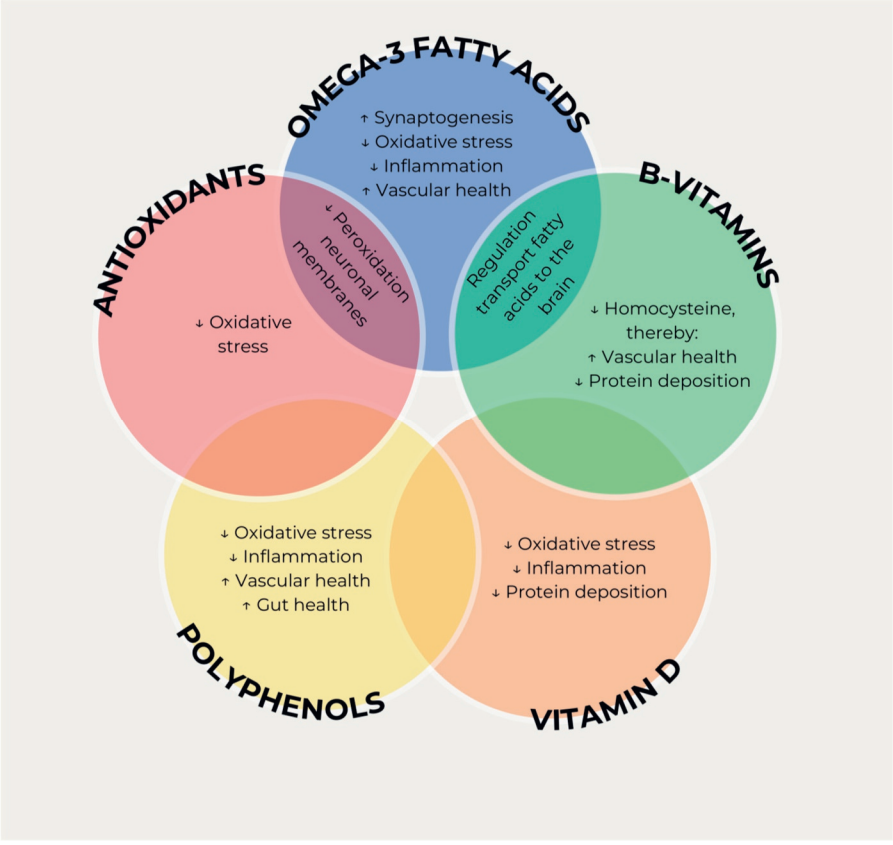


Figure 1: Overview of major single and combined mechanisms of action.

COMPARISON WITH PREVIOUS RESEARCH

In the first part of this thesis, we focused on the interaction between certain B-vitamins and omega-3 fatty acids (**chapters 2** and **3**) and these nutrients in combination with vitamin D (**chapter 4**). The literature on the interaction between these specific nutrients has already been extensively discussed in the respective chapters. In this part, we will adopt a broader view on multiple-nutrient interventions.

Further proof for the interaction between omega-3 fatty acids and B-vitamins comes from research on Souvenaid®, a medical food designed to support nutritional needs for individuals with early Alzheimer's disease (AD). Souvenaid® contains various precursors of synapse membranes, including omega-3 fatty acids and B-vitamins, as well as vitamins C and E, choline, phospholipids and selenium. Daily consumption of this medical food has demonstrated beneficial effects in mild stage AD patients on

synaptic connectivity [4] and brain atrophy [5]. The effect on cognitive functioning appeared to depend on the duration of the trial, with no effects being demonstrated after 2 years [5], but positive effects after 3 years of daily consumption [6].

Multiple-nutrient intervention studies lacking either omega-3 fatty acids or B-vitamins, however, demonstrate mixed results. The effect of multivitamin supplementation (including vitamins A, B, C, D, E and K, and other micronutrients, but without omega-3 fatty acids) has been investigated in two intervention studies. A first large (n=2,262) trial demonstrated that 3-year multivitamin supplementation improved various aspects of cognition in cognitively healthy older adults [7]. However, the effectiveness of multi-vitamin supplementation was not confirmed in another large trial (n=5,947), with long-term use of a multivitamin not affecting cognitive functioning in older male physicians [8]. Further null-results come from interventions that combine omega-3 fatty acids and polyphenols. Combined supplementation with fish oil and cocoa flavanols for one year in older adults with memory complaints did not result in cognitive improvements [9]. In line with these findings, older adults with self-perceived cognitive decline did not experience cognitive benefits from daily consumption of fish oil and anthocyanin rich blueberry powder for 24 weeks [10].

It could be hypothesized that the lack of supplementation with omega-3 fatty acids and/or B vitamins in these interventions is responsible for the null-findings. This would imply that these nutrients are essential in slowing brain ageing. This is further supported by studies that adopted a dietary pattern perspective, as only dietary patterns rich in plant-based foods and fish (**chapters 6-8**), and thus rich in both omega-3 fatty acids and B-vitamins, seemed beneficial for brain ageing. Our observation is in line with results from previous research on the widely investigated Mediterranean diet. Observational research has consistently demonstrated that better adherence to the Mediterranean diet is associated with better cognition, lower risk of cognitive impairment, and dementia [11]. Also, interventional research, though limited in number, supports the benefits of the Mediterranean diet for the ageing brain [12]. Finally, even though other dietary patterns have not been investigated as extensively as the Mediterranean diet, protective associations have been demonstrated for a wide variety of dietary patterns rich in plant foods and fish, including the Dietary Approaches to Stop Hypertension (DASH) diet, the MIND diet and dietary patterns based on local dietary guidelines [13-15].

Alternatively, it could be that the null-findings of the multiple-nutrient studies referred to above are the result of methodological limitations.

METHODOLOGICAL CONSIDERATIONS

Shifting the research focus from single- to multiple-nutrient strategies seems a promising approach. However, it is likely only one piece of the puzzle, as not all multiple-nutrient interventions have demonstrated positive results. In **chapters 2 to 8**, methodological considerations specific for the respective chapters have been addressed. In the following paragraphs, we will present a general overview of the methodological considerations essential to advance the field of nutrition and brain ageing. We will do this by reflecting on issues related to exposures and interventions, followed by discussing cognitive functioning as outcome measure. Subsequently, we will reflect on which populations may benefit from nutritional interventions to slow brain ageing.

EXPOSURES AND INTERVENTIONS

The main finding of this thesis relates to exposures and interventions, namely that we should shift from single- to multiple-nutrient strategies. In this part, we will discuss further aspects to consider with respect to multiple-nutrients and dietary patterns. Also, we will touch upon multi-domain interventions.

MULTIPLE-NUTRIENTS

The main finding of this thesis primarily relates to what kind of exposure/intervention is effective to slow brain ageing (multiple-nutrient). In addition to the 'what', it is also important to consider 'how much', i.e. the dose of a nutritional intervention.

With respect to omega-3 fatty acids, it has been hypothesized that doses of at least 2 grams per day are required to achieve beneficial effects on cognition. Previous omega-3 supplementation trials have primarily used doses of ~1 gram of DHA per day and have mainly demonstrated negative results [16], possibly because these doses are too low to increase brain DHA levels. The few studies that have been done on brain DHA delivery indicate that high dose DHA supplementation of ~2 gram per day is effective in modestly increasing brain DHA levels [17, 18]. Further research will show if the effects of high-dose DHA supplementation are also beneficial for cognition (NCT03613844).

Yet, it is important to note that higher doses are not always advantageous, as demonstrated by literature on B-vitamins. In this thesis, we made use of data from

the B-proof study, a randomized controlled trial on the effect of daily supplementation with 400 µg folic acid and 500 µg vitamin B12 versus placebo. While supplementation was effective in slowing down cognitive decline in a subgroup of participants with higher DHA levels (**chapter 3**), supplementation with these B-vitamins had some adverse effects. In a long-term follow-up of the participants, it appeared that allocation to the B-vitamin intervention was associated with a higher risk of cancer [19]. These results show that careful monitoring is required to make sure status does not exceed healthy thresholds.

DIETARY PATTERNS

Strategies based on dietary patterns naturally provide combinations of nutrients. The fact that different nutrients/foods within a dietary pattern may have cumulative beneficial effects may explain why research often shows stronger effect sizes for dietary patterns compared to single nutrients [15]. However, methods should be applied in a correct manner. In this part, we will touch upon important aspects to consider when working with dietary patterns, such as the food components to include, and the scoring and assessment of these components.

COMPONENTS

In this thesis, we demonstrated that consumption of a plant-rich diet without fish was not associated with cognitive functioning (**chapter 5** and **6**) while better adherence to plant-rich diets combined with fish, such as the EAT-Lancet (**chapter 7**) and MIND (**chapter 8**) diets, were associated with improved cognitive ageing. As discussed in the respective chapters, this makes sense from a nutrient perspective, as plant-rich diets combined with fish contain all nutrients crucial for healthy brain ageing. This underlines the concept that the food components included in a dietary pattern should cover all beneficial nutrients for the ageing brain.

In addition to the food components providing beneficial nutrients, it is important to consider which nutrients and foods to avoid, as consumption of unhealthy foods may attenuate the effects of a healthy diet on brain ageing. For example, higher consumption of foods belonging to a Prudent diet, such as vegetables, fruits and fish, was associated with less cognitive decline. However, additional consumption of foods belonging to a Western diet, rich in red and processed meat, sugar, and refined grains, attenuated the beneficial association [20]. Similarly, another study showed that an association between adherence to a Mediterranean diet with cognitive decline was present in individuals with low adherence, but not in individuals with high adherence to a Western dietary pattern rich in red and

processed meat, sugar, and refined grains [21]. The *a priori* dietary patterns discussed in this thesis all penalize high intakes of unhealthy foods. For example, the healthful plant-based diet index negatively scored intake of animal fat, refined grains, and sweets and desserts (**chapter 6**), the EAT-Lancet diet rated added sugar negatively (**chapter 7**), and in the MIND diet negative scores were given to red meat and products, pastries and sweets and fast and fried foods (**chapter 8**). Taking these detrimental foods and/or nutrients into account may be crucial to the demonstration of associations.

SCORING

Methods of scoring adherence to a specific diet varied largely between studies and dietary patterns, with differences in how cut-offs are set, how scoring is applied, and if weighting factors are used.

With respect to the cut-offs, we can distinguish two approaches in this thesis. The cut-offs for the plant-based diet index were based on population intake distribution (**chapter 6**) and the cut-offs for the EAT-Lancet and MIND diets were set *a priori* based on literature (**chapter 7** and **8**). Both methods have their advantages and disadvantages. Cut-offs based on population intake distributions are better able to capture contrasts in intake, which is particularly relevant when there is little variation in intake between participants. However, this method does not take into consideration the non-linear dose-response relationship between intake of a food group and healthier brain ageing. For example, the consumption of one portion of fish per week has been associated with lower risk of dementia, with higher consumption providing no additional benefits [22]. Also, the use of cut-offs based on population intake distribution limits comparison of results to other study populations.

It is also important to consider how scoring is applied. Both the number of components and the number of levels for scoring varied between the dietary patterns part of this thesis. The plant-based diet index included a total of 18 components that were scored into quintiles of intake, adding up to a hypothetical score range from 18 – 90 points (**chapter 6**). The EAT-Lancet diet comprised 14 components that were assigned a score ranging from 0 to 3 points, resulting in a score range from 0 – 42 points (**chapter 7**). The MIND diet is traditionally scored for 15 food components with scores 0, 0.5 or 1, and has a narrower score range of 0 – 15 points with a 0.5-point interval (**chapter 8**). A broader hypothetical score range

better allows to capture contrasts in intake, while a narrow hypothetical score range is easier to implement and understand.

Among the dietary patterns investigated in this thesis, none applied a weighting factor to the food components, thus assuming that all components are equally important to slowing brain ageing. This is likely not the case, which is substantiated by our finding in **chapter 6** that fish seemed crucial in the association between a plant-rich diet and cognitive ageing. One could apply weighting factors based on effect sizes, in line with the methods applied by Neuffer and colleagues [23]. Even though the adding of weights to food components adds complexity, it would be valuable to gain insight in the relative importance of food components in slowing brain ageing.

ASSESSMENT OF DIETARY INTAKES

In this thesis, we made use of two different methods to assess food intake: a 7-day food diary (**chapter 5**) and a food frequency questionnaire (FFQ) (**chapter 6** and **7**). Both methods are based on self-reporting, which is prone to bias due to social desirability and difficulties in estimation of portion sizes. Additionally, food frequency questionnaires rely on the participant's memory, and are limited by the foods being queried. These limitations are less relevant for 7-day food diaries, as foods are recorded when consumed and the open-ended questions allow high level of specificity. However, a 7-day food diary is less representative of usual intake and puts more burden on the participant compared to food frequency questionnaires [24]. Both dietary assessment methods have their limitations and the nutritional epidemiology field has been criticized because of this [25]. However, we have used the method that was most appropriate to answer our research questions. In **chapter 5**, we studied the cross-sectional association between intake of foods, gut microbiota and cognitive functioning. As gut microbiota composition rapidly responds to changes in diet [26], a method capturing current intake, such as a food record, is appropriate. In **chapters 6** and **7**, we studied the association between two dietary patterns and cognitive ageing, for which a method that reflects longer term intake, such as an FFQ, is deemed most appropriate. Additionally, we have taken approaches to limit bias. For example, we have adjusted for energy intake to increase validity, and our analyses were adjusted for confounders that have been associated with over- and underreporting, such as BMI [25].

Multi-domain interventions

The main focus of this thesis is on nutrition. However, as discussed in **chapter 1**, an unhealthy diet is not the sole risk factor for brain ageing. How the brain ages is influenced by a variety of lifestyle factors, including but not limited to physical inactivity, social isolation and smoking. By targeting multiple risk factors simultaneously, one can achieve an even greater effect on slowing brain ageing. This strategy is referred to as 'multi-domain interventions'.

In the field of dementia prevention, the FINGER study is the best-known example of a multi-domain intervention. In this Finnish trial, participants at risk for dementia underwent a variety of interventions including dietary guidance, exercise, cognitive training and management of vascular risk factors. Over a 2-year follow-up period, the multi-domain intervention improved cognitive functioning compared to the control condition [27]. If these encouraging findings can be extended to multiple populations and settings around the world is currently being tested within the world-wide FINGERS network [28].

OUTCOME MEASURE: FROM COGNITIVE TESTS TO BIOMARKERS

Cognitive functioning can be measured directly through various methods, such as generic tests or a neuropsychological test batteries. Alternatively, biomarkers can be used as an indirect proxy for cognitive functioning. Selecting the appropriate method is crucial for obtaining reliable results. In the paragraphs below, we will discuss each of these methods.

Generic tests

Cognitive functioning can be assessed using generic tests, such as the Mini-Mental State Examination (MMSE), Telephone Interview for Cognitive Status modified (TICS-m), or Montreal Cognitive Assessment (MoCA). These generic tests have been originally developed as screening tools for cognitive impairment or dementia and are widely used because of their affordability and ease of administration. However, despite these advantages, these tests lack sensitivity. Generic tests are not sensitive to subtle differences in cognition and cannot be used to assess cognition in individuals without cognitive impairment due to ceiling effects. Moreover, they may not adequately capture subtle effects of dietary interventions [29]. This lack of sensitivity is likely responsible for the null-finding we observed in **chapter 8** on the association between adherence to the MIND diet and cognition as measured with generic tests, with positive associations being demonstrated in only 2 of 6 cross-sectional and 2 of 5 longitudinal studies. Thus, while generic tests serve as valuable

initial screening tool, more sensitive assessments may be needed for a comprehensive evaluation of cognitive function and to capture effects of dietary interventions.

NEUROPSYCHOLOGICAL TEST BATTERY

A more sensitive method to measure cognitive functioning involves the use of a wide variety of different cognitive tests that measure different cognitive concepts prone to decline with ageing. The outcomes of these tests can be combined into cognitive domain composite scores to establish robust measures. We have applied this approach in **chapters 2, 3, 5, 6,** and **7**, where we established a global cognition composite as an overarching measure of cognitive functioning, and/or composites for domain-specific cognitive functioning, such as episodic memory, information processing speed, or executive functioning.

Our findings mainly point towards associations or effects on global cognition, with positive findings in all chapters that included this measure (**chapters 2, 3, 6, 7, 8**). These positive findings are likely because this measure is most robust. Small benefits on all separate cognitive domains may have a cumulative effect on global cognition, thereby providing greater statistical power to detect effects.

With respect to domain-specific cognitive functioning, one could expect the most prominent effects on executive functioning and episodic memory, as these domains primarily decline during the preclinical stage of AD [30]. However, our findings are mixed. For executive functioning, we only demonstrated positive associations with higher adherence to the EAT-Lancet diet (**chapter 7**), but not in any other chapter (**chapters 2, 3, 5, 6,** and **8**). For episodic memory, our systematic review on the MIND diet demonstrated positive associations between higher adherence to the MIND diet and episodic memory in the majority of included cohorts (**chapter 8**), although we did not demonstrate positive findings in any of our own analyses (**chapters 2, 3, 5, 6,** and **7**). These mixed findings may be explained by the specific choice of cognitive tests, as the sensitivity to cognitive changes and their validity varies between tests [31]. Alternatively, differences in construction of cognitive domains may limit comparability of findings. This highlights the need for a standardized cognitive test battery that is sensitive to capture nutritional associations and effects in individuals during the preclinical stage of dementia. In addition, harmonized rules on how to construct cognitive domains are required, to facilitate comparison between studies.

BIOMARKERS OF DEMENTIA

In addition to measuring cognitive function with a neuropsychological test battery, biomarkers of AD could provide indirect information about cognitive functioning. These biomarkers hold significance as they become abnormal before cognitive decline is detectable by cognitive tests [32]. According to a model of the temporal pattern of biomarker abnormalities for AD-related pathophysiological processes as visualized in **figure 2** [33], the earliest indicator of the preclinical state of AD is the accumulation of β-amyloid, which can be measured in cerebrospinal fluid (CSF) or in plasma. This is followed by an increase in CSF and plasma tau levels. Subsequently, β-amyloid can be measured via PET imaging, and markers of glial activation (CSF sTREM2) and synaptic dysfunction (CSF neurogranin) increase. Then, hippocampal atrophy as measured with MRI becomes apparent, along with other neurodegeneration (CSF neurofilament light) and synaptic dysfunction (FDG and SV2A PET) biomarkers. Not long thereafter, cognitive functioning starts to decline. It is thought that the first indicator (β-amyloid accumulation) precedes the decline in cognitive function by more than a decade [34], highlighting the potential of using these biomarkers as outcome measure. However, it should be noted that a causal relationship between preventing biomarker levels to become abnormal and slowing of cognitive decline has not been demonstrated to date.

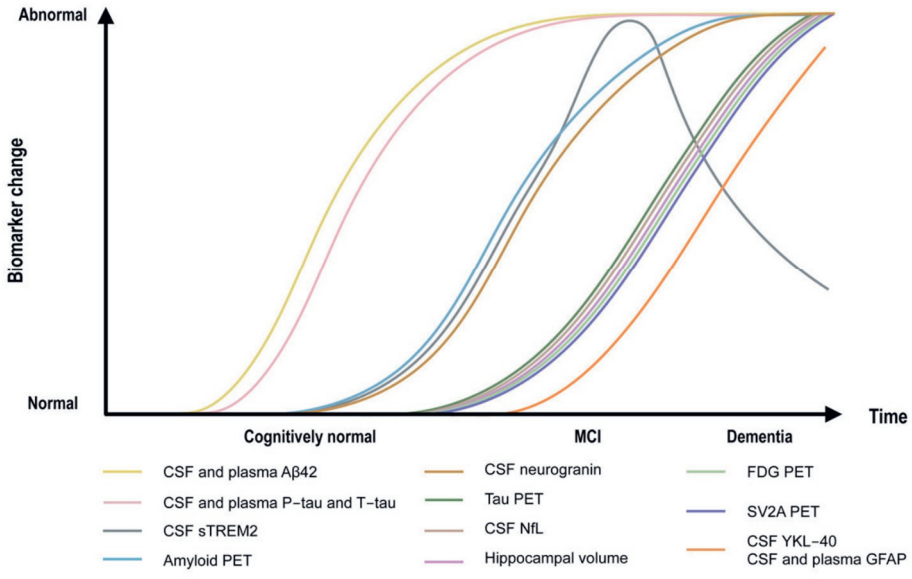


Figure 2: Model of the temporal pattern of biomarker abnormalities for Alzheimer's disease related pathophysiological processes, adapted from Zetterberg 2021 [33].

The field of AD biomarker research is quickly advancing. While some of the biomarkers mentioned have only been developed recently, others have been around for a longer time. Among these biomarkers, brain atrophy and β-amyloid and tau accumulation have already been investigated in relation to diet. A systematic review summarizing data from 9 longitudinal studies on the association between dietary patterns and brain imaging outcomes, suggested protective associations between healthy dietary patterns and reduced brain atrophy and β-amyloid accumulation [35]. Similarly, a second systematic review that also included cross-sectional and interventional data confirms these findings [36] and a third systematic review extends these findings to plasma biomarkers of AD [37]. These systematic reviews did not include studies on the MIND diet. According to our review on this diet (**chapter 8**), biomarkers of cognition including CSF tau and brain volume had only been addressed in few studies (n=5) that so far did not hint towards protective associations. It should be noted that the majority of these studies had a cross-sectional design, which may explain the null-findings.

When investigating the association between nutritional factors and biomarkers of dementia, it is important to be aware of the concept of cognitive reserve: the brain's resilience to cope with neurological damage. This level of resilience is not the same for everyone: individuals with higher cognitive reserve may show better cognitive functioning despite presence of biomarker pathology, while others with lower cognitive reserve may experience faster cognitive decline in the presence of a similar level of biomarker pathology [38]. Research on the role of diet in cognitive reserve is very limited [39], though a recent study demonstrated that higher adherence to the MIND diet was associated with better cognitive reserve [40], demonstrating the importance of taking this factor into account.

STUDY POPULATION: TOWARDS A PERSONALIZED APPROACH

When selecting a study population for research on nutrition and brain ageing, four aspects should be taken into account: 1) baseline nutritional intake and/or status; 2) biological susceptibility of the study population to the nutritional intervention (i.e. mechanism of action); 3) baseline cognitive status, and 4) ApoE genotype.

Baseline nutritional intake and/or status

One possible explanation why many intervention studies have demonstrated null-findings may be the lack of considering baseline nutrient intake and/or status. Even though researchers have already raised concerns about this issue over a decade ago

[41], still many nutritional interventions are designed to test the hypothesis that the intervention is beneficial for health, irrespective of baseline nutrient intake and/or status. However, when a participant already consumes a healthful diet (and thus is in the adequate range of intake) or when a participant does not have a nutritional deficiency (thus is in the adequate range of status), further nutritional intervention is unlikely to yield any additional benefit (**figure 3**).

Several trials have presented their results stratified by baseline status, and demonstrated the importance of considering this factor in the design of future studies. For example, the MAPT trial demonstrated null-findings for the effect of supplementation with EPA and DHA for 3 years in frail older adults. Interestingly, there was a trend towards effect of supplementation in individuals with low baseline omega-3 index at baseline [42]. Likewise, B-vitamin supplementation in women with cardiovascular disease or cardiovascular disease risk factors was not effective in slowing cognitive decline, whereas a subgroup of participants with low dietary intake of B-vitamins did benefit [43].

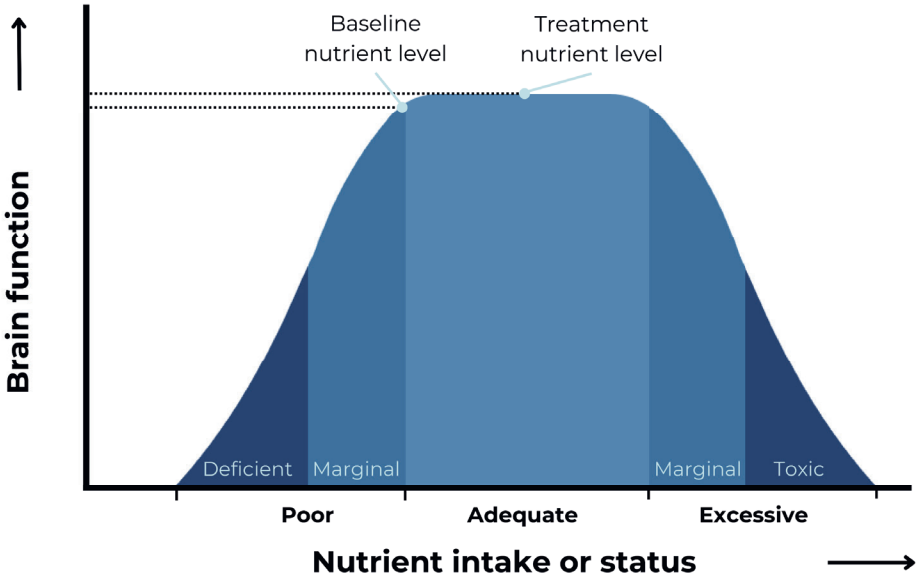


Figure 3: Relation between level of nutrient status or intake and brain function, adapted from Morris 2011 [41]. In individuals who are already at adequate baseline nutrient level, further improvements of intake or status provide no additional benefit for brain function.

All in all, further research should select participants based on their baseline nutritional intake or status. Preferably participants with suboptimal intake and/or

status of multiple nutrients are selected, considering that individuals with multiple nutritional deficiencies have a substantially increased risk of cognitive decline [44] and developing dementia (**chapter 4**; [23]). The nutrient status index that we developed in **chapter 4** may be used as screening tool to identify individuals likely to benefit from a nutritional intervention.

For observational research it is also important to consider nutritional intake or status. Generally, a wide variation in intake and/or status is preferred, as this creates contrast which allows the demonstration of associations. Our finding in **chapter 6**, that better adherence to a more plant-based diet was not associated with cognitive function or the rate of cognitive decline, may be explained by this lack of contrast. The level of adherence to a plant-based diet was measured with the plant-based diet index. Scores for this index could theoretically range from 18 (worst adherence) to 90 (best adherence) points, but in our study population scores ranged from 34 to 70 points, covering only 50% of the possible range. Contrary, a study that did demonstrate a beneficial association between adherence to a more plant-based diet with risk of cognitive impairment had a wider variation in plant-based diet index scores. In this study, scores ranged from 22 to 60 points with a theoretically possible range from 15 to 75 points, covering 73% of the possible range [45].

BIOLOGICAL SUSCEPTIBILITY TO THE NUTRITIONAL INTERVENTION

Another crucial factor to consider when selecting participants for nutrition trials is their biological susceptibility to the nutritional intervention. For example, if a nutritional intervention is expected to slow cognitive ageing by reducing low-grade inflammation, it would be prudent to select participants with elevated inflammation markers. While this seems a very straightforward approach, many intervention trials have not considered this aspect due to practical difficulties, i.e. having to take and analyse blood samples for screening of participants.

Some intervention studies that did take biological susceptibility into account, have demonstrated positive findings. For example, the FACIT trial, in which the effect of folic acid supplementation was tested in middle-aged adults with elevated homocysteine levels but without vitamin B12 deficiency, demonstrated improved cognitive functioning over 3 years [46]. Additionally, secondary analyses of intervention studies stratifying for biological susceptibility *a posteriori* support the importance of considering this factor. For instance, a secondary analysis of the VITACOG trial showed that B-vitamin supplementation to slow brain atrophy was

only effective in a subpopulation of participants with elevated homocysteine levels [47].

In this thesis, we further examined data from two intervention trials: the FACIT trial (**chapter 2**) and the B-proof trial (**chapter 3**). Both trials investigated the effect of supplementation with specific B-vitamins (folic acid with or without vitamin B12) and took biological susceptibility into account by selecting participants with elevated homocysteine levels. This may have been essential to the positive findings in these chapters, as in both we demonstrated an interaction between B-vitamin supplementation and omega-3 fatty acid status.

Baseline cognitive status

Considering baseline cognitive status when selecting a study population is crucial for two main reasons: 1) cognitive status is closely linked to biological susceptibility; and 2) selecting individuals at risk of cognitive decline is essential to effectively capture the effects of an intervention.

As discussed in the paragraph above, selecting participants based on biological susceptibility is difficult from a practical point of view. As a more feasible approach, many studies have chosen to select participants based on their cognitive state, as this gives an indication on the processes occurring in the body. An example where this has been successfully implemented involves trials on ketones, which are an alternative energy source to glucose. Uptake of glucose by the brain is unimpaired in cognitively healthy individuals, but reduced in individuals with mild cognitive impairment (MCI) or AD [48]. As a consequence, supplementation with ketones has shown to benefit cognition in individuals with MCI [49]. On a similar note, supplementation with probiotics to slow cognitive decline is mainly effective in cognitively impaired individuals (MCI, AD) [50-52] while effectiveness in cognitively healthy older adults is inconsistent [53, 54]. This observation may be attributed to the decreased microbial diversity in cognitively impaired, but not cognitively healthy individuals [55]. This may be a possible explanation why we did not demonstrate an association between gastro-intestinal microbiota composition and cognitive functioning in our sample of cognitively healthy older adults (**chapter 5**).

A second reason supporting the importance of considering baseline cognitive status involves the likelihood to decline over the intervention period. Cognitively healthy individuals may not deteriorate to an extent that is detectable by cognitive tests, making it impossible to demonstrate effects of an intervention. Therefore, it is

important to select participants who are likely to cognitively decline over the intervention period, such as persons who already experience subjective memory complaints or have been diagnosed with MCI. This is substantiated by a trial that showed that the effect of a polyphenol extract from grape and blueberry was dependent on baseline cognitive status. While daily consumption of the extract did not slow cognitive decline in the total study sample, a positive effect was demonstrated in participants with poorer memory performance at baseline [56].

The relevance of ApoE Genotype

In the field of nutrition and brain ageing research, ApoE genotype is an important but complex modifying factor. This complex role of ApoE genotype is reflected in the contradictory findings in this thesis. In **chapter 4**, we presented that ApoE genotype influences the association between multi-nutrient deficiencies and dementia risk, with a strong association in carriers but not in non-carriers of the ApoE4 allele. ApoE carrier status also emerged as an effect modifier in our systematic review on the MIND diet in relation to brain ageing (**chapter 8**). However, whether carriers or non-carriers benefit from better adherence to the MIND diet remains unclear, as both improved MIND-diet related brain ageing had been demonstrated among carriers and non-carriers. At the same time, no interaction was found in **chapter 2**, where ApoE4 carrier status did not influence the interaction between folic acid supplementation and omega-3 fatty acid status. Possibly, a lack of statistical power may have been responsible for this null-finding.

It has been hypothesized that the interaction with ApoE genotype depends on the clinical state of the participant, with ApoE4 carriers being susceptible to interventions in preclinical stages while benefits in the clinical stages are limited to non-carriers. This pattern has both been observed for preventive lifestyle strategies [57] and omega-3 fatty acids [58, 59]. However, it remains to be confirmed whether this pattern applies for other nutrients as well. Consequently, it is too early to make recommendations on how we should deal with ApoE genotype in the design of future trials. However, for further research it is strongly encouraged to determine ApoE genotype to be able to stratify for this factor.

FURTHER RESEARCH

There is increasing support for the idea that multiple nutrients, acting in conjunction, are crucial for healthy brain ageing. Strategies based on this concept, combining omega-3 fatty acids, folic acid and vitamin B12, vitamin D, antioxidants, and polyphenols, are a promising way to advance the field. However, many aspects of the field remain unexplored: not all nutrients relevant to brain ageing have been studied to the same extent, optimal nutrient thresholds required are unknown, and the relative importance of individual nutrients in combination with others is still largely unknown. Moreover, there is a considerable need for intervention studies to establish causal relationships between multiple-nutrients and brain ageing.

To advance the field, we propose a 2-step approach for further research. The first step focuses on obtaining a complete view of the interactive properties of nutrients, and in a second step this knowledge should be implemented in the design of intervention studies.

The first step should comprise secondary analyses of existing single-nutrient interventions, to test to what extent effects of their supplementation may depend on status and/or intake of other nutrients. This could be a practical and cost-effective approach to gain more insight in the interactive properties between nutrients, and to define the optimal thresholds of nutrient statuses and/or intakes. Additionally, observational research on dietary patterns could focus on investigating relative weights of nutrients on slowing brain ageing, to gain more insight in the relative importance of nutrients.

In the second step, intervention studies could be designed based on these and previous findings. These proposed studies should have an ambitious setup: participants may either undergo multiple-nutrient supplementation or a dietary intervention with due attention to intake of all nutrients relevant to brain ageing. The methodological considerations as discussed in this chapter should be accounted for. This means that it is crucial to select a study population that is likely to benefit from the intervention, such as individuals with low nutritional status or intake, and/or individuals with biological susceptibility to the intervention. Additionally, the study population and the study duration should be chosen based on the likelihood to exhibiting a measurable decline in cognition during the follow-up period, and the outcome measure should be a sensitive neuropsychological test battery capable of capturing this decline as well as effects of the nutritional intervention. Alongside the cognitive tests, it is recommended to include the assessment of early biomarkers.

Finally, as already touched upon in chapters 6 and 7, it is important to broaden the perspective from brain ageing to ageing in general. Ageing not only affects the brain, but also other organs including the bone and the muscle. These organs may benefit from a diet rich in high-quality proteins, likely a more animal-based diet. Further research should consider investigating multiple ageing-related outcomes at once, to gain insight in the preferred diet for the ageing population.

CONCLUSION

To conclude, this thesis further substantiated the importance of the interplay between nutrients in relation to healthy brain ageing, with due attention to omega-3 fatty acids, folic acid and vitamin B12, vitamin D, antioxidants, and polyphenols. Based on secondary analyses of intervention studies, we demonstrated that efficacy of supplementation of folic acid with or without vitamin B12 is dependent on omega-3 fatty acid status. Based on observational research, we showed that concomitant deficiencies of omega-3 fatty acids, homocysteine and vitamin D were associated with considerably increased dementia risk. Additionally, plant-centred diets that include regular fish consumption, thus rich in all nutrients relevant to brain ageing, were associated with healthier cognitive ageing. The work in this thesis emphasizes the urgent need of shifting from single- to multiple-nutrient strategies. However, whether the relation between multiple-nutrients and brain ageing is causal remains to be investigated in intervention studies. In such studies, we should not only shift the focus from single- to multiple nutrient strategies, but also take a more personalized approach when it comes to selecting participants, and carefully select outcomes sensitive to capture the cognitive decline of these participants and effects of interventions. Further advancing the field will hopefully result in healthier cognitive ageing and fewer dementia cases, thereby reducing the personal, social and economic burden associated with cognitive decline.

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SUMMARY

Cognitive decline leading to dementia is a major public health problem. The enormous social impact and economic costs, along with the absence of curative treatment, highlight the urgent need for prevention. Nutritional interventions, particularly those with omega-3 fatty acids, vitamins B and D, antioxidants, and polyphenols, are considered promising preventive strategies. However, while mechanistic and observational research have demonstrated benefits of these individual nutrients on brain ageing, interventional research has not yet been able to confirm the positive effects. This discrepancy might stem from the fact that interventional studies have thus far focussed on single nutrients, not taking into account that different nutrients are involved in processes that are intertwined and dependent on each other. Therefore, the aim of this thesis was to shift the focus from single- to multiple-nutrient strategies and to thereby investigate to what extent combinations of specific nutrients are beneficial for brain ageing.

Chapter 2 and **3** describe the results of two secondary analyses of supplementation trials involving folic acid and vitamin B12, in which we investigated if the effects of these B-vitamins on cognition related to the omega-3 fatty acid status of the participants. In **chapter 2**, 791 adults aged 50-70 years with elevated homocysteine levels from the FACIT trial received daily supplementation with folic acid for 3 years. The effect of folic acid on cognitive functioning was found to be related to baseline omega-3 fatty acid (EPA + DHA) status; individuals in the lowest tertile of omega-3 fatty acid status benefitted from folic acid supplementation, while individuals in the highest tertile did not. The mean difference in treatment effect of folic acid on global cognition between these two groups was 0.16±0.06 standardized units. Furthermore, the interaction was mainly driven by EPA.

Chapter 3 included 191 adults aged ≥65 years with elevated homocysteine levels of the B-proof trial. The efficacy of daily supplementation with folic acid and vitamin B12 for 2 years on cognitive decline did not relate to total omega-3 fatty acid (EPA+DHA) or EPA status, but did relate to DHA status. Individuals in the highest, but not in the lowest tertile of DHA status benefitted from folic acid and vitamin B12 supplementation, with a mean difference in treatment effect of 0.24±0.06 standardized units. Despite the contrasting findings between chapters 2 and 3 regarding which subgroup would benefit from B-vitamin supplementation, findings from both chapters highlight the importance of considering interactions between nutrients.

Chapter 4 further confirms the importance of considering interactions between single nutrients. Using data from 968 non-demented adults aged 50 years or older from the Framingham Heart Study, Offspring cohort, we developed a nutrient status index that captured the number of suboptimal statuses of omega-3 fatty acids, vitamin D and homocysteine, the latter as marker of B-vitamin status. Suboptimal status of one or more of these nutrients was associated with dementia risk, with each additional suboptimal status elevating this risk by 50%. Individuals with a suboptimal status for all three nutrients showed a 4-fold higher dementia risk compared to those with optimal levels for all nutrients.

In **chapter 5**, we explored cross-sectional associations between diet, gastro-intestinal microbiota, and cognition in a sample of 226 Dutch healthy adults aged 65-79 years from the NU-AGE study. By performing multivariate analyses, we demonstrated a link between the inflammatory potential of the diet and the gastro-intestinal microbiota composition. An anti-inflammatory diet rich in plant foods such as fresh fruits and nuts was linked to a microbiota profile with higher anti-inflammatory potential, while a pro-inflammatory diet high in animal-based foods was associated with a higher pro-inflammatory gastro-intestinal microbial potential. Despite the prominent role of inflammation in cognitive ageing, we did not demonstrate associations with cognitive functioning.

In **chapter 6**, we studied the association between adherence to a more plant-based diet with cognitive functioning in cognitively healthy older adults aged ≥65 years, using data from the B-proof trial. Better adherence to a more plant-based diet or a healthy plant-based diet were both not associated with better cognitive function (n=658) or slower rates of cognitive decline (n=314), possibly because individuals consuming a more plant-based diet had lower intakes of vitamin B12, EPA and DHA. Interestingly, fish consumption appeared to influence the association, with only individuals who consume at least one portion of fish per week seeming to benefit from adhering to a more plant-based diet. These benefits equalled to being 2.5 and 4.6 years younger in cognitive age for each standard deviation increase in overall and healthy plant-based diet adherence score, respectively.

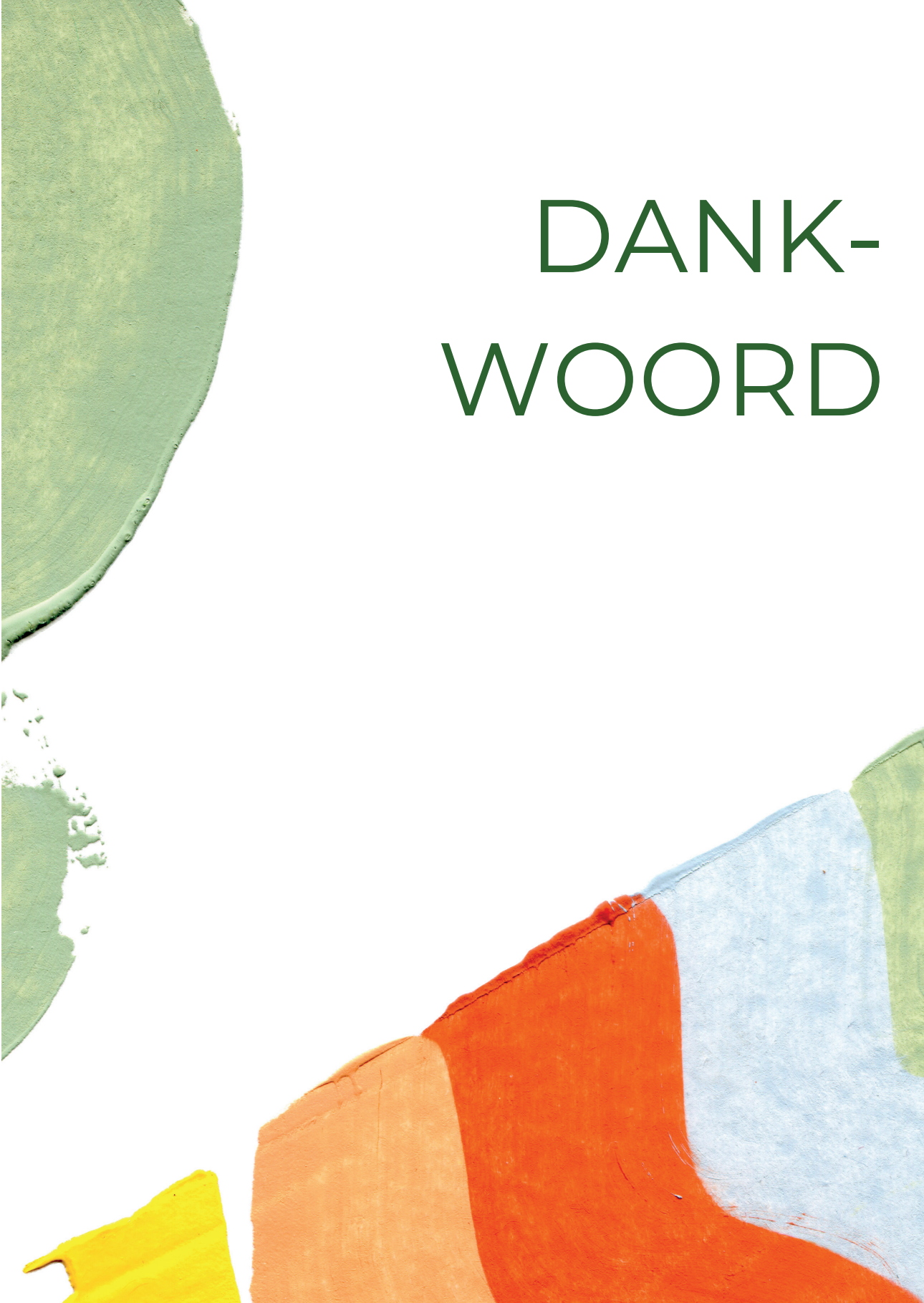
Chapter 7 examines another plant-focused dietary pattern: the EAT-Lancet diet. We investigated the association between the level of adherence to this healthy and sustainable dietary pattern with cognitive ageing using data from the same cohort as chapter 6. Higher adherence to the EAT-Lancet diet was associated with improved global cognition and a slower rate of cognitive decline, equivalent to a cognitive age

4.5 years younger for each standard deviation increase in EAT-Lancet diet adherence score. These findings further support the importance of plant-rich dietary patterns with fish for healthy cognitive ageing.

Chapter 8 provides a systematic review of the literature on the MIND diet in relation to brain ageing. A total of 38 observational and 2 interventional articles were included that examined a variety of brain health-related outcomes: global cognition, domain-specific cognition, cognitive decline, cognitive impairments, dementia, Parkinson's disease, brain volume and brain neuropathology. Observational studies indicated protective associations between higher adherence to the MIND diet with global cognition (3 of 4 cohorts), episodic memory (4 of 6 cohorts) and dementia risk (7 of 10 cohorts), with protective associations primarily reported for North American cohorts. Observational evidence for other brain outcomes and interventional evidence was mixed and/or limited.

Collectively, the findings presented in this thesis support our hypothesis that a shift from single- to multiple-nutrient strategies is necessary, with due attention to B-vitamins, omega-3 fatty acids, vitamin D, antioxidants and polyphenols. To further advance the field of nutrition and brain ageing research, secondary analyses of existing single-nutrient interventions could be performed to gain more insight into the interactive properties between nutrients and to define optimal thresholds for nutrient statuses and/or intake. Subsequently, these findings can serve as the basis for the design of intervention studies, in which participants with suboptimal nutrient statuses undergo personalized multiple-nutrient supplementation or a brain-healthy dietary intervention. Eventually, these advances should provide more insight into the most optimal combination of nutrients for healthy brain ageing, supporting dietary guidelines for maintaining good cognitive health into old age.

DANK-WOORD



Het zit erop! Na een periode van 5 hele leerzame en uitdagende jaren is mijn promotietraject toch echt ten einde. En dit was alles behalve een individuele prestatie. Want net zoals verschillende nutriënten samenwerken om hersenveroudering te vertragen, was het voltooien van dit promotietraject een echte gezamenlijke prestatie. In dit laatste hoofdstuk van mijn thesis wil ik graag mijn oprechte dank uitspreken aan iedereen die hieraan heeft bijgedragen!

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Een promotietraject bestaat uit hoogtepunten maar misschien nog wel meer tegenslagen. Eén daarvan is dat de BrainBerries trial er uiteindelijk niet van is gekomen. Ik heb echter wel veel hulp gehad met de voorbereidingen, waar ik erg dankbaar voor ben. **Henriette** en **Els**, dank jullie wel voor het meedenken over de opzet, planning, en alle logistiek rondom de bessen. **Wilma**, die helaas niet meer bij ons is, dank je wel voor de bijdragen aan het gut-brain deel van het onderzoek. Ik vond het ontzettend fijn met je samenwerken met jouw oprechte interesse en enthousiasme.

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ABOUT THE AUTHOR

CURRICULUM VITAE

Annick Pauline Marie van Soest was born on April 11th, 1994 in Nieuwegein, the Netherlands. After obtaining her secondary school diploma from Staring College Lochem in 2012, she started her bachelor studies Nutrition and Health at Wageningen University and Research. In her bachelor, she focussed on courses in nutritional physiology and health status and wrote a thesis about sensory decline in ageing. In 2015, she proceeded with the master Nutrition and Health with a specialization in Sensory Science and Eating Behaviour at the same university. For her MSc thesis, she studied the development of taste preferences in babies. During her internship at BioActor in Maastricht, she studied the effect of flavonoids on cognition. During her masters and directly thereafter, Annick worked as research assistant. Among others, she assisted in the writing of a grant proposal on protein intake and osteoporosis, assisted in the data collection of a trial on protein intake and resistance exercise for the prevention of sarcopenia, and she applied for medical ethical approval for a trial on fatty acids for healthy cognitive ageing.

After a small break on nutrition research when she worked as data manager paediatric oncology at Prinses Máxima Centrum, she started as PhD candidate Nutrition and Cognitive Ageing in May 2019. Under supervision of dr. Ondine van de Rest, prof. Lisette de Groot and prof. Renger Witkamp, Annick studied to what extent combinations of specific nutrients are beneficial for healthy brain ageing. The results of this work are described in this thesis. Besides research, she was involved in teaching in the BSc and MSc program Nutrition and Health, supervising thesis students, and as content coordinator in the Massive Open Online Course Nutrition for Healthy Ageing.

LIST OF PUBLICATIONS

van Soest APM, de Groot LCPGM, Witkamp RF, Melo van Lent D, Seshadri S, van de Rest O. Concurrent nutrient deficiencies are associated with dementia incidence. *Alzheimer's & Dementia.* In press.

van Soest APM, van de Rest O, Witkamp RF, de Groot LCPGM. The association between adherence to the EAT-Lancet diet and cognitive ageing. *Age and Ageing*. 2024;53(supplement 2), ii39-ii46. DOI: 10.1093/ageing/afae032

van Soest APM*, Beers S*, van de Rest O, de Groot LCPGM. The Mediterranean-Dietary Approaches to Stop Hypertension Intervention for Neurodegenerative Delay (MIND) Diet for the Aging Brain: A Systematic Review. *Advances in Nutrition*. 2024;15(3):100184. DOI: 10.1016/j.advnut.2024.100184

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* *shared first authorship*

OVERVIEW OF COMPLETED TRAINING ACTIVITIES

Discipline specific activities	Organizing institute	Year
Food and cognition event	Donders, NL	2019
Symposium Nutrition, exercise and sports	WUR, NL	2019
Symposium Pioneering Nutrition	WUR, NL	2019
Intestinal Microbiome of Humans and Animals	VLAG, NL	2019
Alzheimer's Association International Conference 2020	Alzheimer's association, online	2020
Nutrition and metabolism PIA meetings	Alzheimer's association, online	2020-2024
Symposium n-3 fatty acids, cognition and mental health	Rank Prize Funds, online	2021
Alzheimer's Association International Conference 2021	Alzheimer's association, online	2021
Nutritional Science Days	NAV, NL	2021
Alzheimer's Association International Conference 2022	Alzheimer's association, online	2022
Nutrition and the ageing brain	ILSI, PT	2022
Nutritional Science Days	NAV, NL	2022
Symposium n-3 fatty acids, cognition and mental health	Rank Prize Funds, UK	2023
Alzheimer's Association International Conference 2023	Alzheimer's association, NL	2023
Nutritional Science Days	NAV, NL	2023

General courses	Organizing institute	Year
VLAG PhD week	VLAG, NL	2019
Good clinical practice course	Profess medical consultancy, NL	2019
Repeated measures mixed models	The analysis factor, online	2019
Reviewing a scientific manuscript	WGS, online	2021
Workshop carousel	WGS, online	2021
Mindful productivity for scientists	WGS, online	2022
Career orientation	WGS, NL	2023
Workshop carousel	WGS, NL	2023
Effective and efficient communication in academia and beyond	WGS, NL	2023

Teaching and supervision activities	Organizing institute	Year
Interventions for healthy ageing in humans and model species (HNH51806)	WUR, NL	2019-2020
Introduction to the field of nutrition and health (HNH11804)	WUR, NL	2019
Nutrition and the brain (HNH31706)	WUR, NL	2020-2023
Nutrition and the ageing body (HNH34106)	WUR, NL	2022-2023
Supervising BSc and MSc students	WUR, NL	2019-2023

Other activities	Organizing institute	Year
Preparation of research proposal	WUR, NL	2019
Weekly group meetings Nutritional Biology	WUR, NL	2019-2024
Member of the PhD committee	WUR, NL	2019-2022
MOOCs understanding dementia and preventing dementia	Wicking Dementia Centre, online	2020
Interview Gezondheidsgids	n/a	2020
Peer reviewing	n/a	2021-2023
PhD study tour	WUR, CH&IT	2022

COLOPHON

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