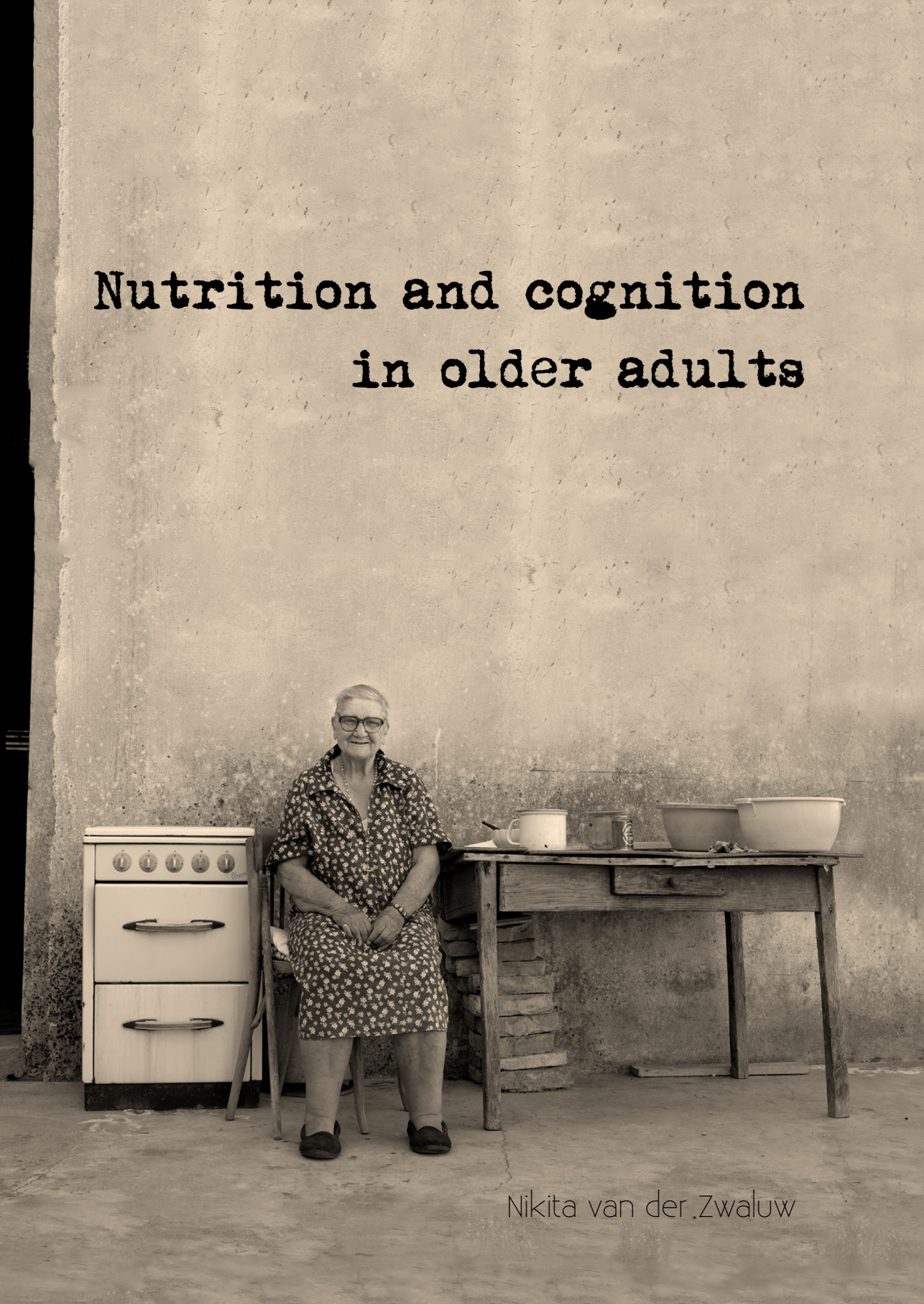
Nutrition and cognition in older adults



Nikita van der Zwaluw

Nutrition and cognition in older adults

Studies on the role of glucose, sucrose, protein, vitamin B_{12} and folic acid

Nikita van der Zwaluw

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Nikita van der Zwaluw

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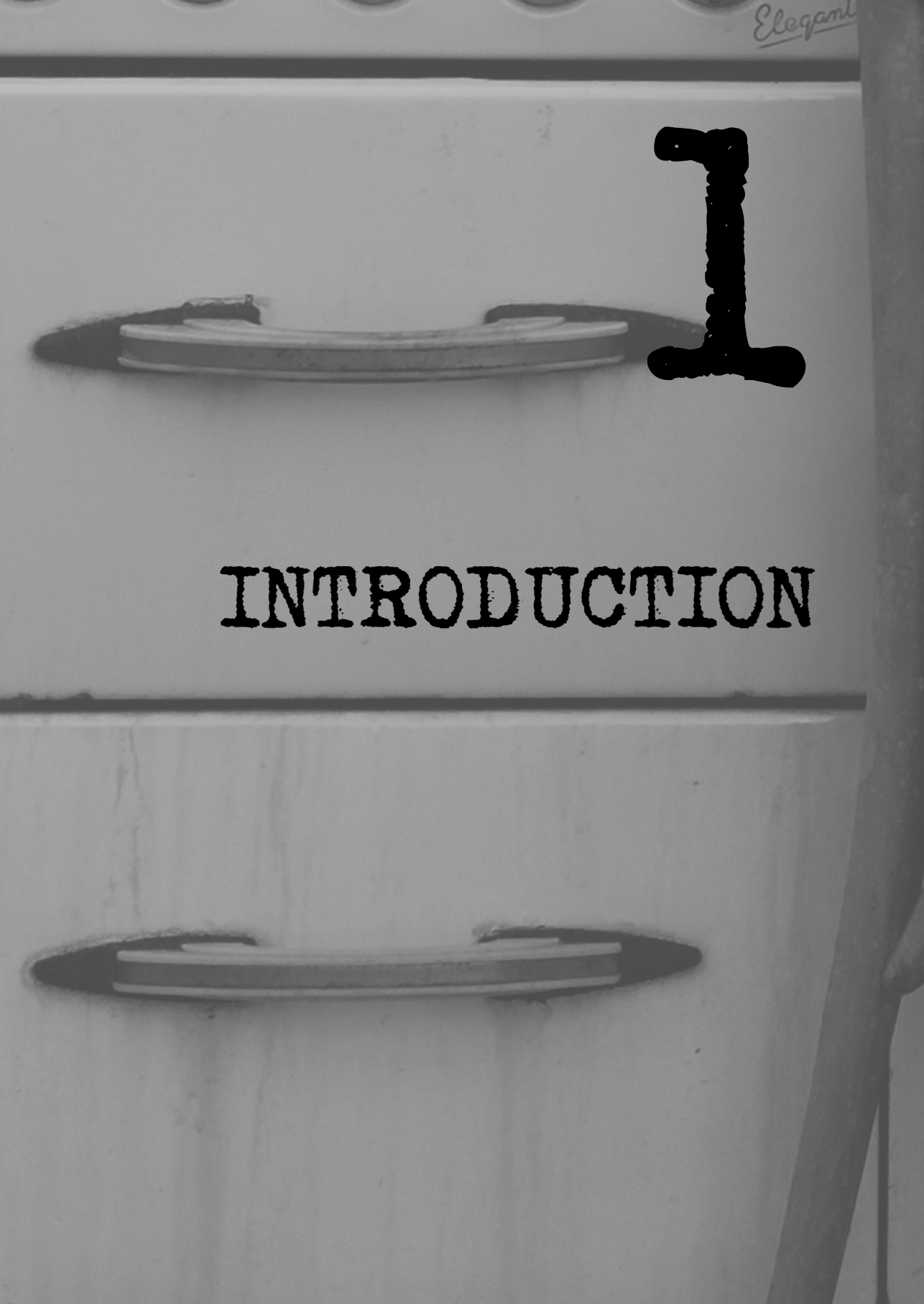

1

INTRODUCTION

Cognitive decline and dementia

With the improvements in medical care and the increase in life expectancy, the older population is expanding, especially the group of the oldest-old (aged>85 years).[1] The expansion in the number of older adults will consequently result in an increase in age-related health problems and diseases).[1] One of the functions that gradually declines during normal aging is cognitive function.[2]

Cognitive function refers to the processes involved in receiving, processing, storing, and using information that enters the brain via sensory input, and the complex mental functions that enable individuals to exert control over their environment.[3] Cognitive functions are often grouped as cognitive domains, including memory, learning, attention and concentration, language, executive functions (i.e. higher-order cognitive functions, such as planning, self-monitoring, problem solving), and visual and spatial skills. Growing old is regularly accompanied by problems related to speed of processing, solving complex problems, and remembering new information.[4] However, not all cognitive functions are affected; those requiring higher mental effort, speed demands, novelty, and information complexity deteriorate more than functions related to behavioural patterns and previously acquired routines and expertise.[2, 5] The rate of decline differs between people.[6] Mild cognitive impairment (MCI) is a rate of decline that goes beyond the range of the normal aging process, and may eventually convert to dementia,[7] as is illustrated in **Figure 1**. Within 5 years after diagnosis, approximately half of the diagnosed MCI patients develop dementia.

Dementia refers to the progressive deterioration of cognitive functions and loss of functional independence as a result of neuronal loss or damage to the connections between neurons.[8] Other neuropsychiatric symptoms may be present as well, such as apathy, agitation,

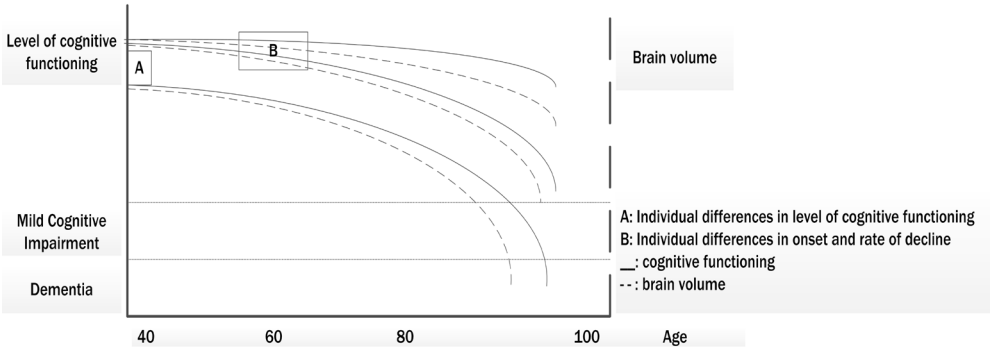

Figure 1 Illustration of decline in cognitive functioning and brain volume, which is different between individuals (not based on absolute numbers)(figure adapted from Dik, 2002)[9]

and depression. Prevalence of dementia is around 0.5% in the worldwide population, and total numbers are expected to nearly double from 35.6 million people in 2011 to 65.7 million in 2030, and to around 115 million in 2050.[10] The presence of dementia increases after the age of 85 years; 22% of the 85-years and older and even 43% of the 90-years old suffer from dementia.[10] In the Netherlands, around 260.000 people are diagnosed with dementia.[11] Dementia is a major cause of disability and dependency among elderly people and has an enormous impact on the quality of life of the patient, caregivers and family. In addition, the costs of the disease are huge, reaching €177 billion per year in Europe.[12] The present medications focus on slowing down declining processes and the effectiveness is uncertain.[10] Although research in finding a cure for dementia is progressing and new therapies are being tested in various clinical trial stages, a long journey is still ahead.

Dementia subtypes

Dementia subtypes are based on different pathologies and symptoms. Alzheimer's disease (AD) is the most diagnosed type of dementia, responsible for 60-70% of the dementia cases.[10] Other major subtypes are vascular dementia, Lewy body dementia and frontotemporal dementias.

- AD is characterized by extra-neuronal β-amyloid plaques, which are abnormal protein deposits, and intra-neuronal neurofibrillary tangles, which consist of phosphorylated tau fibers. While the disease can start many years before symptoms are apparent, the disease often manifests at an older age (late onset dementia). The first symptoms are usually related to memory problems and acquiring new information, which are especially related to damage in the hippocampus.[13]
- Vascular dementia is related to a reduced or blocked blood flow to the brain. The brain is one of the most perfused organs and is sensitive to changes in blood flow, influencing the supply of oxygen and nutrients to brain cells. Small strokes or transient ischemic attacks can therefore cause damage to the brain. Vascular dementia often starts in the prefrontal area, causing particularly problems with higher-order cognitive functions.[14]
- Lewy body dementia is characterized by so-called Lewy bodies and is closely related to Parkinson's disease. Lewy bodies are abnormal circular protein structures and are associated with the depletion of the neurotransmitters dopamine and acetylcholine, resulting in problems with thinking, executive functions, and behaviour.[15]
- Frontotemporal dementias affect mainly the frontal and temporal lobes and therewith disturb social behaviour and personality; onset often appears to be at a younger age, around 40 to 65 years.[16]

Often a mixture of pathologies is seen in a brain autopsy,[17, 18] which makes the contribution of different pathologies to the total number of dementia patients somewhat ambiguous. Although mixed dementia is often suspected, diagnosis whilst the patient is alive is infrequent.[19,10]

Neuropsychological assessment

Cognitive performance comprises many different functions and can be measured by neuropsychological tests. These measure cognitive processes that are important for functions in daily life and should be able to distinguish different states and individuals' competencies.[3, 20, 21] In almost all studies discussed in this thesis, widely used and well-validated classic paper-pencil tests were administered. In addition, computer tests were used to measure reaction times. Cognitive domains that are represented are episodic memory, attention and working memory, information processing speed, and executive functions. **Table 1** gives an overview of all neuropsychological tests that were used in the research described in this thesis and which cognitive function domain they cover.

Structural MRI

In addition to the cognitive function tests, we used another non-invasive method as a measure of brain health; brain volume was assessed by Magnetic Resonance Imaging (MRI). MRI scanners use a strong magnetic field and radiofrequencies to obtain images of the body, based on hydrogen atoms that generate a signal that is different for various bodily tissues. Structural brain scans give information on the volumes of grey and white matter and cerebrospinal fluid of the total brain and of more specific brain regions. Grey matter predominantly contains neuronal bodies, whereas white matter contains mainly long myelinated axons. Whole brain atrophy is an obvious prominent change during normal aging that already starts slowly around the age of 30.[5, 22] Hippocampal and total brain atrophy can be valid markers for the progression of AD.[23] Associations or effects of nutrients observed in structural MRI scans may be expected to have long-term consequences on central nervous system functions.[24]

Table 1 Overview of neuropsychological tests that were used in this thesis

Domains and tests	Explanation	Scoring	Chapter
Global cognitive function			
Mini-Mental State Examination [31]	Basic screening tool with a variety of questions and tasks.	Number of correct answers, max 30 points.	6
Episodic memory	The ability to encode and retrieve events and experiences with a specific temporal or spatial context		
RAVLT, direct recall [32]	Recall as many words from a 15-word list that is read aloud to the participant. This is repeated five times (three times in Chapter 3)	Number of correct reproduced words, max 75 (and 45 in chapter 3)	3, 4, 5, 6
RAVLT, delayed recall	Twenty minutes after the last repetition of RAVLT, the participant is asked to recall the words again.	Number of correct reproduced words, max 15 points	3, 4, 5, 6
RAVLT, delayed recognition	Recognize words from a list with words of the RAVLT and other words.	Number of correct words recognized, max 30 points	4, 5, 6
Story Recall, direct recall	Recall as many details of short story that is read aloud to the participant.	Number of correct sentences reproduced, max 21 points	3
Story recall, delayed recall	After a 20-25 minutes delay, the participant is asked to recall as many details of the story again.	Number of correct sentences reproduced, max 21 points	3
Paired Associate Learning (associate memory)	A list of word pairs is read out by the examiner. This is followed by the examiner reading the first word of the pair, which the participant has to complete with the other word of the pair.	Number of correct pairs formed, max 21 points	3
Attention and Working memory	Resembles the ability of online maintenance of information for a brief period of time		
Digit Span forward (attention) [33]	The examiner verbally presents a series of digits, and the participant has to repeat the digits verbatim. The series of digits increases by one, until the participant consecutively fails two trials of the same digit span length	Number of correct recalls, max 16 points	3, 4, 5, 6
Digit Span backward (working memory)	The same as the Digit Span forward test, but now the participant has to repeat the digits in reversed order.	Number of correct recalls, max 14 points	3, 4, 5, 6
Information processing speed	The ability to process information and to respond accurately under time pressure		
Symbol-Digit and Letter-Digit Substitution Test [34]	Nine different symbols are assigned a unique digit (1–9), presented in a key at the top of the test form. The participants are presented with a random series of symbols in cells and are instructed to add the corresponding digit to the symbols. In Chapter 3, letters, not symbols, had to be recoded into digits.	The number of correctly copied corresponding digits in 90 seconds (60 seconds in Chapter 3) will be recorded, max 125	3, 6

Table continues on the next page

Table 1 Overview of neuropsychological tests that were used in this thesis (continued)

Domains and tests	Explanation	Scoring	Chapter
Trail Making Test part A [35]	Participant has to connect shuffled numbers in ascending order.	Time (s) needed to finish the task, lower scores indicate better performance	3, 4, 5, 6
Stroop part I and II [36]	Part I consists of reading a card with the words of colors "red", "blue", "yellow" and "green" as fast as possible. Part II consists of naming strips of these colors.	Time (s) needed to finish the task, lower scores indicate better performance	3, 4, 5, 6
TAP – alertness (computer task) [37]	The participant has to react as fast as possible when the stimulus (an 'X') appears on the computer screen. Prior to half of the stimuli, a beep was played	Time to react (ms). Lower scores indicate better performance	3
TAP – Letters and digits (computer task)	Per trial, a letter and a digit appear on both sides of the computer screen. The participant has to react as fast as possible on the side where the stimulus (either a letter or a digit) appears on the computer screen.	Time to react (ms). Lower scores indicate better performance	3
Reaction time test (computer task) [38]	This test was a four-choice reaction task, with index and middle fingers of both hands operating four response keys. Goal was to react as quickly and accurately as possible to a single plus-sign on the screen, matching one of the four fingers.	Time to react (ms). Lower scores indicate better performance	4, 5
Executive functions	Resembling the higher-order functions that are needed for among others planning, organizing, strategizing, and problem solving		
Trail Making Test part B (concept shifting interference)	Participant has to connect numbers and letters in alternating order (1-A-2-B etcetera)	Time (s) needed to finish the task, lower scores indicate better performance	3, 4, 5, 6
Stroop III (susceptibility to behavioral interference)	Names of colors are printed in a different color of ink, interchangeably. The participant has to name the colors of the ink, instead of reading the words, which lead to a so called "color-word interference effect".	Time (s) needed to finish the task, lower scores indicate better performance.	3, 4, 5, 6
Verbal Fluency (language) [39]	Participants have to name as many words as possible in 1 minute, starting with a specific letter, or words of a specific category.	Number of correct words	3, 4, 5, 6
TAP-alternating (computer task) (flexibility)	Per trial, a letter and a digit appears on both sides of the computer screen. The participant has to react as fast as possible alternating on the side of the letter and on the side of the digit. This involves both reaction time and attention.	Time to react (ms). Lower scores indicate better performance	3

Notes: RAVLT, Rey Auditory Verbal Learning Test; TAP, Test for Attentional Performance battery.

Risk factors for dementia and cognitive decline

Dementia is a major challenge of this century and it is therefore of great importance to identify risk factors for dementia that can be modified. It has been suggested that an array of interrelated mechanisms between genetics and environment are the cause of the multifactorial disease dementia.[25] Several factors have been proposed to influence the risk of dementia and cognitive decline (**Figure 2**). Many of those factors are based on the cognitive reserve theory. This theory suggests that a reserve capacity can be built during life, with more brain volume and neuronal connections that can be used later in life to compensate for cognitive decline, and with a better resilience (coping with stressors or adverse events). Environmental factors during specific periods of the lifespan may, for instance, stimulate the production of new neurons, which can be sustained when one gets older.[2, 26] The reserve theory may explain why some people with pathologies of dementia do not experience cognitive complaints, whereas others already manifest many cognitive deficits with only few pathologies.[26]

Endogenous factors

Endogenous factors for cognitive decline and dementia include age and being female. The incidence of AD is 1.5-3 times higher in women than in men,[27] and especially women after the age of 80 years have a higher risk compared to men (hazard ratio of 1.7).[28] Having the apolipoprotein E-ε4 allele increases the risk for dementia, in particular AD, ranging from an odds ratio (OR) of 2.6 with one copy of the ε4 allele to an OR of 14.9 with two copies.[29, 30]

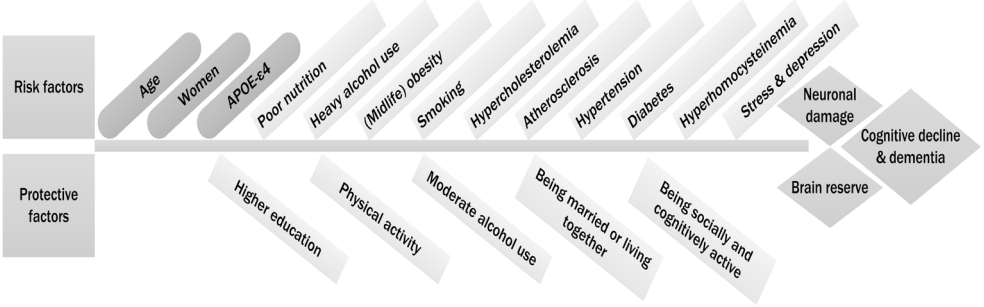

Figure 2 Risk and protective factors for cognitive decline and dementia. Round shapes are non-modifiable factors, square shapes are modifiable, and triangles are the proxy measures and outcome.

Socio-demographic factors

Socio-demographic factors include marital status, living situation, and social contacts.[40, 41] Married men and women [40, 42] and people who live together [43] have a lower risk of developing dementia. It has been suggested that being surrounded by other people enhances socially and mentally stimulating activities, and that these stimulating activities may be protective for neuronal degeneration by maintenance or even an increase of growth of neurons.[42, 44] In addition, high education, high IQ, and occupational attainment can increase brain reserve and therewith decrease the risk on dementia.[26]

Lifestyle-related factors

Vascular health may affect cerebral blood flow and brain health, and therefore, cardiovascular risk factors also affect the risk of cognitive decline and dementia. These factors include midlife hypertension, diabetes, hypercholesterolemia, atherosclerosis, and hyperhomocysteinemia.[14, 45, 46] Age-related differences in risk size have been shown.[47] For instance, hypertension in midlife confers a higher risk for dementia than hypertension in late life.[48] Other risk factors include smoking, midlife overweight and obesity, physical activity, stress and depression.[49, 45] Heavy alcohol use increases the risk on dementia,[50] whereas moderate alcohol use may be protective.[51] A recent report showed that half of the dementia patients can be explained by lifestyle-related factors, including diabetes, hypertension, obesity, depression, physical inactivity, smoking, and cognitive inactivity/education.[45]

Nutrition

Nutrition is another lifestyle factor that may be important for cognitive development and cognitive decline and is the main focus of this thesis. Epidemiological studies have shown possible beneficial associations between fish intake and omega-3 fatty acids (EPA, DHA and α-linoleic acid), B-vitamins, vitamin D, iron, anti-oxidants such as flavonoids, vitamin C and E, magnesium, selenium, zinc, and copper with cognitive performance or decline.[52, 53 54] Negative associations have been shown for high caloric intake and high intake of saturated fatty acids.[55, 56] However, these observational data have not yet been confirmed by randomized controlled trials (RCTs) because the trials are heterogeneous, often of short duration, include small numbers of participants, and therefore rarely show significant beneficial effects on cognitive performance.

Possible mechanisms of nutritional factors

Nutrients pass the blood-brain barrier by active transport, facilitated diffusion, binding receptors, and ion channels, in order to achieve homeostasis in the brain.[57] Nutritional factors may alter brain function and cognitive decline in different ways, and mechanisms are assumed to be different for various nutrients. The basic mechanisms are energy provision (glucose), building blocks (e.g. lipids and amino acids), delivery of essential micronutrients for enzymatic and endocrine processes (e.g., B-vitamins, iron), and as a source of bio- or psychoactive molecules, such as neurotransmitters, synaptic transmission, membrane fluidity,

and signal-transduction pathways.[20, 58] Other mechanisms may act on:[59-61]

- Vascular health and cerebral blood flow
- Metabolic health, e.g., diabetes, insulin insensitivity, high blood glucose levels, and obesity
- Oxidation: anti-oxidants can remove free radicals from the blood
- Inflammation: specific nutrients (e.g. B-vitamins) may reduce levels of the pro-inflammatory cytokine interleukin-1, whereas omega-3 unsaturated fatty acids may stimulate the production of the anti-inflammatory cytokine interleukin-10
- Atrophy: nutrition may play a role in the loss of brain tissue by influencing the capacity of neurogenesis in the hippocampal area
- β-amyloid and phosphorylated tau deposition: the production, accumulation, and clearance of those proteins may be influenced by nutrition.

Nutritional factors and their role in cognitive performance

In the current thesis, different nutrients that might enhance cognitive performance or slow down cognitive decline have been investigated. These nutritional factors include glucose and sucrose, protein and resistance-type exercise with or without protein, and vitamin B_{12} and folic acid. Their possible role in cognitive performance will be introduced separately.

Glucose and sucrose

Glucose is the main fuel for many processes in the body through the generation of adenosine triphosphate (ATP). Pure glucose is directly absorbed into the bloodstream, starting already in the mouth. Sucrose can be absorbed after hydrolysis into glucose and fructose. In the cytosol of a cell, glucose is broken down via glycolysis to pyruvate, which goes to the mitochondria where it is broken down to acetyl-coenzyme-A and ATP. The brain uses approximately 20% of glucose in the body, despite the fact that the brain constitutes just 2% of the total body weight.[62] In the brain, only a limited amount of glucose can be stored as glycogen in astrocytes, and a constant supply of glucose is needed to cover the needs of metabolic processes in the brain. The importance of a good glucose supply to the brain is reflected in the associations between low and high blood glucose levels and negative outcomes on cognitive performance in diabetic patients.[63] In rats, it has been shown that performing a difficult task increases glucose utilization in the brain,[64] and glucose availability can then become a limiting factor.[65] It has been suggested that the supply of extra glucose inhibits this decrease and improves cognitive functioning. A glucose load has shown to improve cognitive performance in humans as well (summarized by Smith et al.).[66] Elderly people may especially benefit from extra glucose. The decrease in extracellular glucose, mainly in the hippocampus – an important brain structure for episodic memory - is larger in

older rats compared to younger rats.[67] Furthermore, peripheral glucose regulation can change with aging.[68]

To obtain an overview of studies that have investigated the effects of a glucose load on cognitive performance in elderly populations, we executed a comprehensive literature review (**Chapter 2**), and the mechanisms are described in depth. Based on the research gap that we identified, we then designed and performed an intervention study examining the acute effects of glucose and sucrose on various cognitive functions (**Chapter 3**).

Protein intake and exercise training

Dietary protein

Proteins consist of amino acids, which are essential compounds for several functions in the body, as structural components and for different processes related to, among others, enzymes or hormones. Amino acids and small proteins are absorbed in the small intestine. The recommended dietary allowance for protein intake in the Netherlands is 0.8 g per kg bodyweight per day for adults.[69] Around 3-10% of community-dwelling elderly who are older than 70 years consume less than the average requirements (0.66 g per kg bodyweight per day) for protein.[70, 71] Low intakes of protein may negatively affect all sorts of functions in the body, such as the loss of skeletal muscle mass, weight management, bone health,[72] and possibly also cognitive functions. Elderly people are especially vulnerable to the possible negative health effects of low dietary protein intake, due to their relatively low body protein stores compared to younger adults.[73, 74]

A potential effect of protein intake on cognitive performance may be attributed to the effects that single amino acids can have on the production of neurotransmitters. A continuous supply of amino acids is essential for the rate of synthesis and release of neurotransmitters.[75, 76] In older age, the production and release of neurotransmitters may be hampered, which calls for extra amino acids at the possible site of action.[77, 78] Tryptophan and tyrosine are of special interest, as these are precursors for serotonin (tryptophan), epinephrine, norepinephrine, and dopamine (tyrosine). Studies have shown that tryptophan loading improved memory performance, but worsened speed and motor performance, presumable because of sleep promotion effects.[79] Tyrosine administration counteracted the detrimental effects of stressed conditions on cognitive performance, especially working memory. Other extra dietary amino acids, in particular L-cysteine, L-glutamic acid, and glycine, may increase the production of glutathione,[61, 80] which is an enzyme that may protect against the damage of reactive oxygen species. Furthermore, low protein intake is associated with an increased risk of frailty,[81] and frailty on the other hand is associated with an increased on dementia.[82-84]

The effects of protein intake on brain functions have hardly been studied. Results thus far are mainly based on observational studies and are inconclusive.[79] The number of RCTs is limited, with only two studies observing an acute enhancing effect of protein intake on cognitive performance,[85, 86] and one showing a beneficial effect on reaction time after a period of a high protein diet in young men.[87] A possible role for dietary protein in cognitive

performance needs to be further investigated, especially in frail individuals.

Resistance-type exercise

Physically activity is one of the suggested modifiable protective factors for cognitive decline and dementia, from observations in cross-sectional, prospective, and retrospective studies.[49, 88] In addition, evidence from RCTs investigating the effects of physical exercise on cognitive performance is accumulating.[89, 90] However, results are not consistent due to the heterogeneity between studies and the different types of training that have been investigated, e.g., aerobic, resistance type, balance, and flexibility training, or a combination of two or more.[89, 90] Several mechanisms have been proposed for physical activity and cognitive functioning. First, better cardiorespiratory health due to the (mainly aerobic) physical exercise has been suggested to be an important mechanism behind the positive effect on cognitive function. Other possible mechanisms include indirect pathways, e.g., via sleep or stress, social engagement, insulin sensitivity, or reducing heart disease.[89, 91] Evidence for possible direct effects are mainly derived from animal studies and includes the induction of angiogenesis, neurogenesis, neural cell proliferation, synaptogenesis, changes in neurotransmitter systems, and changes in the growth factors brain-derived neurotrophic factor [92] and insulin-like growth factor-1.[89]

Although the underlying mechanisms of resistance-type exercise training on brain health are not fully understood, this type of exercise is receiving more attention nowadays as it appears to be a promising means to promote cognitive performance. Resistance-type exercise training combined with a nutritional intervention has not been performed up till now. Because protein supplementation is suggested to benefit cognitive performance, the combination of resistance-type training with protein supplementation may have a synergistic enhancing effect on cognitive performance. Studying this in frail individuals, with an often low physical activity level,[93] low protein intake and a higher risk for dementia, would be interesting. We therefore investigated first the single effects of protein supplementation (**Chapter 4**), followed by the effects of resistance-type training with and without protein supplementation (**Chapter 5**) in a frail elderly population.

Vitamin B_{12}, folate and folic acid

Vitamin B_{12}

Vitamin B_{12} is an essential co-factor for two enzymes that are important for the formation of red blood cells and for the nervous system: methionine synthase and methylmalonyl coenzyme A mutase.[94] In the Netherlands, the recommended dietary daily intake is 2.8 μg for adults. Prevalence of vitamin B_{12} deficiency increases with age and is common among older adults; 6% of the 60 years and older population suffer from a deficiency (serum vitamin B_{12} <148 pmol/L) and >20% have suboptimal levels of vitamin B_{12} (148-221 pmol/L).[95] The two main causes of vitamin B_{12} deficiency are inadequate intake and malabsorption, but malabsorption is the main cause of deficiency in older adults. Due to atrophy of the gastric mucus layer and a decrease of gastric acid that accompanies aging, the absorption of vitamin B_{12} becomes impaired. Hence, vitamin B_{12} is primarily absorbed through passive diffusion in

the small intestine. Suboptimal levels of vitamin B_{12} are related to fatigue, malaise, vertigo, and memory problems.[96] Severe deficiency is characterized by pernicious anaemia, nerve damage in the spinal cord as a results of failure of methylation (myelopathy), and peripheral neuropathy.[97]

Folate

Folate is a carrier of one-carbon fragments for a variety of reactions, and is essential for DNA methylation and synthesis. Folate is absorbed in the jejunum, mostly after reduction and methylation in the intestinal mucosa. Subsequently, 5-methyltetrahydrofolate is the compound that enters the blood stream and is the most important substrate for body tissues. In the Netherlands, the recommended daily folate intake is 300 μg. Folate deficiency is mainly a result of poor intake, but it can also be caused by the use of certain medications or alcohol abuse. Around 10-20% of people aged >65 years are deficient, identified by <10 nmol/L serum folate or 340 nmol/L red blood count folate.[96] Folate deficiency is characterized by megaloblastic anaemia. Supplementation with folic acid, the oxidized and synthetic form of folate, decreases the risk on congenital neural tube defects, and therefore fortification of flour with folic acid is mandatory in some countries. However, high intake of folic acid can mask vitamin B_{12} deficiency and its neurological consequences by preventing the production of megaloblasts.

Interplay between vitamin B_{12} and folate

Observational studies have shown associations between a higher folate and vitamin B_{12} status, improved cognitive function and lower cognitive decline; however, these associations are not consistent among studies.[53] A poor vitamin B_{12} status has also been associated with less brain volume, cross-sectionally [98, 99] and prospectively,[100] and with brain integrity.[101] An important intermediate in the possible associations between B-vitamin status, cognitive decline and brain volume is the amino acid homocysteine. Low vitamin B_{12} or folate intake or status can increase levels of homocysteine.[94] High homocysteine levels are associated with negative health outcomes, in particular cardiovascular disease [102] and fracture risk.[103] In addition, cross-sectional and prospective studies have shown rather consistent associations between homocysteine, cognitive decline, dementia, and brain volume.[46, 104, 105, 106 107]

There are different pathways by which vitamin B_{12}, folate and homocysteine may influence brain health, as shown in **Figure 3**. Elevated homocysteine levels may negatively affect vascular lesions, may increase phosphorylated tau,[108] and high homocysteine and low S-adenosylmethionine levels appear to be neurotoxic [109] and can damage white matter integrity.[110] Vitamin B_{12} and folate by itself may influence methylation reactions in the central nervous system,[111] and consequently affect the methylation of myelin and damage white matter.[110-112]

Supplementation with folic acid and vitamin B_{12} decreases homocysteine levels, and may therefore be beneficial for cognitive performance. Evidence for a beneficial effect of B-vitamin supplementation derived from intervention studies, however, is limited,[113] with only a few studies observing beneficial effects, whereas most of the studies did not observe

any effect at all. The studies up till now were predominantly done in small study populations and often with follow-up times of only one year or shorter. To induce a beneficial effect of lowering homocysteine, longer studies are warranted in larger study populations, preferably in populations who would benefit the most, e.g. people with low vitamin B_{12} or folate status, or with elevated homocysteine levels. Therefore, we investigated the effects of vitamin B_{12} and folic acid supplementation on cognitive functioning by performing a large, two-year intervention study in elderly people with elevated homocysteine levels (**Chapter 6**). We furthermore investigated the associations of folate, vitamin B_{12} status, and brain volumes (**Chapter 7**).

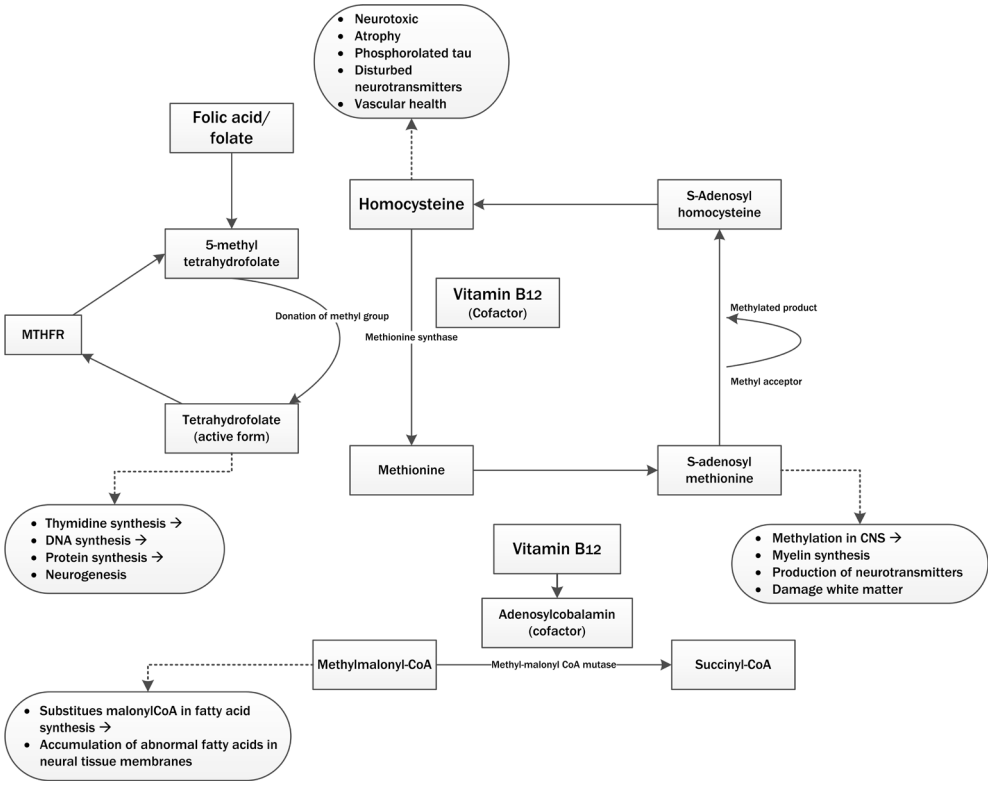

Figure 3 Vitamin B_{12} is needed for the two enzymes involved in the conversion of methylmalonyl-CoA into succinyl-CoA (mehtylmalonyl coenmzyme A mutase) and for the remethylation of homocysteine into methionine (methionine synthase). For this last conversion, folate is required as a methyl donor. Low levels of methionine result in low levels of S-adenosylmethionine (SAM), which is important for methylation reactions in the central nervous system,[111] and consequently affects the methylation of myelin and damage to white matter.[110-112] High levels of methylmalonyl-CoA might substitute malonyl-CaO in fatty acid metabolism, which might result in abnormal fatty acids in neural membranes.[114]

Rationale and outline of this thesis

The general objectives of this thesis are to investigate the short-term (glucose and sucrose), median long-term (protein and exercise training with and without protein supplementation), and long-term effects (vitamin B_{12} and folic acid) of different nutrients on cognitive performance in cognitively healthy, community-dwelling elderly people. **Table 2** gives an overview of the studies that address the aim to investigate these effects. **Chapter 2 and 3** cover the research area of the short-term effects of glucose and sucrose by means of a comprehensive literature overview and a cross-over intervention study. In **Chapter 4 and 5**, the results derived from the ProMuscle study are described. This was an RCT primarily designed to investigate the effects of protein supplementation, with or without resistance-type exercise training, on muscle mass and muscle function, but with cognitive performance as a secondary outcome. **Chapter 4** focuses solely on the effect of protein supplementation, whereas in **Chapter 5** the effects of combined protein supplementation and exercise training are described. In **Chapter 6 and 7** results of the B-PROOF study are presented, a 2-year intervention study that was primarily designed to examine the efficacy of vitamin B_{12} and folic acid supplementation on fracture incidence. An extensive neuropsychological test battery was included, enabling us to investigate the effects of the B-vitamin supplementation on cognitive performance (**Chapter 6**). After two years of supplementation, brain MRI measures were done to study cross-sectionally the associations between vitamin B_{12} and folate status with brain MRI volume (**Chapter 7**). In **Chapter 8**, the Discussion, all results that are presented in this thesis will be discussed and are put in a broader perspective.

Table 2 Overview of used projects in this thesis

Chapter	Project	Study design	Nutrient	Measurements	Study population
2	Sweet Thoughts	Literature review	Glucose and sucrose	Neuropsychological tests	Studies selected that were done in elderly people
3	Sweet Thoughts	Cross-over, acute	Glucose and sucrose	Neuropsychological test battery	43 elderly individuals (≥65 yrs) with light self-reported memory complaints
4	ProMuscle	Intervention, 24 wks	Protein supplementation	Neuropsychological test battery	65 frail and pre-frail elderly individuals (≥65 yrs)
5	ProMuscle	Intervention, 24 wks	Exercise training with and without protein supplementation	Neuropsychological test battery	62 and 65 frail and pre-frail elderly individuals (≥65 yrs)
6	B-PROOF	Intervention, 2 yrs	Vitamin B_{12} and folic acid	Neuropsychological test battery	2,919 elderly people (≥65 yrs) with slightly elevated Hcy levels
7	B-PROOF	Cross-sectional	Vitamin B_{12} and folic acid, and blood markers for vitamin B_{12} status and folate status	MRI brain volumes	218 elderly people (≥65 yrs) with slightly elevated Hcy levels

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2

Effects of a glucose load on cognitive functions in elderly people: A literature review

Nikita L. van der Zwaluw, Ondine van de Rest, Roy P.C. Kessels, and Lisette C.P.G.M. de Groot. Effects of a glucose load on cognitive functions in elderly people: A literature review. Nutrition Reviews, in press.

Abstract

Glucose is the main fuel for the brain and as such, manipulation of glucose supply may affect brain function. The main objective of this review was to give an overview of studies that investigated the acute effects of a glucose load on memory and other cognitive functions in elderly people. Furthermore, the effects of sucrose on cognition and suggested mechanisms are described.

In total, twenty studies met our inclusion criteria. The majority of studies investigated episodic memory and suggested a beneficial role for glucose in that specific cognitive domain. Other cognitive domains, e.g. working memory, semantic memory, visual memory, information processing speed, attention, executive functions, and visual/spatial function, have been studied less frequently and evidence for a beneficial effect of glucose was equivocal. Mechanisms are mainly based on the fact that glucose is needed as metabolic substrate for physiological mechanisms in both central as peripheral processes.

Introduction

Glucose is the main source for metabolic processes in the brain and therefore, it is important to have a constant supply. As such, manipulation of the glucose supply may play a role in the modulation of cognitive processes. An increasing number of studies has been published about the effects of a glucose load on cognitive functions in different study populations, such as young adults, patients with Alzheimer's Disease (AD) and healthy older adults (summarized by Smith et al.).[1]

Aging is accompanied with cognitive decline, resulting in memory loss and a decline in attention, processing speed and executive functions.[2, 3] This suggests that elderly people have more room for cognitive improvement compared to younger adults. Moreover, elderly people often have an impaired glucose metabolism, which includes a reduced ability to normalize elevated blood glucose levels and a decreased peripheral and central glucose utilization.[4, 5] A poor glucose regulation is associated with memory impairment and age-related cognitive deficits;[6] people with diabetes showed to have more difficulties with attention, speed of information processing and memory tasks and they performed worse on verbal fluency tasks than healthy older adults.[6, 7] Also individuals without diabetes, but with a poorer glucose regulation performed worse on cognitive performance tests compared to those with a better regulation.[8, 9] Individual differences in glucose regulation as well as in baseline cognitive performance may influence the effects of a glucose load on cognitive functions. These may partly explain differences in results between young, elderly, and cognitively impaired people.[1] Also mechanisms underlying the effect of glucose may differ between young and older adults.

Many studies have been conducted regarding the beneficial effect of a glucose load on cognitive functions in older adults. In this review we will give an overview of studies that investigated the facilitating effect of glucose on memory and other cognitive functions, performed in healthy older adults, and elderly people with mild cognitive impairment (MCI) and AD. Moreover, the effects of fructose and sucrose will be discussed, as well as possible underlying mechanisms.

Methods

Medline databases were searched for suitable articles up to May 2013. Combinations of the following search terms were used: glucose, sugar, carbohydrates, sucrose, fructose, brain, memory, attention, cognition, cognitive, mental performance. Titles, abstracts and keywords were carefully examined to select articles. Furthermore, reference lists of identified publications were checked. Only papers covering original research, placebo-controlled, written in the English language and performed in elderly people were included. Selected outcome measures were performance on cognitive function tasks after a glucose or sucrose drink compared to a placebo drink.

In accordance with our inclusion criteria, 42 studies were excluded because they were not

performed in older populations (34 studies in younger or middle-aged adults, eight studies in children or adolescents), and four studies because these focused on cognitive deficits other than age-related cognitive decline (for instance, patients with schizophrenia). In total, twenty studies were included, of which nineteen studies investigated glucose and memory functions, and fifteen studies investigated glucose in relation to other, non-memory cognitive functions.

Results

Glucose and memory in elderly people

A detailed overview of studies addressing the effect of a glucose drink compared to a placebo drink on different aspects of memory in elderly people is given in **Table 1**.[9-27] Per study, often more than one memory task was administered in order to capture different aspects of memory function. Although the effects varied among studies, the majority of studies showed a positive effect of glucose on at least one memory task.[10-12, 14, 16-19, 21-24, 26] Contrarily, all studies with more than one test reported also no effects of glucose on other memory tasks that were performed, and five studies reported no beneficial effects of glucose on any of the performed memory tasks.[9, 13, 15, 25, 27]

The domain of episodic memory was studied most frequently, which is the memory for events and experiences with a specific temporal or spatial context. The majority of studies showed an improvement in one of the episodic memory tasks, most often tested with story recall, paired associate learning, or word-list learning tests. There were no clear differences in effects on performance on long-term episodic memory, that is, tasks with a delay between presentation and test, or immediate recall (presentation directly followed by the test); some studies showed a larger effect on long-term memory,[10, 14, 23, 24] others on immediate recall.[11, 18, 22] Riby et al. showed that task difficulty played a role in glucose facilitation; only the easier tasks improved after glucose.[11] In contrast to these free- or cued-recall tests, however, performance on recognition tests ('yes/no' decisions about previously presented stimuli) did not improve.[10, 12] This is probably because recognition tests are not very sensitive and ceiling performances were present. When tests are too difficult, though, a glucose load may be insufficient to improve performance.[11]

Two studies showed that only individuals with a poorer glucose regulation improved their episodic memory performance; differences in memory function between people with poorer and better glucose regulations became smaller after glucose ingestion,[13, 15] mainly in the older participants and only in some of the tests.[13] Another study though, showed that glucose impaired performance of episodic memory in better glucose regulators.[17] Two studies showed that a smaller peak change in glucose levels was associated with larger memory improvement after glucose,[23, 24] whereas two other studies did not observe such an association.[19, 22] Together with glucose regulation, baseline performance might also play a role. Healthy participants with a poorer baseline performance and with a poorer β-cell function were more sensitive for the facilitating effects of glucose.[15] Craft et al. observed similar beneficial effects in patients with mild AD, who had a poorer glucose recovery,[20] but

baseline performance did not always play a role.[21]

Another memory domain that was investigated is working memory, the memory related to the online maintenance of information for a brief period of time, after which it will not be stored into long-term memory. In the studies discussed here, working memory was mostly measured by digit span forward and backward tests (brief maintenance of sequences of digits which increase in length). Overall, working memory performance was not affected after glucose intake. One study showed a beneficial effect on the digit span forward in women with a poor glucose regulation, but women with a better glucose regulation performed worse.[17] Another study observed a lower score on the digit span backwards test after glucose.[27]

Visual memory was not extensively examined, and only one study showed a beneficial effect on the delayed Rey/Taylor Complex Figure test.[26] Also the domain of semantic memory, that is, memory for general knowledge in which the spatial or temporal context of learning has been lost, did not improve after glucose intake. One study showed significant improvements after glucose on verbal fluency [26] and one study on word-stem completion task,[19] but these effects can also be a result of a general improvement in information processing, which may have resulted in a quicker response. An explanation for the lack of effect of glucose may be because abilities for verbal reasoning and semantic memory are not heavily affected by normal aging.[28]

Two of the discussed studies included younger participants as well [11, 24] and one of them showed a more pronounced effect of glucose in the older participants compared to the youngsters.[24] The studies that have investigated the differences between healthy elderly versus cognitively impaired older people showed mixed results. One study showed a difference between early-stage AD and healthy older adults with respect to the effect of glucose administration in relation to their glucose recovery; in healthy participants episodic memory improved in those individuals with a better glucose recovery, while in people with a poor recovery memory was impaired. This pattern was reversed in the AD patients.[20] In another study the enhancing effects of glucose were not more pronounced in patients with MCI compared to healthy elderly people.[10]

In sum, based on the results of nineteen studies, it may be concluded that a glucose load selectively enhances performance on episodic memory, but results on other memory functions are inconclusive. In general, it seems that glucose ingestion is more effective on sensitive cognitive tests with a certain level of task complexity, although the effects of glucose may be limited to a certain extent.

Table 1 Overview of studies investigating the effect of glucose on memory functions in elderly people

Reference	Population description: N, age (mean±SD (range)); and study design	Cognitive tests	Results
Episodic memory			
Van der Zwaluw et al. (2014)[27]	N=43, 78±8 yrs (range >65). 50 g glucose, 100 g sucrose vs. placebo	Word list recall immediate (RAVLT)	Δgl-plac = 0.1, n.s.
		Word list recall delayed (RAVLT)	Δgl-plac = -0.4, n.s.
		Story recall immediate (RBMT)	Δgl-plac = -0.5, n.s.
		Story recall delayed (RBMT)	Δgl-plac = -0.6, n.s.
		PAL easy (WMS-R)	Δgl-plac = 0, n.s.
		PAL difficult (WMS-R)	Δgl-plac = -0.2, n.s.
		Compound score	Δgl-plac = -0.12, n.s.
Messier et al. (2010)[9]	N=93, 70.1±8.3 yrs (range 55-88). 50 g glucose vs. placebo	Story recall immediate (WMS-III)	n.s.
		Story recall delayed (WMS-III)	n.s.
		Verbal free recall	n.s.
		Order recall	n.s.
Riby et al. (2009)[10]	N=24 patients with MCI, 73±5.4 yrs; N=24 healthy elderly, 71±5.6 yrs. 25 g glucose vs. placebo	Story recall immediate (RBMT)	MCI: Δgl-plac = 0.0; Healthy: Δgl-plac = 0.9; n.s.
		Story recall delayed (RBMT)	MCI: Δgl-plac = 0.8; Healthy: Δgl-plac = 1.5; $p<0.05$
		Picture recognition delayed recognition	MCI: Δgl-plac = 0.1; Healthy:Δgl-plac = -0.6; n.s.
Riby et al. (2006)[11]	N=13 elderly, 68±5.9 yrs.[a] 25 g glucose vs. placebo	PAL, easy and difficult, direct recall	Easy: Δgl-plac = 0.8, $p<0.05$; Difficult: Δgl-plac = -0.1, n.s.
		PAL, easy and difficult, delayed recall	Easy: Δgl-plac = -0.3, n.s.; Difficult: Δgl-plac = -0.4, n.s.
		Word learning, easy and difficult, immediate recall	Easy: Δgl-plac = 1.6, $p<0.05$; Difficult: g-Δp = -0.4, n.s.
		Word learning, easy and difficult, delayed recall	Easy: Δgl-plac = 1.7, $p<0.05$; Difficult: Δgl-plac = 0.0, n.s.
Riby et al. (2004)[12]	N=20, 68.8±6 yrs (range 60-80). 25 g glucose vs. placebo	PAL, easy and difficult, immediate recall	Easy: Δgl-plac = 1.3; Difficult: Δgl-plac = 1.3; n.s. overall, but 2(drink)x2(delay) x2(difficulty)ANOVA showed greater recall after glucose ($p<0.05$)
		PAL, easy and difficult, around 30 min delayed recall	Easy: Δgl-plac = 0.4; Difficult: Δgl-plac = -0.3; n.s.
		PAL recognition, easy and difficult, around 30 min delayed	Easy: Δgl-plac = -0.2; Difficult: Δgl-plac = -0.2; n.s.
		PAL recognition, easy and difficult, 1 week delayed	Easy: Δgl-plac = -1.7; Difficult: Δgl-plac = -0.9; n.s.

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Table 1 Overview of studies investigating the effect of glucose on memory functions in elderly people (continued)

Reference	Population description: N, age (mean±SD (range)); and study design	Cognitive tests	Results
Messier et al. (2003)[13]	N=57, 71.9±7.2 yrs (range 55-84). 50 g glucose vs. placebo	Story recall immediate (WMS-III)	n.s.
		Story recall delayed (WMS-III)	n.s.
		Verbal free recall	n.s.
		Order reconstruction recall	n.s.
Kaplan et al. (2001)[14]	N=22, 71.2±1.3 yrs (range 61-79). 50 g glucose vs. fat, protein, and placebo.	Word list recall (RAVLT)	p=0.03, impaired performance after glucose in men (n.s. with Bonferroni correction)
		Story recall immediate (WMS-R)	p=0.02
		Story recall delayed (WMS-R)	p=0.001
Kaplan et al. (2000)[15]	N=20, 72.3±1.4 yrs (range 60-82). 50 g glucose vs. mashed potatoes, barley, and placebo	Word list recall (RAVLT)	n.s.
		Story recall immediate (WMS-R)	n.s. overall, but people with poor GR performed better after glucose intake, while good GR performed worse.
		Story recall delayed (WMS-R)	
Manning et al. (1998)[16]	N=20, 67 yrs (range 60-83). 50 g glucose vs., placebo. Pre-training: glucose on day 1; pre-testing: glucose on day 2	Story recall, 24h delay (WMS-R)	$F(1.19)=11.60$, $p<0.01$ overall. Pre-training glucose gave better recall than pre-testing glucose.
Messier (1997)[17]	N=15, 62.3±6.5 yrs (range ≥55). 50 g glucose vs. placebo	Story recall immediate (WMS-R)	n.s. Tendency for better recall after glucose, but worse in men with poor GR
		Story recall delayed (WMS-R)	n.s.
		PAL (WMS-R)	n.s.
		Word recall (ADAS)	Glucose improved performance in better recovery males
		Word recognition (ADAS)	n.s.
Craft et al. (1993)[18]	N=20 patients with very mild to mild AD, 69.1±6.5 yrs. N=12 age- and sex matched healthy controls 65.3±10.5 yrs. Intravenous raise in glucose levels to 9,7 mmol/L, or 12,5 mmol/L	Story recall immediate (WMS-R)	Significant effect in the 12,5 mmol/L condition, especially seen in the very mild AD patients
		Story recall delayed (WMS-R)	n.s.
		PAL immediate (WMS-R)	n.s.
		PAL delayed (WMS-R)	n.s.
Manning et al. (1993)[19]	N=23 patients with AD, 82 yrs (range 68-94). 75 g glucose vs. placebo	Story recall (WMS-R)	Δgl-plac=1.18, p=0.001
		Word list learning (modified RAVLT)	Δgl-plac=2.53, p=0.003

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Table 1 Overview of studies investigating the effect of glucose on memory functions in elderly people (continued)

Reference	Population description: N, age (mean±SD (range)); and study design	Cognitive tests	Results
Craft et al. (1992)[20]	N=21 mild and very mild patients with AD, 71.5±8.5 yrs; N=14 age- and sex matched controls, 73.5±4.7 yrs. 50 g glucose vs. placebo	Story recall immediate (WMS-R)	Treatment n.s.
		Story recall delayed (WMS-R)	Treatment n.s.
		PAL immediate (WMS-R)	Healthy: Δgl-plac = 2.4, AD: Δgl-plac = 1.6. Treatment n.s.
		PAL delayed (WMS-R)	Healthy: Δgl-plac = 0.4, AD: Δgl-plac = 0.7, treatment n.s.
Manning et al. (1992)[21]	N=22, 67 yrs (range 60-81). 50 g glucose vs. placebo ingested before or after cognitive test	Story recall delayed (24h) (WMS-R)	$F(2.42)=6.7$, $p<0.01$
Parsons and Gold (1992)[22]	N=10, 67.6 yrs (range 60-82). 10, 25 and 50 g glucose vs. placebo.	Story recall 5-min delay (WMS-R)	25g glucose: $t=3.44$, $p<0.01$. N.s. effects after 10 g and 50 g glucose
		Story recall 40-min delay (WMS-R)	25g glucose: $t=2.27$, $p<0.05$. N.s. effects after 10 g and 50 g glucose
Manning et al. (1990)[23]	N=17, 73 yrs (range 62-84). 50 g glucose vs. placebo	Word list learning immediate (Selective Reminding test)	Δgl-plac = 0.75, n.s
		World list learning delayed (Selective Reminding test)	Δgl-plac = 8.63, p=0.05
		Story recall immediate (WMS-R)	Δgl-plac = 2.12, p=0.004
		Story recall delayed (WMS-R)	Δgl-plac = 2.75, p=0.002
Hall et al. (1989)[24]	N=11 elderly, 67.4 yrs (range 58-77).[b] 50 g glucose vs. placebo	PAL easy and difficult, immediate (WMS-R)	n.s.
		PAL 24h-delayed (WMS-R)	n.s.
		Story recall delayed (WMS-R)	Δgl-plac = 2.0, p=0.024
Working memory			
Van der Zwaluw et al. (2014)[27]	N=43, 78±8 yrs (range >65). 50 g glucose, 100 g sucrose vs. placebo	Digit span forward (WAIS-III)	Δgl-plac = -0.1, n.s.
		Digit span backward (WAIS-III)	Δgl-plac = -0.6, p<0.05
Messier et al. (2010)[9]	N=93, 70.1±8.3 yrs (range 55-88). 50 g glucose vs. placebo	Digit span forward (WMS-R)	n.s.
		Digit span backward (WMS-R)	n.s.
		Arithmetic (WAIS-III)	n.s.
		Letter-number sequencing (WAIS-III)	n.s.
		Modified Brown-Peterson	n.s.
Riby et al. (2004)[12]	N=20, 68.8±6 yrs (range 60-80). 25 g glucose vs. placebo	Digit span forward (WMS-III)	Δgl-plac = 0.0, n.s.
		Digit span backward (WMS-III)	Δgl-plac = 0.0, n.s.

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Table 1 Overview of studies investigating the effect of glucose on memory functions in elderly people (continued)

Reference	Population description: N, age (mean±SD (range)); and study design	Cognitive tests	Results
Messier et al. (2003)[13]	N=57, 71.9±7.2 yrs (range 55-84). 50 g glucose vs. placebo	Digit span forward (WAIS-III)	n.s.
		Digit span backward (WAIS-III)	n.s.
		Arithmetic (WAIS-III)	n.s.
		Letter-number sequencing (WAIS-III)	n.s.
		Modified Brown-Peterson	n.s.
Knott et al. (2001)[25]	N=10, 63±5 yrs. 50 g glucose vs. placebo	Sternberg memory scanning – accuracy	n.s.
		Memory scanning – reaction time	n.s.
Messier et al. (1997)[17]	N=15, 62.3±6.5 yrs (range ≥55). 50 g glucose vs. placebo	Digit Span forward (WMS-R)	Women with poor GR improved performance after glucose, while performance in women with good GR was impaired.
		Digit Span backward (WMS-R)	n.s.
Manning et al. (1990)[23]	N=17, 73 yrs (range 62-84). 50 g glucose vs. placebo	Digit span forward (WAIS-R)	Δgl-plac = -0.33, n.s.
		Digit span backward (WAIS-R)	Δgl-plac = -0.14, n.s.
Hall et al. (1989)[24]	N=11 elderly, 67.4 yrs (range 58-77).[b] 50 g glucose vs. placebo	Digit span forward (WMS-R)	n.s.
		Digit span backward (WMS-R)	n.s.
Visual Memory			
Messier et al. (2010)[9]	N=93, 70.1±8.3 yrs (range 55-88). 50 g glucose vs. placebo	Rey/Taylor complex figure test immediate recall	n.s.
		Rey/Taylor complex figure test delayed recall	n.s.
		Spatial span forward (WMS-III)	n.s.
		Spatial span backward (WMS-III)	n.s.
Messier et al. (2003)[13]	N=57, 71.9±7.2 yrs (range 55-84). 50 g glucose vs. placebo	Spatial span forward (WMS-III)	n.s.
		Spatial span backward (WMS-III)	n.s.
Messier et al. (1997)[17]	N=15, 62.3±6.5 yrs (range ≥55). 50 g glucose vs. placebo	Visual memory span forward (WMS-R)	n.s.
		Visual memory span backward (WMS-R)	n.s.
Allen et al. (1996)[26]	N=28, 73 yrs (range 61-87). 50 g glucose vs. placebo	Rey/Taylor Figure, delayed	Δgl-plac = 3.42, $p<0.001$

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Table 1 Overview of studies investigating the effect of glucose on memory functions in elderly people (continued)

Reference	Population description: N, age (mean±SD (range)); and study design	Cognitive tests	Results
Craft et al. (1992)[20]	N=21 mild and very mild patients with AD, 71.5±8.5 yrs; N=14 age- and sex matched controls, 73.5±4.7 yrs. 50 g glucose vs. placebo	Pattern recall immediate	Healthy: Δgl-plac = 0.2; AD: Δgl-plac = 1.0, Treatment n.s.
		Pattern recall delayed	Healthy: Δgl-plac = 0.0; AD: Δgl-plac = 0.8, Treatment n.s.
		Pattern recall recognition	Healthy: Δgl-plac = -0.6; AD: Δgl-plac = -0.4, Treatment n.s.
Manning et al. (1990)[23]	N=17, 73 yrs (range 62-84). 50 g glucose vs. placebo	Rey Complex figure test	Δgl-plac = -0.06, n.s.
Hall et al. (1989)[24]	N=11 elderly, 67.4 yrs (range 58-77).[b] 50 g glucose vs. placebo	Visual memory test (WMS-R)	n.s.
Semantic memory/Verbal fluency			
Van der Zwaluw et al. (2014)[27]	N=43, 78±8 yrs (range >65). 50 g glucose, 100g sucrose vs. placebo	Verbal Fluency	Δgl-plac = 1.4, n.s.
Riby et al. (2006)[11]	N=13 elderly, 68±5.9 yrs.[a] 25 g glucose vs. placebo	Verbal fluency, easy and difficult letters	Easy: Δgl-plac = -1.0, n.s. Difficult: Δgl-plac = 0.9, n.s.
		Category fluency, easy and difficult	Easy: Δgl-plac = -2.6, n.s. Difficult: Δgl-plac = -1.9, n.s.
		Computerized semantic verification task accuracy, easy + hard	Easy: Δgl-plac = 0.13, n.s. Difficult: Δgl-plac = 0.08, n.s.
		Categorical verification response time, easy + hard	Easy: Δgl-plac = 29, n.s. Difficult: Δgl-plac = 36, n.s.
Riby et al. (2004)[12]	N=20, 68.8±6 yrs (range 60-80). 25 g glucose vs. placebo	Category fluency	Δgl-plac = 2.9, n.s.
Allen et al. (1996)[26]	N=28, 73 yrs (range 61-87). 50 g glucose vs. placebo	Verbal fluency	Δgl-plac = 1.68, $p<0.01$
		Boston naming test	Δgl-plac = -0.14, n.s.
Manning et al. (1993)[19]	N=23 patients with AD, 82 yrs (range 68-94). 75 g glucose vs. placebo	Priming: word-stem completion task, recognition	Δgl-plac = 1.63, $p=0.002$
		Priming: word-stem completion task, completion	Δgl-plac = 0.52, n.s.

Notes: Δgl-plac, difference in performance after glucose and placebo drink; n.s., no significant differences between glucose and placebo drink; PAL, Paired Associate Learning; MCI, mild cognitive impaired; WMS(-R), Wechsler Memory Scale (Revised); (S)DAT, (Senile) dementia of the Alzheimer type. [a] n = 14 young, 30±5 yrs, only results in older participants are shown here; [b] n = 12 young, 20 yrs (range 18-23), only results in older participants are shown here.

Glucose and other non-memory cognitive functions in elderly people

Less research has been done on the influence of glucose on cognitive functions other than memory. Fifteen studies investigated the effects of glucose on various other cognitive functions and we divided the performed neuropsychological tests into the following cognitive domains: information processing speed, executive function, attention, visual/spatial function, and other functions (**Table 2**).[9, 10, 12-15, 17, 19, 20, 22, 23, 26, 27, 29] Seven studies showed a positive effect of glucose on various tasks in different cognitive domains.[14, 17, 19, 20, 26, 27, 29]

Information processing speed and executive functioning were mostly measured with different parts of the trail making test, letter symbol digit test, or Stroop color word test, and performance was improved in three studies.[14, 27, 29] In one study, the improvements in performance after glucose were only seen in men.[14] Glucose improved divided attention, measured by a computerized dual reaction time task,[29] but episode-watching, another attention test, did not improve. Visual/spatial function was determined with a facial recognition task, Rey Taylor figure test, figural fluency, or a one-feature target detection task, and three studies observed glucose-enhancing effects,[19, 20, 26] although one study only showed improvement in participants with AD.[20] AD patients also improved their performance on an orientation task after a glucose load.[19]

Poor β-cell function was associated with higher improvements in the trail making test, whereas participants with better β-cell function performed worse.[15] In a study of our own research group we likewise observed an improvement in information processing speed only in participants with a poorer glucose regulation and not in those with a better regulation.[27] The same effect was seen in a study of Messier et al.[17]

Due to the limited number of studies and the variety of tests covering different cognitive domains we are unable to draw any conclusions regarding a possible facilitating effect of glucose on other domains than memory. The higher-order executive functions, which are mainly controlled processes such as activation, inhibition and switching, are influenced by normal aging and require more mental effort. Glucose may mediate the processes, but more research in larger samples is needed to replicate the results.

Table 2 Overview of studies investigating the effect of glucose on other cognitive functions in elderly people

Reference	Population description: N, age (mean ±SD (range); and study design	Cognitive tests	Results
Information processing speed			
Van der Zwaluw et al. (2014)[27]	N=43, 78±8 yrs (range >65). 50 g glucose, 100 g sucrose vs. placebo	Stroop reading and color naming	Δgl-plac = -0.9, n.s.
		Letter digit substitution test	Δgl-plac = 1.1, p=0.04
		TAP Flexibility, letters	Δgl-plac = -4, n.s.
		TAP Flexibility, digits	Δgl-plac = -11, n.s.
		Compound scores	Δgl-plac = -0.1, n.s.
Gagnon et al. (2010)[29]	N=44, 67.7±5 yrs (range 60-80). 50 g glucose vs. placebo	TMT A	F(1, 41)=6.81, p<0.05
		Stroop reading	F(1,41)=3.64, n.s.
		Stroop color naming	n.s.
Messier et al. (2010)[9]	N=93, 70.1±8.3 yrs (range 55-88). 50 g glucose vs. placebo	Digit symbol-coding (WAIS-III)	n.s.
		Symbol search (WAIS-III)	n.s.
Riby et al. (2004)[12]	N=20, 68.8±6 yrs (range 60-80). 25 g glucose vs. placebo	Digit symbol substitution test (WAIS-III)	Δgl-plac = 1.5, n.s.
Messier et al. (2003)[13]	N=57, 71.9±7.2 yrs (range 55-84). 50 g glucose vs. placebo	Digit symbol-coding (WAIS-III)	n.s.
		Symbol search (WAIS-III)	n.s.
Kaplan et al. (2001)[14]	N=22, 71.2±1.3 yrs (range 61-79). 50 g glucose vs. fat, protein and placebo.	TMT A	p=0.02 in men
Messier et al. (1997)[17]	N=15, 62.3±6.5 yrs (range ≥55). 50 g glucose	Digit symbol-coding (WAIS-R)	n.s.
		Cancellation H test	Improved by glucose, but not in men with a good GR.
Allen et al. (1996)[26]	N=28, 73 yrs (range 61-87). 50 g glucose vs. placebo	TMT A	Δgl-plac = -9.4, n.s.
		Grooved pegboard	Δgl-plac = 1.2, n.s.
Manning et al. (1990)[23]	N=17, 73 yrs (range 62-84). 50 g glucose vs. placebo	Finger Oscillation Test	Δgl-plac = -1.72, n.s.
Executive function			
Van der Zwaluw et al. (2014)[27]	N=43, 78±8 yrs (range >65). 50 g glucose, 100 g sucrose vs. placebo	Stroop interference	Δgl-plac = 2.3, n.s.
		TAP Flexibility	Δgl-plac = 27, n.s.
		Compound scores	Δgl-plac = -0.02, n.s.
Gagnon et al. (2010)[29]	N=44, 67.7±5 yrs (range 60-80). 50 g glucose vs. placebo	TMT B ratio ((B-A)/A)	F(1, 41)=0.15, n.s.
		Stroop inhibition	F(1, 39)=4.61, p<0.05
		Stroop switching	F(1, 41)=10.47, p<0.01

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Table 2 Overview of studies investigating the effect of glucose on other cognitive functions in elderly people (continued)

Reference	Population description: N, age (mean ±SD (range); and study design	Cognitive tests	Results
Kaplan et al. (2001)[14]	N=22, 71.2±1.3 yrs (range 61-79). 50 g glucose vs. fat, protein, and placebo.	TMT A+B	p=0.02 in men
Kaplan et al. (2000)[15]	N=20, 72.3±1.4 yrs (range 60-82). 50 g glucose vs. mashed potatoes, barley, and placebo	TMT B	n.s. overall, but carbohydrate intake improved performance in those with a poor β cell function and impaired in those with better β cell function
Allen et al. (1996)[26]	N=28, 73 yrs (range 61-87). 50 g glucose vs. placebo	TMT B	Δgl-plac = -15.1, n.s.
Attention			
Van der Zwaluw et al. (2014)[27]	N=43, 78±8 yrs (range >65). 50 g glucose, 100 g sucrose vs. placebo	TAP Alertness, simple RT	Δgl-plac = -6, n.s.
		TAP Alertness, cued RT	Δgl-plac = -10, n.s.
Gagnon et al. (2010)[29]	N=44, 67±5 yrs (range 60-80). 50 g glucose vs. placebo	Computerized dual-task – RT	$F(1, 38)=8.49$, $p<0.01$
		Computerized dual-task - accuracy	$F(1, 38)=4.61$, $p<0.05$
Riby et al. (2009)[10]	N=24 patients with MCI, 73±5.4 yrs; N=24 healthy elderly, 71±5.6 yrs. 25 g glucose vs. placebo	SART hit rate	MCI: Δgl-plac = 0.03; Healthy: Δgl-plac = -0.02; n.s.
		SART reaction time	MCI: Δgl-plac = -0.03; Healthy: Δgl-plac = 0.00; n.s.
		SART false alarm rates	MCI: Δgl-plac = 0.04; Healthy: Δgl-plac = -0.01; n.s.
Kaplan et al. (2001)[14]	N=22, 71.2±1.3 yrs (range 61-79). 50 g glucose vs. fat, protein and placebo.	Episode-watching (sustained attention)	n.s.
Kaplan et al. (2000)[15]	N=20, 72.3±1.4 yrs (range 60-82). 50 g glucose vs. mashed potatoes, barley, and placebo	Episode-watching (sustained attention)	n.s.
Allen et al. (1996)[26]	N=28, 73 yrs (range 61-87). 50 g glucose vs. placebo	Dichotic listening	Δgl-plac = -0.15, n.s.
Visual/spatial function			
Messier et al. (2010)[9]	N=93, 70.1±8.3 yrs (range 55-88). 50 g glucose vs. placebo	Rey/Taylor complex figure test copy	n.s.

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Table 2 Overview of studies investigating the effect of glucose on other cognitive functions in elderly people (continued)

Reference	Population description: N, age (mean ±SD (range); and study design	Cognitive tests	Results
Allen et al. (1996)[26]	N=28, 73 yrs (range 61-87). 50 g glucose vs. placebo	Meier visual test	Δgl-plac = -0.79, n.s.
		Rey/Taylor Complex Figure test, copy	Δgl-plac = 0.74, n.s.
		Figural fluency	Δgl-plac = 1.43, p<0.05
Craft et al. (1992)[20]	N=21 mild and very mild patients with AD, 71.5±8.5 yrs; N=14 age- and sex matched controls, 73.5±4.7 y. 50 g glucose vs. placebo	One-feature target detection and localization task	Healthy: Δgl-plac = 0.4; AD: Δgl-plac = 3.7, in AD significant improvement after glucose
		Two-feature target detection and localization task	Healthy: Δgl-plac = -2.36; AD: Δgl-plac = -0.58, n.s.
Manning et al. (1990)[23]	N=17, 73 yrs (range: 62-84). 50 g glucose vs. placebo	Letter Cancellation Test	Δgl-plac = -4.29, n.s.
Other			
Riby et al. (2004)[12]	N=20, 68.8±6 yrs (range: 60-80). 25 g glucose vs. placebo	Mental control test (WMS III)	Δgl-plac = 0.8, n.s.
Manning et al. (1993)[19]	N=23 patients with AD, 82 yrs (range 68-94). 75 g glucose vs. placebo	Randt Memory test - Orientation	Δgl-plac = 0.91, p=0.045
Parsons and Gold (1992)[22]	N=10, 67.6 yrs (range 60-82). 10, 25 and 50 g glucose vs. placebo	Ammons Quick test - Intelligence	n.s.
Manning et al. (1990)[23]	N=17, 73 yrs (range 62-84). 50 g glucose vs. placebo	Ammon's Quick Test - Intelligence	Δgl-plac = 2.00, n.s.

Notes: Δgl-plac, difference in performance after glucose and placebo drink; n.s., no significant differences between glucose and placebo drink; MCI, mild cognitive impaired; TAP, Test for Attentional Performance; TMT, Trail Making Test; RT, reaction time; SART, Sustained Attention to Response Task; WMS, Wechsler Memory Scale; AD Alzheimer dementia.

Fructose, sucrose and cognitive performance

Fructose is, just like glucose, a mono-saccharide and together with glucose it forms sucrose. Only two studies were found that investigated the effects of sucrose on cognitive functions in an adult human population.[27, 33] In a study of our group, we showed that after a sucrose drink elderly participants performed better on speed of information processing tasks compared to a placebo drink.[27] Another study showed that sucrose in combination with nicotine may be beneficial for performance on a continuous performance task in young adults.[33]

Studies performed in rats showed that fructose can improve memory performance, although extrapolating results from animal studies to humans has to be done with caution. In a series of rat experiments of Messier and White [31] both glucose and fructose enhanced memory with the same dose and also Rodriguez et al. showed that peripheral injected fructose may affect cognitive functioning.[34] Another study observed that a combination of fructose and glucose affected memory less than a single dose of one of these mono-saccharides.[35] This might be a result of the hepatic output of glucose, which may be attenuated when both fructose and glucose are administered.[36] White [37] only observed an effect of fructose after a higher dose (2 g/kg), and not after a smaller dose (100 mg/kg), whereas two other studies showed an improvement after 100 mg/kg.[34, 35] In contrast to glucose, fructose cannot cross the blood-brain barrier,[30] does not raise blood glucose levels, and is metabolized by the liver. This can indicate that there are other mechanisms responsible than raised glucose levels to enhance cognition, probably of a peripheral nature.[31, 32]

Due to the limited amount of studies done in human and the equivocal results of rat studies, no conclusions can be drawn about the effects of fructose and sucrose on cognitive functions in older humans.

Methodological issues

Study design

All studies but one [29] used a repeated-measures design to minimize the influence of substantial variability in memory function of elderly people on test scores, which is probably the most optimal study design for this research area that focuses on acute effects. To counteract the risk of learning effects in such a design, the order of test drinks was mostly counterbalanced, and parallel versions for cognitive tests sensitive for learning effects were used. Other inter-individual variability, such as glucose recovery abilities, stress, sex and baseline performance may still have influenced the results.[15] The majority of studies was done in the morning, after an overnight fast, to control for circadian rhythm, but this questions the usability of a glucose load in daily life.[12] In younger adults it has already been shown that a glucose-enhancing effect already is present after only two hours of fasting,[38] but in elderly people this has not been investigated yet.

Neuropsychological testing

A variety of neurocognitive tests was used, and the number of different tests within a study was sometimes large, which may have increased the risk of chance findings. Nevertheless, the effect of glucose on episodic memory was present in most of the studies, indicating that glucose may be beneficial for improving episodic memory. It has been discussed that level of task difficulty is important to show an enhancing effect of glucose; an often mentioned assumption is that tasks have to be difficult enough to detect a glucose effect. Arguments that may confirm this thought are results in which delayed recall tasks profit more from glucose than immediate recall tasks.[10, 14, 23] However, Riby et al. [11] observed a beneficial effect in the easier task only, and not under high-demanding conditions. The authors suggested that the capacity of an individual is limited when the conditions are too demanding, and that glucose would not be able to counteract these high costs. Nonetheless, what exactly can be labeled as 'cognitively demanding' has not been clearly defined, as both duration and task complexity play a role in this.

Test conditions and glucose dose

All studies used glucose dissolved in water, compared to a placebo solution (250 ml) that contained saccharine, aspartame, or a combination of artificial sweeteners, and cognitive testing started around 5-15 minutes after ingestion. Drinks were matched for sweetness and taste by adding a lemon or orange flavor to the drink. Artificial sweeteners are assumed not to alter blood glucose levels and thus that they would not affect cognition. However, small effects of saccharin on several brain regions have been shown,[39] which can be explained by the effect of taste on memory processes, as activated taste receptors produce small amounts of insulin.[32, 40] No difference, though, was observed when a saccharin solution was compared to water.[41] One study in young adults only observed a beneficial effect of glucose when participants were told that they received the glucose drink and not when was told that they drank the placebo drink.[42]

A discrepancy between studies was the used dosage of glucose; either 25 g or 50 g of glucose was used for the glucose drinks, and once a dose of 10 g (no effect) [22] and a dose of 75 g was used (which was beneficial in AD patients).[19] For memory functions, it was shown that all studies using a dose of 25 g glucose showed a positive effect on episodic memory, where three-quarter of the studies that used a dose of 50 g showed a positive effect. In the non-memory cognitive domains the opposite was shown; in the studies that used a dose of 25 g glucose, only three though, no facilitating effects were observed,[10, 12, 22] whereas the studies using a dose of 50 g glucose did show effects on different cognitive functions. It is plausible that the optimum glucose dose differs for different cognitive functions. In rats it has been shown that every brain region differs in extracellular glucose level.[43, 44] Glucose transporters are not homogeneously distributed throughout the brain and this may suggest that the effects of glucose vary between brain regions.[45-47] Moreover, brain regions may vary in the degree of increasing glucose metabolism to the maximum capacity.[48] Furthermore, because every individual has a different body weight, the amount of glucose per kilogram body weight was different, which may, partly, explain the different results.[38] Dose-response studies

in young and older adults showed that the effect of glucose follows an inverted U-shaped dose-response function indicating 25 g as the most optimum dose,[22, 38, 49] which is comparable to what was found in a rat study.[50] The optimum blood glucose concentration to facilitate memory lay between 8 to 10 mmol/L, around 8.9 mmol/L in healthy elderly individuals. In patients with AD, higher concentrations are probably warranted to induce an enhancing effect of glucose; Craft et al. showed that 75 g of glucose was needed to reach an optimal blood glucose level of 225 mg/dL (12.5 mmol/L).[18]

The optimal dose may also depend on peripheral glucose regulation, but results are inconclusive. The different findings may be explained by the mostly used method, a median split, to divide the study population into better and poorer glucose recoverers. This is based on the study population and not on a reference value, and therefore, reliable determination of poor glucose regulators classified as such in a different study population has been questioned. Nevertheless, possible explanations for the effect of glucose regulation are present for both better and poorer glucose recovery conditions. The improvements for the better regulators could be that a good glucose regulation is needed to use the extra glucose in an effective way. Insulin sensitivity is probably also better, and consequently it may have its effects on the brain. On the other hand, people with a poorer glucose regulation perform worse and thus have more room for improvements.[1]

Possible mechanisms for the effect of glucose on cognitive functions

Glucose enters the brain through the blood-brain barrier by using GLUT1 glucose transporters, which are located in the endothelial cells of blood vessels.[51] A concentration gradient drives glucose to the extracellular fluid of the brain, and therefore extracellular glucose levels fluctuate slightly with blood glucose levels, whereas intracellular levels are more controlled.[52] Only a limited amount of glucose can be stored as glycogen in astrocytes, but not in neurons.[53, 54] When a deficit of neuronal glucose occurs, lactate, derived from glycogen, goes from the astrocytes to the extracellular fluid to the neurons, where it can be used as energy substrate. This drive of lactate is dependent on the level of adenosine triphosphate (ATP) within the neuron: low levels of ATP increase the transport.[32]

Several possible neurochemical systems have been implicated underlying the glucose facilitating effect on cognition. Mechanisms are mainly based on the rationale that glucose is needed as metabolic substrate for physiological mechanisms in both central as peripheral processes, such as ion pumps involved in neurotransmission, cell mechanisms, and for the synthesis of the neurotransmitters serotonin, noradrenalin, glutamate, gamma-aminobutyric acid (GABA) and acetylcholine (ACh).[43]

A general proposed mechanism is that the supply of a glucose load may prevent the depletion of brain glucose levels when performing a cognitive task;[55] because of the limited storage capacity of glucose in the brain, a continuous peripheral supply of glucose is

necessary.[56] Increased neural activity is associated with increased use of glucose in localized brain areas,[44] resulting in a decrease of glucose in extracellular brain areas after learning a task. Particularly more difficult tasks may lead to more depleted storages of glucose and supply of extra glucose may counteract these depletion effects especially when astrocytes cannot deliver enough lactate. Recently, suggestions have been put forward that effects of glucose on cognitive performance are mediated by the control of metabolic substrates by astrocytes.[54] In normal conditions, glucose is taken up by astrocytes to form glycogen. During learning or a cognitive task, glycogenolysis and production of lactate are induced by activation of membrane receptors on astrocytes. Glucose is then mainly taken up by astrocytes rather than neurons.[57] This may indicate that lactate from astrocytes may provide an additional energy substrate, next to glucose.[54, 58]

An important brain area regarding the glucose-facilitating effects is the hippocampus. This is a key structure in learning and memory modulation and is especially important in mediating episodic memory function. As we concluded from the discussed study results, performance on episodic memory benefitted the most from glucose ingestion. When learning a new task, hippocampal activity increases and extra glucose can help sustain hippocampal activity, which may lead to a better storage of new memories.[59] Hippocampal extracellular glucose levels fluctuate depending on cognitive demands;[55] levels decrease during memory tasks, as is shown in rats, and these changes appeared to be larger in older than in younger rats.[60] A functional magnetic resonance imaging (fMRI) study in patients with schizophrenia showed that glucose ingestion was associated with greater parahippocampal activation during verbal encoding.[61] These results indicate that the medial temporal region may be involved in memory facilitating due to glucose.

In the hippocampus, another possible mechanism can be identified; the neurotransmitter ACh is produced in the hippocampus and is part of the cholinergic system, a neurotransmitter system involved in the regulation of memory and learning.[62] The release of ACh is partly regulated by glucose availability. In rat studies, a relationship has been shown between glucose availability and brain ACh levels; intra-hippocampal and intra-septal glucose injections increased ACh release and improved alternation scores in rats.[63, 64] In highly demanding situations, such as difficult memory tasks, increased glucose levels may enhance the production of acetyl coenzyme A and therewith facilitate the production of ACh.[65] ACh also plays a role in prefrontal lobe functioning [66] and as such, the increase of ACh following a glucose load may explain the possible effects of glucose on executive function and attention.[67]

Throughout the brain, although not homogenously, insulin receptors are found, with higher concentrations of receptors in, among others, the hippocampus.[68, 69] This indicates another possible site of action of glucose intake because of a glucose-mediated secretion of insulin. Insulin can enter the brain by receptor-mediated transport in the blood-brain barrier. Via insulin receptors, insulin may be important in memory formation by activating specific signaling pathways that may modulates synaptic plasticity, density, and neurogenesis.[70, 71] It has been shown that intra-nasal infusion with insulin improves memory in patients with AD.[72] Under euglycemic hyperinsulinemic conditions, insulin has shown to improve verbal

memory and selective attention.[73] However, when an oral glucose load is given, it is difficult to discriminate between the possible mechanism of the glucose-mediated insulin secretion and pure sole glucose effects, because of the narrow relationship between an increase in glucose levels and the subsequent increase in insulin levels.

Another possible mechanism of glucose facilitation is via ATP-sensitive potassium channels, which seems to be sensitive for glucose metabolism. The presence of glucose is needed to increase intra-neuronal ATP levels, which results in depolarization of neurons. This again mediates the release of neurotransmitters (nicely explained by Stefani et al.),[74] which can then alter cognitive performance.

The action of glucose seems not only to occur in the central nervous system, but also in the peripheral nervous system.[43] Peripheral mechanisms might go via the gut hormone cholecystokinin and its receptors in the celiac ganglion.[15, 32] In addition, glucose may also affect cognition via the liver, where a mechanism may send a neural signal by stimulating the vagus nerve, to the central nervous system to influence the physiological processes underlying memory.[32] Findings in vagotomized rats substantiate that there is a peripheral mechanism as well; L-glucose, which does not cross the blood-brain barrier, enhanced task performance in healthy rats, but not in vagotomized rats.[75] This may also explain the possible effects of fructose, which does not cross the blood-brain barrier but has been shown to enhance memory in rats. The metabolite pyruvate, a common metabolite of glucose and fructose, may be a key in this mechanism of improving memory.[35] Pyruvate can cross the blood-brain barrier in a substantial amount. In the brain, pyruvate undergoes reversible carboxylation and can then act as an energy substrate for neurons in case of energy deficiency.[76]

The facilitating effects of glucose on memory and partly on other cognitive functions are shown in younger and older populations, but the effects seem to be more pronounced in older individuals.[24] Underlying causes of aging can be changes in neuron components in a structural, chemical, or in an electrical way.[56] Moreover, neuroendocrine regulation may be changed whereby key hormones involved in memory storage are disturbed, as well as the age-related impairments in glucose metabolism.[51] Circulating blood glucose levels increase with age while overall cerebral glucose metabolism decreases with age, and insulin sensitivity decreases. Aged rats showed a larger decrease in extracellular hippocampal glucose during training than younger rats [60, 77] and extracellular glucose levels in the hippocampal areas are lower compared to younger rats,[77] which may, partly, explain why the effect of glucose is larger in elderly people. Furthermore, insulin receptors in the brain may change due to aging and might play a role in memory formation.[78]

Mechanisms that explain the glucose-enhancing effects by the raise in blood glucose levels have been contradicted by a few studies. Kaplan et al. investigated the effects of other nutrients besides glucose on cognition and showed that protein [14] and barley,[15] which have low glycemic indexes, also improved cognition. Blood glucose levels rose minimally and it was suggested that the supply of energy may be more important to enhance cognitive functions, pointing to other possible evolutionary mechanisms. Furthermore, Manning et al.

showed a beneficial effect of glucose at a 24-hour delayed recall, when glucose levels were already at normal levels again.[21] This indicates that once the memory has been formed, this outlasts the acute effects of glucose on the brain.

Summary and conclusions

In this review we discussed the effects of a glucose load on various cognitive functions in healthy elderly people and patients with MCI and AD. Based on the selected studies, we conclude that glucose may improve episodic memory in elderly people. On other cognitive domains, however, the beneficial effect was doubtful; effects have been studied less abundant with only a few studies that observed beneficial effects on different cognitive domains. The limited number of studies is surprisingly, because attentional functions and higher executive functions are particularly vulnerable for decline due to aging.[79] Nevertheless, it is also possible that glucose has domain-specific actions and thus, may only slightly improve functions other than episodic memory. Fructose and sucrose might also improve cognitive performance, but most evidence was derived from studies in rats and it has hardly been investigated in humans.

To the best of our knowledge, we collected all available studies performed in elderly individuals concerning the glucose effects on cognitive performance. Our focus was on studies done in the elderly, because it is assumed that elderly people have more room for improvements than younger individuals and therefore might benefit more from glucose. Furthermore, it has been suggested that elderly people have different needs because their brain metabolic rate is different than younger adults.[56] Also dysfunction of neurons containing ACh, especially in the hippocampus, as a result of aging supports the interest in the glucose-enhancing effects in older individuals.[66] A review done in 2011 discussed results not only in older populations, but also in younger individuals, and concluded that glucose can have beneficial effects in younger populations as well.[1] Also in younger populations, the focus was mainly on episodic memory, and especially when there was a divided attention condition by dual tasking, glucose improved memory performance.[1] Glucose may also have an effect on non-memory functions, but tasks have to be challenging. In a study done in both elderly and younger participants, the facilitating effect of glucose was more pronounced in the older adults,[24] but contradictory results were found in a meta-analysis, showing a smaller effect size in older adults than in younger adults. The authors, however, mentioned to treat the effects with caution, as the studies in both populations were heterogeneous and more effect sizes were available from studies done in younger populations.[12] By focusing on only the elderly population in the current review, we were able to discuss the results extensively, and by showing the broad spectrum of neuropsychological tests that were done in those studies, we had a distinctive look at the results of all cognitive functions that were examined.

In conclusion, a glucose load seems to improve episodic memory in elderly people, but limited evidence is available for the effects of glucose on other cognitive domains. The effects of fructose or sucrose on cognitive functioning have hardly been studied and

therefore no conclusions can be made. Recommendations for the regular dietary use of pure glucose, sucrose, or products containing high amounts of added sugars, are, however, hard to make due to the negative health effects it has on the long term, as is also stated in the guidelines for healthy nutrition for American people.[80] In addition, a recent draft report of the World Health Organization proposes to reconsider the guidelines for free sugar use, as limiting the consumption of free sugars may reduce the risk of obesity and dental caries.[81] For difficult memory tasks, though, extra glucose might be a useful tool to improve performance. A quick rise in glucose levels may be beneficial, but on the longer-term stable glucose levels are warranted. To get a more complete picture, future research has to focus on a better understanding of the mechanism behind the effect of glucose and sucrose on cognitive performance, which would also be useful in understanding neurological processes. Neuroimaging studies will be of great use for determining specific brain regions involved in the enhancing effects of glucose, whereas animal models can be useful in entangling different mechanisms of neuronal functions.

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3

Short-term effects of glucose and sucrose on cognitive performance and mood in elderly people

Nikita L. van der Zwaluw, Ondine van de Rest, Roy P.C. Kessels, and Lisette C.P.G.M. de Groot. Short-term effects of glucose and sucrose on cognitive performance and mood in elderly people. Journal of Clinical and Experimental Neuropsychology, 2014, 36(5); 517-527.

Abstract

Objective: In this study we determined the short-term effects of a glucose drink and a sucrose drink compared to a placebo on cognitive performance and mood in elderly people with subjective, mild memory complaints using a randomized crossover study design.

Methods: In total, 43 non-diabetic older adults with self-reported memory complaints were included. Drinks consisted of 250 mL with dissolved glucose (50 g), sucrose (100 g), or a mixture of artificial sweeteners (placebo). Multiple neuropsychological tests were performed and combined by means of Z-scores into four cognitive domains: episodic memory, working memory, attention and processing speed, and executive functioning. Mood was assessed with the s-POMS questionnaire. Blood glucose concentrations were measured at five time points to divide participants into those with a better or poorer blood glucose recovery.

Results: Performance on the domain of attention and information processing speed was significantly better after consuming the sucrose drink (domain score of 0.06 ± 0.92) than after the placebo drink (-0.08 ± 0.91, $p = 0.04$). Sucrose had no effect on the other three domains, and glucose had no effect on any of the domains compared to the placebo. When dividing participants into poorer or better glucose recoverers, the beneficial effect of sucrose on attention and information processing speed was only seen in participants with a poorer recovery. After sucrose consumption, depressive feelings and tension were slightly higher than after the placebo.

Conclusions: To conclude, 100 g sucrose, but not 50 g glucose, optimized attention and information processing speed on the short-term in this study in elderly people with subjective, mild memory complaints.

Introduction

Glucose is the main metabolic fuel for the brain and as such, it plays a role in the modulation of cognitive processes. In rats, it has been shown that the use of brain glucose is enhanced when neural activity related to high cognitive demands increases.[1] Brain glucose availability can then become a limiting factor for optimal brain functioning.[2] In rats, it has also been shown that blood glucose concentrations and glucose levels in extracellular brain fluid change with aging.[3] Aging is accompanied with cognitive decline and neuro-endocrine dysregulation, which includes a disturbed regulation of key hormones and neurotransmitters that are involved in glucose regulation and memory storage, such as adrenalin and acetylcholine.[4] Therefore, elderly people are a highly relevant population to study the effects of manipulating blood glucose concentration on cognitive performance.

The amount of evidence that the intake of a glucose load may have beneficial effects on cognitive performance is growing, in particular on the domain episodic memory. In rodents and human populations with cognitive deficits (i.e., Alzheimer's disease, Down syndrome and schizophrenia) enhancing effects of glucose on episodic memory have been consistently demonstrated (see Smith et al. for review),[4] and also in relatively healthy elderly people beneficial effects have been observed. Aging also affects attentional mechanisms,[5] but the few studies that focused on the effect of glucose on this cognitive domain showed mixed results. Enhancing effects of glucose on attention have been reported in younger [6, 7] and in older populations,[8] but other studies, did not find effects.[9, 10]

Next to cognitive performance, blood glucose concentrations may influence behavioral functions and mood, such as for instance a less energetic feeling when blood glucose levels decrease.[11] Glucose intake can increase the synthesis of the neurotransmitter serotonin, which is involved in mood regulation and sedation.[12] Evidence on the effect of a glucose load on mood is, however, limited and inconsistent, with only a few studies that reported inconsistent and small effects,[13, 14] and others that did not find an effect.[15]

Whether sucrose, which consists of half glucose and half fructose, has the same effect on cognitive performance as glucose, has hardly been investigated in older adults. Sucrose in combination with nicotine may be beneficial for performance on a continuous performance task [16] and in rodents, a positive effect of fructose on memory performance has been demonstrated.[17, 18] In contrast to glucose, fructose cannot cross the blood-brain barrier and only slightly raises blood glucose concentrations.[18] Thus, it has to act through another mechanism, probably of a peripheral nature,[19] like via the gut hormone cholecystokinin and its receptors in the celiac ganglion.[20] Because sucrose may have a direct effect on the brain via glucose, as well as an effect on a peripheral mechanism via fructose, we hypothesized that sucrose may have a stronger effect than only glucose on cognitive performance and mood.

The objectives of the current study were to investigate the short-term effects of glucose and sucrose on cognitive performance and mood in healthy elderly people with self-reported mild memory complaints. Furthermore, we studied whether there was a differential effect of glucose and sucrose on cognitive performance between participants who had a better or

poorer glucose recovery, since the ability to normalize blood glucose concentrations after a glucose load depends on an individual's glucose recovery abilities.

Methods

Participants

Participants were recruited in the area of Wageningen, the Netherlands, by sending an invitation letter to volunteers from an existing database. Potentially eligible participants were invited for a screening session at the Wageningen University. Inclusion criteria were: elderly men and women (≥70 years), non-diabetic (fasting blood glucose <7.0 mmol/L),[21] non-depressed (score on the Center for Epidemiologic Studies Depression Scale (CES-D) ≤16, range 0-60; a higher score indicates more depressive symptoms),[22] non-demented (Mini-Mental State Examination (MMSE) score >25, range 0-30; a lower score indicates more cognitive impairment),[23] and mild memory complaints. The latter was specified by the Cognitive Failure Questionnaire (CFQ),[24] that assesses self-reported memory complaints. The total score ranges from 0-100 and a higher score indicates more memory complaints. An individual was included when the CFQ score was >21, and when the participant reported to experience memory complaints and was hampered by those complaints.[25] Exclusion criteria were pharmacological antidepressants or suffering from liver disease, Parkinson's disease or Phenylketonuria. This study was approved by the Wageningen University Medical Ethical Committee. Written informed consent was obtained from all participants. This trial was registered in the NIH clinical trial database (ClinicalTrials.gov number NCT01427231).

The required sample size was calculated based on the results on the Paired Associate Learning task after 50 g of glucose and a placebo drink in elderly participants.[26] The calculated effect size was 0.40,[27] and with a power of 80% and a significance level (α) of 0.05, a sample size of at least 41 participants was required to find a difference between glucose and placebo drink, as was calculated by the program G*Power 3.0.10.[28]

In total, 311 volunteers were invited of which 119 were interested to receive more information, 79 did not respond to the invitation letter, and 113 were not interested in participating. Of the 80 volunteers who returned the medical questionnaire, 71 were screened and 45 fulfilled the inclusion criteria. In the end, 43 participants were included in the study. Only one woman withdrew from the study, because of dizziness and heart palpitations after consuming the test drinks.

Design and procedure

A randomized repeated-measures crossover design was used. Each participant consumed three test drinks on three different moments, with at least one week in between to minimize carryover effects. The order of drinks was randomized. Test sessions took place during the morning after an overnight fast (10-12h).

During each test session, blood glucose response was measured using a finger prick

(HemoCue Glucose 201) at t=0 (fasting), t=15, t=30, t=60, and t=90 minutes. Immediately after the first blood glucose measurement, the participant had to consume the test drink, which had to be finished within five minutes. Cognitive testing was started fifteen minutes after consumption of the test drink, allowing a good absorption of the sugar into the blood stream.

Materials

All three test beverages were presented in a non-transparent cup, in a serving of 250 mL that contained either 50 g of glucose, 100 g of sucrose (consisting of 50 g glucose and 50 g fructose), or a mixture of artificial sweeteners as placebo condition (see **Supplementary Table 1** for all ingredients). All drinks were matched for color, flavor and sweetness (by adding artificial sweeteners) and were lemon-flavored to mask differences in taste. Drinks were provided by United Soft Drinks (Utrecht, the Netherlands).

After each test drink, participants completed a questionnaire on sensory properties to assess differences in taste. Pleasantness, sweetness, lemon taste, sourness, viscosity, pleasantness of the after taste, artificial taste, and hunger and thirst were rated on 10-point scales (e.g. 1 = "totally not pleasant" and 10 = "totally pleasant").

Cognitive performance

Cognitive performance was measured using an extensive battery of neuropsychological tests, which was performed in a quiet room by well-trained research assistants who followed a strict protocol. In 74% (n = 31) of the participants, all three test sessions were performed by the same research assistant. Tests were always performed in the same order. To minimize learning effects, parallel versions were used for the tests measuring episodic memory (Rey Auditory Verbal Learning Test (RAVLT), Story recall, Paired Associate Learning (PAL) test and for the Verbal Fluency).

A test session started with the RAVLT direct recall (three-trial version) to measure episodic memory.[29] Twenty minutes later, RAVLT delayed recall was tested. In between, Wechsler's Digit Span Task forward and backward were performed to measure working memory and attention.[30] Furthermore, Letter Digit Substitution Test (LDST) [31] and the Alertness subtest from the Test for Attentional Performance (TAP) were performed to measure information processing speed and reaction time.[32] After the RAVLT delayed recall, the TAP subtest Flexibility was done measuring complex reaction times (parts Letters and Digits), and mental flexibility (Letter-Digit Alternation) as part of executive function. This was followed by another episodic memory test: the Story Recall subtest of the Rivermead Behavioral Memory Tests direct recall and delayed recall (approximately 20 minutes after direct recall).[33] The Stroop Color-Word Test was used to measure response inhibition as part of executive function.[34] For Verbal Fluency three different letter combinations were used for each test session; 'D-A-T', 'K-O-M' and 'P-G-R'. This test measures semantic memory and language production.[35] Each test session was finished with the PAL test to assess associative memory (see **Supplementary Table 2** for the order of cognitive tests).[36] To control for the effect of

motor speed on performance we calculated an interference measure for Stroop part 3 (Time needed for Stroop part 3 – (mean time needed for Stroop part 1 and 2)).

Mood

Mood was assessed immediately after consuming the test drink and after each test session by the short Profile Of Mood Status (s-POMS) questionnaire. The s-POMS focuses on how participants are feeling at that particular moment. It consists of 32 items, covering five components of mood: depression, fatigue, anger, tension, and vigor.[37]

Other measurements

Information about smoking habits, alcohol use, marital status and highest education level was obtained using a questionnaire. Education was categorized according to Statistics Netherlands (CBS; low, moderate, or high educated). During the first test session, body weight was measured to the nearest 0.1 kg with a digital scale (Seca Delta-707), with participants wearing light clothes and without shoes. Body height and waist circumference were measured to the nearest 0.1 cm. Furthermore, a fasting blood sample was collected in an EDTA-tube to determine plasma insulin concentrations. Insulin was analyzed by radioimmunoassay (Insulin RIA Kit; LINCO Research Inc, St Charles, MO).

Statistics

Data are expressed as *n* (%), means ± SD, or as median [interquartile range (IQR)] for non-normal distributed data. Repeated-measures ANOVA was used to determine differences in effect of the three test drinks on cognitive test scores and mood scores at t=0 and t=90. Cognitive test scores acted as dependent factor, whereas drinks and time (test day) were set as fixed variables. Tukey post-hoc tests were used to compare mean scores between the three drinks and to correct for multiple testing.

To compare the results of individual cognitive test scores and to limit the number of dependent variables, individual cognitive tests were transformed into Z-scores and clustered into compound scores for four neuropsychological domains: episodic memory; working memory; attention and information processing speed; and executive functioning.

$$\text{Episodic memory} = (Z_{\text{RAVLT, direct}} + Z_{\text{RAVLT, delayed}} + Z_{\text{PAL}} + Z_{\text{Story recall, direct}} + Z_{\text{Story recall, delayed}})/5$$

$$\text{Working memory} = (Z_{\text{DigitSpan forward}} + Z_{\text{DigitSpan backward}})/2$$

$$\text{Attention and information processing speed} = (-Z_{\text{Stroop mean part 1+2}} + Z_{\text{LDST}} + -Z_{\text{TAP Flexibility letters}} + -Z_{\text{TAP Flexibility digits}} + -Z_{\text{TAP Alertness simple}} + -Z_{\text{TAP Alertness cued}})/6$$

$$\text{Executive functioning} = (-Z_{\text{Stroop (part 3-(part 1+part 2)/2)}} + Z_{\text{Verbal Fluency Letters}} + Z_{\text{TAP Flexibility letter-digit alternating}})/3$$

Previous research showed that facilitating effects of glucose may be influenced by blood glucose recovery. We therefore determined the rate at which blood glucose concentrations returned to baseline level, by calculating the incremental area under the curve (AUC) for

each participant.[38] A median split divided the study population into two groups; participants with a better or poorer glucose recovery. Participants with values above the median split were indicated as having a poorer recovery, whereas participants with values below the median split were classified as having a better recovery.

A two-sided p-value of 0.05 was used to determine statistical significance. All statistical analyses were performed using SPSS Statistics v19 (SPSS Inc. Chicago, IL).

Results

Participants

Baseline characteristics of the 43 participants can be found in **Table 1**. Mean age was 78 ± 6 years and slightly more women (63%) than men participated in the study. Median MMSE score was 29 (IQR 28-30, range 26-30).

Table 1 Characteristics of Dutch older adults with subjective, mild memory complaints (n = 43)

Characteristic	
Age (yrs)[a]	77.7 ± 5.6
Sex, men[b]	16 (37%)
BMI (kg/m^2)	25.6 ± 2.9
Waist circumference (cm)	92 ± 13
Education low / middle / high	3 (7%) / 20 (47%) / 20 (47%)
Smokers	3 (7%)
Number of cigarettes/cigars per day[c]	4.5 ± 0.7
Alcohol consumers	31 (72%)
Alcohol consumption (glasses/ week)[d]	8.1 ± 7.1
MMSE[e]	29 [28-30]
CES-D	7.7 ± 4.2
CFQ	33.2 ± 8.8
Baseline glucose (mmol/L)	5.2 ± 0.7
Baseline insulin (mU/L)	4.8 ± 2.4
HOMA Insulin resistance	1.1 ± 0.7
HOMA beta cell function	66.9 ± 37.6
AUC better glucose recovery[f]	332 ± 52
AUC poorer glucose recovery[g]	168 ± 57

Notes: BMI, Body Mass Index; MMSE, Mini-Mental State Examination; CES-D, Center for Epidemiologic Studies Depression Scale; CFQ, Cognitive Failure Questionnaire; HOMA, Homeostasis Model Assessment; AUC, incremental Area Under the Curve. [a] mean ± SD (all such values); [b] n (%)(all such values); [c] Mean use in smokers only; [d] Mean use in alcohol consumers only; [e] median [IQR]; [f] n = 21; [g] n = 22.

Sensory properties

Sensory properties of the three test drinks are shown in **Table 2**. Participants rated the glucose drink as most pleasant (5.8 ± 2.0) and the placebo drink as least pleasant (4.8 ± 2.2), p<0.01 with Tukey's post-hoc test) on a palatability scale 1 (not at all pleasant) to 10 (very pleasant). The sucrose drink was rated as sweeter (7.7 ± 1.7) compared to the placebo drink (6.6 ± 2.3, p = 0.02). Other sensory aspects did not differ between test drinks and participants were equally hungry and thirsty at all test days.

Table 2 Sensory properties of three test drinks, and feelings of hunger and thirst (*n* = 43, mean values ± SD)

Variable	Glucose (50 g)	Sucrose (100 g)	Placebo (n = 42)	(df) F-ratio	p
Pleasantness	5.8 ± 2.0[a]	5.5 ± 2.2	4.8 ± 2.2[a]	(2,81) 6.00	<0.01
Sweetness	7.3 ± 1.9	7.7 ± 1.7[b]	6.6 ± 2.3[b]	(2,81) 3.92	0.02
Lemon taste	5.1 ± 2.2	5.0 ± 2.3	4.8 ± 2.5	(2,81) 0.39	0.68
Sourness	3.7 ± 2.3	3.8 ± 2.1	3.8 ± 2.4	(2,81) 0.06	0.93
Viscosity	2.9 ± 1.9	3.5 ± 2.1	3.2 ± 2.1	(2,81) 2.29	0.11
Pleasantness aftertaste	5.5 ± 1.8	5.1 ± 2.1	4.7 ± 2.2	(2,81) 2.49	0.09
Artificial taste	4.2 ± 2.3	4.2 ± 2.4	4.7 ± 2.6	(2,81) 1.20	0.31
Hunger	4.1 ± 2.0	4.0 ± 2.3	3.6 ± 2.0	(2,81) 1.38	0.26
Thirst	4.1 ± 2.3	3.5 ± 2.2	3.8 ± 2.4	(2,81) 1.15	0.32

Notes: Differences between the three test conditions were measured with repeated-measures ANOVA. All scores were on a scale of 1-10, whereas a higher score indicates a stronger sensory property. [a, b] Within a row, groups with differing superscripts differ at p < 0.05, tested with Tukey post-hoc test

Cognitive performance

Almost all test scores, except for the working memory tasks and three tasks of the TAP, improved significantly over time (p < 0.01), which indicates there was a learning effect. Post-hoc analyses showed that the largest improvement was seen at test day 2 compared to test day 1 (data not shown).

Table 3 presents the scores of the cognitive performance tests and the scores for the four cognitive domains per drink. After the sucrose drink, the cognitive domain attention and information processing speed was significantly better (mean 0.06 ± 0.92) than after the placebo drink (mean -0.08 ± 0.91, p = 0.04). Within this domain, performance of the TAP Flexibility-letters test was best after the sucrose drink (mean 541 ± 89 ms) compared to the placebo drink (mean 566 ± 92 ms, p = 0.01) and compared to the glucose drink (mean 562 ± 100 ms, p = 0.04). Furthermore, LDST score was better after the sucrose drink (mean 30.4 ± 6.8) and after the glucose drink (mean 30.6 ± 7.6) compared to the placebo drink (mean 29.3 ± 5.5, p = 0.10 and p = 0.04 respectively).

No other differences were observed between the glucose or sucrose drink compared to the placebo drink. Working memory, however, was better after sucrose compared to glucose (p = 0.04), due to better performances on the Digit Span Test forward and backward after

sucrose. Executive function was also slightly better after the sucrose drink (mean 0.09 ± 0.81) compared to the glucose drink (mean -0.02 ± 1.03), but this difference was not significant (p = 0.08). Episodic memory performance did not differ between test drinks.

Table 3 Performance on cognitive tests and domain scores of 43 Dutch older adults with subjective, mild memory complaints after three test drinks (mean ± SD)

Cognitive measures	Glucose (50 g)	Sucrose (100 g)	Placebo[#]	(df) F-ratio	p
Episodic memory					
RAVLT, immediate recall, max. 45 words[*]	21.4 ± 7.6	21.4 ± 7.0	21.3 ± 6.1	(2,79) 0.14	0.87
RAVLT, delayed recall, max. 15 words[*]	6.4 ± 3.1	6.0 ± 2.8[a]	6.8 ± 3.0[a]	(2,79) 3.00	0.06
Story recall, immediate recall, max. 21 points[*]	9.8 ± 3.6	10.2 ± 3.5	10.3 ± 3.6	(2,79) 0.84	0.44
Story recall, delayed recall, max. 21 points[*]	8.2 ± 3.7	9.0 ± 3.2	8.8 ± 4.3	(2,76) 0.69	0.50
PAL easy, max. 9 points[*]	8.0 ± 1.2	7.9 ± 1.6	8.0 ± 1.3	(2,79) 0.10	0.90
PAL difficult, max. 12 points[*]	5.4 ± 3.0	5.9 ± 3.2	5.6 ± 3.3	(2,79) 1.13	0.33
Working memory					
Digit Span Forward, max. 16 points[*]	8.1 ± 2.0	8.6 ± 1.8	8.2 ± 1.5	(2,79) 1.41	0.25
Digit Span Backward, max. 14 points[*]	5.8 ± 1.8[b]	6.1 ± 1.9	6.4 ± 2.0[b]	(2,79) 1.81	0.17
Attention and information processing speed					
Stroop Color Word Test I (s)[†]	52.0 ± 11.1	52.3 ± 12.2	52.9 ± 11.5	(2,81) 0.35	0.71
Stroop Color Word Test II (s)[†]	64.3 ±14.9	64.9 ± 17.6	65.4 ± 14.6	(2,79) 0.31	0.74
Letter Digit Substitution test	30.6 ± 7.6[c]	30.4 ± 6.8	29.3 ± 5.5[c]	(2,81) 2.61	0.08
TAP Alertness, simple RT (ms)[†]	280 ± 47	279 ± 63	286 ± 64	(2,81) 0.59	0.56
TAP Alertness, cued RT (ms)[†]	279 ± 59	276 ± 62	289 ± 69	(2,81) 1.77	0.18
TAP Flexibility, letters (ms)[†]	562 ± 100[d]	541 ± 89[d,e]	566 ± 92[e]	(2,81) 4.30	0.02
TAP Flexibility, digits (ms)[†]	571 ± 118	559 ± 96	560 ± 108	(2,81) 0.88	0.42
Executive functioning					
Stroop Color Word Test interference (s)[†]	48.2 ± 21.0[f]	43.5 ± 16.3[f]	45.9 ± 19.9	(2,81) 4.85	0.01
TAP Flexibility, letter-digit alternating (ms)[†]	915 ± 405	871 ± 322	888 ± 298	(2,79) 1.44	0.24
Word fluency (total words)	41.9 ± 13.6	41.4 ± 12.7	40.5 ± 12.3	(2,81) 0.42	0.66
Domain scores					
Episodic memory	0.03 ± 0.96	0.15 ± 0.91	0.15 ± 0.94	(2,76) 0.37	0.69
Working memory	-0.11 ± 1.09[g]	0.15 ± 1.00[g]	0.10 ± 0.89	(2,79) 1.23	0.30
Attention and information processing speed	0.02 ± 0.91	0.06 ± 0.92[h]	-0.07 ± 0.91[h]	(2,81) 2.31	0.11
Executive functioning	-0.02 ± 1.03	0.09 ± 0.81	-0.00 ± 0.85	(2,79) 2.23	0.11

Notes: Differences between the three test conditions were measured with repeated-measures ANOVA.
RAVLT, Rey Auditory Verbal Learning Test; PAL, Paired Associate Learning; TAP, Attention Test Battery; RT, reaction time. [#] $n = 42$; [*] $n = 42$ for the glucose and sucrose condition because of one participant with hearing difficulties, in the placebo condition $n = 41$; [†] A higher score indicates more time was needed to complete the task, indicating a poorer performance. [a-h] Within a row, groups with differing superscripts differ at $p < 0.05$, tested with Tukey post-hoc test.

Glucose recovery

Blood glucose response is presented in **Figure 1**. Baseline blood glucose concentrations were similar for all test drinks. As expected, plasma glucose concentrations did not increase after the placebo drink, whereas the glucose drink and sucrose drink caused an increase in the first 30-60 minutes, followed by a decrease during the next 30 minutes. No differences were observed between the glucose recovery rate of glucose and sucrose.

Domain scores for participants with a better or poorer glucose recovery are shown in **Table 4**. Participants with a better glucose recovery tended to have an enhanced performance than poorer glucose recoverers on all but one domain after the placebo drink; attention and information processing speed was better in participants with a poorer glucose recovery. In the group with a poor glucose recovery, attention and information processing speed improved significantly after glucose (mean 0.13 ± 0.87, p = 0.04) and sucrose (mean 0.15 ± 0.96, p = 0.02) compared to the placebo (mean -0.06 ± 1.09). The facilitating effects of glucose or sucrose on this domain were not observed in participants with better glucose recovery.

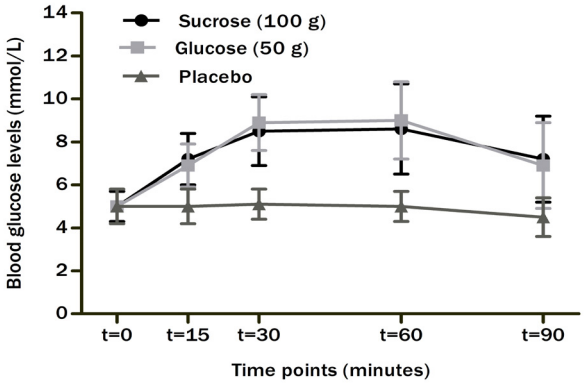

Figure 1 Mean (SD) blood glucose response in mmol/L for three test drinks measured at five time points

Mood

Five components of mood were measured right after consumption of the test drink (t=0), and again after the cognitive tests (t=90, **Table 5**). After sucrose consumption, negative feelings were higher than after the placebo drink at t=90 (depression p = 0.03; tension p = 0.03). Mood components after glucose consumption were not different than after the placebo drink at t=0 or at t=90. Compared to sucrose, however, mood components vigor at t=0 (p = 0.02) and at t=90 (p = 0.05), and depression at t=0 (p = 0.02) were better after the glucose drink.

Table 4 Performance per cognitive domain, measured in 43 Dutch older adults with subjective, mild memory complaints, split up by glucose recovery index (mean ± SD)

Cognitive domains	Glucose (50g)	Sucrose (100g)	Placebo	(df) F-ratio	p
Better glucose recovery*					
Episodic memory	0.07 ± 0.97	0.12 ± 0.73	0.21 ± 0.89	(2,37) 0.51	0.60
Working memory	-0.08 ± 1.25[a]	0.25 ± 1.11[a]	0.19 ± 0.97	(2,38) 0.68	0.51
Attention and information processing speed	-0.07 ± 0.95	-0.03 ± 0.89	-0.08 ± 0.74	(2,40) 0.42	0.66
Executive functioning	-0.03 ± 0.80[b]	0.15 ± 0.71[b]	0.02 ± 0.78	(2,38) 4.15	0.02
Poorer glucose recovery†					
Episodic memory	-0.02 ± 0.97	0.17 ± 1.09	0.09 ± 1.02	(2,35) 0.02	0.98
Working memory	-0.15 ± 0.92	0.04 ± 0.89	-0.00 ± 0.81	(2,37) 0.99	0.38
Attention and information processing speed	0.13 ± 0.87[c]	0.15 ± 0.96[d]	-0.06 ± 1.09[c,d]	(2,37) 3.25	0.05
Executive functioning	-0.01 ± 1.12	0.03 ± 0.92	-0.03 ± 0.93	(2,37) 0.00	1.00

Notes: Differences between the three test conditions were measured with repeated-measures ANOVA.
* $n = 22$, but $n = 21$ for the placebo condition; † $n = 21$, but $n = 20$ for episodic memory because of one participant with hearing difficulties. [a-d] Within a row, groups with differing superscripts differ at $p < 0.05$, tested with Tukey post-hoc test.

Table 5 Mood scores per component measured by the s-POMS in 43 Dutch older adults, immediately measured after consuming the test drink (t=0) and after the cognitive functioning tests (t=90) (mean values ± SD)

Mood factors	Glucose (50 g)		Sucrose (100 g)		Placebo#		(df) F-ratio	p	(df) F-ratio	p
	t=0	t=90	t=0	t=90	t=0	t=90	t=0	t=0	t=90	t=90
Depression	1.4 ± 3.4[a]	1.8 ± 4.1	2.7 ± 4.6[a]	2.6 ± 4.6[b]	2.3 ± 4.8	1.3 ± 3.1[b]	(2,79) 4.6	0.01	(2,76) 2.3	0.11
Anger	1.0 ± 2.1	1.4 ± 3.4	1.5 ± 3.0	1.6 ± 3.8	1.2 ± 3.2	1.0 ± 2.9	(2,81) 0.8	0.46	(2,75) 1.0	0.37
Fatigue	2.9 ± 4.1	5.0 ± 5.4	3.8 ± 4.9	4.7 ± 5.1	2.7 ± 4.0	4.2 ± 4.3	(2,80) 3.3	0.04	(2,76) 0.7	0.48
Tension	2.5 ± 3.4	2.3 ± 3.9	2.9 ± 3.9	3.0 ± 4.3[c]	2.9 ± 3.7	1.9 ± 2.6[c]	(2,81) 1.1	0.34	(2,76) 2.8	0.07
Vigor	12.1 ± 3.7[d]	11.5 ± 4.0	10.4 ± 4.5[d]	10.3 ± 4.2	11.0 ± 4.3	10.9 ± 4.2	(2,81) 3.9	0.02	(2,76) 3.6	0.03

Notes: Per component, differences between the three test conditions were measured with repeated-measures ANOVA. Higher scores mean stronger feelings of that mood component. # $n = 42$. [a-d] Within a row, groups with differing superscripts differ at $p < 0.05$, tested with Tukey post-hoc test.

Discussion

In this study, short-term effects of glucose and sucrose on cognitive performance in healthy elderly men and women with mild, self-reported memory complaints were investigated. Our findings showed that a sucrose drink had a beneficial effect on attention and information processing speed in this study population, whereas glucose improved attentional performance only in participants having a poorer glucose recovery. In contrast to previous studies, no beneficial effects of glucose or sucrose were observed on episodic memory function.

One of the strengths of this study was the use of an extensive neuropsychological test battery to measure cognitive performance, with sufficiently challenging tests to be able to detect subtle changes. Moreover, the tests we used have been applied in other studies as well (e.g. [8, 39]). We clustered data of individual test scores into four cognitive domains - episodic memory, working memory, attention and information processing speed, and executive function - to obtain more robust cognitive scores. The number of dependent variables was thereby limited and the possibility of chance findings is decreased. By assessing the four cognitive domains, we were able to investigate the effect of glucose and sucrose on a broad spectrum of cognitive functions that decline with aging.

Another strength was the crossover design, which was used to minimize the influence of substantial variability in memory function of elderly people on test scores.[40, 41] Test days were at least one week apart to minimize carryover effects, as was done in other studies applying a similar design (e.g. [42-44]). Furthermore, validated parallel versions were used for those cognitive tests that are prone to learning effects, and by randomizing the order of the drinks, a time effect was supposed to be diminished. However, still an effect over time was seen as participants improved performance on most tests after the first test day, independently of the test drink. Although the tests were carefully explained and most tests were practiced beforehand, it would have been better to include a complete training session for all tests.

Unlike many previous studies (reviewed by Smith et al.),[41] we did not observe a beneficial effect of glucose on episodic memory. Comparable to other studies, cognitive tests were always started fifteen minutes after consuming the test drink, assuming that this time interval would be sufficient to elevate glucose concentrations.[44] After fifteen minutes, blood glucose concentrations were indeed elevated, but most optimal blood glucose concentrations for achieving cognitive effects, which lay between 8 and 10 mmol/L,[45] were found after 30-60 minutes. Because a test session always started with a memory test, it might be that glucose concentrations at that time point were not sufficiently elevated to enhance memory function. Next to the timing, the used glucose dose can be discussed. We assumed 50 g glucose to be the proper dose for older adults to enhance memory based on previous studies,[39, 40, 45-47] but studies using 25 g of glucose also observed a facilitating effect on memory.[9, 45, 48] This suggests that other factors than dose may play a role;[45] body size for instance, may influence circulating blood volume and differences in glucose utilization as an energy substrate, which may affect the quantity of glucose delivered to the brain.[41, 49] It is also possible

that the optimum dose differs for different cognitive tasks; in rats it has been shown that brain regions differ in extracellular glucose levels.[2] The dose to improve memory might be somewhat lower than 50 g, which may explain why we did not find any positive effects of glucose and sucrose on memory, but that we did find a positive effect on attention and information processing speed.

The dose of 100 g sucrose was chosen to have an equal amount of glucose in the glucose and in the sucrose drink (in both test drinks 50 g of glucose). In the total study population, we observed a small effect of sucrose, but not of glucose, on attention and information processing speed. This may indicate that the combination of glucose and fructose is a better enhancer for this cognitive domain, but we should take into account that the sucrose drink contained a double amount of energy as the glucose drink. It is therefore not possible to assign the beneficial effect of sucrose only to fructose. To investigate the exclusive effect of fructose, an extra treatment group investigating 50 g of fructose should have been included. Due to limitations in organizational aspect and to keep the burden for participants acceptable, we decided to only investigate the 100 g sucrose. Moreover, other studies that investigated the effects of glucose also encountered the aspect of energy differences, since the placebo drinks almost never contained any calories.

The positive effects of glucose and sucrose on attention and information processing speed were shown in particular in participants having a poorer glucose regulation. This finding is in accordance with a study of Kaplan et al.,[44] in which older adults with a poorer glucose recovery had worse cognitive abilities, which was counteracted by the ingestion of glucose. People with a poorer glucose regulation tend to score worse on cognitive tests,[19] so it is plausible that more effect can be achieved in these persons. Other studies, though, have shown a stronger beneficial effect in better than in poorer glucose recoverers.[43, 50] This discrepancy may be due to the commonly used method of dividing a study population by a median split, which is rather arbitrary, since it depends on a particular study population. Furthermore, it has been suggested that younger adults with a poorer glucose regulation have a similar response as elderly people with a better glucose regulation.[4]

By including older individuals with self-reported memory complaints, we aimed to include a study population that would be sensitive for changes in cognitive functioning. MMSE scores, however, were still high, which may be partly due to the fact that we excluded real cognitively impaired (MMSE ≤25) participants. MMSE, however, is a global measure for cognitive performance and impairments may only be picked up in a later stage, while memory complaints are usually the first signs of cognitive decline. Mol et al. showed that people with subjective memory complaints had a lower score on information processing speed.[51] However, other causes than a poor cognitive performance are also known for reporting memory complaints, such as anxiety, depression,[52] low self-esteem and neuroticism.[53]

We did not find a clear effect of glucose or sucrose on mood. Carbohydrates can increase brain serotonin concentrations and therewith decrease stressed feelings.[11] In our study, mood was worst after the sucrose drink, the drink with the highest amount of carbohydrates, which can be attributed to the low score on pleasantness of the sucrose drink. The few studies

that are known, reported inconsistent and small effects of a glucose drink on mood.[11] In a study of Bentone & Owen,[14] a very small effect was observed in a large study population, and the authors already mentioned that it would be difficult to replicate these subtle findings in a smaller study population. In our study, mood was a secondary outcome, and as such, the study population was probably too small to observe an effect. Also the timing of the questionnaires could have played a role in that serotonin concentration was not elevated enough at the first time point of measurement, and that it was maybe depleted at the second time point.[11] However, it was also suggested that mood would be more influenced by the supply of glucose when performing cognitively demanding tasks, as this will increase brain's need for glucose.[11] We therefore expected a positive effect of glucose or sucrose on mood after the cognitive tests.

The best-known mechanism of the glucose facilitating effect on memory is the possible effect on the hippocampus, which is the key structure in mediating episodic memory. Due to fluctuations in hippocampal extracellular glucose concentrations after a high-demanding cognitive task, replenishing these glucose concentrations could be beneficial to optimize performance. Also, insulin can play a role on the hippocampus; its receptors are densely concentrated in the hippocampus. In the absence of a raise in plasma glucose, it has been found that insulin has a direct effect on memory.[43] However, after a glucose load, it is difficult to distinguish between the effects of a raise in blood glucose concentrations and the effects of insulin. Other mechanisms that may play a role in the possible facilitating effect of glucose on cognitive performance may be related to the effect on neurotransmitters, such as epinephrine, glutamate, serotonin and acetylcholine, all involved in cognitive functioning.[19, 54] Synthesis of acetylcholine is dependent on glucose and in rats, improved memory was shown after glucose ingestion together with an increase in hippocampal neurotransmitter acetylcholine synthesis.[55]

A limitation of this study was the lack of an extra arm of fructose drink. Another important consideration of glucose use and the consecutive elevated blood glucose levels points to the negative long-term effects on neurons, and cognitive decline and dementia.[56] As such, recommendations to take extra glucose are hard to make, but in case of a high-demanding task, it might be useful to raise your glucose levels to improve performance.

To conclude, a sucrose drink - which contained twice as much energy as the glucose drink - optimized attention and information processing speed on the short-term in elderly participants with mild memory complaints. Glucose also showed a beneficial effect on attention and information processing speed, but this effect was only observed in people with poorer glucose recovery.

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Supplementary material

Supplementary Table 1 Ingredients (in g) per 250 mL test drink

Test drink	Sucrose	Dextrose	Sodium cyclamate	Aspartame	Acesulfame potassium	Citric acid Anhydrous
Sucrose drink	100	-	-	-	-	1.00
Glucose drink	-	50	0.04	0.10	0.10	0.75
Placebo drink	-	-	0.10	0.25	0.25	0.44

Notes: To all drinks potassium sorbate (0.15 g), drink essence (0.30 g), and ascorbic acid (0.10 g) was added.

Supplementary Table 2 Order of measurements on a test day

Time	Measurements
	Blood glucose measurement
t=0	Drink
	Questionnaire sensory properties
	s-POMS
t=15	Blood glucose measurement
	RAVLT
	Digit Span
	LDST
t=30	Blood glucose measurement
	TAP-Alertness
	Delayed recall RAVLT
	TAP-Flexibility
	Story Recall
t=60	Blood glucose measurement
	Stroop-Color Word test
	Letter Fluency
	Delayed recall Story recall
	PAL
t=90	Blood glucose measurement
	s-POMS

Notes: s-POMS, short-Profile Of Mood Status; RAVLT, Rey Auditory Verbal Learning Test; LDST, Letter-Digit Substitution Test; TAP, Test for Attentional Performance; PAL, Paired Associate Learning.


4

Protein supplementation and cognitive performance in frail elderly people

Nikita L. van der Zwaluw, Ondine van de Rest, Michael Tieland, Jos J. Adam, Gert Jan Hiddink, Luc J.C. van Loon, and Lisette C.P.G.M. de Groot. The impact of protein supplementation on cognitive performance in frail elderly. European Journal of Nutrition, 2014, 53; 803-812

Abstract

Objective: Maintenance of cognitive abilities is important for elderly to stay independent. With the aging of the population, the call for modifiable factors is emerging. Dietary protein might improve cognitive performance; however, this has hardly been studied. Therefore, we studied the impact of 24 weeks dietary protein supplementation on cognitive performance in pre-frail and frail elderly people.

Methods: Pre-frail and frail elderly subjects, according to the Fried-criteria, randomly received a protein drink containing 15 g protein or a non-caloric placebo drink twice a day. Cognitive performance was measured at baseline and after 24 weeks by means of a sensitive neuropsychological test battery. In addition, reaction time was assessed after both 12 and 24 weeks of intervention. Domain scores were calculated for the domains episodic memory, attention and working memory, information processing speed, and executive functioning. ANCOVA was used to determine differences between groups. Linear Mixed Models were used to determine differences in reaction time over time and per treatment.

Results: In total, 65 subjects (79 ± 8 years) with a median MMSE score of 28 (interquartile range 26-30) were included. Reaction time improved more in the protein group (68 ms) than in the placebo group (18 ms, $p = 0.03$). Dietary protein had no significant effect on any of the cognitive domain scores.

Conclusions: Protein supplementation might improve reaction time performance in pre-frail and frail elderly, but did not improve other cognitive functions.

Introduction

The prevalence and incidence of dementia is an increasing problem worldwide. Almost 66 million people will suffer from dementia in the year 2030, and this number will nearly be doubled till 115 million in 2050.[1] Cognitive decline, which usually accompanies aging, is associated with loss of independency and autonomy.[2, 3] Maintaining or improving cognitive performance is therefore of major importance to elderly. With the aging population, the call for modifiable factors to stay cognitive healthy, such as nutrition, is emerging.[4, 5]

The recommendations for protein intakes are based on short-term nitrogen balance. However, in order to create an optimum for a long-term health and well-being, these recommendations are nowadays being discussed.[6] A possible beneficial role of protein on several health outcomes, such as sarcopenia, weight management and bone health, has been suggested by an increasing number of studies.[7-10] The effect of protein intake on cognitive performance, however, has barely been studied. A few observational studies reported an association between protein intake and cognitive performance. Goodwin and colleagues showed that older adults with lower protein intakes had a lower score on verbal memory tests and nonverbal abstract reasoning than adults who were better nourished.[11] Positive correlations between protein intake and several cognitive tests were also found by La Rue et al. [12] and Roberts et al. [13] showed that higher protein intakes were associated with a decreased risk on mild cognitive impairment or dementia 3.7 years later. Furthermore, acute beneficial effects of high protein intake on memory functions compared to a high-carbohydrate meal [14] and compared to a placebo [15] were observed. A high-protein diet improved cognitive performance in healthy young men after three weeks of intervention,[7] but the authors suggested that the beneficial effect also may have been due to elevated levels of vitamin D, B_2, B_6 and B_{12} intake compared to the control group.[7] On the other hand, an 8-weeks intervention trial with exercise training and whey-protein and leucine supplementation did not show any difference between the protein and the placebo group.[16]

Acute beneficial effects were also found of the single amino acid tyrosine on cognitive performance in a stressful condition.[17] Tyrosine is a precursor for the neurotransmitters dopamine, norepinephrine and epinephrine [18] and its dietary intake influences the synthesis and release of neurotransmitters. As such, it may alter brain functions.[19] Also the presence of the amino acid tryptophan, the precursor for serotonin, may be influenced by diet and can affect cognitive performance.[19-21] Tryptophan loading may have a detrimental effect on psychomotor speed, but it has also been shown to improve memory, especially in stress-vulnerable or depressed participants who have suboptimal levels of serotonin.[22, 23] Also elderly may have suboptimal serotonin activity.[24] The effect and uptake into the brain of both tryptophan and tyrosine, however, is dependent on other amino acids, because they share and compete for a transporter in the blood brain barrier.

Cognitive performance is positively related to physical fitness.[25] Physical frailty on the other hand is a predictor for cognitive decline and Alzheimer's disease.[26-28] Frailty comes with age and is seen as a biological syndrome associated with decreased reserves and resistance to

stressors, which increases the risk of adverse health outcomes, such as the onset of disability, morbidity, and institutionalization.[29, 30] Beasley et al. showed that higher protein intakes are associated with a lower risk of frailty, which suggests a preventive role of protein on frailty.[10] Furthermore, previous work of our group showed that protein supplementation might be a promising nutritional strategy to improve functional performance.[31]

We hypothesized that frail elderly are susceptible to a possible beneficial effect of protein supplementation on cognitive performance. Because no long-term studies have been conducted about the effect of protein on cognition in elderly, our aim was to investigate the effect of 24 weeks protein supplementation on cognitive performance in frail and pre-frail elderly.

Methods

Participants

Participants were men and women who participated in the ProMuscle study, a study that investigated the effect of protein supplementation on muscle mass and muscle strength.[31] Subjects' selection and attrition have extensively been described elsewhere.[31] In short, subjects were recruited through an existing database of volunteers, distribution of information flyers and letters, and local information meetings. Inclusion criteria were: being ≥65 years old and being pre-frail or frail according to the criteria from Fried et al.[32] The five criteria to define frailty were: [1] unintentional weight loss, [2] weakness (low handgrip strength), [3] self-reported exhaustion, [4] slow walking speed, and [5] low physical activity. Pre-frailty was classified when one or two of these criteria were present, and frailty was classified when three or more criteria were present. Exclusion criteria were diabetes mellitus type I or II (as measured by a fasted plasma glucose level ≥7,0 mmol/L),[33] cancer, COPD, participation in any structured exercise training program in the past two years, and/or renal failure (estimated Glomerular Filtration Rate (eGFR) <60 mL/min/1.73 m^2).[34] All subjects gave their written informed consent and the study was approved by The Wageningen University Medical Ethical Committee.

Study design

After enrollment, subjects were randomized to receive either the protein supplementation or the placebo for a period of 24 weeks. Randomization was done by an independent person by means of computer-generated random numbers in stratified permuted blocks of size four, stratified by gender. All outcome measures were collected at baseline, after 12 weeks (except most cognitive function tests) and after 24 weeks of intervention.

Protein supplementation

During the intervention period, volunteers either received a 250 mL protein-supplemented beverage that contained 15 g protein (MPC80; milk protein concentrate), 7.1 g lactose, 0.5

g fat, and 0.4 g calcium, or a non-caloric placebo supplement that contained no protein (FrieslandCampina Consumer Products Europe, the Netherlands). All beverages were in a non-transparent package and were vanilla flavored to mask the contents of the drinks. Participants were instructed to consume one beverage after their breakfast and one beverage after their cold lunch meal (time depended on timing of lunch: ~12:00 p.m. or ~18:00 p.m.). Compliance was judged by returned ticked calendars and non-consumed beverages. Staff members and participants were blinded towards treatment allocation until completion of data analyses.

Cognitive performance

At baseline, the Mini-Mental State Examination (MMSE) was performed to assess overall cognitive status.[35] Cognitive performance was measured at baseline and after the 24-week intervention period by well-trained research assistants. Reaction time test was also performed after 12 weeks.

An extensive neuropsychological test battery was used to cover four cognitive domains: episodic memory, attention and working memory, information processing speed, and executive functioning. To measure episodic memory, Word Learning Test (WLT) was used, consisting of fifteen words that were read aloud for five times. To assess direct recall, the participant had to recall as many words as possible after every trial; WLT delayed recall and WLT recognition were measured 20 minutes after the last direct recall.[36] Wechsler Digit Span (DS) forward and backward tests were used to determine attention and working memory.[37] For the DS forward test participants had to immediately repeat strings of numbers that range from two to eight numbers, whereas for the DS backward test, the strings had to be repeated in reserve order. Trail Making Test (TMT) part A and B measure information processing speed and concept shifting interference, respectively.[38] For part A, numbers in ascending order had to be connected by a line as fast as possible, whereas for part B alternating numbers and letters had to be connected (1-A-2-B etcetera). Stroop Color-Word Test determines selective attention and susceptibility to behavioral interference.[39] Stroop I and II consisted of a sheet of color names in black, and blocks of colors resp. that had to be read by the participant. Stroop III consisted of a sheet of color names printed in a different color; the participant had to name the color of the ink. The Verbal Fluency test was used to measure executive functioning; participants had to name as many words starting with the letter 'P', and words in the category 'animals'.[40] This test battery was always performed in the afternoon in the same quiet room following a standard protocol. Coffee and tea were not allowed before and during the test session.

Reaction time was measured by the computerized finger-cuing task,[41, 42] after an overnight fast following a strict protocol. This test was a four-choice reaction task, with index and middle fingers of both hands operating four response keys. Goal was to react as quickly and accurately as possible to a single plus-sign on the screen, matching one of the four fingers. The screen always showed a reference row consisting of four plus signs indicating the four possible stimulus locations. A trial was started by showing a cue signal, which could be four plus signs in all four positions (neutral uncued condition) or only two plus signs, i.e.: the

hand-cued condition in which the cue specifies two fingers on the same hand; the finger-cued condition in which the cue specifies the same finger on two hands; or the neither-cued condition in which the cue specifies different fingers on two hands. Those cues reduce the number of possible stimulus locations from four to two and would be of benefit for the reaction time. After a preparation interval of 2000 ms, the target signal (one plus sign) appeared in one of the cued positions. Participants had to react as quickly as possible to the target signal by pressing the corresponding response key. Outliers were defined as reaction time less than 150 ms or in excess of 2000 ms and were excluded from data analysis (mean percentage of outliers was 1.4% based on 160 trials per test session). Reaction time was based only on correct trials.

Blood sampling

Fasted blood samples were collected at baseline to determine glucose homeostasis and renal function. Plasma glucose concentrations were analyzed with a COBAS FARA analyzer (Uni Kit III; Roche, Basel, Switzerland). Plasma insulin was analyzed by radioimmunoassay (Insulin RIA Kit; LINCO Research Inc, St Charles, MO). Serum creatinine was measured by using Roche Modular System P (Roche Diagnostics GmbH, Mannheim, Germany).

Dietary intake

Participants recorded their food intake for three days at baseline, after 12 weeks, and after 24 weeks to assess potential changes in daily food intake during the intervention period. Trained dieticians gave oral and written instructions about recording the type of foods and estimating portion sizes in household measures. Furthermore, they checked the food records for completeness, obtained additional information about unclear items or amounts and used household measures to improve the estimation of portion size. Dietary intake data were coded (type of food, time of intake, and amount) and energy and macronutrient intakes were calculated using a nutrient calculation program (Komeet, BaS nutrition software, 2004, Arnhem, the Netherlands) and the Dutch food composition database.[43]

Other measurements

Height was measured at baseline with a wall-mounted stadiometer to the nearest 0.1 cm. Weight was measured at baseline, after 12 weeks and after 24 weeks in a fasted state to the nearest 0.1 kg with a calibrated digital scale (ED-6-T; Berkel, Rotterdam, The Netherlands). Information about the highest education level, smoking habits, alcohol intake, depression (Center for Epidemiologic Studies Depression Scale), and medical history was obtained by questionnaires. Blood pressure was measured in the morning after 10 minutes of rest with an Omron HEM-907 (Lake Forest, IL, USA) device. At baseline, 12 weeks and 24 weeks, four measurements were done, with an interval of two minutes, of which the first measurement was discarded. The mean value of the three subsequent measurements for systolic and diastolic blood pressure was taken.

Statistics

Data analyses were performed by the intention-to-treat principle. Data are expressed as means ± SD, or as median [interquartile range (IRQ)] for non-normal distributed data. Baseline characteristics were compared between treatment groups using independent samples T-test or Chi-square test.

WLT decayed recall was calculated as the number of words recalled after approximately 15 minutes following the fifth session of the WLT minus the number of words recalled during the fifth session of the WLT. To control for the effect of motor speed on performance we calculated interference measures for TMT (TMT B/TMT A) and Stroop III (Time needed for Stroop III – (mean time needed for Stroop I and II)). To take into account errors made during the Stroop Test, accuracy and speed-accuracy trade-offs (Stroop-SAT) were calculated. Accuracy = ((maximum right answers – amount of errors) / maximum right answers). Speed-accuracy trade-off = accuracy / time needed to complete the task.[44] The three cued conditions of the reaction time test (hand-cued, finger-cued and neither-cued) were averaged to one value (reaction time test cued).

To compare the results of the individual cognitive tests and to limit the number of dependent variables, crude test scores were clustered into compound scores for four neuropsychological domains: Episodic memory; Attention and working memory; Information processing speed; and Executive functioning. Data of baseline were used as norm data to create individual Z-scores for baseline and 24-week data (Z-score = (score test – mean baseline)/SD baseline). The following domains in formula form were formed:

$$\text{Episodic Memory} = (Z_{\text{WLT total immediate recall}} + Z_{\text{WLT decayed recall}} + Z_{\text{WLT recognition}})/3$$

$$\text{Attention and working Memory} = (Z_{\text{Digit Span forward}} + Z_{\text{Digit Span backward}})/2$$

$$\text{Information processing speed} = (Z_{\text{Stroop-SAT mean I and II}} + -Z_{\text{Trail making test part A}} + -Z_{\text{Reaction time test uncued}} + -Z_{\text{Reaction time test cued}})/4$$

$$\text{Executive Functioning} = (Z_{\text{Stroop-SAT interference}} + Z_{\text{Verbal Fluency Animtal}} + Z_{\text{Verbal Fluency Letter P}} + -Z_{\text{Trail making ratio}})/4$$

Differences between treatments overtime were analyzed using analyses of covariance (ANCOVA), in which baseline data acted as the covariate factor, the outcome measures as the dependent factor and treatment as the fixed between-subject factor. To assess the effect of protein supplementation on reaction time, which was measured at three time points, Linear Mixed Models were used with the unstructured covariance type. Time, treatment and their interaction were defined as fixed factors, whereas subject was defined as random factor.

An α-level of 0.05 was used to determine statistical significance. All statistical analyses were performed using SPSS Statistics v19 (SPSS Inc. Chicago, IL).

Results

Participants

At baseline, 65 participants were enrolled. Eight subjects, four in each treatment group, discontinued the study because of the following reasons: side-effects of the drink (diarrhea, nausea; *n* = 3), dislike of the drink (*n* = 1), medical reasons (*n* = 2), depression (*n* = 1), and burden too high (*n* = 1). After 12 weeks, reaction time data were missing from all drop-outs, whereas after 24 weeks reaction time data were obtained from 60 participants. For the other cognitive tests data from 59 participants were collected. Compliance to the treatment, counted as returned full drinks and ticked calendars, was very high (92%, with only five subjects <80%).

Baseline characteristics did not differ between treatment groups (**Table 1**). Mean age was 78 ± 8 years in the protein group and 81 ± 7 in the placebo group. Percentage of lower educated was slightly higher in the protein group (9%) than in the placebo group (0%, p = 0.21), as was the percentage of smokers (15 vs. 3%, p = 0.20). MMSE-score was high in both groups (median of 29 in the protein group and 28 in the placebo group [IQR: 26-30 in both groups]), indicating no impaired cognitive performance of most participants. Ten subjects had a score <25 indicating mild cognitive impairment (6 in protein and 4 in placebo group).[45] Habitual protein intake did not differ between groups and did not significantly change over time (only baseline data shown).

Cognitive performance

Mean cognitive baseline scores did not differ between the two treatment groups (**Table 2**). **Table 3** shows the changes in cognitive test scores after 24 weeks. A significant larger improvement in reaction time was found in the protein group than in the placebo group (without taking the measurements at week 12 into account). Reaction time in the uncued condition improved with 68 ± 157 ms in the protein group and 18 ± 142 ms in the placebo group (p = 0.03), and in the cued condition, a similar improvement was found (p = 0.05). Overall error rate was very low (2.5% for the protein group and 2.3% for the placebo group), with small, non-significant reductions in error rate for both groups at the end of the treatment compared to baseline.

No significant differences were found on the other cognitive tests. Subjects in the protein group recalled one more word at the WLT-direct recall (Δend-baseline 2.9 ± 7.6) than subjects in the placebo group (Δend-baseline 2.1 ± 5.5), whereas they recognized one word less on WLT-recognition (Δend-baseline = -0.7 ± 1.9) than the placebo group (Δend-baseline 0.3 ± 2.8), but these differences were not significant. Participants in the protein group became slightly slower at the TMT part A; time to complete the task increased 3.7 ± 20.1 s over time in the protein group and decreased 3.5 ± 14.6 s in the placebo group (p = 0.11).

Domain scores did not differ between treatment groups after 24 weeks (**Table 3**). After 24 weeks, scores in the domains episodic memory and information processing speed were slightly better in the placebo group, though these effects were not significant. Executive

functioning improved by 0.19 ± 0.43 in the protein group and 0.09 ± 0.42 in the placebo group (p = 0.41).

Table 4 shows the scores of all three time points that reaction time task was measured. After 12 and 24 weeks, both intervention groups improved their reaction time, but treatment effects were larger after 24 weeks than after 12 weeks. Significance however, was not reached in neither the uncued condition (treatment*time p = 0.25, treatment p = 0.14) nor in the cued conditions (treatment*time p = 0.34, treatment p = 0.17 for the mean cued condition). In both groups, reaction time was fastest in the hand-cued condition and slowest in the neither-cued condition. In all cuing conditions, a similar trend in treatment*time effect was observed.

Table 1 Characteristics of 65 Dutch pre-frail and frail elderly people

	Protein	**Placebo**
Age (yrs)[a]	78 ± 8	81 ± 7
Sex, men, *n* (%)	14 (41)	15 (48)
Education Low/ Middle/ High (%)	9 / 59 / 32	0 / 55 / 45
BMI (kg/m^2)	27.0 ± 4.6	26.2 ± 3.2
Smokers, *n* (%)	5 (15)	1 (3)
Number of cigarettes/cigars per day	12	10
Alcohol consumers (%)	68	71
Alcohol consumption (g/day)[b]	12 ± 14	9 ± 11
MMSE[c]	29 [26-30]	28 [26-30]
Range	21-30	22-30
CES-D	6.6 ± 5.7	7.0 ± 4.1
Pre-frail / frail, *n* (%)	27 (79) / 7 (21)	20 (65) / 11 (35)
Baseline glucose (mmol/L)	5.2 ± 0.4	5.3 ± 0.4
Baseline insulin (mU/L)	18.0 ± 7.4	18.2 ± 7.1
eGFR (mL/min/1.73 m^2)	84.4 ± 20.2	77.6 ± 12.5
Blood pressure, diastolic (mmHg)	77 ± 10	75 ± 9
Blood pressure, systolic (mmHg)	152 ± 25	150 ± 23
Energy intake, kJ	8335 ± 2457	8114 ± 1748
Protein intake, g/day (En%)	78 ± 26 (16 ± 3)	74 ± 14 (16 ± 3)
Fat intake, g/day (En%)	83 ± 30 (36 ± 5)	73 ± 23 (33 ± 7)
Carbohydrate intake, g/day (En%)	211 ± 63 (44 ± 8)	228 ± 65 (48 ± 8)
Protein intake g/kg body weight/day	1.1 ± 0.3	1.0 ± 0.2

Notes: BMI, Body Mass Index; MMSE, Mini-Mental State Examination; CES-D, Center for Epidemiologic Studies Depression Scale; eGFR, estimated Glomerular Filtration Rate. [a] Mean ± SD (all such values); [b] Mean use in consumers only; [c] Median [IQR].

Table 2 Baseline scores of cognitive tests (mean ± SD) in a Dutch pre-frail and frail elderly population (n = 65)

	Protein	Placebo
Episodic memory		
Word learning test – Immediate recall, max. 75 words	34.6 ± 11.8	33.5 ± 10.5
Word learning test – Decayed recall (delayed-WLT trial 5)	-2.8 ± 1.5	-2.6 ± 2.0
Word learning test – Recognition, max. 30 words	28.2 ± 1.9	27.1 ± 2.7
Attention and working memory		
Digit Span forward, max. 16 points	7.9 ± 1.4	8.3 ± 2.1
Digit Span backward, max. 14 points	5.2 ± 1.3	6.0 ± 2.3
Information processing speed		
Trail Making Part A (s)[a]	61.6 ± 27.2	62.6 ± 23.0
Stroop 1[b, c]	1.91 ± 0.36	1.80 ± 0.33
Stroop 2[b, c]	1.54 ± 0.35	1.44 ± 0.28
Reaction time, uncued (ms)[a]	764 ± 301	830 ± 243
Reaction time, cued (ms)[a]	753 ± 330	821 ± 263
Executive functioning		
Stroop Interference (Part 3 – (Part 1 + Part 2/2))[b,c]	-0.90 ± 0.24	-0.79 ± 0.20
Word Fluency – Animals[b]	21.4 ± 6.4	20.6 ± 5.8
Word Fluency – Letter P[b]	15.2 ± 5.8	14.1 ± 3.8
Trail Making Test (Part B/Part A)[a]	1.77 ± 0.61	1.83 ± 0.62
Domain scores		
Episodic memory	0.08 ± 0.56	-0.08 ± 0.75
Attention and working memory	-0.15 ± 0.63	0.17 ± 1.12
Information processing speed[b]	-0.08 ± 0.94	-0.27 ± 0.72
Executive functioning[b]	0.05 ± 0.67	-0.02 ± 0.60

Notes: [a] Higher scores indicate more time was needed to complete the task, so it indicates a poorer performance; [b] Placebo group n = 30; [c] Time corrected for errors (time needed / accuracy).

Table 3 Changes in cognitive test scores and domain scores (mean ± SD) of 58 Dutch pre-frail and frail elderly (only participants with baseline and 24 weeks data)[a, b]

	Baseline	24 wks	Change	p
Episodic memory				
Word learning test - Immediate recall, max. 75 words				0.58
Protein	35.9 ± 11.1	38.8 ± 12.0	2.9 ± 7.6	
Placebo	34.4 ± 9.8	36.5 ± 10.4	2.1 ± 5.5	
Word learning test – Decayed recall (delayed-WLT trial 5)				0.47
Protein	-2.7 ± 1.6	-2.6 ± 2.1	0.1 ± 2.4	
Placebo	-2.7 ± 2.1	-2.2 ± 2.0	0.5 ± 2.6	
Word learning test – Recognition, max. 30 words				0.43
Protein	28.4 ± 1.9	27.7 ± 2.2	-0.7 ± 1.9	
Placebo	27.4 ± 2.4	27.7 ± 2.6	0.3 ± 2.8	
Attention and working memory				
Digit Span forward, 16 points				0.46
Protein	8.0 ± 1.4	8.1 ± 1.7	0.1 ± 1.4	
Placebo	8.3 ± 2.2	8.0 ± 2.1	-0.3 ± 1.7	
Digit Span backward, 14 points				0.92
Protein	5.3 ± 1.3	5.4 ± 1.5	0.0 ± 1.2	
Placebo	6.2 ± 2.3	6.0 ± 2.4	-0.1 ± 1.7	
Information processing speed				
Trail Making Part A (s)[c,d]				0.11
Protein	57.2 ± 21.8	61.7 ± 36.6	3.7 ± 20.1	
Placebo	63.5 ± 24.0	60.0 ± 24.6	-3.5 ± 14.6	
Stroop 1[c]				0.89
Protein	1.96 ± 0.33	1.96 ± 0.29	-0.01 ± 0.20	
Placebo	1.80 ± 0.33	1.83 ± 0.38	0.03 ± 0.20	
Stroop 2[c]				0.71
Protein	1.60 ± 0.31	1.61 ± 0.28	0.00 ± 0.11	
Placebo	1.44 ± 0.27	1.45 ± 0.30	0.01 ± 0.16	
Reaction time, uncued (ms)[c,f]				0.03
Protein	739 ± 246	671 ± 183	-68 ± 157	
Placebo	826 ± 233	808 ± 238	-18 ± 142	
Reaction time test, cued (ms)[c,f]				0.05
Protein	724 ± 265	635 ± 208	-89 ± 149	
Placebo	818 ± 257	775 ± 266	-44 ± 130	
Executive functioning				
Trail Making Ratio (Part B/Part A)[c]				0.95
Protein	1.75 ± 0.63	1.77 ± 0.57	0.01 ± 0.60	
Placebo	1.83 ± 0.62	1.77 ± 0.66	-0.05 ± 0.78	
Stroop Interference (Part 3 – (Part 1 + Part 2/2))[c]				0.43
Protein	-0.92 ± 0.24	-0.84 ± 0.21	0.08 ±0.18	
Placebo	-0.79 ± 0.21	-0.80 ± 0.21	-0.01 ± 0.17	

Table continues on next page

Table 3 Changes in cognitive test scores and domain scores (mean ± SD) of 58 Dutch pre-frail and frail elderly (only participants with baseline and 24 weeks data)[a, b] (continued)

	Baseline	24 wks	Change	p
Word Fluency – Animals				0.19
Protein	21.8 ± 6.2	24.2 ± 6.5	2.4 ± 4.1	
Placebo	20.7 ± 5.7	22.0 ± 6.2	1.3 ± 3.6	
Word Fluency – Letter P				0.83
Protein	15.7 ± 5.6	15.7 ± 6.7	0.0 ± 4.9	
Placebo	14.3 ± 3.8	14.8 ± 5.5	0.5 ± 4.0	
Domain scores				
Episodic memory				0.40
Protein	0.15 ± 0.56	0.16 ± 0.81	0.01 ± 0.57	
Placebo	-0.03 ± 0.67	0.17 ± 0.86	0.20 ± 0.78	
Attention and working memory				0.54
Protein	-0.08 ± 0.62	-0.04 ± 0.78	0.04 ± 0.56	
Placebo	0.23 ± 1.14	0.11 ± 1.11	-0.12 ± 0.68	
Information processing speed				0.39
Protein	0.19 ± 0.58	0.25 ± 0.75	0.05 ± 0.30	
Placebo	-0.24 ± 0.74	-0.14 ± 0.81	0.10 ± 0.34	
Executive functioning				0.41
Protein	0.01 ± 0.62	0.20 ± 0.68	0.19 ± 0.45	
Placebo	0.02 ± 0.59	0.11 ± 0.61	0.09 ± 0.42	

Notes: Differences between the two groups were measured using ANCOVA (with test scores at 24 weeks as dependent variable and baseline cognitive test scores as covariate). [a] $n = 30$ for the protein group; [b] $n = 28$ for the placebo group; [c] Higher scores indicate more time was needed to complete the task, so it indicates a poorer performance; [d] $n = 31$ for protein group; [e] Corrected for errors (time needed / accuracy); [f] $n = 32$ for protein group.

Table 4 Reaction time measured at baseline, after 12 weeks and at the end, in a pre-frail and frail elderly population (mean ± SD)

	Baseline[a]	12 wks[b]	24 wks[c]	treatment * time effect (p)	treatment effect (p)	time effect (p)
Reaction time, uncued (ms)				0.25	0.14	0.01
Protein	764 ± 301	663 ± 151	671 ± 183			
Placebo	830 ± 243	778 ± 196	808 ± 238			
Reaction time, hand-cued (ms)				0.29	0.21	0.01
Protein	716 ± 318	597 ± 170	612 ± 210			
Placebo	767 ± 263	726 ± 208	740 ± 260			
Reaction time, finger-cued (ms)				0.22	0.13	<0.01
Protein	772 ± 333	640 ± 179	643 ± 208			
Placebo	840 ± 261	773 ± 215	799 ± 291			
Reaction time, neither-cued (ms)				0.50	0.16	<0.01
Protein	771 ± 342	648 ± 184	650 ± 213			
Placebo	857 ± 275	781 ± 221	785 ± 251			
Reaction time, mean cued (ms)				0.34	0.17	<0.01
Protein	753 ± 330	628 ± 176	635 ± 208			
Placebo	821 ± 263	760 ± 212	775 ± 266			

Notes: Higher scores indicate more time was needed to complete the task, so it indicates a poorer performance. Differences among the two groups were measured using Linear Mixed Models. [a] $n = 34$ for the protein group, $n = 31$ for the placebo group; [b] $n = 28$ for the protein group, $n = 29$ for the placebo group; [c] $n = 32$ for the protein group, $n = 28$ for the placebo group

Discussion

We investigated the impact of 24 weeks protein supplementation on cognitive performance in pre-frail and frail elderly people. Dietary protein did not improve the cognitive domains of episodic memory, attention and working memory, information processing speed, and executive functioning. Performance on the reaction time task however, improved significantly more in the protein group than in the placebo group. In fact, after 24 weeks of treatment, the improvement in reaction time was more than twice as large in the protein compared with the placebo group. This finding suggests that the protein supplementation improved reaction time performance above and beyond the improvement, which may have occurred due to learning (i.e., repeated task performance) in the placebo group. Furthermore, the percentages of errors were stable over time, which indicates that reaction time improved without losing accuracy. Looking at the number of tests analyzed, however, it is possible that it was a chance finding.

To the best of our knowledge, this was the first intervention study investigating the impact of long-term protein supplementation on cognitive performance in frail elderly people. To investigate this, we included a variety of sensitive neuropsychological tests, covering the domains most affected by aging. By clustering the data of individual cognitive tests by means of standardization into Z-scores, we created a more robust cognitive score. Moreover, we

limited the number of dependent variables thereby decreasing the possibility of chance findings. Cognitive performance was measured in a valid and standardized way by following a strict protocol and by not allowing coffee and tea during the tests. However, because of increasing inter-individual differences with aging,[46] the relatively small study population made it difficult to uncover more subtle changes on cognitive performance. Also the duration of the study might have been too short to pick up an intervention effect on cognitive domains. Because cognitive decline is a rather slow process, it is recommended to conduct trials with a duration of at least 18 months to study the effects on cognitive performance.[47] However, by including a population at high risk for cognitive decline, such as our frail elderly population, a trial could be more efficient in finding an effect and therefore, a shorter time period would be sufficient.

Daily protein intake did not change over time in the two treatment groups, which suggested that participants who received the protein supplement did not compensate for the extra protein by eating less daily protein products. Together with the high compliance (92%), this showed that the supplement indeed was an extra protein source. The placebo drink was a non-caloric drink and did not contain any ingredients that may affect cognitive performance. Levels of eGFR were not lower after 24 weeks of supplementation in both intervention groups, indicating no harmful effects of extra protein intake occurred on kidney functioning.[31]

The results of our study were in accordance with the results of Jacobsen and colleagues who investigated the effects of a high-protein diet on several health outcomes, including cognitive performance, in young men.[7] After three weeks following a high-protein diet, reaction time improved significantly; on other cognitive functions however (attention, verbal fluency, memory, language, and visuospatial function), no effect was found. Reaction time, as measured in the present study with the finger-cuing task, is part of the domain information processing speed, together with the Stroop test (part I and II) and TMT part A. These latter tests did not improve after protein supplementation. This may be due to the fact that the finger-cuing task is a computer task, which may minimize external influences such as influence of research assistants. Also, computer tasks have much more trials and therefore the result will be more robust than the result of a test with a single trial. The finger-cuing task reaction time task could therefore be a more sensitive test than the Stroop test and TMT. Nevertheless, it should be taken into account that our positive findings on reaction time may be due to chance. Because of the large number tested, the likelihood of type I error increases. The construction of compound scores would overcome this risk, and the compound scores are therefore preferred over the single test scores. Performance on the reaction time test improved significantly over time and although the test was carefully explained and practiced beforehand, it would have been better to include a complete training session for all neuropsychological tests to forestall effects of training. It is possible that the large time effect may have diluted possible treatment effects.

Previous studies showed associations between cognitive performance, gait speed and balance in elderly people.[44, 48] Functions important for gait speed and balance, such as planning, monitoring and executing complex actions, are also needed for cognitive functions. In our

study population, a beneficial effect of dietary protein supplementation was already shown on physical functioning.[31] The improvement in reaction time we found in the current study might be linked to this improvement in physical functioning.

Protein supplementation may have an effect on blood concentrations of single amino acids,[18, 49] which causes variations in the uptake of amino acids into the brain and therewith influences the production of neurotransmitters.[50] Optimum levels of neurotransmitters for cognitive performance vary for different cognitive domains.[51] We used provided dairy protein as opposed to any mixture of specific amino acids, and as such, we cannot draw conclusions about the effect of specific amino acids. Other possible mechanisms for the effect of protein supplementation on cognitive performance might relate to vascular health. Cardiovascular diseases are a risk factor for cognitive decline; a better vascular health can be beneficial for cerebral perfusion.[52] A small beneficial effect of protein on blood pressure was suggested in a systematic review by Altorf-Van der Kuil et al.[53] This could be due to triggering a vasodilatory response, or via insulin sensitivity.[54] In additional data analyses though, we did not find an effect of protein on blood pressure.[31]

To conclude, in our study population of pre-frail and frail elderly people, protein supplementation did not improve performance on the cognitive domains episodic memory, attention and working memory, information processing speed, and executive functioning. However, our results indicate that extra protein intake might improve reaction time.

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5

Effect of resistance-type exercise training with or without protein supplementation on cognitive functioning in frail and pre-frail elderly people

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Abstract

Objective: Physical activity has been proposed as one of the most effective strategies to prevent cognitive decline. Protein supplementation may exert an additive effect. The effect of resistance-type exercise training with or without protein supplementation on cognitive functioning in frail and pre-frail elderly people was assessed in a secondary analysis.

Methods: Two 24-week, double-blind, randomized, placebo-controlled intervention studies were carried out in parallel. Subjects performed a resistance-type exercise program of two sessions per week ($n = 62$) or no exercise program ($n = 65$). In both studies subjects were randomly allocated to either a protein (2 x 15 g daily) or a placebo drink. Cognitive functioning was assessed with a neuropsychological test battery focusing on the cognitive domains episodic memory, attention and working memory, information processing speed, and executive functioning.

Results and conclusions: In frail and pre-frail elderly resistance-type exercise training in combination with protein supplementation improved information processing speed (changes in domain score 0.08 ± 0.51 versus -0.23 ± 0.19 in the non-exercise group, $p = 0.04$). Exercise training without protein supplementation was beneficial for attention and working memory (changes in domain scores 0.35 ± 0.70 versus -0.12 ± 0.69 in the non-exercise group, $p = 0.02$). There were no significant differences among the intervention groups on the other cognitive tests or domain scores.

Introduction

Current estimates indicate that 35.6 million people worldwide are living with dementia and because of the aging population this number is predicted to double by 2030 and more than triple by 2050.[1] Interventions targeted at risk factors for cognitive decline and Alzheimer's Disease (AD) may offer opportunities for development of an optimal preventive strategy. Dementia is preceded by a decline in cognitive functioning; however, this decline in cognitive functioning is not uniform across all older individuals or across all cognitive domains. One of the factors that have been associated with cognitive decline is physical activity. Several meta-analyses and systematic reviews have summarized the evidence for the impact of physical activity on cognitive function in older individuals that is provided by either observational studies,[2, 3] randomized controlled trials (RCTs),[4-10] or both.[11, 12] Generally, it has been suggested that physical activity improves cognitive function and prevents cognitive decline, but results of observational as well as experimental studies are limited and inconsistent to draw firm conclusions.

The heterogeneous results may be explained by varying study populations and differences in study duration, but also by the different intensities and types of exercise employed. Beneficial effects, though not all significant, of various kinds of exercise programs were observed; aerobic, strength, balance, flexibility, or a combination of these. It is unclear which type of exercise program is most effective, for what aspect of cognition and for which specific population. The importance of resistance training is stressed in the meta-analysis by Colcombe et al., who showed that aerobic-based training exercises together with resistance training exercises had a greater beneficial effect on cognition than programs of aerobic-based exercise training alone [13] and is also postulated by a recent literature review of Bherer et al.[12] Also, the few RCTs that used resistance training alone were largely positive. Study durations ranged from 8-52 weeks and effects were mainly observed on the cognitive domains memory and executive functioning.[7-9, 14, 15] A very recent study that was performed in frail older adults showed that three months of a combination of aerobic and strength exercise significantly improved several domains of cognitive performance.[16] Studies on different types of exercise in relation to brain function, structure and connectivity assessed using magnet resonance imaging (MRI) have also started to emerge and results are promising as has been summarized by Voelcker-Rehage and Niemann.[17]

In addition to exercise, dietary protein might improve cognitive functioning. Van de Rest et al. performed a literature review on the role of protein intake in relation to cognitive functioning and concluded that this has barely been studied.[18] Five observational and three case-control studies were found, showing mixed results. However, all three RCTs that were performed showed some beneficial effects. A population of physically frail older individuals would be a suitable target population for a combined exercise and protein supplementation intervention, as physical frailty has been identified as a risk factor for cognitive decline and AD,[19] frail elderly people generally have a limited habitual physical activity level and moreover, they have a suboptimal protein intake.[20]

We hypothesized that resistance-type exercise training would beneficially affect cognitive functioning, in particular in subjects supplemented with dietary protein, which would augment the exercise effect. Therefore, we examined the effect of 24 weeks resistance-type exercise training with or without protein supplementation on cognitive function in frail and pre-frail elderly people.

Methods

Participants

Individuals ≥65 years of age were recruited between December 2009 and October 2010, by using an existing database of volunteers, through distribution of information flyers, and by local information meetings. Potentially eligible participants were screened for pre-frailty and frailty using the five criteria from Fried et al.: [1] unintentional weight loss, [2] weakness, [3] self-reported exhaustion, [4] slow walking speed, and [5] low physical activity.[21] Pre-frailty was classified when one or two criteria were present and frailty when three or more criteria were present. Individuals who were diagnosed with cancer, chronic obstructive pulmonary disease, muscle disease, type 2 diabetes (plasma glucose concentration ≥7.0 mmol/L) [22] or renal insufficiency (estimated Glomerular Filtration Rate <60 mL/min/1.73 m^2) [23] were excluded. For participants in the resistance-type exercise training program, a resting electrocardiogram was performed to exclude silent ischemia. The Wageningen University Medical Ethical Committee approved the study and all subjects gave their written informed consent.

Study design

For the present study we used data of two randomized, placebo-controlled trials that were carried out in parallel. Both trials covered a 24-week intervention period and investigated the effect of protein versus placebo supplementation; in the absence or presence of additional resistance-type exercise training.[24, 25] Primary outcome in both studies was muscle mass, but one of the secondary outcome measures was cognitive functioning.

Sample size was calculated based on an expected difference in lean body mass of 1.1 kg between groups [26, 27] with a SD of 1.4 kg, a minimum of 24 subjects per treatment group would be required to detect a difference (power = 80%, $\alpha = 0.05$). With an expected dropout rate of 25% [28, 29] a sample size of 30 subjects per treatment group was considered adequate.

After inclusion, 62 of the 127 subjects participated in a 24-week resistance-type exercise training program and 65 did not perform any exercise program. All subjects were randomly allocated to either protein or placebo supplementation for 24 weeks. Allocation was carried out independently for both trials by an independent person using a computer-generated random numbers in stratified permuted blocks of size 4 and was stratified by gender. All outcome measures were collected at baseline and after 12 (except most cognitive function tests) and 24 weeks of intervention.

Resistance-type exercise program

The resistance-type exercise training was performed twice per week under personal supervision for a 24-week period. The sessions were performed in the morning and afternoon with at least 72 hours between sessions. The training consisted of a 5-minute warm-up on a cycle ergometer, followed by 4 sets on the leg-press and leg-extension machines and 3 sets on chest press, lat pulldown, pec-dec, and vertical row machines (Technogym, Rotterdam, the Netherlands). The workload started at 50% of 1-Repetition Maximum (1-RM) (10–15 repetitions per set) to 75% of 1-RM (8–10 repetitions) to stimulate muscle hypertrophy. Resting periods of 1 minute were allowed between sets and 2 minutes between exercises. To evaluate changes in muscle strength, 1-RM was repeated after 4, 8, 12, 16, and 20 weeks of training. Workload intensity was adjusted based on the 1-RM outcomes.

Protein supplementation

Twice daily, the subjects received either a 250-mL protein-supplemented beverage containing 15 g protein (MPC80; milk protein concentrate), 7.1 g lactose, 0.5 g fat, and 0.4 g calcium, or a matching placebo supplement containing no protein, 7.1 g lactose, and 0.4 g calcium (FrieslandCampina Consumer Products Europe, Wageningen, the Netherlands). All beverages were vanilla flavoured to mask the contents of the drinks and packages were non-transparent. The participants consumed one beverage after breakfast and one beverage after lunch. Adherence to the protein and placebo drinks was judged by returned ticked calendars and non-consumed beverages. Staff members and subjects were blinded toward treatment allocation until completion of data analysis.

Cognitive performance

At baseline, the Mini-Mental State Examination (MMSE) was performed to determine general cognitive status.[30] At baseline, after 12 weeks (only reaction time) and after 24 weeks of intervention, a standard battery of neuropsychological tests was performed by well-trained research assistants to assess the cognitive domains episodic memory, working memory and attention, information processing speed, and executive functioning. The Word Learning Test (WLT) measures immediate and delayed memory and retrieval of newly acquired verbal material.[31] The forward test of the Wechsler Digit Span Task measures attention and the backward test measures working memory.[32] The Trail Making Test (TMT) version A measures sensorimotoric speed and version B measures concept shifting interference (executive function).[33] The Stroop Color-Word Test measures selective attention and susceptibility to behavioral interference.[34] The Verbal Fluency test measures semantic memory and language.[35] The finger precuing task is a four-choice reaction time task [36] requiring participants to respond to spatial-location stimuli on a computer screen with discrete button-press responses by index and middle fingers of both hands. The screen always showed a reference row consisting of four plus signs indicating the four possible stimulus locations. A trial was started by showing a cue signal, which could be four plus signs in all four positions (neutral uncued condition) or only two plus signs, i.e.: the hand-cued

condition in which the cue specifies two fingers on the same hand; the finger-cued condition in which the cue specifies the same finger on two hands; or the neither-cued condition in which the cue specifies different fingers on two hands. Those cues reduce the number of possible stimulus locations from four to two. After a preparation interval of 2000 ms, the target signal (one plus sign) appeared in one of the cued positions. Participants had to react as quickly as possible to the target signal by pressing the corresponding response key. Outliers were defined as reaction time less than 150 ms or in excess of 2000 ms and were excluded from data analysis (mean percentage of outliers was 1.5% based on 160 trials per test session). Reaction time was based only on correct trials.

Blood sampling

Fasted blood samples were collected at baseline, after 12 weeks, and directly after the intervention to determine glucose homeostasis and renal function (only baseline data shown). Plasma glucose concentrations were analyzed with a COBAS FARA analyzer (Uni Kit III; Roche, Basel, Switzerland). Insulin was analyzed by radioimmunoassay (Insulin RIA Kit; LINCO Research Inc, St Charles, MO). Serum creatinine was measured by using Roche Modular System P (Roche Diagnostics GmbH, Mannheim, Germany).

Dietary intake

Participants recorded their food intake for three days at baseline, after 12 weeks, and after 24 weeks to assess potential changes in daily food intake during the intervention period (only baseline data shown). Trained dieticians gave oral and written instructions about recording the type of foods and estimating of portion sizes in household measures, and they checked the food records for completeness, obtained additional information about unclear items or amounts and used household measures to improve the estimation of portion size. Dietary intake data were coded (type of food, time of intake, and amount) and energy and macronutrient intakes were calculated using a nutrient computation program (Komeet) and the Dutch food composition database.[37]

Other measurements

Physical performance was assessed by the short physical performance battery (SPPB), which consists of three components: balance, gait speed, and chair rise ability.[38] Scores of 0 to 4 were based on categories of performance in the balance tests, on the time necessary to complete the walk, and on the time needed to perform the chair-rise test. A summary performance score of 0 to 12 was calculated by summing the scores of the tests. Habitual physical activity data were quantified using a tri-axial accelerometer (ActiGraph GTX3, 2009, Pensacola, FL, USA) worn on the hip for 1 week. Change of acceleration per second and epochs of 60 s were used. After 7 days, data were uploaded and analyzed using the MAH/UFFE analyzer, version 1.9.0.3 (MRC Epidemiology Unit, Cambridge, UK). Data files that did not meet 10 hours of monitoring per day on at least 5 days as well as files that included periods of >100 min without activity were excluded from the analysis. Mental health was

assessed using the Center for Epidemiologic Studies Depression Scale (CES-D), a 20-item, self-report scale developed to measure depressive symptoms experienced in the past week.[39] Overall health status and quality of life of the subjects was assessed using the 12-Item Short Form Health Survey (SF-12) [40] and the EuroQol-5D (EQ-5D).[41] The SF-12 is a short form of the widely used SF-36 that generates a physical (PCS12) and a mental (MCS12) composite score. The EQ-D5 defines health in five dimensions and a visual analogue scale indicates health status from 0-100. Height was measured at baseline with a wall-mounted stadiometer to the nearest 0.1 cm. Body weight was measured in the fasted state to the nearest 0.1 kg with a calibrated digital scale (ED-6-T; Berkel, Rotterdam, The Netherlands). Blood pressure was measured in the morning after a 10-minute rest with an Omron HEM-907 (Lake Forest, IL, USA) device. At each visit four measurements were done, with an interval of two minutes, of which the first measurement was discarded. The mean value of the three subsequent measurements for systolic and diastolic blood pressure was taken.

Statistics

Differences in baseline characteristics among the four intervention groups were compared using Analysis of Variance (ANOVA) for continuous variables or chi-square for categorical variables. Z-scores were created for cognitive tests at baseline and 24-week using the baseline data as norm data to calculate the grand mean and SD per test. Individual neuropsychological tests were clustered into compound scores for four neuropsychological domains:

$$\text{Episodic memory} = (Z_{\text{WLT total1-5 immediate recall}} + Z_{\text{WLT decay}} + Z_{\text{WLT recognition}})/3$$

$$\text{Attention and working memory} = (Z_{\text{Digit Span forward}} + Z_{\text{Digit Span backward}})/2$$

$$\text{Information processing speed} = (-Z_{\text{Trail making test part A}} + Z_{\text{Stroop mean I and II}} + -Z_{\text{Reaction time uncued}} + -Z_{\text{Reaction time cued}})/4$$

$$\text{Executive functioning} = (Z_{\text{Verbal Fluency Animal}} + Z_{\text{Verbal Fluency Letter P}} + Z_{\text{Stroop interference}} + - Z_{\text{Trail making interference}})/4$$

To take into account errors made during the Stroop test, accuracy (maximum right answers – amount of errors / maximum right answers) and speed-accuracy trade-off (accuracy / time needed to complete the task) were calculated.[42] To control for the effect of motor speed on performance we calculated interference measures for TMT (TMT B/TMT A) and Stroop part 3 (Stroop part 3-(part 1+part 2)/2).[43] For the finger precuing task trials with errors or outliers (reaction time <150 ms or >2000 ms) were excluded from data analyses. The three cued conditions of the reaction time test (hand-cued, finger-cued, and neither-cued) were averaged to obtain one variable cued reaction time. Analyses were performed for the effect of resistance-type exercise versus non-exercise within both protein supplemented groups and separately for the effect of resistance-type exercise versus non-exercise within both placebo supplemented groups. Differences between interventions overtime were analyzed using analyses of covariance (ANCOVA), in which the baseline data were included as the covariate, the outcome measures as the dependent factor and exercise intervention as the fixed factor. Linear Mixed Models with unstructured covariance structure was used to assess the effect of the intervention on reaction time, which was measured at three time points. Time, treatment

and their interaction were defined as fixed factors, whereas subject was defined as random factor. Data were analyzed by intention-to-treat and an α-level of 0.05 was used to determine statistical significance. All statistical analyses were performed using SPSS Statistics v19.

Results

Participants

Details of the participant flow of recruitment and screening have been published before.[24, 25] In total 127 frail and pre-frail elderly men and women fulfilled to the inclusion criteria and were willing to participate: 62 in the study with and 65 in the study without resistance-type exercise training. Nineteen participants withdrew during the intervention: eleven from the exercise intervention (five from the protein and six from the placebo group) and eight from the non-exercise intervention (four from the protein and four from the placebo group). Of these drop-outs eight were willing to have final measurements, so they could be included in the intention-to-treat analysis. The average adherence to the protein and placebo drinks based on ticked calendars and non-consumed returned beverages was 98% in the exercise study and 93% in the non-exercise study.

Mean age of the participants at baseline was 79 ± 8 years, 39% were men and these distributions were not significantly different among the intervention groups. There were also no significant differences in any of the other baseline characteristics among the intervention groups (**Table 1**). In total, 15 subjects had a MMSE score <25, suggesting a cognitive impairment.[44] However, the overall median MMSE score of 28 showed that most subjects were cognitively healthy.

Cognitive functioning

Baseline mean cognitive function scores for each of the individual tests and for the four cognitive domains composite scores did not differ among the groups (**Table 2**). **Table 3** shows the changes in cognitive tests and domains after 24 weeks of intervention. When comparing the exercise and non-exercise group who received additional protein supplementation for 24 weeks, the non-exercise group improved compared to the exercise group on Verbal Fluency Animals (2.4 ± 4.1 more animals compared to -0.6 ± 4.1 animals respectively, $p < 0.01$). On the domain of information processing speed the exercise group (change in domain score 0.08 ± 0.51) improved significantly compared to the non-exercise group (change in domain score -0.23 ± 0.19) ($p = 0.04$).

After 24 weeks of placebo supplementation, the exercise group significantly improved on the Digit Span forward test (0.7 ± 1.7 versus -0.3 ± 1.7 for the exercise and non-exercise group respectively, $p = 0.04$) (**Table 3**). Accordingly, this significant improvement was also observed in the corresponding cognitive domain of attention and working memory (change in domain score 0.35 ± 0.70 in the exercise group versus -0.12 ± 0.69 in the non-exercise group, $p = 0.02$). On the other cognitive tests or domain scores no significant differences were found among the intervention groups.

Table 1 Baseline characteristics of 127 Dutch frail and pre-frail elderly people by intervention group

	Protein (n = 65)		Placebo (n = 62)	
Characteristic	Exercise (*n* = 31)	Non-exercise (*n* = 34)	Exercise (*n* = 31)	Non-exercise (*n* = 31)
Age (yrs)[a]	77.7 ± 8.8	77.9 ± 8.1	79.2 ± 6.3	81.2 ± 7.4
Sex, men, n (%)	11 (36%)	14 (41%)	10 (32%)	15 (48%)
Education Low/ Middle/ High (%)	10 / 55 / 36	9 / 59 / 32	3 /71 /26	0 / 55 / 45
BMI (kg/m^2)	28.7 ± 4.5	27.0 ± 4.6	28.2 ± 4.6	26.2 ± 3.2
Smokers	2 (7)	5 (15)	1 (3)	1 (3)
Number of cigarettes/cigars per day	8	12	5	10
Alcohol consumers (%)	74	68	77	71
Alcohol consumption (g/ day)[b]	11 (6-22)	15 (5-30)	9 (3-17)	11 (3-22)
MMSE score (0-30)[c]	28 [27-29]	29 [26-30]	28 [27-29]	28 [26-30]
Range	23-30	21-30	23-30	22-30
CES-D score (0-60)	8.2 ± 6.9	6.6 ± 5.7	6.1 ± 6.0	7.0 ± 4.1
PCS12 (0-100 points)	42.6 ± 6.4	43.9 ± 10.0	42.7 ± 9.8	42.7 ± 9.8
MCS12 (0-100 points)	56.0 ± 8.2	54.0 ± 7.9	56.6 ± 7.2	54.9 ± 8.0
EQ-5D (0-1 points)	0.7 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	0.7 ± 0.2
Frailty score (1-5)[d]	1.5 ± 0.7	1.7 (1-2)	1.6 ± 0.8	2.0 (1-3)
Frail/pre-frail, *n*	4/27	7/27	7/24	11/20
Physical activity score (counts/minute)[e]	159 ± 111	153 ± 101	122 ± 69	115 ± 88
Baseline glucose (mmol/L)	5.4 ± 0.5	5.2 ± 0.4	5.2 ± 0.5	5.3 ± 0.4
Baseline insulin (mU/L)	19.6 ± 6.9	18.0 ± 7.4	18.1 ± 6.7	18.2 ± 7.1
eGFR (mL/min/1.73 m^2)	80.6 ± 14.1	84.4 ± 20.2	79.3 ± 13.9	77.6 ± 12.5
Blood pressure, systolic (mmHg)	142 ± 19.2	152 ± 25	143 ± 20.3	150 ± 23
Blood pressure, diastolic (mmHg)	74 ± 8.3	77 ± 10	73 ± 9.8	75 ± 9
SPPB (0-12)	8.0 ± 2.4	8.9 ± 2.8	7.9 ± 2.4	7.8 ± 3.6
Energy intake, kcal	1980 [1687-2226]	1981 [1513-2295]	1698 [1543-2128]	1932 [1593-2261]
Protein intake, g/day (En%)	72 [61-85] (16)	72 [57-92] (16)	75 [62-85] (17)	72 [64-78] (16)
Fat intake, g/day (En%)	76 [49-95] (33)	83 [55-107] (36)	71 [58-92] (35)	73 [57-94] (33)
Carbohydrate intake, g/day (En%)	234 [190-270] (47)	198 [166-256] (44)	184 [162-254] (45)	216 [173-273] (48)

Notes: BMI, Body Mass Index; MMSE, Mini-Mental State Examination; CES-D, Center for Epidemiologic Studies Depression Scale; PCS12, Physical Composite Score; MCS12, Mental Composite Score; EQ-5D, EuroQol-5D; eEGR, estimated Glomerular Filtration Rate; SPPB, Short Physical Performance Battery. [a] Mean ± SD (all such values); [b] Mean use in consumers only; [c] Median [interquartile range] (all such values); [d] Higher scores indicate being more frail according to Fried et al. [21]; [e] Significant difference between the protein and the placebo group ($p < 0.05$).

Both uncued as well as mean cued reaction time (and also all three individual cued conditions) significantly improved over time in all intervention groups ($p < 0.05$) (**Table 4**). However, neither exercise plus protein supplementation nor exercise without protein supplementation showed a significant benefit compared to non-exercise. Overall error rate was very low (1.7%), with small, non-significant reductions in error rate for all groups at the end of the treatment compared to baseline.

Table 2 Baseline cognitive test scores (mean ± SD) of Dutch frail and pre-frail elderly people, by intervention group

	Protein (n = 65)		**Placebo (n = 62)**	
Cognitive tests, maximum score	Exercise (*n* = 31)	Non-exercise (*n* = 34)	Exercise (*n* = 31)	Non-Exercise (*n* = 31)
Episodic memory				
Word learning test - Immediate recall 1-5, 75 words	31.8 ± 10.9	34.6 ± 11.8	33.6 ± 10.0	33.5 ± 10.5
Word learning test - Delayed recall, 15 words	6.1 ± 2.7	6.2 ± 3.1	6.5 ± 3.1	5.9 ± 2.9
Word learning test - Decay (delayed recall – WLT5)	-2.0 ± 1.8	-2.8 ± 1.5	-2.3 ± 1.8	-2.6 ± 2.0
Word learning test - Recognition, 30 words	27.5 ± 2.5	28.2 ± 1.9	28.1 ± 1.8	27.1 ± 2.7
Attention and working memory				
Digit Span forward, 16 points	8.0 ± 1.8	7.9 ± 1.4	7.9 ± 1.7	8.3 ±2.1
Digit Span backward, 14 points	5.2 ± 1.9	5.2 ± 1.3	5.5 ± 1.8	6.0 ± 2.3
Information processing speed				
Stroop 1[a]	1.85 ± 0.33[b]	1.91 ± 0.36	1.91 ± 0.37[c]	1.80 ± 0.33
Stroop 2[a]	1.47 ± 0.28[b]	1.5 ± 0.4	1.51 ± 0.32[c]	1.44 ± 0.28
Trail Making Part A (s)[d]	63.7 ± 29.0	61.6 ± 27.2	54.4 ± 24.3[c]	62.6 ± 23.0
Reaction time uncued (ms)[d]	833 ± 290[b]	764 ± 301	787 ± 221[c]	830 ± 243
Reaction time, mean cued conditions (ms)[d]	832 ± 309	753 ± 330	779 ± 240	821 ± 263
Executive functioning				
Stroop Interference (Part 3 – (Part 1 + Part 2/2))[a]	-0.89 ± 0.29[b]	-0.90 ± 0.24	-0.89 ± 0.25[c]	-0.79 ± 0.21
Trail Making Test (Part B/ Part A)[d]	1.93 ± 0.63	1.77 ± 0.61	1.68 ± 0.49[c]	1.83 ± 0.62
Word Fluency - Animals	20.6 ± 6.3	21.4 ± 6.4	20.0 ± 6.8	20.6 ± 5.8
Word Fluency - Letter P	13.0 ± 6.2	15.2 ± 5.8	14.7 ± 5.5	14.1 ± 3.8
Domain specific z-scores				
Episodic memory	-0.01 ± 0.63	0.04 ± 0.60	0.09 ± 0.62	-0.13 ± 0.77
Attention and working memory	-0.07 ± 0.90	-0.11 ± 0.64	-0.04 ± 0.87	0.22 ± 1.13
Information processing speed	-0.06 ± 0.74[c]	0.10 ± 1.00	0.11 ± 0.81[c]	-0.09 ± 0.75
Executive functioning	-0.14 ± 0.64[c]	0.06 ± 0.60	0.05 ± 0.58[c]	0.08 ± 0.55

Notes: [a] Corrected for errors (time needed / accuracy); [b] *n* = 30; [c] *n* = 29; [d] Higher scores indicate more time was needed to complete the task, so poorer performance.

Table 3 Changes on cognitive test scores (mean ± SD) of Dutch frail and pre-frail elderly people, by intervention group (only participants with baseline and end data)

Cognitive tests/ domains	Exercise (n = 28)			Non-exercise (n = 30)			
Protein	**Baseline**	**24 weeks**	**Change**	**Baseline**	**24 weeks**	**Change**	**p**
Episodic memory							
Word learning test - Immediate recall, 75 words	31.9 ± 10.9	35.1 ± 10.9	3.3 ± 7.4	35.9 ± 11.1	38.8 ± 12.0	2.9 ± 7.6	0.83
Word learning test - Delayed recall, 15 words	6.2 ± 2.6	6.8 ± 3.2	0.6 ± 1.9	6.6 ± 2.9	6.8 ± 3.6	0.2 ± 2.3	0.53
Word learning test - Decay	-2.0 ± 1.8	-2.1 ± 1.5	-0.2 ± 2.6	-2.7 ± 1.6	-2.6 ± 2.1	0.1 ± 2.4	0.37
Word learning test - Recognition, 30 words	27.4 ± 2.5	27.4 ± 2.8	0.0 ± 2.3	28.4 ± 1.9	27.7 ± 2.2	-0.7 ± 1.9	0.54
Attention and working memory							
Digit Span forward, 16 points	7.7 ± 1.6	7.9 ± 1.9	0.1 ± 1.4	8.0 ± 1.4	8.1 ± 1.7	0.1 ± 1.4	0.92
Digit Span backward, 14 points	5.2 ± 1.9	5.8 ± 1.9	0.6 ± 1.5	5.3 ± 1.3	5.4 ± 1.5	0.0 ± 1.2	0.16
Information processing speed[a]							
Trail Making Part A (s)[b]	59.4 ± 21.1	58.2 ± 25.8	-1.1 ± 24.2	57.2 ± 21.8	60.8 ± 36.9	3.7 ± 20.1	0.85
Stroop 1 (s)[c]	1.9 ± 0.3	1.9 ± 0.3	0.0 ± 0.2	2.0 ± 0.3	2.0 ± 0.3	-0.0 ± 0.2	0.95
Stroop 2 (s)[c]	1.5 ± 0.3	1.5 ± 0.3	0.0 ± 0.2	1.6 ± 0.3	1.6 ± 0.3	0.1 ± 0.1	0.60
Reaction time, uncued (ms)[b]	834 ± 295	744 ± 181	-90 ± 162	686 ± 136	644 ± 154	-42 ± 122	0.20
Mean reaction time cued (ms)[b]	830 ± 312	726 ± 226	-104 ± 149	667 ± 147	604 ± 172	-63 ± 107	0.19
Executive functioning[a]							
Stroop Interferencec	-0.9 ± 0.3	-0.9 ± 0.2	0.0 ± 0.2	-0.9 ± 0.2	-0.8 ± 0.2	0.1 ± 0.2	0.42
Word Fluency - Animals	21.2 ± 6.1	20.6 ± 5.8	-0.6 ± 4.1	21.8 ± 6.2	24.2 ± 6.5	2.4 ± 4.1	<0.01
Word Fluency - Letter P	13.0 ± 6.5	13.6 ± 6.1	0.6 ± 4.3	15.7 ± 5.6	15.7 ± 6.7	0.0 ± 4.9	0.99
Trail Making Test (Part B/Part A)[b]	1.86 ± 0.54	1.86 ± 0.57	0.01 ± 0.76	1.75 ± 0.63	1.77 ± 0.57	0.01 ± 0.60	0.75
Domain specific z-scores							
Episodic memory	-0.02 ± 0.64	0.05 ± 0.77	0.07 ± 0.62	0.11 ± 0.57	0.13 ± 0.83	0.01 ± 0.57	0.79
Attention and working memory	-0.16 ± 0.85	0.03 ± 0.97	0.19 ± 0.63	-0.03 ± 0.63	0.01 ± 0.79	0.04 ± 0.57	0.38
Information processing speed[a]	-0.05 ± 0.71	0.03 ± 0.41	0.08 ± 0.51	0.34 ± 0.60	0.10 ± 0.54	-0.23 ± 0.19	0.04
Executive functioning[a]	-0.09 ± 0.64	-0.05 ± 0.52	0.04 ± 0.44	0.07 ± 0.60	0.25 ± 0.64	0.17 ± 0.43	0.09

Table continues on the next page

Table 3 Changes on cognitive test scores of Dutch frail and pre-frail elderly people, by intervention group (only participants with baseline and end data) (mean ± SD) (continued)

	Exercise (n = 27)			Non-exercise (n = 28)			
Placebo	**Baseline**	**24 weeks**	**Change**	**Baseline**	**24 weeks**	**Change**	**p**
Episodic memory							
Word learning test - Immediate recall, 75 words	33.3 ± 10.5	37.0 ± 10.3	3.7 ± 6.2	34.4 ± 9.8	36.5 ± 10.4	2.1 ± 5.5	0.36
Word learning test - Delayed recall, 15 words	6.5 ± 3.3	6.9 ± 3.4	0.4 ± 2.2	6.1 ± 2.8	6.8 ± 3.5	0.7 ± 2.4	0.72
Word learning test - Decay	-2.1 ± 1.7	-2.4 ± 1.8	-0.3 ± 2.3	-2.7 ± 1.8	-2.2 ± 2.0	0.5 ± 2.6	0.58
Word learning test - Recognition, 30 words	28.2 ± 1.8	27.9 ± 2.1	-0.3 ± 1.6	27.4 ± 2.4	27.7 ± 2.6	0.3 ± 2.8	0.78
Attention and working memory							
Digit Span forward, 16 points	8.0 ± 1.7	8.8 ± 2.7	0.7 ± 1.7	8.3 ± 2.2	8.0 ± 2.1	-0.3 ± 1.7	0.04
Digit Span backward, 14 points	5.6 ± 1.8	6.2 ± 2.1	0.5 ± 1.6	6.2 ± 2.3	6.0 ± 2.4	-0.1 ± 1.7	0.22
Information processing speedd,e							
Trail Making Part A (s)[b]	55.9 ± 25.9	51.2 ± 17.6	-4.7 ± 15.5	63.9 ± 24.3	60.1 ± 25.1	-3.8 ± 14.8	0.50
Stroop 1 (s)[c]	1.9 ± 0.4	2.0 ± 0.4	0.0 ± 0.2	1.8 ± 0.3	1.8 ± 0.4	0.0 ± 0.2	0.85
Stroop 2 (s)[c]	1.5 ± 0.3	1.6 ± 0.4	0.1 ± 0.2	1.4 ± 0.3	1.5 ± 0.3	0.0 ± 0.2	0.47
Reaction time, uncued (ms)[b]	792 ± 239	722 ± 189	-69 ± 138	832 ± 236	812 ± 242	-21 ± 144	0.10
Mean reaction time cued (ms)[b]	794 ± 261	696 ± 206	-98 ± 148	813 ± 260	772 ± 271	-41 ± 135	0.10
Executive functioning[d,f]							
Stroop Interference[c]	-0.9 ± 0.3	-0.9 ± 0.3	0.0 ± 0.2	-0.8 ± 0.2	-0.8 ± 0.2	-0.0 ± 0.2	0.84
Word Fluency - Animals	20.6 ± 7.3	20.2 ± 7.7	-0.4 ± 4.4	20.9 ± 5.6	22.0 ± 6.3	1.1 ± 3.7	0.21
Word Fluency - Letter P	15.7 ± 5.5	15.9 ± 6.1	0.2 ± 4.8	14.6 ± 3.8	14.9 ± 5.7	0.3 ± 4.1	0.99
Trail Making Test (Part B/Part A)[b]	1.62 ± 0.47	1.59 ± 0.43	-0.04 ± 0.59	1.83 ± 0.65	1.79 ± 0.69	-0.04 ± 0.81	0.37
Domain specific z-scores							
Episodic memory	0.11 ± 0.65	0.13 ± 0.71	0.02 ± 0.57	-0.08 ± 0.68	0.12 ± 0.87	0.20 ± 0.79	0.51
Attention and working memory	0.05 ± 0.85	0.40 ± 1.27	0.35 ± 0.70	0.28 ± 1.16	0.16 ± 1.13	-0.12 ± 0.69	0.02
Information processing speed[d, c]	0.11 ± 0.87	0.18 ± 0.47	0.07 ± 0.48	-0.14 ± 0.78	-0.01 ± 0.46	0.13 ± 0.50	0.32
Executive functioning[d,f]	0.09 ± 0.62	0.12 ± 0.55	0.02 ± 0.42	0.10 ± 0.58	0.17 ± 0.61	0.07 ± 0.42	0.78

Notes: Differences between the two groups were measured using ANCOVA (baseline cognitive function as covariate). [a] $n = 26$ for the exercise-protein group; [b] Higher scores indicate more time was needed to complete the task, so poorer performance; [c] Corrected for errors (time needed / accuracy); [d] $n = 24$; [e] $n = 27$; [f] $n = 26$.

Table 4 Reaction time (in ms) of Dutch frail and pre-frail elderly measured at baseline, and after 12 and 24 weeks, by intervention group (mean ± SD)

	Baseline	12 weeks	24 weeks	Treatment*time (p)	Treatment effect (p)	Time effect (p)
Protein						
RT uncued				0.69	0.17	<0.01
Exercise	833 ± 290	832 ± 268	769 ± 220			
Non-exercise	764 ± 301	663 ± 151	671 ± 183			
RT hand-cued				0.92	0.86	<0.01
Exercise	798 ± 321	756 ± 267	725 ± 249			
Non-exercise	716 ± 318	597 ± 170	613 ± 210			
RT finger-cued				0.22	0.13	<0.01
Exercise	847 ± 304	844 ± 303	765 ± 262			
Non-exercise	772 ± 333	640 ± 179	643 ± 208			
RT neither-cued				0.90	0.47	<0.01
Exercise	850 ± 310	820 ± 266	762 ± 259			
Non-exercise	771 ± 342	648 ± 184	650 ± 213			
RT mean cued				0.79	1.00	<0.01
Exercise	832 ± 309	807 ± 276	751 ± 255			
Non-exercise	753 ± 330	628 ± 176	635 ± 208			
Placebo						
RT uncued				0.43	0.27	0.01
Exercise	787 ± 221	741 ± 182	722 ± 189			
Non-exercise	830 ± 243	778 ± 196	808 ± 238			
RT hand-cued				0.37	0.41	0.02
Exercise	748 ± 234	689 ± 201	670 ± 196			
Non-exercise	767 ± 263	726 ± 208	740 ± 260			
RT finger-cued				0.25	0.32	<0.01
Exercise	790 ± 239	752 ± 211	706 ± 217			
Non-exercise	840 ± 261	773 ± 215	799 ± 291			
RT neither-cued				0.52	1.00	<0.01
Exercise	800 ± 256	752 ± 218	711 ± 210			
Non-exercise	857 ± 275	781 ± 221	785 ± 251			
RT mean cued				0.37	1.00	<0.01
Exercise	779 ± 240	731 ± 208	696 ± 206			
Non-exercise	821 ± 263	760 ± 212	775 ± 266			

Notes: Differences between the two groups were measured using Linear Mixed Models. Higher scores indicate more time was needed to complete the task, so poorer performance.
Protein with exercise: Baseline n = 30, 12 weeks n = 30, 24 weeks n = 27
Protein non-exercise: Baseline n = 28, 12 weeks n = 32, 24 weeks n = 32
Placebo with exercise: Baseline n = 29, 12 weeks n = 27, 24 weeks n = 24
Placebo non-exercise: Baseline n = 31, 12 weeks n = 29, 24 weeks n = 28

Discussion

In this study performed in frail and pre-frail elderly people, 24 weeks of resistance-type exercise training in combination with protein supplementation improved the cognitive domain information processing speed and the same training without protein supplementation was beneficial for attention and working memory.

To the best of our knowledge, this is the first study that investigated the effect of resistance-type exercise in combination with protein supplementation on cognitive functioning. As cognitive function was not the primary outcome of the study, the total number of participants included was not based on cognition function as outcome measure, but on the primary outcome muscle mass. However, compared to the other interventions studies that have been performed on resistance-type exercise and cognitive function, this was still one of the largest studies [14-16, 45-51] and the only one that combined exercise with protein supplementation. Furthermore, cognitive function was measured in a standardized way with an extensive neurological test battery, which enabled us to thoroughly investigate the effects of the intervention on multiple cognitive domains that are sensitive to age-related cognitive decline.

As frailty has been associated with cognitive impairment [19] we expected this study population to be relatively cognitively impaired and therefore subjective to change. However, except 15 individuals who scored <25 points on the MMSE, the group in general was still cognitively healthy. Still we observed beneficial changes in cognitive functioning on the cognitive domain information processing speed after the exercise plus protein intervention and on attention and working memory after the exercise intervention without additional protein supplementation. In more cognitively impaired elderly people, exercise might benefit other individual neuropsychological tests or cognitive domains as well.

The current study of 24 weeks might also have been too short to pick up an effect of the interventions on the other cognitive domains, because the process of cognitive decline is a rather slow process. According to the IANA taskforce, trials studying effect on cognitive functioning should have a study duration of at least 18 months.[52] Though, compared to the other resistance-type exercise intervention studies that did find effects on cognitive function, our intervention was of average length. Only two studies were longer with respectively 9 and 12 months of intervention [14, 45] and because our study population of frail and pre-frail older individuals was expected to be more sensitive, effects could have occurred faster. Additionally, seen the extensive exercise program that was used in our study and the fact that this was performed by frail and pre-frail individuals, 24 weeks was a substantial period for this target group and a longer study duration could have resulted in a relatively large number of drop-outs and age-related co-morbidities interfering with the effects of the intervention.

The majority of the other intervention studies that focused on the effects of resistance training on cognitive function also observed an effect, mostly on the cognitive domains of memory and executive functioning.[7-9, 14-16] We did observe an effect of the resistance training on attention and working memory, but not on episodic memory. Additionally, we observed

an effect of resistance-type exercise plus protein supplementation on information processing speed. It may be that for improvement in speed related tasks, where the neuromuscular system is involved, additional protein is needed to reinforce the effect of exercise. Also, in our cognitive domain of information processing speed, the finger precuing task was a major component. This is a complex reaction time task, which involves more central processing and may be more likely to show improvements than simple reaction time tasks. More trials of sufficient duration and size, performed in susceptible target groups, and with extensive and sensitive neuropsychological test batteries like ours, are needed to disentangle these effects.

Although the suggestion that physical exercise training is beneficial for cognitive functioning is becoming more and more established, the underlying biological mechanisms are not yet elucidated. In the review of Bherer et al. (2013) the current ideas have been summarized and include direct effects at the supramolecular and molecular level.[12] In animal studies, physical activity has been shown to induce angiogenesis, neurogenesis, neural cell proliferation or synaptogenesis. The evidence for molecular mechanisms also comes from animal studies and include changes in molecular growth factors such as brain-derived neurotrophic factor (BDNF) and insulin-like growth factor (IGF-1).[12] The findings for an exercise-related increase in BDNF have also been shown for the first time in humans and were associated with an increased hippocampal volume and improved memory.[53] Recently, also differences in underlying molecular mechanisms for different types of exercise were postulated; aerobic training increased BDNF in the hippocampus and resistance training increased IGF-1 levels.[54] Something to take into consideration in future studies is that there are also some factors that might moderate the effects of physical activity on brain health, such as apolipoprotein E (APOE), BDNF, catechol-O-methyltransferase (COMT) and the dietary omega-3 fatty acids.[55, 56] Furthermore, studies on physical activity and brain function, structure and connectivity assessed using MRI are still in their early stages, but with these studies could also provide more insight in underlying mechanisms.[17]

In addition to the resistance-type exercise program participants were also supplemented with either protein or placebo. One of the possible links between protein and cognitive functioning may be the fact that dietary protein contains some indispensable amino acids, tryptophan and tyrosine in particular, which act as precursors for several brain neurotransmitters.[57] However, the number of studies on supplementation with protein or some individual amino acids is very small and more research is needed to elucidate the effects on cognitive functioning.

Our main interest was in the effects of resistance-type exercise training, with or without protein supplementation, on cognitive functioning. This is the first time that the effects of a resistance-type exercise intervention could be examined in a frail elderly population. However, to examine this interesting effect, analyses had to be performed across the two trials, which may be a methodological limitation. Although not designed as such, the two trials together also approach a 2x2 factorial design. To examine possible interaction effects between exercise and protein, we explored the results of adding an interaction term to our models, despite the fact that the trials were each powered for main effects and not for interaction effects. There was no significant interaction between exercise and protein

supplementation on any of the cognitive domains, but on the individual cognitive tests a significant interaction was observed for the uncued and cued reaction time tests ($p = 0.05$ for both tests). However, taking into account the limited power, these p-values have to be interpreted with caution. Moreover, this interaction was not observed in the corresponding cognitive domain of information processing speed. Still, a possible interaction between exercise and protein supplementation has to be taken into account in future studies using similar interventions in parallel.

In summary, in this population of frail and pre-frail elderly people 24 weeks of resistance-type exercise training in combination with protein supplementation was beneficial for the cognitive domain information processing speed and the same training without protein supplementation was beneficial for attention and working memory. We were able to investigate the effect of exercise with or without protein supplementation, as an example for a study on interaction or synergy between diet and physical activity, an area which is relatively unexplored so far.

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6.

Results of two-year B-vitamin supplementation on cognitive performance

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Abstract

Objective: We investigated the effects of two-year folic acid and vitamin B_{12} supplementation on cognitive performance in elderly people with elevated homocysteine (Hcy) levels.

Methods: This multicenter, double-blind, randomized, placebo-controlled trial included 2,919 elderly participants (≥65 years) with Hcy levels between 12 and 50 μmol/L. Participants received daily either a tablet with 400 μg folic acid and 500 μg vitamin B_{12} (B-vitamin group), or a placebo tablet. Both tablets contained 15 μg vitamin D_3. Data were available for global cognitive functioning assessed by MMSE (*n* = 2,556), episodic memory (*n* = 2,467), attention and working memory (*n* = 759), information processing speed (*n* = 731), and executive function (*n* = 721).

Results: Mean age was 74.1 ± 6.5 years. Hcy concentrations decreased 5.0 (95% CI -5.3--4.7) μmol/L in the B-vitamin group and 1.3 (95% CI -1.6--0.9) μmol/L in the placebo group. Cognitive domain scores did not differ over time between the two groups, as determined by ANCOVA. MMSE score decreased with 0.1 (95% CI -0.2--0.0) in the B-vitamin group and 0.3 (95% CI -0.4--0.2) in the placebo group (p = 0.05), as determined by an independent t-test.

Conclusions: Two-year folic acid and vitamin B_{12} supplementation did not beneficially affect performance on four cognitive domains in elderly people with elevated Hcy levels. It may slightly slow the rate of decline of global cognition, but the reported small difference may be due to chance.

Introduction

With the aging of populations, the prevalence of dementia is growing dramatically.[1] A possible modifiable risk factor of dementia is an elevated plasma homocysteine level (Hcy). Hcy may be toxic for neurons and vascular endothelial cells,[2] and cross-sectional and prospective studies have shown associations between elevated Hcy levels and cognitive decline and dementia.[2, 3]

Hcy levels can be lowered by supplementation with folic acid and vitamin B_{12}.[4] Serum folate and vitamin B_{12} are involved in the Hcy pathway, acting on the conversion of Hcy into methionine. Deficiencies of these vitamins may result in elevated Hcy levels, impaired DNA synthesis and methylation, and accumulation of abnormal fatty acids in membranes of neural tissue.[2, 5] This may negatively influence cognitive performance. Although observational studies have shown associations between poor vitamin B_{12} and folate status and cognitive performance,[2] evidence from randomized controlled trials (RCTs) is limited and less convincing.[3] A meta-analysis of thirteen trials in healthy older adults did not observe beneficial effects of supplementation with vitamin B_{12}, folic acid, vitamin B_6, or a combination of these vitamins.[3] However, the studies were heterogeneous in their used dosage and study population, which makes comparisons difficult. Furthermore, only four trials had a study period of one year or longer. Therefore, we investigated the effects of two-year folic acid and vitamin B_{12} supplementation on cognitive performance in elderly people with elevated Hcy levels through a double-blind, randomized, placebo-controlled trial.

Methods

Study design and Participants

The study was conducted among Dutch elderly men and women who participated in the B-PROOF (B-Vitamins for the Prevention of Osteoporotic Fractures) study.[6] Primary goal of the B-PROOF study was to assess the efficacy of oral supplementation with 400 μg folic acid and 500 μg vitamin B_{12} in the prevention of fractures, with cognitive performance as secondary outcome. The study was conducted in three research centers in the Netherlands: VU University Medical Center (VUmc, Amsterdam), Erasmus MC (Rotterdam) and Wageningen University (WU, Wageningen).

Primary outcome of the current study was the difference between treatment groups in performance on episodic memory after two years of supplementation (Class I evidence). We hypothesized that supplementation with folic acid and vitamin B_{12} would delay decline in episodic memory by, among others, lowering plasma Hcy levels. Secondary outcomes were the differences in performance at follow-up on attention and working memory, information processing speed, executive function, and single test scores (Class I evidence).

Participant selection and attrition have been extensively described elsewhere.[6] In short, most participants were recruited via the registries of municipalities in the surroundings of

the research centers. The study was carried out between October 2008 and March 2013. Inclusion criteria were being 65 years and older, having an elevated plasma Hcy level (12-50 μmol/L), being competent to make own decisions, and having a compliant tablet intake (≥85%) in the run-in period. Exclusion criteria were: cancer diagnosis within the last five years except for basal cell carcinoma and squamous cell carcinoma, bedridden, serum creatinine level >150 μmol/L, current or recent (<4 months) use of intramuscular injections of vitamin B_{12} or folic acid supplementations (>300 μmol), and participation in other intervention studies.

Standard Protocol Approvals, Registrations, and Patient Consents

The WU Medical Ethical Committee approved the study and the Medical Ethics Committees of Erasmus MC and VUmc gave approval for local feasibility. All participants gave their written informed consent. This trial is registered at clinicaltrials.gov as NCT00696514 and at Netherlands Trial Register as NTR1333.

Randomization and masking

Participants were randomly allocated (1:1 ratio) to receive either a daily tablet with 400 μg folic acid and 500 μg vitamin B_{12}, or a placebo tablet for two years. Both tablets contained 15 μg (600 IU) of vitamin D_3 to ensure normal vitamin D status. Randomization was done by an independent person by means of computer-generated randomization numbers in stratified permuted blocks of size 4, stratified by study center, sex, age (65-80 years, ≥80 years), and Hcy levels (12-18 μmol/L, ≥18 μmol/L). All participants and employees of the study were blinded until data analyses were finished. Every six months participants received new tablets and participants were requested to return any remaining tablets in order to measure compliance.

Descriptive characteristics

Standing height was measured with a stadiometer to the nearest 0.1 cm. Weight was measured to the nearest 0.5 kg with a calibrated scale (Seca, Deventer, the Netherlands). Information about education level, marital status, living situation, smoking habits, alcohol intake, and medical history was obtained by questionnaires. Depressive symptoms were measured with the short Geriatric Depression Scale (GDS-15), in which a score ≥5 indicates being at risk for depression.[7] Physical activity was measured with the LASA Physical Activity Questionnaire; frequency and duration of activities during the past two weeks were assessed to calculate physical activity in kcal/day.[8]

Cognitive tests

Cognitive performance was assessed at baseline and at the end of the intervention period by well-trained research assistants following a standard protocol. We used the Mini-Mental State Examination (MMSE) [9] and an extensive, sensitive, and validated neuropsychological test battery to assess the cognitive domains episodic memory, attention and working

memory, information processing speed, and executive function. The battery consisted of the Rey Auditory Verbal Learning Test (RAVLT),[10] Digit Span forward and backward,[11] Trail Making Test (TMT) part A and B,[12] Stroop Color-Word Test,[13] Symbol Digit Modalities Test (SDMT),[14] and the Letter Fluency (three letters) (see **Appendix 1** for a detailed description).[15] The MMSE and the RAVLT were performed in all three research centers, the other tests were only assessed in the WU participants. For the RAVLT, TMT, and Verbal Fluency test, validated parallel versions were used for baseline and the two-year measurement to minimize learning effects.

RAVLT decayed recall was calculated as the number of words recalled after approximately 15 minutes following the fifth session of the RAVLT, minus the number of words recalled at the fifth session of the RAVLT. To control for the effect of motor speed on performance we calculated interference measures for TMT (TMT B/TMT A) and Stroop part 3 (Time needed for Stroop part 3 – (mean time needed for Stroop part 1 and 2)). The scores on the three letters of Verbal Fluency test were summed.

To compare the results of the individual cognitive tests and to limit the number of dependent variables, crude test scores were clustered into compound scores for the four neuropsychological domains. Data of baseline measurements were used as norm data to create individual Z-scores for baseline and two-year follow-up data (Z-score = (score test – mean baseline)/SD baseline).

Blood markers

Before and immediately after the intervention period blood samples through venapunction were collected in a fasted state or after a restricted breakfast to obtain whole blood, plasma, serum and buffy coats.[6] Plasma Hcy, serum creatinine, vitamin B_{12}, folate, holotranscobalamin (holoTC), and methylmelonic acid (MMA) were determined. DNA was isolated to determine the genotype for Methylenetetrahydrofolate reductase (MTHFR 677T) and Apolipoprotein E (ApoE, rs429358) genotype. Detailed methodology can be found in the **Appendix 1**.

Statistics

Analyses were performed before unblinding of the treatment code. Intention-to-treat analyses, with all participants who completed reliable baseline and follow-up measurements included, and per-protocol analyses, with those participants who were compliant to the study protocol (≥80% of total tablet intake), were performed. Data are reported as *n* (%), means ± SD, or as median [interquartile range (IQR)] for non-normally distributed data. Baseline characteristics were compared between treatment groups using Chi-square, independent-samples t or Mann-Whitney non-parametric tests. Also differences in age, sex, Hcy, and MMSE between dropouts and participants who completed the study were tested.

Differences between treatment groups over time were analyzed using analyses of covariance (ANCOVA), in which the outcome measures at two-year acted as the dependent factor,

baseline data as a covariate factor, and treatment as the fixed between-subject factor. In the fully adjusted model, age, sex, and study center were added as covariates. Because test scores on the MMSE violated normality assumptions of ANCOVA, change over time was calculated and analyzed for treatment effects with an independent-samples t-test.

For all four cognitive domains, predefined interaction terms of treatment with sex, baseline age (<80, ≥80 years), plasma Hcy (<18, ≥18 μmol/L), and ApoE genotype (ApoE-ε4 or not) were tested. A $p<0.10$ for interaction was considered as a justification for stratified subgroup analyses. Exploratory post-hoc analyses were done for MTHFR genotype, baseline levels of vitamin B_{12}, folate, MMA, and holoTC, and for follow-up Hcy concentration (<12.0, ≥12.0 μmol/L).

A two-sided p-value of < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS Statistics v20 (SPSS Inc. Chicago, IL, USA).

Results

Participants

Figure 1 shows the CONSORT participant flow through the study for the total study population. In the placebo group, 200 participants (14%) discontinued use of tablets, whereas this number was 222 in the B-vitamin group (15%, n.s.). Dropouts were older (77.1 years, $p < 0.01$), had a higher median Hcy level (15.2 μmol/L, $p < 0.01$), had a lower MMSE score (median 27, $p < 0.01$), and were more likely to be women (16% versus 13% men, $p = 0.01$) compared to persons who completed the study. Average compliance to treatment was 90%, and 84% of all participants had an overall compliance ≥80%. Compliance was equal for both treatment groups.

Mean age of the total population was 74.1 ± 6.5 years, 50% were men, and MMSE score was 28 (IQR 27-29, range 14-30) (**Table 1**). Baseline characteristics were equal for both treatment groups, except for serum holoTC ($p = 0.03$, data not shown). In the WU subpopulation with extensive cognitive tests ($n = 856$), baseline characteristics were all similar for both treatment groups. In this subgroup, mean age was 72.6 ± 5.8 years and 58% were men (**Table 1**).

Plasma Hcy levels decreased more in the B-vitamin group compared to placebo ($p<0.01$) (total population). In the B-vitamin group, levels decreased from a median of 13.9 [IQR 12.9-16.2] to 9.3 [IQR 8.2-11.2] μmol/L (mean change of -5.0, 95% CI -5.3--4.7), versus 14.4 [IQR 12.9-16.4] to 13.3 [IQR 11.5-16.0] μmol/L (mean change of -1.3, 95% CI -1.6--0.9) in the placebo group.

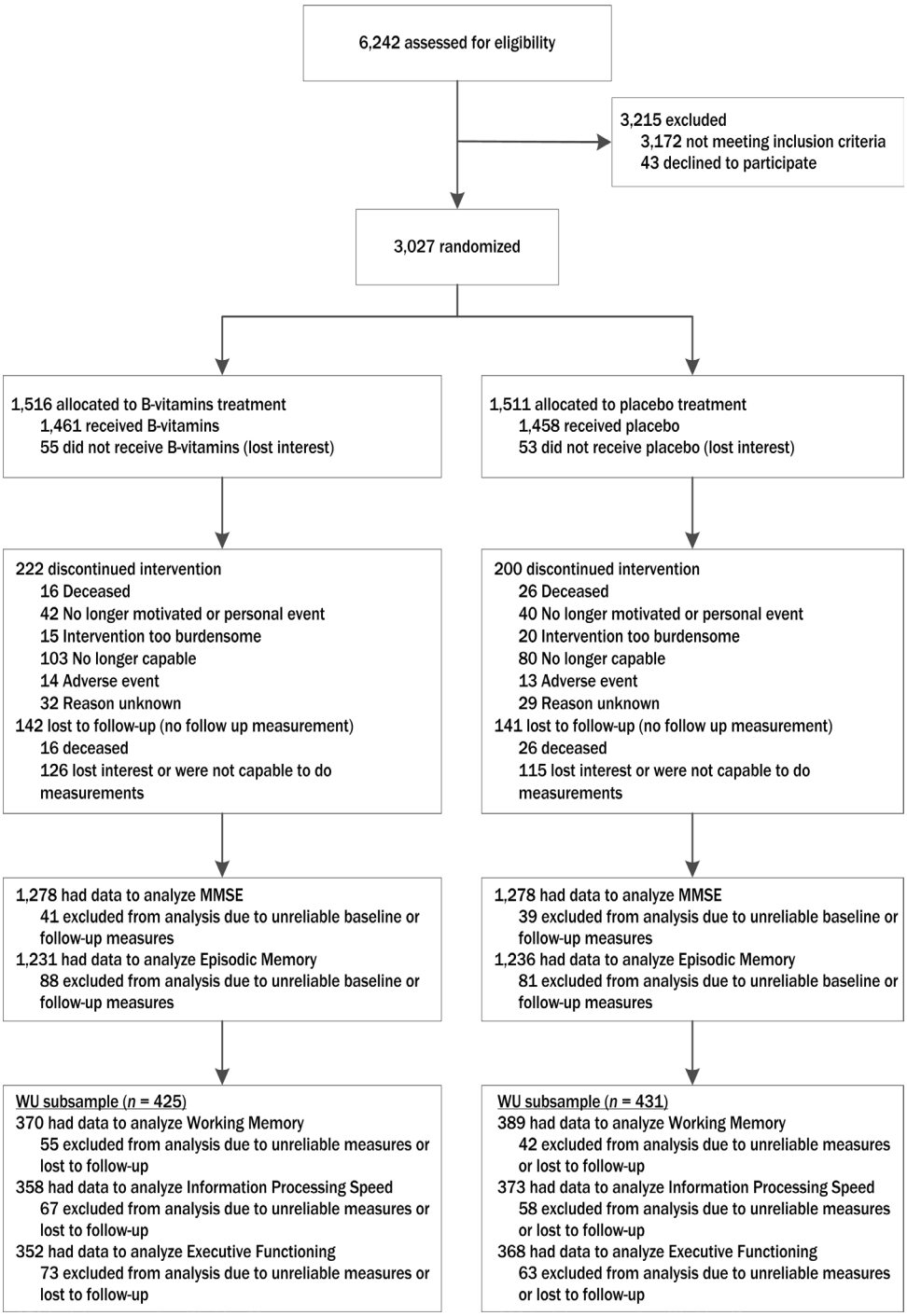

Figure 1 Screening, randomization and follow-up in the B-PROOF study concerning the cognitive assessment.

Table 1 Baseline characteristics of the cognitive subsample per treatment group (n = 856) and the total B-PROOF population (n = 2,919)

	Cognitive subsample		Total population
	Placebo (n = 431)	B-vitamin (n = 425)	n = 2,919
Descriptive measures			
Age (yrs)[a]	72.6 ± 5.8	72.6 ± 5.7	74.1 ± 6.5
Sex, men[b]	255 (59%)	244 (57%)	1459 (50%)
Education			
Low	184 (43%)	184 (43%)	1545 (53%)
Middle	99 (23%)	91 (21%)	615 (21%)
High	148 (34%)	150 (35%)	757 (26%)
Marital status			
Living together or married	295 (68%)	296 (70%)	1845 (63%)
Widow	81 (19%)	75 (18%)	712 (24%)
Unmarried or divorced	55 (13%)	54 (13%)	361 (12%)
Smoker behavior			
Current smoker	49 (11%)	38 (9%)	281 (10%)
Former smoker	249 (58%)	253 (60%)	1649 (57%)
Never smoked	133 (31%)	134 (32%)	989 (34%)
Alcohol consumers	381 (88%)	385 (91%)	2511 (86%)
BMI (kg/m^2)	27.2 ± 3.8	27.2 ± 4.0	27.1 ± 4.0
MMSE score[c]	29 [8-30]	29 [27-30]	28 [27-29]
Score <25	21 (5%)	24 (6%)	177 (6%)
GDS score	1 [0-2]	1 [0-2]	1 [0-2]
Score≥5	21 (5%)	26 (6%)	200 (7%)
Total physical activity (kcal/day)	661 ± 483	669 ± 471	650 ± 479
Biochemical measures			
Plasma Hcy (µmol/L)	14.4 [12.9-16.4]	14.0 [12.9-16.2]	14.4 [13.0–16.6]
Serum vitamin B_{12} (pmol/L)	263 [200-345]	257 [200-326]	266 [208-343]
Serum folate (nmol/L)	18.9 [14.2-22.7]	19.2 [14.0-22.6]	18.7 [14.7-24.2]
Serum holoTC (pmol/L)	60 [42-80]	59 [46-78]	64 [46-85]
Serum MMA (µmol/L)	0.23 [0.19–0.31]	0.23 [0.19-0.30]	0.23 [0.18-0.30]
Genetic polymorphism			
MTHFR genotyped			
677 TT	47 (12%)	57 (15%)	334 (13%)
677 CT/CC	344 (88%)	318 (85%)	2236 (87%)
ApoE-ε4[c]			
TT	301 (73%)	276 (68%)	2023 (73%)
TC	105 (25%)	117 (29%)	692 (25%)
CC	7 (2%)	10 (2%)	60 (2%)

Notes: BMI, Body Mass Index; MMSE, Mini-Mental State Examination; GDS, Geriatric Depression Scale; Hcy, Homocysteine; holoTC, holotranscobalamin; MMA, Methylmelonic acid; MTHFR, Methylenetrahydrofolate reductase; ApoE-ε4, apolipoprotein E-ε4. [a] Mean ± SD; [b] n (%); [c] median [IQR]; [d] data available for n = 766 in the cognitive subsample and n = 2,570 in the total population; [e] Data available for n = 816 in the cognitive subsample and n = 2,721 in the total population.

Cognitive performance

Mean baseline cognitive test scores did not differ between treatment groups (**Supplementary Table 1**). In the intention-to-treat analyses (**Table 2**), both treatment groups improved performance on episodic memory (total population) and executive functioning (subpopulation) after two years, but no effect of B-vitamin treatment was observed. More specifically, episodic memory improved with 0.08 (95% CI 0.05-0.12) in the placebo group and with 0.11 (95% CI 0.07-0.14) in the B-vitamin group (p = 0.42). Performance by the subpopulation on attention and working memory, and information processing speed declined over time, but there was no treatment effect.

Table 3 shows the performance on single cognitive tests at baseline and after two years. Performance on the MMSE, measured in the total population, decreased slightly less in the B-vitamin group compared to the placebo group (p-value for difference between groups = 0.05). None of the other single cognitive tests at two years were significantly different between the treatment groups. Verbal fluency tended to improve more in the B-vitamin group (change 2.6, 95% CI 1.8-3.4) compared to the placebo group (change 1.6, 95% CI 0.9-2.3), but this was not significant (p = 0.10). The per-protocol analyses revealed slightly larger differences between groups, but no significant effects were observed (n = 2,284 for episodic memory and $n \approx 688$ for the other domains) (data not shown).

Subgroup analyses

The predefined variables that were tested for interaction effects with treatment only revealed a significant interaction effect for age on information processing speed. Stratified analyses in participants <80 years (n = 686) and ≥80 years (n = 45), however, did not show any treatment effect (see **Supplementary Table 2 and Table 3**). Results of explorative post-hoc subgroup analyses did not reveal beneficial effects of B-vitamin treatment, except that participants with a low holoTC concentration in the B-vitamin group improved more (0.13, 95% CI 0.08-0.18) on their episodic memory performance than the placebo group (0.08, 95% CI 0.03-0.13, p = 0.04) (**Supplementary Table 3**).

Table 2 Changes in cognitive domain scores of elderly people (only participants with baseline and two-year data)

	Unadjusted mean ± SD		Mean change (95% CI)	Model 1	Model 2
	Baseline	2 yr		p	p
Episodic memory (*n* = 2,467)[a]					
Placebo	0.04 ± 0.69	0.13 ± 0.75	0.08 (0.05-0.12)	0.27	0.42
B-vitamins	0.05 ± 0.69	0.16 ± 0.75	0.11 (0.07-0.14)		
Attention and working memory (*n* = 759)					
Placebo	0.02 ± 0.86	-0.04 ± 0.88	-0.06 (-0.12-0.01)	0.38	0.37
B-vitamins	-0.01 ± 0.84	-0.10 ± 0.82	-0.09 (-0.16--0.02)		
Information processing speed (*n* = 731)					
Placebo	0.08 ± 0.75	0.06 ± 0.79	-0.02 (-0.06-0.01)	0.65	0.51
B-vitamins	0.04 ± 0.75	0.01 ± 0.77	-0.03 (-0.07-0.00)		
Executive functioning (*n* = 720)					
Placebo	0.04 ± 0.54	0.10 ± 0.68	0.06 (-0.00-0.12)	0.20	0.26
B-vitamins	-0.01 ± 0.52	0.13 ± 0.66	0.13 (0.07-0.19)		

Notes: Differences between the two groups over time were measured using ANCOVA. Model 1: Adjusted for baseline domain scores; Model 2: Adjusted for baseline domain scores, age, sex ([a] and study center)

Discussion

This large randomized, placebo-controlled trial did not reveal beneficial effects of two years supplementation with folic acid and vitamin B_{12} on the cognitive domains of episodic memory, attention and working memory, information processing speed, and executive function in elderly people with elevated Hcy levels. However, decline of global cognitive performance was slightly lower in the B-vitamin group, but the effect would have disappeared when correcting for multiple testing.

Although cross-sectional studies have often demonstrated positive associations between folate or vitamin B_{12} status and cognitive performance, results from RCTs do not convincingly support this.[2] We add evidence to this with our study, which is one of the largest intervention study in elderly people up till now that investigated the effects of long-term B-vitamin supplementation on global cognitive function, episodic memory, attention and working memory, information processing speed, and executive functioning. Only a few studies have shown beneficial effects of combined supplementation of folic acid and vitamin B_{12} on cognitive performance; in elderly people with elevated psychological distress on overall cognitive functioning, immediate and delayed recall,[16] and in elderly people with mild cognitive impairment (MCI).[17] Like our study, most other studies that were performed in non-demented elderly with a study duration of at least one year did not show significant effects of B-vitamin supplementation.[18-20] One study even showed that two-year supplementation with 500 μg vitamin B_{12} and 1000 μg folic acid resulted in worse information processing speed and overall cognitive function compared to the placebo group.[21]

Table 3 Changes in cognitive test scores of Dutch elderly people (only participants with baseline and two-year data) (unadjusted means ± SD)

	Baseline	2 yr	Change (95% CI)	p
Global cognition (n = 2,556)				
MMSE, max 30 points[a]				
Placebo	28.2 ± 1.8	27.9 ± 2.0	-0.3 (-0.4--0.2)	0.05
B-vitamins	28.1 ± 1.8	28.0 ± 1.9	-0.1 (-0.2--0.0)	
Episodic memory (n = 2,485)				
RAVLT – Immediate recall, max. 75 words				
Placebo	37.2 ± 10.0	38.5 ± 10.4	1.3 (0.9-1.8)	0.47
B-vitamins	37.4 ± 9.8	38.9 ± 10.1	1.5 (1.0-1.9)	
RAVLT – Decayed recall (delayed-WLT trial 5)				
Placebo	-2.2 ± 1.9	-1.9 ± 1.9	0.3 (0.2-0.5)	0.44
B-vitamins	-2.2 ± 1.9	-1.8 ± 2.0	0.4 (0.2-0.5)	
RAVLT – Recognition, max. 30 words				
Placebo	28.0 ± 2.2	27.9 ± 2.5	-0.1 (-0.3--0.0)	0.38
B-vitamins	28.0 ± 2.2	27.9 ± 2.5	-0.1 (-0.2-0.1)	
Attention and working memory (n = 760)				
Digit Span forward, max. 16 points				
Placebo	8.1 ± 1.8	8.0 ± 1.8	-0.1 (-0.3-0.0)	0.31
B-vitamins	8.2 ± 1.7	8.0 ± 1.7	-0.2 (-0.4--0.1)	
Digit Span backward, max. 14 points				
Placebo	6.0 ± 1.8	5.9 ± 1.8	-0.1 (-0.2-0.1)	0.64
B-vitamins	5.9 ± 1.8	5.8 ± 1.7	-0.1 (-0.2-0.1)	
Information processing speed (n = 755)				
Trail Making Part A, sec[b]				
Placebo	43.7 ± 16.2	44.1 ± 17.1	0.4 (-1.0-1.8)	0.54
B-vitamins	44.3 ± 16.4	45.1 ± 16.3	0.8 (-0.6-2.1)	
Stroop 1 and 2 mean, sec[b]				
Placebo	58.5 ± 11.7	59.3 ± 11.6	0.8 (0.1-1.6)	0.48
B-vitamins	58.6 ± 11.2	59.0 ± 11.2	0.4 (-0.4-1.3)	
Symbol Digit Modalities Test, correct number				
Placebo	47.0 ± 9.5	46.4 ± 9.9	-0.6 (-1.2--0.1)	0.97
B-vitamins	46.4 ± 8.8	45.9 ± 9.6	-0.5 (-1.1-0.1)	
Executive functioning (n = 754)				
Trail Making Test (Part B/Part A)[b]				
Placebo	2.4 ± 0.8	2.4 ± 0.8	-0.0 (-0.1-0.1)	0.41
B-vitamins	2.4 ± 0.8	2.3 ± 0.7	-0.0 (-0.1-0.0)	
Stroop Interference (Part 3 – (Part 1 + Part 2/2))[b]				
Placebo	57.9 ± 30.2	59.6 ± 34.1	1.6 (-0.6-3.7)	0.31
B-vitamins	57.4 ± 31.2	57.4 ± 31.7	0.0 (-2.5-2.5)	
Verbal Fluency, total number				
Placebo	37.2 ± 11.9	38.8 ± 12.2	1.6 (0.9-2.3)	0.10
B-vitamins	36.2 ± 10.5	38.8 ± 11.4	2.6 (1.8-3.4)	

Notes: MMSE, Mini-Mental State Examination; RAVLT, Rey Auditory Verbal Learning Test. Difference between groups over time were measured using ANCOVA, adjusted for baseline values. [a] Difference in change between groups was measured with independent-samples t-test; [b] Higher score indicates that more time was needed to complete the task, reflecting a poorer performance.

We showed a small beneficial effect of B-vitamin supplementation on global cognitive performance, as measured with the MMSE. In a different study with MCI patients, and only in participants with elevated Hcy levels, a beneficial effect of moderate doses of vitamin B_{12} and folic acid supplementation on the MMSE was observed (placebo group decreased with 1.2 point, B-vitamin group with 0.3 point).[17] Although the MMSE is a widely used and validated cognitive screening tool, it has limitations because of ceiling effects and its sensitivity to pick up subtle changes has been questioned,[22] especially in well-educated populations like ours. Our significant finding was therefore somewhat unexpected and the results may be due to the large study population. The difference between treatment groups in our study was only 0.2 on a maximum score of 30 and this difference is far from clinical relevance. Furthermore, based on the number of statistical tests we performed, it should also be taken into account that our finding might be a chance finding. Adjustments for multiple testing would have resulted in non-significant results.

Because effects of supplementation may be more pronounced in people with higher baseline Hcy levels [17, 23] and low dietary folate and vitamin B_{12} intake,[18] we analyzed predefined interaction terms. These analyses did not reveal significant results for sex, age, plasma Hcy, and ApoE-ε4 genotype. Post-hoc analyses, however, showed that participants with low HoloTC levels, the active form of vitamin B_{12}, had more benefits of the B-vitamin supplementation on the domain of information processing speed.

In addition to our large sample size as strength of our study, is the use of validated neuropsychological tests, which enabled us to study the effects of B-vitamins on a broad spectrum of cognitive functions that decline with aging.[24] By combining tests measuring the same cognitive concept, we were able to create sensitive and reliable domain compound scores and to decrease the risk of chance findings or ceiling performances. Furthermore, compliance to the treatment was high, as was also reflected by the decreased plasma Hcy levels in the B-vitamin group compared to a much smaller decrease in the placebo group. Additionally, the duration of two years is adequate in order to induce a change in cognitive performance; according to the IANA taskforce a minimum of 18 months would be required.[25] Nevertheless, even though our study population was hypothesized to be at a higher risk for cognitive decline due to the elevated Hcy levels and therefore would be expected to be even more sensitive for changes, it is possible that the decreased plasma Hcy levels may not have revealed their possible beneficial effects yet at time of the measurements.

A limitation of the study design was the lack of intermediate measurements, which made intention-to-treat analyses in participants who did not come to their two-year visit not possible. Another relevant point is that both treatment groups received vitamin D_3. High serum 25-OH-vitamin D concentrations have been associated with better cognitive performance.[26] RCTs investigating the effects of vitamin D supplementation in elderly people, however, are scarce. Although vitamin D supplementation is recommended for elderly people in the Netherlands, still 44% of our total population was vitamin D deficient at baseline (< 50 nmol/L).[27] Possibly, supplementation of vitamin D may have diluted potential effects of vitamin B_{12} and folic acid on cognitive performance, although these nutrients may have different mechanisms that account for a possible effect.

Recently, concerns have been put forward on folic acid supplementation and increased risk of cancer. Post-hoc analyses of adverse events in the B-PROOF population showed that participants in the B-vitamin group reported cancer more often than those in the placebo group.[28] Nevertheless, a recent meta-analysis did not observe a significant negative effect based on thirteen studies.[29] Our dose of folic acid was well below the upper tolerable limit that is used in the Netherlands (1 mg/day).[30] Although our finding could be attributed to chance, we should interpret it with care and it makes daily supplementation with folic acid in the given dose questionable.

Based on the current results and results from previous RCTs, we conclude that supplementation with folic acid and vitamin B_{12} in healthy elderly people did not beneficially affect cognitive performance on four cognitive domains in elderly people with elevated Hcy levels. We cannot extrapolate this to persons who already have cognitive impairments. Furthermore, cross-sectional and prospective magnetic resonance imaging (MRI) studies [31, 32] as well as one RCT in MCI patients,[33] observed beneficial effects of these vitamins on brain atrophy. To unravel the effects on B-vitamins, a closer look into early brain pathologies would be useful to uncover subtle effects that are difficult to pick up with paper-pencil tests. Within the B-PROOF study we have anticipated on these innovative developments and results with respect to MRI are awaited.

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We gratefully acknowledge all participants in the B-PROOF study. Furthermore, we want to thank our colleagues, and all students for their help and dedication.

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Supplementary material

Appendix 1

Cognitive performance tests

The Rey Auditory Verbal Learning Test (RAVLT) was used to measure direct recall, delayed recall and recognition for word lists, as indices of immediate and delayed episodic memory. The Digit Span forward and backward from the Wechsler Adult Intelligence Test (WAIS-III) measures attention and working memory, respectively. Trail Making Test (TMT) part A measures information processing speed and part B assesses concept shifting interference. Stroop Color-Word Test determines selective attention and susceptibility to behavioral interference. The Symbol Digit Modalities Test (SDMT) measures information processing speed. Letter Fluency (three letters) measures language. The following domains in formula form were formed:

Episodic memory = $(Z_{\text{RAVLT total immediate recall}} + Z_{\text{RAVLT decayed recall}} + Z_{\text{RAVLT recognition}})/3$

Attention and working memory = $(Z_{\text{Digit Span forward}} + Z_{\text{Digit Span backward}})/2$

Information processing speed = $(-Z_{\text{Stroop mean I and II}} + -Z_{\text{Trail making test part A}} + Z_{\text{Symbol Digit Modalities Test}})/3$

Executive functioning = $(-Z_{\text{Stroop interference}} + -Z_{\text{Trail making ratio}} + Z_{\text{Verbal Fluency}})/3$.

Blood parameters

Plasma Hcy was measured using the Architect i2000 RS analyzer (VUmc), HPLC method (WU), and LCMS/ MS (EMC). Cross-calibration showed that outcomes of the three centers did not differ significantly. Serum creatinine was measured using the enzymatic colorimetric Roche CREA plus assay (CV=2%). Serum vitamin B_{12} and serum folate were analyzed using immunoelectrochemiluminescence assay (Elecsys, 2010, Roche GmbH, Mannheim, Germany) (CV vitamin B_{12} 5.1% at 125 pmol/L and 2.9% at 753 pmol/L; CV folate: 5.9% at 5.7 nmol/L and 2.8% at 23.4 nmol/L). Serum holotranscobalamin (holoTC) was determined by the Abbott AxSYM analyser (Abbott Diagnostics, Hoofddorp, The Netherlands) (CV <8% at 20, 40 and 80 pmol/L). Serum methylmelonic acid (MMA) was determined using LC-MS/MS (CV = 6.7%, 5.0% and 5.0% at 0.15, 0.36 and 0.65 μmol/L, respectively).

Supplementary Table 1 Cognitive performance on baseline per treatment group in a Dutch elderly population (mean ± SD)

	Placebo group	B-vitamin group
Episodic memory	*n* = 1410[a]	*n* = 1407[a]
RAVLT – Immediate recall, max. 75 words	36.5 ± 10.1	36.7 ± 10.0
RAVLT – Delayed recall, max. 15 words	7.0 ± 3.0	7.1 ± 3.1
RAVLT – Decayed recall (delayed-RAVLT- trial 5)	-2.2 ± 1.9	-2.2 ± 1.9
RAVLT – Recognition, max. 30 words	27.8 ± 2.3	27.8 ± 2.4
Attention and working memory	*n* = 426[a]	*n* = 418[a]
Digit Span forward, max. 16 points	8.1 ± 1.8	8.2 ± 1.7
Digit Span backward, max. 14 points	6.0 ± 1.8	5.8 ± 1.8
Information processing speed	*n* = 422[a]	*n* = 415[a]
Trail Making Test Part A (s)[b]	44.1 ± 16.4	45.1 ± 17.0
Stroop 1 (s)[b]	51.8 ± 11.3	51.7 ± 10.7
Stroop 1, # corrections	0.1 ± 0.4	0.1 ± 0.3
Stroop 2 (s)[b]	65.6 ± 14.2	66.5 ± 15.1
Stroop 2, # corrections	0.4 ± 0.8	0.4 ± 0.8
Symbol Digit Modalities Test, correct number	46.7 ± 9.6	45.7 ± 9.3
Symbol Digit Modalities Test, incorrect number	1.3 ± 1.5	1.4 ± 1.7
Executive functioning	*n* = 421[a]	*n* = 414[a]
Trail Making Test Part B (s)[b]	103.6 ± 48.0	104.7 ± 47.6
Trail Making Test ratio (Part B/Part A)[b]	2.4 ± 0.8	2.4 ± 0.8
Stroop 3 (s)[b]	117.3 ± 36.6	117.4 ± 35.9
Stroop 3, number of corrections	1.1 ± 1.8	1.0 ± 1.8
Stroop Interference (Part 3 – (Part 1 + Part 2/2))[b]	58.6 ± 30.6	58.3 ± 31.6
Verbal Fluency, total words	37.2 ± 11.7	35.9 ± 10.6

Notes: Abbreviations: RAVLT, Rey Auditory Verbal Learning Test. [a] Number of participants may deviate a little between tests for all these values. [b] Higher scores indicate poorer performance.

Supplementary Table 2 Interaction p-values between treatment and predefined and post-hoc terms (intention-to-treat)

	Episodic memory	Working memory	Information processing speed	Executive function
Predefined terms				
Age, <80 years≥	0.27	0.11	0.07	0.46
Gender, men/women	0.59	0.17	0.67	0.25
Homocysteine, <14.4 μmol/L≥	0.44	0.75	0.31	0.99
APOE-ε4, TT / CT&CC	0.58	0.53	0.31	0.52
Post-hoc terms				
MTHFR genotype, CC&CT / TT	0.74	0.09	0.17	0.47
Serum vitamin B_{12}, <266.4 pmol/L ≥	0.42	0.68	0.11	0.59
Serum folic acid, <18.73 nmol/L ≥	0.40	0.75	0.05	0.61
Serum holotranscobalamin, <64 μmol/L ≥	0.09	0.84	0.08	0.86
Serum methylmalonic acid, <0.23 pmol/L ≥	0.90	0.06	0.57	0.68

Notes: ApoE-ε4, apolipoprotein E-ε4; MTHFR, Methylenetrahydrofolate reductase

Supplementary Table 3 Stratified analyses for those interaction terms that were significant (p<0.10) in the intention-to-treat analyses

	Adjusted means ± SE at 2-year	Change over 2 years (95%CI)	p
Episodic memory			
HoloTC, <64 pmol/L (*n* = 1,212)			
Placebo	0.09 ± 0.02	0.08 (0.03-0.13)	0.04
B-vitamins	0.16 ± 0.02	0.13 (0.08-0.18)	
HoloTC, ≥64 pmol/L (*n* = 1,255)			
Placebo	0.17 ± 0.02	0.09 (0.04-0.14)	0.70
B-vitamins	0.16 ± 0.02	0.08 (0.03-0.14)	
Working memory			
MTHFR genotype, CC/CT (*n* = 601)			
Placebo	-0.04 ± 0.04	-0.05 (-0.12-0.02)	0.22
B-vitamins	-0.10 ± 0.04	-0.10 (-0.18--0.02)	
MTHFR genotype, TT (*n* = 88)			
Placebo	-0.08 ± 0.08	-0.14 (-0.35--0.00)	0.13
B-vitamins	0.10 ± 0.08	0.03(-0.13-0.18)	
MMA, <0.23 μmol/L (*n* = 367)			
Placebo	-0.11 ± 0.04	-0.10 (-0.19--0.01)	0.45
B-vitamins	-0.06 ± 0.04	-0.06 (-0.15-0.04)	
MMA, ≥0.23 μmol/L (*n* = 392)			
Placebo	0.01 ± 0.04	-0.01(-0.10-0.07)	0.06
B-vitamins	-0.11 ± 0.05	-0.12 (-0.22--0.01)	
Information processing speed			
Age, <80 yrs (*n* = 686)			
Placebo	0.09 ± 0.02	-0.01 (-0.05-0.02)	0.30
B-vitamins	0.06 ± 0.02	-0.03(-0.07-0.00)	
Age, ≥80 yrs (*n* = 45)			
Placebo	-0.74 ± 0.08	-0.20(-0.38--0.02)	0.23
B-vitamins	-0.58 ± 0.10	-0.03(-0.29-0.22)	
Folic acid, <18.73 nmol/L (*n* = 419)			
Placebo	-0.01 ± 0.02	-0.01(-0.06-0.03)	0.11
B-vitamins	-0.07 ± 0.02	-0.06 (-0.11--0.02)	
Folic acid 18.73≥ nmol/L (*n* = 312)			
Placebo	0.10 ± 0.03	-0.04 (-0.10-0.01)	0.26
B-vitamins	0.15 ± 0.03	0.01 (-0.05-0.06)	
HoloTC, <64 pmol/L (*n* = 409)			
Placebo	-0.03 ± 0.03	-0.05 (-0.10-0.00)	0.43
B-vitamins	-0.01 ± 0.03	-0.02 (-0.07-0.04)	
HoloTC, ≥64 pmol/L (*n* = 322)			
Placebo	0.13 ± 0.03	0.01 (-0.05-0.06)	0.08
B-vitamins	0.04 ± 0.03	-0.05 (-0.10-0.00)	

Notes: Differences between the two groups over time were measured using ANCOVA, adjusted for baseline performance. HoloTC, holotranscobalamin; MTHFR, Methylenetrahydrofolate reductase; MMA, methylmalonic acid.


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Folate and vitamin B_{12}-related biomarkers in relation to brain MRI volumes

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Abstract

Objective: We investigated the cross-sectional associations between circulating homocysteine (Hcy), folate and biomarkers of vitamin B_{12} status with brain volumes, and we compared brain volumes of participants who received daily folic acid and vitamin B_{12} supplementation for two years with participants who did not.

Methods: Participants of the B-PROOF study (n = 2,919) were assigned to 400 μg folic acid and 500 μg vitamin B_{12}, or a placebo. After two years of intervention, T1-weighted MRI scans were made in a random subsample (n = 218) to obtain volumes of grey and white matter, and a sum score of total brain volume (TBV). Follow-up plasma Hcy, serum folate, vitamin B_{12}, holotranscobalamin, and methylmalonic acid concentrations were measured. ANCOVA was used to determine group differences. Multiple linear regression analyses were used to assess the associations between these blood biomarkers and brain volumes.

Results: Mean age differed significantly between the group with B-vitamin supplementation (mean ± SD 72.7 ± 5.3 years) and without supplementation (mean ± SD 74.5 ± 6.3 years, p = 0.02). In the fully adjusted ANCOVA model, the group that received B-vitamins had a lower TBV (mean ± SE 1064 ± 3 mL) than the non-supplemented group (mean ± SE 1072 ± 3 mL, p = 0.03). Cross-sectionally, inverse associations between plasma Hcy with TBV (β ± SE of -0.89 ± 0.48, p = 0.06) and between serum folate and TBV (β ± SE of -0.20 ± 0.09, p = 0.03) were observed. No significant associations were observed for serum vitamin B_{12} and holotranscobalamin.

Conclusions: Results were contradictory, with both higher Hcy and folate levels associated with lower TBV. Furthermore, participants who had received B-vitamin supplementation had a slightly lower TBV, which might be explained by the age difference between groups.

Introduction

Elevated homocysteine (Hcy) levels have been associated with faster cognitive decline, cognitive impairment, and dementia [1] by being neurotoxic or via other, probably vascular, pathways.[2] Remethylation of Hcy into methionine is dependent of vitamin B_{12} and folate. Observational studies have shown associations between vitamin B_{12} and folate with cognitive performance, but the majority of intervention studies does not show an effect of supplementation with vitamin B_{12} and folic acid, despite a lowering effect on Hcy.[3]

Another method to investigate the role of Hcy, vitamin B_{12} and folate in brain health, is by studying brain volumes. Cross-sectional studies have shown associations of Hcy levels with ventricle-brain ratios [2] and white-matter lesions as an index of the integrity of white matter.[11] Additionally, inverse associations were observed between vitamin B_{12} status and white-matter lesions.[11, 12] Positive associations were observed between vitamin B_{12} status and total brain volume, cross-sectionally [2, 4] and prospectively.[5] Higher folate levels have been associated with less white matter lesions, but not with more hippocampal or amygdalar volume.[6]

We investigated the associations of levels of plasma Hcy, serum folate and three markers related to vitamin B_{12} status (serum B_{12}, MMA, and holoTC) with volumes of grey and white matter, and total brain volume as a derivative, measured by magnetic resonance imaging (MRI) scans in the B-PROOF study. We also studied the difference between participants who received a daily supplement with folic acid and vitamin B_{12} for two years and those who did not receive this.

Methods

Study design and Participants

This study was part of the B-PROOF (B-vitamins for the PRevention Of Osteoporotic Fractures) study that was conducted between October 2008 and April 2013 in three research centers in the Netherlands; VU medical center, Erasmus Medical Center, and Wageningen University. Primary objective of this randomized, double-blind, placebo-controlled intervention study was to assess the efficacy of two years oral supplementation with 400 μg folic acid and 500 μg vitamin B_{12} in the prevention of osteoporotic fractures. Both the placebo tablet and the B-vitamin tablet contained 15 μg (600 IU) vitamin D. Participants were people aged ≥65 years with mildly elevated plasma Hcy levels (12-50 μmol/L). Participant selection has been extensively described elsewhere.[7] MRI scans were made in a random subsample of the population of Wageningen University. Participants who came for their two-year follow-up measurement between July 2012 and April 2013 and who had not dropped out during the intervention period were invited to participate in the MRI study (see **Figure 1** for the participant flow). MRI scans were made within one month after completion of the intervention period, before unblinding the treatment allocation. Participants were carefully screened on contra-indications for MRI scans. The Wageningen University Medical Ethical Committee approved the study and all participants gave their written informed

consent. This trial is registered at clinicaltrials.gov as NCT00696514 and at Netherlands Trial Register as NTR1333.

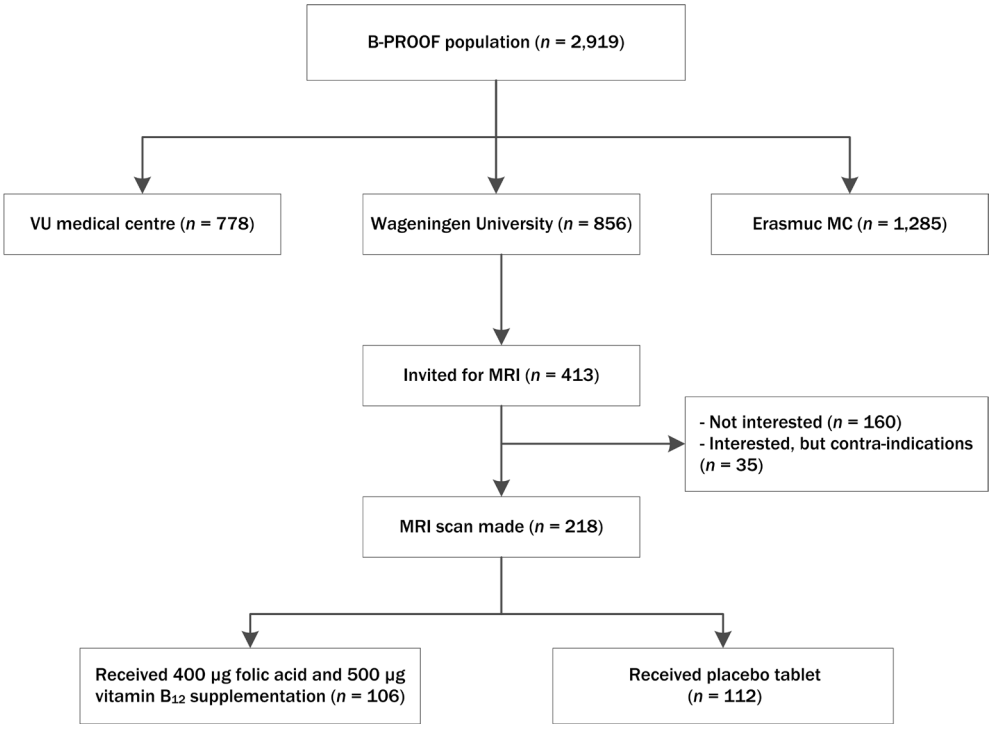

Figure 1 Flowchart of participants that were enrolled in the MRI study, a sub-study of the B-PROOF trial

Descriptive measures

Global cognitive performance was measured with the Mini-Mental State Examination (MMSE).[8] Depressive symptoms were assessed with the 15-item Geriatric Depression Scale (GDS-15),[9] in which a score ≥5 is indicated as being likely to be depressed. Standing height was measured with a stadiometer to the nearest 0.1 cm and weight was measured to the nearest 0.5 kg with a calibrated scale (Seca, Deventer, the Netherlands). Body mass index (BMI) was calculated by dividing the weight by the squared height (kg/m^2). Information about highest educational level, smoking habits, alcohol intake (Garrett index),[10] marital status, and living situation was obtained by structured questionnaires. Physical activity was measured with the LASA Physical Activity Questionnaire (LAPAQ).[11] Self-reported frequency and duration of activities during the past two weeks were checked by a research assistant and were used to calculate physical activity in kcal/day.

MRI scans

Cranial volumetric MRI scans were made after 2 years of intervention at the Hospital Gelderse Vallei (Ede, the Netherlands) on a 3-Tesla Siemens Magnetom Verio (Siemens, Erlangen, Germany), with a 32-channel head coil. Here, we analyzed the T1-weighted scan (MPRAGE, repetition time = 2300 ms, echo time = 3.0 ms, inversion time = 900 ms, 9º flip angle, field of view = 256x256mm, 192 sagittal slices, voxel size = 1 x 1 x 1mm, acceleration factor (GRAPPA) = 2).

The voxel-based morphometry (VBM8) toolbox within the SPM8 software (Wellcome Department of Imaging Neuroscience, London, UK, http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) and FSL-VBM v6.0 (FMRIB Software Library, Oxford, UK, http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM) [12] were used for segmentation. T1-weighted images were first reoriented to match the standard template images in FSL-VBM. VBM8 spatially normalizes participants' brain images to a standard space, and then automated segments into grey matter, white matter, and cerebrospinal fluid, using a unified tissue segmentation approach.[13] These three measures were summed to calculate intracranial volume. Grey and white matter volumes were summed to calculate total brain volume.

Blood measurements

Blood samples were obtained in the morning, when participants were fasted or had consumed a restricted breakfast. For the current study, follow-up levels of serum folate and vitamin B_{12} biomarkers were used. Plasma Hcy was measured using the HPLC method (intra assay CV = 3.1%, inter assay CV = 5.9%). Serum vitamin B_{12} and serum folate were analyzed by using electrochemiluminescence immunoassay (Elecsys, 2010, Roche GmbH, Mannheim, Germany) (CV vitamin B_{12} 5.1% at 125 pmol/l and 2.9% at 753 pmol/l; CV folate: 5.9% at 5.7 nmol/l and 2.8% at 23.4 nmol/l). Serum holoTC was determined by the AxSYM analyser (Abbott Diagnostics, Hoofddorp, The Netherlands) (intra assay CV<8%) and serum MMA was measured by LC-MS/MS (intra assay CV = 8.1% at 0.18 μmol/L, inter assay CV = 1.6% at 0.24 μmol/L). DNA was isolated from buffy coats to determine the genotype for

methylenetetrahydrofolate reductase (MTHFR 677TT) using the Illumina Omni-express array and to determine Apolipoprotein E (ApoE) genotype by using Taqman. All analyses were done in the biochemical laboratory of Erasmus MC, except Hcy, which was measured at Wageningen University.

Statistics

Population characteristics are reported as *n* (%), means ± SD, or as median [interquartile range (IQR)] for non-normally distributed data. Comparisons between treatment groups were made using Chi-square test, independent-samples t-test or Mann-Whitney non-parametric test. Differences between treatment groups were analyzed using analyses of covariance (ANCOVA), in which the brain volumes acted as dependent factor, treatment as fixed between-subject factor, and intracranial volume, age and sex as covariates (model 1). Model 2 included the covariates from model 1 plus BMI, smoking, alcohol, education, and physical activity.

Linear regression analyses were used to examine the associations between serum folate, different markers of vitamin B_{12} status (serum B_{12}, holoTC, MMA), and plasma Hcy, with volumes of grey and white matter and total brain volume. The crude model was adjusted for intracranial volume, in model 1 additional adjustments were made for age and sex, and model 2 included the covariates of model 1 plus BMI, smoking, alcohol, education, and physical activity. Because we expected different regression slopes for the two treatment groups, we tested for interaction and stratified the regression analyses for treatment.

Results are presented as adjusted means ± SE for the ANCOVA analyses, and as β-coefficients±SE for the regression analyses. Alpha was set at 0.05 and two-tailed analyses were performed. All statistical analyses were performed using SPSS Statistics v22 (SPSS Inc. Chicago, IL, USA).

Results

Participants

The general characteristics of the total subgroup at time of the MRI measurement are presented in **Table 1**. The group that received two years of B-vitamin supplementation (n = 106) and the group that did not receive B-vitamins (n = 112) contained both 57% men. Participants in the B-vitamin group were significantly younger and had a slightly higher MMSE score (age: 72.7 ± 5.3; median MMSE 29, IQR 27-30, range 19-30) than the non-supplemented group (age: 74.5 ± 6.3, p = 0.02; median MMSE 28, IQR 27-29, range 24-30, p = 0.09). Follow-up concentrations of folate, vitamin B_{12} and holoTC were higher in the group that received B-vitamin supplementation than in the group that did not, whereas MMA and Hcy were lower (all p-values < 0.001), as a consequence of the two-year supplementation.

Table 1 Population characteristics at time of MRI measurements in participants who received two years of B-vitamin supplementation and participants who did not receive this supplementation.

Variable	**Total population (n = 218)**	**With B-vitamin supplementation (n = 106)**	**Without B-vitamin supplementation (n = 112)**	**p**
Age (yrs)[a]	73.6 ± 5.9	72.7 ± 5.3	74.5 ± 6.3	0.02
Sex, men[b]	124 (57%)	60 (57%)	64 (57%)	0.94
Body mass index (kg/m^2)	27.7 ± 4.2	27.3 ± 4.4	28.1 ± 4.1	0.21
Education				0.12
Low	90 (41%)	49 (46%)	41 (37%)	
Medium	52 (24%)	19 (18%)	33 (30%)	
High	76 (35%)	38 (36%)	39 (34%)	
Smoking				0.67
Never	64 (29%)	27 (26%)	37 (33%)	
Former	139 (64%)	70 (66%)	69 (62%)	
Current	15 (7%)	9 (9%)	6 (5%)	
Alcohol				0.72
Light	152 (70%)	73 (69%)/	79 (71%)	
Moderate	60 (28%)	31 (29%)/	29 (26%)	
Excessive	6 (3%)	2 (2%)	4 (3%)	
MMSE, max 30 points[c]	28 [27-29]	29 [27-30]	28 [27-29]	0.09
Low MMSE <25	6 (3%)	4 (4%)	2 (2%)	0.37
GDS, max 15 points	1 [0-2]	1 [0-2]	1[0-2]	0.89
Physical activity (kcal/day)	664 ± 416	642 ± 288	683 ± 440	0.47
ApoE-ε4, carrier	60 (28%)	32 (30%)	28 (25%)	0.45
MTHFR, 677TT	28 (13%)	17 (15%)	11 (10%)	0.40
Grey matter (mL)	574 ± 56	577 ± 57	572 ± 55	0.51
Grey matter / ICV	0.42 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.98
White matter (mL)	493 ± 61	495 ± 63	492 ± 60	0.73
White matter / ICV	0.36 ± 0.02	0.36 ± 0.02	0.36 ± 0.02	0.74
Cerebrospinal fluid (mL)	304 ± 52	306 ± 51	302 ± 53	0.62
Cerebrospinal fluid / ICV	0.22 ± 0.03	0.22 ± 0.03	0.22 ± 0.03	0.81
Serum folate (nmol/L)	36.7 [23.4-54.2]	53.1 [42.5-68.3]	24.1 [19.5-31.8]	<0.001
Serum vitamine B12 (pmol/L)	404 [262-558]	558 [459-715]	274 [222-373]	<0.001
Vitamin B12<258 pmol/L	49 (23%)	1 (1%)	48 (43%)	<0.001
Serum HoloTC (pmol/L)	81 [56-111]	111 [89-147][d]	63 [44-80]	<0.001
HoloTC <30 pmol/L	12 (6%)	1 (1%)	11 (10%)	0.01
Serum MMA (µmol/L)	0.20 [0.16-0.25]	0.17 [0.15-0.21]	0.24 [0.19-0.30]	<0.001
MMA >0.30 µmol/L	35 (16%)	5 (5%)	30 (27%)	<0.001
Plasma Hcy (µmol/L)	11.4 (8.9-14.4)	9.1 (7.8-10.6)[e]	13.9 (12.0-16.3)[f]	<0.001

Notes: MMSE, Mini-Mental State Examination; GDS, Geriatric Depression Scale; ApoE-ε4, Apolipoprotein E-ε4 ; MTHFR, methylenetetrahydrofolate reductase; ICV, intracranial volume; HoloTC, holotranscobalamin; MMA, Methylmalonic acid; Hcy, homocysteine. [a] Mean ± SD (all such values); [b] *n* (%) (all such values); [c] median [IQR] (all such values); [d] *n* = 96; [e] *n* = 103; [f] *n* = 109.

Differences between supplementation groups

Table 2 presents the differences in brain volumes between the group that received B-vitamin supplementation and the group that did not. Unadjusted analyses did not show a difference between groups. The fully adjusted model, however, revealed a lower total brain volume in the B-vitamin group (1063.6 ± 2.7 mL) compared to the non-supplemented group (1072.3 ± 2.6 mL, p = 0.03). This was also reflected by a non-significant (p = 0.07) lower volume of white matter in the B-vitamin group (490.2 ± 2.6 mL) compared to the non-supplemented group (496.7 ± 2.5 mL). Grey matter volume did not differ between groups.

Table 2 Differences in brain volumes (mL) between participants who received two-year B-vitamin supplementation (*n* = 106) and participants who did not receive this supplementation (*n* = 112)

	Grey matter volume		White matter volume		Total brain volume	
	Adjusted means ± SE	**p**	**Adjusted means ± SE**	**p**	**Adjusted means ± SE**	**p**
Crude model		0.78		0.67		0.93
Without B-vitamin supplementation	573.6 ± 2.9		493.8 ± 2.6		1067.4 ± 3.5	
With B-vitamin supplementation	574.7 ± 3.0		492.2 ± 2.7		1066.9 ± 3.6	
Model 1		0.40		0.13		0.03
Without B-vitamin supplementation	575.7 ± 2.6		495.6 ± 2.4		1071.3 ± 2.6	
With B-vitamin supplementation	572.5 ± 2.7		490.3 ± 2.5		1062.8 ± 2.7	
Model 2		0.57		0.07		0.03
Without B-vitamin supplementation[a]	575.6 ± 2.8		496.7 ± 2.5		1072.3 ± 2.6	
With B-vitamin supplementation[a]	573.4 ± 2.8		490.2 ± 2.6		1063.6 ± 2.7	

Notes: Differences between groups were tested with ANCOVA, adjusted for intracranial volume (crude model), age, sex (model 1), BMI, alcohol, smoking, education, and physical activity (model 2). [a] *n* = 111 for the group without B-vitamin supplementation; [b] *n* = 103 for the group with B-vitamin supplementation.

B-vitamin status and brain MRI volumes

Fully adjusted linear regression models of follow-up biomarkers and brain volumes in the total population (**Table 3**) showed an inverse association for serum folate with total brain volume (β ± SE = -0.20 ± 0.09, p = 0.03), and a borderline significant association with white matter volume (β ± SE = -0.17 ± 0.09, p = 0.06). Furthermore, plasma Hcy was borderline significantly inversely associated with total brain volume (β ± SE = -0.89 ± 0.48, p = 0.06). No significant associations were observed for serum B_{12}, holoTC, or MMA with any of the brain volumes in the fully adjusted models.

Associations differed between the group that received B-vitamin supplementation and the group that did not (**Table 4**), more specifically, interaction terms with treatment were significant or tended towards significance for MMA and grey matter (p = 0.04), MMA and

total brain volume (p = 0.10), and Hcy and total brain volume (p = 0.10). In both groups, serum MMA was inversely associated with total brain volume, but only in the B-vitamin group it remained significant after adjustment for covariates (B-vitamin group: β ± SE -126.8 ± 53.1, p = 0.02; group without B-vitamins: β ± SE -27.2 ± 15.4, p = 0.08). Only in the B-vitamin group, MMA was also associated with grey matter volume (β ± SE -136.2 ± 53.1, p = 0.01). Inverse associations were observed for Hcy and total brain volume in both groups, with stronger associations in the supplemented group (β ± SE -4.24 ± 1.31, p < 0.01) than in the non-supplemented group (β ± SE -1.78 ± 0.65, p = 0.01). In the B-vitamin group, a trend was observed for Hcy and grey matter volume (β ± SE -2.53 ± 1.40, p = 0.07), but not for white matter volume. In the non-supplemented group, a trend was observed for Hcy and white matter volume (β ± SE -1.00 ± 0.59, p = 0.09), but not for grey matter volume.

Table 3 Associations between follow-up blood values and brain MRI measures (*n* = 218)

Models	Grey matter volume		White matter volume		Total brain volume	
	β-coefficients ± SE	p	β-coefficients ± SE	p	β-coefficients ± SE	p
Serum folate (nmol/L)						
Crude	0.04 ± 0.10	0.71	-0.09 ± 0.09	0.31	-0.06 ± 0.12	0.64
Model 1	-0.05 ± 0.09	0.61	-0.14 ± 0.08	0.09	-0.19 ± 0.09	0.04
Model 2[a]	-0.03 ± 0.09	0.71	-0.17 ± 0.09	0.06	-0.20 ± 0.09	0.03
Serum vitamin B12 (pmol/L)						
Crude	0.00 ± 0.01	0.76	0.01 ± 0.01	0.28	0.01 ± 0.01	0.29
Model 1	-0.01 ± 0.01	0.41	0.00 ± 0.01	0.80	-0.01 ± 0.01	0.56
Model 2[a]	-0.01 ± 0.01	0.42	-0.00 ± 0.01	0.95	-0.01 ± 0.01	0.39
Serum holotranscobalamin (pmol/L)						
Crude[b]	0.07 ± 0.05	0.17	0.07 ± 0.04	0.10	0.14 ± 0.06	0.02
Model 1[b]	-0.00 ± 0.04	0.97	0.02 ± 0.04	0.66	0.02 ± 0.05	0.71
Model 2[c]	0.01 ± 0.04	0.90	0.01 ± 0.04	0.81	0.02 ± 0.05	0.73
Serum methylmalonic acid (µmol/L)						
Crude	-27.2 ± 14.5	0.06	-18.0 ± 13.3	0.18	-45.2 ± 17.4	0.01
Model 1	-13.2 ± 13.2	0.32	-7.7 ± 12.4	0.54	-20.9 ± 13.5	0.12
Model 2[a]	-14.5 ± 13.2	0.27	-7.2 ± 12.7	0.58	-21.7 ± 13.4	0.11
Plasma homocysteine (µmol/L)						
Crude[d]	-1.36 ± 0.48	<0.01	-1.19 ± 0.44	<0.01	-2.55 ± 0.57	<0.001
Model 1	-0.33 ± 0.47	0.49	-0.36 ± 0.44	0.42	-0.69 ± 0.48	0.15
Model 2[c]	-0.53 ± 0.48	0.26	-0.36 ± 0.45	0.43	-0.89 ± 0.48	0.06

Notes: Crude: Adjusted for intracranial volume; Model 1: Adjusted for intracranial volume, age, sex; Model 2: Adjusted for model 1 and BMI, alcohol, smoking, education, physical activity. [a] *n* = 214; [b] *n* = 208; [c] *n* = 205; [d] *n* = 212; [e] *n* = 208.

Table 4 Stratified analyses follow-up blood values and MRI brain volumes for the group with (n = 106) and without a history of B-vitamin supplementation (n = 112)

Models	Grey matter volume		White matter volume		Total brain volume	
	β ± SE	p	β ± SE	p	β ± SE	p
Without B-vitamin supplementation (n = 112)						
Serum folate (nmol/L)						
Crude	0.16 ± 0.26	0.53	-0.20 ± 0.23	0.37	-0.04 ± 0.31	0.90
Model 1	0.29 ± 0.23	0.22	-0.08 ± 0.21	0.71	0.20 ± 0.24	0.39
Model 2[a]	0.19 ± 0.23	0.43	-0.06 ± 0.22	0.79	0.13 ± 0.24	0.60
Serum vitamin B_{12} (pmol/L)						
Crude	0.04 ± 0.03	0.13	0.04 ± 0.02	0.11	0.08 ± 0.03	0.02
Model 1	0.02 ± 0.02	0.47	0.02 ± 0.02	0.26	0.04 ± 0.02	0.08
Model 2[a]	0.01 ± 0.02	0.75	0.02 ± 0.02	0.31	0.03 ± 0.02	0.22
Serum holotranscobalamin (pmol/L)						
Crude	0.08 ± 0.12	0.52	0.19 ± 0.10	0.07	0.26 ± 0.14	0.07
Model 1	0.00 ± 0.11	0.98	0.15 ± 0.10	0.11	0.16 ± 0.11	0.15
Model 2[a]	-0.04 ± 0.11	0.74	0.16 ± 0.10	0.12	0.12 ± 0.11	0.28
Serum methylmalonic acid (µmol/L)						
Crude	-16.9 ± 16.8	0.32	-23.4 ± 14.5	0.11	-40.3 ± 19.9	0.05
Model 1	-8.9 ± 15.0	0.55	-19.9 ± 13.5	0.14	-28.9 ± 15.0	0.06
Model 2[a]	-10.5 ± 15.1	0.49	-16.7 ± 13.9	0.23	-27.2 ± 15.4	0.08
Plasma homocysteine (µmol/L)						
Crude	-1.70 ± 0.69	0.02	-1.77 ± 0.58	<0.01	-3.47 ± 0.77	<0.001
Model 1[b]	-0.67 ± 0.66	0.31	-1.08 ± 0.58	0.07	-1.75 ± 0.64	0.01
Model 2[c]	-0.78 ± 0.66	0.24	-1.00 ± 0.59	0.09	-1.78 ± 0.65	0.01
With B-vitamin supplementation (n = 106)						
Serum folate (nmol/L)						
Crude	-0.01 ± 0.16	0.93	-0.10 ± 0.16	0.54	-0.11 ± 0.20	0.58
Model 1	-0.07 ± 0.16	0.66	-0.14 ± 0.15	0.37	-0.20 ± 0.16	0.19
Model 2[d]	-0.03 ± 0.17	0.88	-0.24 ± 0.16	0.15	-0.26 ± 0.16	0.11
Serum vitamin B_{12} (pmol/L)						
Crude	-0.01 ± 0.02	0.51	0.02 ± 0.01	0.21	0.01 ± 0.02	0.66
Model 1	-0.01 ± 0.01	0.48	0.02 ± 0.01	0.15	0.01 ± 0.01	0.50
Model 2[d]	-0.01 ± 0.01	0.37	0.02 ± 0.01	0.22	0.00 ± 0.01	0.76
Serum holotranscobalamin (pmol/L)						
Crude[e]	0.10 ± 0.07	0.16	0.11 ± 0.07	0.12	0.21 ± 0.09	0.02
Model 1[e]	0.05 ± 0.07	0.49	0.06 ± 0.07	0.39	0.10 ± 0.07	0.13
Model 2[f]	0.06 ± 0.07	0.38	0.05 ± 0.07	0.49	0.11 ± 0.07	0.12
Serum methylmalonic acid (µmol/L)						
Crude	-179.5 ± 53.0	<0.01	-18.8 ± 54.1	0.73	-198.3 ± 66.0	<0.01
Model 1	-136.6 ± 50.7	0.01	29.9 ± 50.5	0.55	-106.7 ± 51.5	0.04
Model 2[d]	-136.2 ± 53.1	0.01	9.4 ± 54.1	0.86	-126.8 ± 53.1	0.02
Plasma homocysteine (µmol/L)						
Crude d	-3.44 ± 1.30	0.01	-3.56 ± 1.26	<0.01	-7.00 ± 1.51	<0.001
Model 1[d]	-1.88 ± 1.30	0.15	-2.10 ± 1.25	0.10	-3.98 ± 1.26	<0.01
Model 2[g]	-2.53 ± 1.40	0.07	-1.71 ± 1.35	0.21	-4.24 ± 1.31	<0.01

Notes: Crude: Adjusted for intracranial volume; Model 1: Adjusted for intracranial volume, age, sex; Model 2: Adjusted for model 1 and BMI, alcohol, smoking, education, physical activity. [a] n = 111; [b] n = 109; [c] n = 108; [d] n = 103; [e] n = 96; [f] n = 93; [g] n = 93.

Discussion

Our cross-sectional analyses showed that higher levels of folate were associated with lower brain volume. After dividing the study population into those who received two-year supplementation with vitamin B_{12} and folic acid and those who did not, the association was not present anymore in the latter group, whereas a trend was still observed in the B-vitamin group. In contrast, higher levels of Hcy and MMA were associated with lower total brain volumes, and with stronger associations in the B-vitamin group than in the non-supplemented group. No associations were observed for serum vitamin B_{12} or holoTC with brain volumetric measures. Furthermore, directly comparing the supplementation groups with respect to brain volumes does not point towards a beneficial effect of B-vitamin treatment; after adjustment for important covariates the group that received the B-vitamin supplementation had a lower brain volume compared to the placebo group.

The data add to two other findings on cognitive function within the B-PROOF study. Cross-sectionally, we observed baseline associations between MMA, Hcy and folate, indicating a better vitamin B_{12} and folate status, with episodic memory and information processing speed.[14] Two-year supplementation with folic acid and vitamin B_{12}, however, did not show beneficial effects on specific cognitive function domains.[15] Most other randomized controlled trials (RCTs) investigating the effects of B-vitamin supplementation with at least one year follow-up also failed to show a beneficial effect on cognitive performance.[16, 17] Potentially, study populations were not sensitive enough to induce an effect, study durations were too short to significantly slow down the development of cognitive decline, or the effects were too subtle to be detected by the neuropsychological testing. Neuropsychological tests may be susceptible to practice effects or short-term fluctuations in test performance,[18] while structural MRI may be less susceptible to these fluctuations. Brain volume can therefore act as a predictor for brain health,[18-20] as rate of brain volume loss is correlated with performance on cognitive tasks and it may predict the conversion of mild cognitive impairment (MCI) into dementia.[20-22]

We observed a negative association between folate and brain volume in the total population. After stratification, the association was not present in the group that did not receive B-vitamin supplementation, whereas a trend was still seen in the supplemented group. The non-supplemented group might reflect a sample with blood levels closer to normal instead of being intervened by folic acid supplementation, and thus it might be that these associations come closer to normal values. Folic acid, the synthetic form used in supplements, has a higher bioavailability than folate naturally present in food. Because the conversion of folic acid to metabolized folate is low as a result of low activity of the enzyme dihydrofolate reductase, high intake of synthetic folic acid may cause an accumulation of unmetabolized folic acid. Studies have suggested that high folate levels, in combination with and without vitamin B_{12} deficiency are detrimental for cognitive health.[23-26] Especially older adults who took folic acid supplements containing >400 μg folic acid supplements, had a higher risk for cognitive decline.[26] Hence, it is possible that folate levels follow an inverted U-shape regarding optimal cognitive performance and brain health; cross-sectional and

intervention studies, however, are inconclusive in their findings.[6, 27, 24] We cannot speculate on the possible negative effects on brain volume in our study population because we do not have data on unmetabolized folic acid. To unravel the role of folate and folic acid, in combination with and without low vitamin B_{12} levels, in brain health requires more research.

Our cross-sectional findings regarding Hcy and vitamin B_{12} status and brain volumes are similar to other studies. Studies that investigated Hcy concentrations and brain atrophy showed clear associations, with higher levels associated with more total atrophy [2, 28, 29] and lower hippocampal volume.[30] In line with our findings, associations of MMA and Hcy with total brain volume measured 4.6 years later are shown, but not with serum vitamin B_{12}.[4] A prospective study (n = 107, mean age 73 years), however, showed that lower serum concentrations of vitamin B_{12} and holoTC, but not folate, Hcy or MMA, were associated with smaller brain volumes after 5 years of follow-up.[5] The only RCT currently published was performed in 168 patients with MCI, showing that B-vitamin (B_{12}, B_6 and folic acid) supplementation slowed down total brain atrophy and grey matter atrophy, but only in those with the highest Hcy levels.[31] We observed a borderline significant association between Hcy and grey matter in our healthier study population, but only in the group that received B-vitamin supplementation. Interestingly, the observed associations were thus stronger in those with lower Hcy levels as a consequence of the B-vitamin treatment. It might be that the stronger associations between vitamin B_{12} status, as reflected by Hcy and MMA, and brain volumes in the B-vitamin group are the results of the intervention, but since baseline MRI data are lacking, no conclusions can be drawn about the causality of the relation between the intervention and the MRI measures.

In contrast to our hypothesis, we observed that participants who received B-vitamin supplementation had lower brain volumes than the non-supplemented group. Participants who received B-vitamins were significantly younger than participants who did not receive the supplements. As age is a major predictor for brain atrophy,[32] it is possible that our findings may be the result of this two-year age difference between the two groups. This hypothesis is supported by the fact that the unadjusted model did not show differences on brain volumes between treatment groups, whereas a difference was expected due to the age difference. When adjustments for age were made, this resulted in a significant difference in brain volume between the two groups.

To put our findings into perspective, some methodological issues need to be discussed. Limitations include the lack of baseline data on brain volumes, which makes it impossible to draw conclusions on the effects of two years B-vitamin supplementation on grey and white matter volume. Furthermore, one of the inclusion criteria of the B-PROOF study was an elevated Hcy level, which makes the generalizability to the total elderly population difficult. Last, we performed multiple statistical tests, which increased the risk on chance findings. Strengths of our study are the use of a 3T scanner to make high-precision images, our relatively large study population for conducting MRI scans, the possibility to adjust for multiple confounders, and the available data of folate, Hcy and three markers of vitamin B_{12} status. Due to the two-year supplementation with vitamin B_{12} and folic acid, we created measureable variation between participants regarding their folate and vitamin B_{12} status.

The observed cross-sectional associations of higher Hcy concentrations and lower brain volume may be explained by several mechanisms. Hcy may be neurotoxic, which can induce brain atrophy and hamper neurogenesis.[2, 28] Furthermore, elevated Hcy levels may lead to an increase of phosphorylated tau, which is associated with atrophy in specific brain regions.[33, 34] Higher Hcy levels have also been associated with more white matter hyperintensities [35] and decreased myelination and consequently on the integrity of white matter.[36, 37]

The results of the current study suggest that lower levels of Hcy and the vitamin B_{12}-metabolite MMA may be important in order to attenuate brain atrophy in elderly people. Brain volume reduction in healthy older people (>60 years) is around 0.5 to 1.1 % per year,[5] which is equivalent to about 5 to 10 mL. In the B-PROOF study, Hcy levels decreased with 5 μmol/L after two years of B-vitamin supplementation.[38] This decrease can be considered as one to three years less brain atrophy based on the observational results in the current study, which might be considered substantial. However, the negative associations of folate and the lack of a baseline measurement withhold us from giving recommendations on whether folic acid and vitamin B_{12} supplementation will be beneficial above and beyond normal dietary intake. Nevertheless, in relation to brain health a lower Hcy level seems to be beneficial for healthy elderly people.

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Elegant

8

DISCUSSION

Main findings

The effects of different nutrients, i.e. glucose and sucrose, protein, resistance-type exercise with and without protein, and vitamin B_{12} and folic acid on cognitive performance in different older study populations were investigated in this thesis. **Table 1** presents the main findings. In general, the described interventions caused small effects on cognitive performance and different cognitive domains were affected. Yet, processing speed seems to be the most susceptible cognitive domain to the nutritional interventions presented in this thesis.

There are multiple plausible mechanisms on how these nutrients could influence cognitive performance. Observational studies have often suggested a certain role of nutrition in the process of cognitive decline. Despite an appreciation of the possible biological mechanisms and associations from epidemiological studies, adequate evidence from randomized controlled trials (RCTs) is lacking.[1] This is not only due to the smaller number of RCTs compared to observational studies, but also because many RCTs did not confirm the expected effects of a dietary intervention on cognitive performance. Our limited effects do not provide sufficient evidence in the field of nutritional neurosciences, but does this imply that the role of nutrition in cognitive decline is limited? Or are other, for instance methodological issues, the underlying explanation for the limited effects? In this final chapter, methodological issues relating to the studies are described in the context of nutritional neurosciences. This is followed by a discussion on the implications of the investigated nutrients, proposed future research and summary remarks.

Table 1 Summary of main findings

Nutrient	Design	Findings		Chapter
		Beneficial effects/ associations	No/negative effects/ associations	
Sweet Thoughts				
Glucose (acute effects)	Literature review	Glucose may improve episodic memory in elderly people	Limited number of studies regarding other cognitive domains; results were inconclusive	2
	Cross-over intervention trial in 43 adults ≥70 y with light self-reported memory complaints		No effects on episodic memory, working memory, attention and information processing speed, or executive functions	3
Sucrose (acute effects)	Literature review		Very limited number of studies done; little evidence of a beneficial effect	2
	Cross-over intervention trial in 43 adults ≥70 y with light self-reported memory complaints	Beneficial effect on information processing speed	No effect on episodic memory, working memory, or executive functioning	3
ProMuscle				
Protein supplementation	24-week intervention trial in 65 pre-frail and frail adults ≥65 y	Improved reaction time	No effects on episodic memory, attention and working memory, information processing speed, executive functioning	4
Exercise training without protein supplementation	24-week intervention trial in 62 pre-frail and frail adults ≥65 y	Improved attention and working memory	No effects on episodic memory, information processing speed, executive functioning Unfavourable effect on Letter Fluency test	5
Exercise training with protein supplementation	24-week intervention trial in 65 pre-frail and frail adults ≥65 y	Improved information processing speed	No effects on episodic memory, attention and working memory, executive functioning	5
B-PROOF				
Supplementation with folic acid and vitamin B_{12}	2-year intervention in 2,919 adults ≥65 y with elevated homocysteine levels	Small beneficial effect on global cognitive performance	No effects on episodic memory, attention and working memory, information processing speed, executive functioning (n = 856)	6
Serum levels vitamin B_{12}-related markers	Cross-sectional study in 218 adults ≥65 y	Higher methylmalonic acid levels were inversely associated with total brain volume and grey matter in the group with a 2-yr history of B-vitamin supplementation	No associations of serum B_{12} or holotranscobalamin with brain volumes	7
Serum levels folate			Folate levels were inversely associated with white matter volume and total brain volume	7
Plasma levels homocysteine		Homocysteine levels were inversely associations with total brain volume		7

Methodological considerations

The great complexity of nutritional neurosciences is challenging. Specifically for the topics in the previous chapters, several strengths and limitations have already been discussed. Despite the fact that the presented studies were diverse in their exposure, design, duration and population, which makes comparisons slightly difficult, some generic methodological issues and considerations about study design, target population, study duration, outcome measures, and the investigated nutrients will be discussed.

Study design

The core of this thesis (**Chapter 3, 4, 5 and 6**) consists of placebo-controlled intervention trials, and except for one study (**Chapter 5**, effect of resistance-type exercise) all were randomized and double-blind. RCTs can confirm causal relationships and therefore stand high in the evidence pyramid. Limitations of RCTs include the generalizability of the observed effects and the feasibility of the translation of a controlled environment into daily life.[2]

When investigating the slow process of cognitive decline and dementia, another drawback of RCTs becomes apparent. As cognitive functions gradually decline over multiple years, it is difficult to halt this process in the often short time span of an RCT. Additionally, dietary intake during the entire life or during specific sensitive periods in life may be more important than dietary or supplement intake during an intervention period. In order to identify possible risk factors for cognitive decline, long-term cohort studies can be used to define dietary intake during life and to associate this with the gradual decline in cognitive performance. Certainly, observational studies have their limitations as well. It is, for instance, challenging to adjust for all possible confounders, which results in residual confounding. Furthermore, the assessment of dietary intake is prone to errors and misclassifications, among others, due to self-reported measures and the lack of accurate biomarkers.[3] And finally, reverse causality prevents the drawing of conclusions on the causal relationship of nutrients on cognitive health, although with prospective studies this limitation can be more or less overcome.

Prospective studies with multiple measurements over time can be initiated in middle age, as brain abnormalities and cognitive decline may already be present by then.[4] People who are at higher risk of developing dementia, for instance people with the apolipoprotein E-ε4 (ApoE-ε4) allele, or having a family history of dementia are preferably included. It still remains that observational evidence needs to be confirmed with data from intervention trials.[5] Consensus statements on nutritional neurosciences still advocate long-term intervention trials as the gold standard.[6] Based on results of prospective studies, intervention trials can be designed, with regard to target populations and duration.

Target populations

Intervention studies provide the highest level of evidence, but at the same time they are costly, and it is therefore important to consider and select the right circumstances in which to

perform a trial. We undertook our trials in elderly people older than 65 or 70 years without known diagnosis of mild cognitive impairment (MCI) or dementia. Several changes in the brain occur when growing older and this makes the group of elderly people an interesting group to study. Based on the inclusion criteria, the study populations that were used for our studies were at risk of cognitive decline [7-9] and therefore, they were assumed to be more susceptible to the possible effects of nutritional interventions. Although we aimed to include vulnerable individuals, our populations were well educated, generally healthy, had high scores on the Mini-Mental State Examination (MMSE), and consequently may have had good brain reserves. The inclusion of healthy individuals is a common feature of response bias, as it is likely that people who are willing to participate are, in general, healthier, higher educated, more conscious about a healthy lifestyle, and more willing to change their habits than those who do not volunteer for scientific studies. This may explain the small observed changes in their cognitive performance during the intervention periods, as was clearly observed in the B-PROOF study after two-year follow-up. Consequently, it may be the reason why we observed few effects in our nutritional intervention studies. Therefore, our findings cannot be generalized to the entire elderly population.

Nutrient deficiencies

Older adults are more susceptible to nutrient deficiencies than younger populations, because of impaired absorption or inadequate diet.[10] More than 20% of older adults have vitamin B_{12} deficiency, and around 10% of community-dwelling and frail older individuals have an insufficient protein intake.[11] Supplementation may therefore be useful or even necessary to treat nutritional deficiencies, although, whether there is an additional effect on health, e.g. cognitive performance, beyond normal homeostasis levels is still uncertain.[12] Nutritional status is often not taken into account in trials, while well-nourished older individuals may not benefit from nutritional supplements.[13] It is suggested that research in deficient participants will yield larger effects, but it has to be considered that when choosing this target population not treating the control group may have ethical concerns. With the inclusion criteria of elevated homocysteine levels, it was suspected that the B-PROOF population was at risk of vitamin B_{12} and/or folate deficiency, and subsequently responsive to the effects of supplementation. Depending on which marker and cut-off values are used, vitamin B_{12} deficiency ranged from 8-30% in this population, indicating that the majority had adequate vitamin B_{12} levels.

MCI patients

Populations who already have some cognitive problems, such as patients with depressive symptoms,[14] mild cognitive impairment (MCI),[15] or dementia,[16] may be more susceptible to nutritional interventions than cognitively healthy older adults. They are suggested to be suitable to study the effects of nutrition in the process of cognitive decline, as these populations will decline more over time than healthy older individuals.

Studies in patients with MCI or dementia do, however, not have consistent findings.[1, 10] Concerns have been raised whether brain damage that may be present in patients with MCI

or dementia, and even already in healthy older adults, is reversible. When a clinical diagnosis of dementia is made, underlying brain damage leading to the disease has been present for many years [6, 12] and at the moment of diagnoses, it might be too late to undo the existing damage or to prevent further decline.[17] Therefore, suggestions have been put forward to conduct research in younger populations, for instance in midlife adults, aiming to build more cognitive reserve and minimize neuronal damage. A debate is ongoing concerning the age at which cognitive decline presents, with a meta-analysis showing decline starting at the age of 60 years. However, another large study that was not included in the meta-analysis showed the presence of decline already at the age of 45 years.[4] Younger populations less often have a comprised nutrition status, making them less susceptible to nutritional interventions. In addition, intervention trials in midlife adults require very long study duration before an effect, if any, can be detected using neuropsychological test batteries. As brain pathologies may be present at a younger age than cognitive decline,[18] early biomarkers, including imaging studies or markers in cerebrospinal fluid (CSF), should be considered. Till biomarkers are further validated, MCI patients and elderly populations who are at high risk for either malnutrition, cognitive decline, or both are the preferred target populations for nutritional interventions. Cognitive decline can still be reversible in these populations, as is reflected by the fact that almost half of the MCI patients return to "normal" in the first year after diagnosis.[19] Patients with severe-stage dementia on may, on the other hand, have too much brain damage to counteract this with nutrition or medication.

Study duration

To induce changes in cognitive functions and to be able to detect them, an intervention study should be at least 18 months long according to consensus guidelines of the IANA.[20] The follow-up period should be based on the mechanisms of action [6] and can be slightly shorter when very vulnerable participants are included, such as patients with MCI or frail older adults.[20] With its 24 months, the B-PROOF study should have been long enough to induce an effect of B-vitamin supplementation. Nevertheless, it is possible that the main expected mechanism, namely reducing homocysteine levels, had not revealed its effects yet, and as such, no beneficial effects were observed. Homocysteine levels increase with age [21] and, in addition, the negative effects of homocysteine on cognitive performance may accumulate.[22] If homocysteine already has damaged the brain, it is difficult to undo the damage. The ProMuscle study was shorter, being only 24 weeks long, but we observed beneficial effects of the exercise programme with and without protein supplementation. An intervention with physical exercise might induce faster or larger effects than nutritional compounds, and can therefore be detected after a shorter study duration.[23, 24] In addition, with the exercise programme we used in the ProMuscle study, it may be questionable whether participants would still have complied to the treatment if the study had a longer follow-up.

The recommended guidelines are an indication for all nutritional intervention studies on cognitive functioning in cognitively healthy people, but whether it is really possible to induce and detect an effect of any nutritional intervention within 18 months, remains uncertain. Currently, some longer-term studies have been performed, including the EPDI studies,

with a follow-up time of 6 years in older adults without a diagnosis of MCI or dementia.[25] Longer-term intervention studies are, however, difficult to perform in view of manpower, participants, compliance, and they often do not fit into subsidy programs regarding costs and time. By including populations who are at higher risk of cognitive decline than normal, as suggested in the previous section, studies of a slightly shorter duration might be feasible.

Measuring brain health

Neuropsychological tests

Cognitive performance can be measured in various ways. Global measures for cognitive performance, such as the MMSE and the Clinical Dementia Rating scale have often been used in observational studies. These measures are easy to use, cheap, and are mainly developed as screening tools to detect MCI or dementia. They are, however, prone to ceiling effects, and they are not very sensitive tools to discriminate performance in healthy elderly persons or to detect an effect of a nutritional intervention.[26] Therefore, the MMSE as an outcome measure has been considered questionable, although we used it as a rough measure for global cognitive change over a two-year period and were able to observe a small effect of B-vitamin supplementation. A better method to measure cognitive performance is by applying neuropsychological test batteries, as were used in the studies described in this thesis (see Table 1 in **Chapter 1**).[26] The tests we used have been validated and have more often been used in comparable studies in older populations. They were supposed to be difficult enough to avoid ceiling effects, but also not too demanding to avoid stress or to affect motivation. They covered a wide range of cognitive functions that are vulnerable to age-related decline. Single test scores were combined into cognitive domain composition scores in order to create robust measures. The clustering was based on the cognitive concepts that were measured, similar to other studies.[27, 28] A limitation of this method, however, is that the clustering is not always straightforward, as some of the cognitive tests assess more than one function. In addition, certain functions are needed to perform other functions, e.g. to remember a list of words you have to pay attention to this list. As a result, it is possible that a compound score did not change after an intervention, while one of the tests was affected by the treatment, but another test was not due to a difference in sensitivity or difficulty. We therefore always investigated the single cognitive scores as well.

An abundance of different neuropsychological tests is available, which makes the choice of the most accurate tests difficult. A known or hypothesized relationship between a nutrient and a cognitive function should be the lead in selecting cognitive tasks, rather than the availability or easiness of the tests.[26] The use of different tests and the subsequent difficulties comparing them is a problem and suggestions have therefore been put forward for a standard neuropsychological test battery that can be used in nutritional neurosciences.[26] This will enable firm conclusions to be drawn on postulated nutritional benefits.

Imaging techniques

The impairment in cognitive performance has been suggested to be one of the last features of brain dementia pathologies.[26, 29] According to a hypothetical model of biomarkers of Alzheimer's disease (AD), preclinical AD starts with an elevation of β-amyloid accumulation. This is followed by synaptic dysfunction, tau-mediated neuronal damage, changes in brain structures, and only then by problems with cognitive functions.[29] Imaging techniques that measure these changes could therefore be of great use to investigate early effects of nutrition. However, to date they have not often been applied in nutritional studies.[1]

One of the imaging techniques includes structural MRI scans to measure brain volumes. Total grey and white matter volume, and volume of cerebrospinal fluid (CSF) are relatively rough measures for the rate of atrophy.[1] Together with hippocampal atrophy, total brain atrophy can be a predictor for cognitive decline.[30, 31] At the population level, brain volumes correlate with neuropsychological performance and daily functioning,[32, 33] although these relations are not always present at an individual level. Within the B-PROOF study, we obtained different types of images to study brain volume (**Chapter 7**), and to be able to investigate more specific brain regions volumes, white matter integrity, and associate these measures with cognitive performance measures and B-vitamin status.

Other structural imaging techniques include the presence of white matte integrity by measuring white matter hyperintensities, diffusion tensor imaging (DTI), and Positron emission tomography (PET). Functional imaging techniques include functional MRI (fMRI), fluorine-18 fluorodeoxyglucose PET (FDG-PET), proton magnetic resonance spectroscopy (MRS), phosphorous MRS, near-infrared spectroscopy (NIRS), electroencephalonography (EEG), magnetoencephalography (MEG). Molecular biomarkers in CSF are also available, such as Aβ42, Tau, and p-tau,[1] but the measurements are rather invasive. Research on biomarkers in blood, which has a lower burden for the participants than a lumbar puncture and is more accessible, is developing.[34, 35]

Not everyone with pathologies will end up with dementia due to greater brain reserve and better compensation strategies. Brain imaging and CSF biomarkers may predict cognitive decline at group level,[18, 36] and the use of these methods can give more understanding about possible mechanisms. However, the predictive values of the mentioned biomarkers for cognitive decline or dementia have not yet been established for individual diagnosis.[37] It is furthermore debatable what it means when a nutritional intervention affects these brain biomarkers, but when cognitive performance measurements or daily activities are not changed.[2] What benefit does it give to people when they do not notice it in daily life, even though on the longer-term it may be beneficial? The use of combinations of biomarkers, imaging techniques and cognitive performance measures in research should be encouraged to increase the understanding and predictive value of biomarkers.[5]

Nutritional factors

Single nutrients, multi-nutrients, dietary patterns, or multi-domain studies

The rationale for the investigated nutrients in this thesis has extensively been discussed in the previous chapters. The interventions covered different dietary approaches, including single macronutrients (glucose and protein), combinations of nutrients (sucrose, folic acid and vitamin B_{12}), and a multi-domain intervention (exercise and protein). A topic of interest in nutritional (neuro)science is that people consume whole diets rather than single nutrients. By investigating single nutrients, interactions with other nutrients are not being taken into account and it may be questionable to what extent intervening with one or two nutrients may influence brain health when the normal diet has not been adjusted. As such, a single tablet with a certain nutrient without a controlled diet might be ineffective.[12] An advantage of the single-nutrient approach is that when observing an effect, it is clear this effect was caused by the particular nutrient and it consequently gives a clue to potential mechanisms. Possible interactions of nutrients and other lifestyle factors may have additional effects compared to single nutrients. Therefore, micronutrient combinations, dietary patterns, and multi-domain studies are receiving more attention nowadays.

Multi-nutrients combinations

The combination of vitamin B_{12} and folic acid, and sometimes with added vitamin B_6, has often been used in other trials. It is suggested that these B-vitamins work on at least one common mechanism, via lowering homocysteine, to affect brain health. Studies on other combinations of multi-nutrient supplements are heterogeneous and show mixed effects. A meta-analysis in older adults without dementia suggested a possible effect of multivitamin supplements on free recall performance, but not on other cognitive domains.[38] A study in older men that was not yet included in the meta-analysis but with a follow-up of 12 years did not observe any beneficial effect at all.[39] The multi-nutrient drink Souvenaid® contains a combination of nutrients that is hypothesized to target one mechanism, namely synaptic loss. Beneficial effects were observed on memory performance in MCI patients and mild AD patients,[40, 41] but not in mild-to-moderate AD patients.[42] The combination of nutrients may yield larger effects, but alternatively, it is important to take into account that different nutrients may have opposing directions because of different mechanisms, which can cause conflicting results. Targeting one specific mechanism with a multi-nutrient approach is an opportunity for future research.

Dietary patterns

A step further in investigating combinations of nutrients is studying whole dietary patterns. It has been shown that people with AD adhere less to the Mediterranean diet,[43] and prospective studies have shown that this type of diet may be protective for dementia.[43, 44] A meta-analysis of longitudinal studies with follow-up times ranging from 2.2 to 8 years showed a 33% lower risk of cognitive impairment (MCI or AD) in the tertile who complied most to a Mediterranean diet than those in the lowest tertile, and also the conversion of

MCI to AD was lower in the highest Mediterranean diet tertile.[44] Other healthy diet indexes, such as the DASH (Dietary Approaches to Stop Hypertension) [45] also showed associations with cognitive performance. A difficulty with these diet scores is that cut-offs are not based on absolute values, but on relative levels for the study populations. This makes comparisons between studies difficult and generic recommendations to the public somewhat challenging. To our knowledge, only one RCT (the PreDimed-Navarra study) has been done thus far with Mediterranean components, i.e. olive oil or nuts, and showed a beneficial effect of olive oil on cognitive performance after 6.5 years in older adults initially without cardiovascular disease but with a high risk on cardiovascular diseases.[46] More trials are needed to investigate the efficacy of Mediterranean diet on cognitive performance.

Multi-domain studies

To gain a better understanding of the role of lifestyle factors in relation to the multifactorial process of cognitive decline, components other than nutrition should be taken into account. The potential synergistic effects of combined interventions, addressing multiple factors, might affect the process of cognitive decline more than one factor alone.[47-49] The combination of nutrition and physical exercise, as we applied in **Chapter 5** with protein supplementation and resistance-type exercise, is an example of this type of research. Another example of a multi-domain intervention study is the MAPT (Multi-Domain Intervention in the Prevention of Age-related Cognitive Decline) study in France, which investigates the combination of nutrition (omega-3 fatty acids), and a combination of cognitive training, nutritional advice and physical activity advice on cognitive decline.[50] Performing a multi-domain intervention trial is challenging, and preferably it includes groups with the single intervention as well as the combined intervention.

A broader perspective and health implications of the investigated nutrients

Glucose and sucrose

Effects on cognitive performance

To the best of our knowledge, the literature review in **Chapter 2** captured all studies (n = 20) performed in the field with acute effects of glucose and sucrose on cognitive performance in non-demented older adults. We concluded that a glucose load may enhance episodic memory performance in the short-term in elderly people. This conclusion was in line with another review, which was conducted in younger and older adults, and patients with AD.[51] In our intervention study (**Chapter 3**) we did not observe an effect on episodic memory, although we observed an effect of sucrose on information processing speed, which was in accordance with the effect of glucose in two other studies out of eight that investigated this cognitive domain.

Similar to other published studies, our study was performed when participants were in a fasted state, and the effects of glucose or other food products might be different during the day when people are allowed to eat.[52] Furthermore, not only does a glucose load seem to influence cognitive performance; enhancing effects were also observed after the intake of carbohydrates with a lower glycaemic index (barley and potato),[53] and after a meal rich in protein and fat.[54] This may suggest that beneficial effects are not limited to a high-glycaemic product, such as glucose or sucrose, but that it is the supply of energy that is important for optimizing cognitive functions in the short-term.

Health considerations

Based on the available research, it seems that a rapid rise in glucose levels enhances episodic memory in the short-term. In the longer-term, however, stable blood glucose levels are preferred. Prolonged elevated blood glucose levels, insulin insensitivity and diabetes may harm vessel walls and induce neuronal brain death, and consequently increase the risk of cognitive decline and dementia.[55] The consumption of products containing a considerable amount of refined sugar may be unfavourable for a person's health in the long term. The Dutch guidelines for healthy nutrition state to be careful with sugar intake.[56] In addition, a recent draft report of the WHO proposes to reconsider the guidelines for free sugar use, as limiting the consumption of free sugars may reduce the risk of obesity and dental caries.[57] This makes it challenging to set a recommendation for the regular dietary use of pure glucose, sucrose, or products containing high amounts of added sugars to improve cognitive performance.

Protein supplementation and resistance-type exercise

Dietary protein and cognitive performance

We have added an intervention study (**Chapter 4**) to the limited existing literature on dietary protein and cognitive performance, which mainly consisted of observational studies and only a few RCTs.[58] Although our findings were heterogeneous, our study does provide leads for future studies. Our positive finding on reaction time can be attributed to the effects of extra supply of precursors for neurotransmitters or may be related to a better physical performance as a result of the protein supplementation.[59] However, the finding could also be a chance finding, as no effects were observed on the associated domain of information processing speed. Therefore, to unravel the possible role of protein supplementation in cognition replication studies are warranted.

Studies on the effects of single amino acids, more particularly tyrosine and tryptophan, have shown acute effects on cognitive performance.[58] Whether a change in amino acid supply impacts cognitive performance in the longer-term is unknown. Examining this hypothesis is challenging, as the uptake of amino acids is modified by the presence of other amino acids due to the competitive transport across the blood-brain barrier. Furthermore, when amino acids are consumed together with other dietary components, part of the amino acids is absorbed by other body tissues, such as muscles. These crossing pathways complicate the prediction of how much of the amino acids will be taken up by the brain and can subsequently be used for neurotransmitter synthesis. It would be interesting to investigate changes in amino acid concentrations in CSF after a longer-term intervention of amino acid or protein supplementation to unravel a part of the mechanism. The optimal dose, supplementation method (protein drink vs. amino acid supplement), timing (during a meal, presence of carbohydrates) and the burden of measurements in CSF should be considered.

Physical exercise and cognitive performance

Physical activity has been indicated as one of the modifiable risk factors in the process of cognitive decline, but intervention studies with exercise programmes do not consistently observe beneficial effects.[60, 61] Only one study has investigated the effects of a combination of aerobic and resistance-type exercise in a frail population, and observed beneficial effects on executive functions and processing speed, although the effects were not limited to the frail part of the study group.[24] We added to this that resistance-type exercise improved attention and working memory in a pre-frail and frail population of older adults. Moreover, the combined exercise and protein supplementation revealed benefits on information processing speed. One would expect that the combination exercise and protein would, at least, be beneficial also for attention and working memory. We cannot explain this, but it may be that mechanisms are slightly different when a nutritional compound is added to exercise training. Imaging studies have already shown that exercise may influence prefrontal areas, white matter integrity, and functional connectivity,[61] and this can relate to our findings on speed-related functions and working memory.

Other exercise types, including aerobic and flexibility training have been suggested to

be good strategies to enhance cognitive performance, but especially the combination of different types of exercise seems to be most beneficial.[60] Results of trials, however, are not totally equivocal. These mixed results may be a result of differences in study design, follow-up time, efficiency of training, and study population. A challenge in physical exercise studies is a good control group, namely social interaction has been shown to be an important factor regarding cognitive function. This aspect of social interaction is often lacking in a non-exercise group. Social engagement has been shown to improve cognitive performance, and with training sessions twice a week this may interact with the much smaller social interaction contribution in the control group. In our study (**Chapter 5**), the control group visited the university on test days, which may already have had a beneficial effect on their well-being and social interactions, but for a perfect control group more social activities should have been included. Another aspect that needs to be considered is whether older adults, frail or non-frail, can comply with extensive exercise training, for a longer period of time. Future research preferably includes measures that can shine a light on the underlying mechanisms involved in physical exercise and cognitive performance.

Vitamin B_{12} and folic acid

Within the B-PROOF study, the role of B-vitamins in brain health has been extensively investigated. Cross-sectional baseline data showed that higher methylmalonic acid (MMA) and homocysteine levels, but not serum vitamin B_{12} and holotranscobalamin, were associated with poorer performance on episodic memory (homocysteine and MMA) and information processing speed (homocysteine).[62] In accordance, these biomarkers were also inversely associated with brain volume after two years supplementation with vitamin B_{12} and folic acid (**Chapter 7**). Higher folate concentrations at baseline were associated with better performance on episodic memory. In contrast, folate was also associated with lower brain volumes. Two-year supplementation with vitamin B_{12} and folic acid significantly decreased homocysteine and MMA levels. This did, however, not result in beneficial effects on cognitive domain performance (**Chapter 6**), whereas a small beneficial effect was observed on the incidence of cerebrovascular events in women.[63] In contrast to our hypothesis, brain volumes were slightly smaller in the group that received B-vitamin supplementation compared to those who did not. To put the somewhat heterogeneous results of the B-PROOF study in perspective and to draw conclusions on the role of B-vitamins in brain health, we have to look at evidence from other studies as well.

Evidence for the role of homocysteine and vitamin B_{12} and folate in brain health is mainly derived from pre-clinical [64, 65] and observational studies that examined cross-sectional and prospective associations between vitamin B_{12} markers and folate with cognitive performance,[10, 66] brain volume [67-71] and brain integrity.[72, 73] Our null-findings of the two-year supplementation are in concordance with the conclusions of different reviews,[74, 75] a meta-analysis of studies conducted in elderly people,[9] and with one other large study that was not yet included in the meta-analysis.[76] Another study that was also not included in the meta-analysis showed beneficial effects on cognitive performance in older adults with depressive symptoms,[14] as well as a study in MCI patients showing beneficial effects on cognitive

performance and grey matter atrophy.[77]

Wrapping up the evidence, we suggest that there is no strong evidence for the use of vitamin B_{12} and folic acid supplementation to affect cognitive performance in healthy elderly adults who are not vitamin B_{12} or folate deficient. However, the observational data on vitamin B_{12} and folate status on cognitive performance and brain parameters cannot be neglected, as well that in other populations, e.g. MCI and dementia patients, the effects may be different. This calls for further research.

Future perspectives

In this chapter, various ideas are suggested as recommendations for the nutritional neurosciences field. In **Box 1**, a summary of recommendations is given. Nutritional interventions should aim at prevention rather than treating cognitive impairment. Main messages are:

1) There is a need for long-term prospective studies with multiple measurements over time with both neuropsychological tests and biomarkers that reflect risk factors for brain health;
2) To confirm observational data, RCTs are needed in high-sensitive populations, such as MCI patients, people with suboptimal nutritional status, ApoE-ε4 carriers or family history of dementia;
3) To get a complete picture of the influence of lifestyle, not only single nutrients need to be investigated, also dietary patterns and combinations of different domains should be studied.

It should be taken into account that it is difficult to get people to change their lifestyle, including dietary habits. Taking a supplement in the form of a tablet or capsule may be easy, but to change whole dietary patterns can be difficult. To be able to estimate the effects of a change in lifestyle, it is important to study both the efficacy and effectiveness of an intervention.

Concluding remarks

This thesis described the results of a literature review, one cross-sectional study and four RCTs to investigate the role of different nutrients in cognitive performance and brain volumes in healthy Dutch elderly people. In general, limited effects of the different nutritional interventions, e.g. glucose and sucrose, protein and resistance-type exercise with and without extra protein, and vitamin B_{12} and folic acid, on cognitive performance were observed. Based on our results, we cannot yet recommend the use of supplemental extra dietary protein, vitamin B_{12} and folic acid, or glucose or sucrose in the prevention of cognitive decline. Given the large problem of dementia, research on modifiable risk factors, including nutrition, should continue. Future research should use well thought out research methods, including large and long-term observational and intervention studies with high-

sensitive study populations and early biomarkers (e.g. brain imaging techniques) for cognitive decline in combination with neuropsychological tests. In this way, nutrition can be added to the list of lifestyle factors that can fight dementia.

Box 1: Future perspectives for nutritional neurosciences

- There is a need for long-term, large-scale studies, both prospective cohorts and intervention studies.
- Prospective cohorts should include multiple follow-up measurements, and preferably start as early as in midlife with a long follow-up time. Accurate assessments of nutrient intake, total dietary intake, and patterns, are therefore needed.
- Especially populations high at risk should be included in studies, such as people with a family history of dementia and people with ApoE-ε4 genotype. Those people who are at highest a priori risk, are also the most likely to benefit from risk reduction strategies.[78] This may be of special interest when studies are done in younger participants.
- In addition, it would be valuable to include patients with diagnosed MCI or self-reported memory complaints, especially in RCTs. Although brain damage may probably already be present, it is still possible that people return to normal again. This would be good for quality of life and healthcare costs. Also people with a nutritional deficiency are of interest, as they have room to improve their nutritional status and cognitive performance.
- RCTs should last at least 18 months, but preferably longer to be sure that the mechanism can induce its suggested effect.
- For a better comparability of studies, a standardized neuropsychological test battery is warranted and consensus is needed on the diagnosis of normal cognitive decline, MCI and dementia. The use of global measures, such as the MMSE, as an outcome measure is discouraged.
- Neuropsychological test batteries are suggested to be the primary outcome to define brain health, but it will be valuable to use other, earlier markers of cognitive decline next to the cognitive tests. Early markers include imaging techniques as well as biomarkers in blood or cerebrospinal fluid, but the predictive value has not yet been established and further research is needed.
- When multi-arm interventions are performed, combinations of nutrients and single nutrients should be included to be able to disentangle the possible mechanisms. Also whole diet studies are needed, as synergy between nutrients seems to be important.[12] Long-term RCTs investigating the effects of a whole dietary change, however, are expensive and difficult to perform.[6]
- Besides nutrition, other lifestyle factors can play a role in the development of cognitive decline. In particular, physical activity may have synergic effects with nutrition. Other multi-domain options that may interfere with nutritional effects are cognitive and social engagement, and treatment of diseases.[61]

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Summary

The age-related cognitive decline and the increase in dementia patients are large problems in societies with growing aging populations. No cure is present for dementia, while the available medication only focuses on alleviating symptoms. It is therefore of major importance to find risk factors that can modify the development of cognitive decline and dementia. Pre-clinical and observational studies suggest a role for nutrients. Evidence derived from randomized controlled trials (RCTs) is, however, limited and equivocal with most studies showing no effect and only a few studies showing a beneficial effect of a nutritional intervention. In the current thesis, we investigated the acute and longer-term effects of different nutrients, i.e. glucose and sucrose, protein, resistance-type exercise training with or without protein, and vitamin B_{12} and folic acid in order to optimize and preserve cognitive functions in non-demented elderly people.

A comprehensive literature review was performed on the acute effects of glucose and sucrose on cognitive performance (**Chapter 2**). Glucose is the most important fuel for the brain, and as such, manipulation of the supply of glucose may affect cognitive functions. The main conclusion of our review was that a glucose load may have a short-term beneficial effect on episodic memory. Enhancing effects on other cognitive domains were less clear, partly due to the small number of studies examining these effects. Limited research was also done on the possible effects of sucrose on cognitive functions. Therefore, we investigated the acute effects of 50 g of glucose and 100 g of sucrose on a broad spectrum of cognitive functions reflecting performance on episodic memory, working memory, attention and information processing speed, and executive functions (**Chapter 3**). This was done by a cross-over study in 43 elderly participants who had self-reported memory complaints. In contrast to the conclusion of our review, we did not observe an effect of glucose or sucrose on episodic memory, though we showed a beneficial effect of sucrose on attention and information processing speed.

Protein supplementation was the next nutritional intervention that was investigated. Several amino acids are precursors for neurotransmitters, and their supply may affect the synthesis and release of these neurotransmitters, and may consequently affect cognitive performance. A 24-week randomized placebo-controlled trial was carried out in 65 frail and pre-frail elderly people (**Chapter 4**). The protein supplementation included twice a day 15 grams of protein in the form of a drink. Reaction time improved more in the protein group compared to the placebo group, but the scores on the cognitive domains, i.e. episodic memory, attention and working memory, information processing speed, and executive functions, or the other single test scores, did not differ between treatment groups. We furthermore investigated the effects of 24 weeks resistance-type exercise training with and without protein supplementation in pre-frail and frail elderly people (**Chapter 5**). Exercise training without extra protein (n = 62) improved performance on the domain attention and working memory. Exercise training together with protein supplementation (n = 65) improved performance on information processing speed.

Last, the role of vitamin B_{12} and foic acid on cognitive health was investigated. Low levels of these nutrients can increase homocysteine levels, which is a suggested risk factor for cognitive decline. The effect of daily supplementation with 500 μg vitamin B_{12} and 400 μg folic acid was investigated in 2,919 participants for two years (**Chapter 6**). Global cognitive function and episodic memory were assessed in the total study population, whereas extensive neuropsychological testing was done in a subpopulation (n = 856). B-vitamin supplementation did not improve cognitive domain scores. Only a small, though significant, effect was observed on global cognitive performance, measured by the Mini-Mental State Examination, but this was suggested to be due to chance. Brain MRI scans were made in a subgroup (n = 218) after two years of intervention to obtain volumetric measures of grey and white matter, and total brain volume (**Chapter 7**). We investigated the cross-sectional associations between follow-up levels of folate, homocysteine and three vitamin B_{12} status biomarkers, i.e. methylmalonic acid, holotranscobalamin and serum vitamin B_{12}, and brain volumes. Fully adjusted regression models showed a borderline significant association between plasma homocysteine and total brain volume, with a stronger association in the group that received B-vitamin supplementation. Serum B_{12} and holotranscobalamin were not associated with brain volumes, whereas high methylmalonic acid levels were associated with lower brain volumes in the group that received B-vitamins. In contrast, higher folate levels were associated with lower total brain volumes. In addition, when comparing the group that received two years of B-vitamin supplementation and those who did not, we observed lower brain volumes in the B-vitamin group, which might be a result of a difference in age between the two groups.

To conclude, the nutritional intervention studies showed little evidence for a beneficial effect on cognitive performance in relatively healthy older adults. Given the large problem of dementia, research on modifiable risk factors, including nutrition, should continue, with well thought out research methods, including large and long-term observational and intervention studies with high-sensitive study populations and early biomarkers (e.g. imaging techniques) for cognitive decline in combination with neuropsychological tests. In this way, nutrition can be added to the list of lifestyle factors that can fight dementia.

Samenvatting

Met de toename van het aantal ouderen groeit ook het aantal patiënten met dementie. Omdat er nog geen genezing van dementie mogelijk is, is het belangrijk dat er leefstijlfactoren worden geïdentificeerd die van invloed zijn op het proces van cognitieve achteruitgang, zoals voeding. Preklinische en epidemiologische studies laten een mogelijke rol zien voor verschillende nutriënten. Bewijs van gerandomiseerde gecontroleerde onderzoeken is echter schaars en niet overtuigend, waarbij de meeste studies geen of kleine effecten laten zien. Dit proefschrift richt zich op de acute en langere termijn effecten van verschillende nutriënten (glucose en sucrose, eiwit, krachttraining met en zonder eiwit, en vitamine B_{12} en foliumzuur), om nader te onderzoeken of deze nutriënten een rol spelen in het optimaleseren en het behouden van cognitieve functies in gezonde ouderen.

In **hoofdstuk 2** is er een uitgebreid literatuuronderzoek gedaan naar de acute effecten van glucose en sucrose op cognitieve functies. Glucose is de belangrijkste brandstof voor de hersenen, en manipulatie van de glucosetoevoer zou daarom cognitieve functies kunnen beïnvloeden. De belangrijkste conclusie van deze literatuurstudie was dat de inname van een glucosedrank positieve effecten kan hebben op het episodisch geheugen op de korte termijn. Op de andere cognitieve domeinen was het effect van glucose minder duidelijk, wat gedeeltelijk verklaard zou kunnen worden door een kleiner aantal studies. Onderzoeken naar een mogelijk effect van sucrose op cognitieve functies waren ook schaars. In **hoofdstuk 3** hebben we daarom de effecten van 50 g glucose en 100 g sucrose op verschillende cognitieve functies onderzocht in een cross-over studie. De bestudeerde domeinen in deze studie waren episodisch geheugen, werkgeheugen, aandacht en informatieprocessnelheid, en uitvoerende functies. De onderzoekspopulatie bestond uit 43 oudere deelnemers met lichte zelf-gerapporteerde geheugenklachten. In tegenstelling tot de conclusie van het literatuuronderzoek werd er geen effect van glucose of sucrose gevonden op episodisch geheugen. Wel was het cognitieve domein van aandacht en informatieprocessnelheid beter na de sucrosedrank vergeleken met de placebo.

In **hoofdstuk 4** is het effect van eiwitsuppletie op cognitief functioneren onderzocht. Verschillende aminozuren zijn nodig voor de aanmaak van neurotransmitters, en daarom suggereren studies dat extra toevoer van deze aminozuren mogelijk de aanmaak van neurotransmitters kan beïnvloeden. Dit zou effect kunnen hebben op cognitief functioneren, maar dit is nog maar weinig onderzocht. In een gerandomiseerde, placebo-gecontroleerde studie van 24 weken kregen 65 kwetsbare ouderen tweemaal daags een eiwitdrank met 15 g eiwit, of een placebodrank. Na 24 weken was de reactietijd in de eitwitgroep meer verbeterd dan in de placebogroep. Het functioneren op de andere cognitieve taken en domeinen (episodisch geheugen, aandacht en werkgeheugen, informatieprocessnelheid en uitvoerende functies) verschilde niet tussen de twee groepen. Ook werden de effecten van 24 weken krachttraining met en zonder eiwitsuppletie in kwetsbare ouderen onderzocht (**hoofdstuk 5**). Krachttraining zonder extra eiwit (n = 62) verbeterde de scores op het domein van aandacht en werkgeheugen. Daarnaast verbeterde krachttraining met eiwitsuppletie (n = 65) de informatieprocessnelheid.

In **hoofdstuk 6 en 7** is de rol van vitamine B_{12} en folaat onderzocht. Lage concentraties van deze nutriënten kunnen het homocysteïne gehalte in het bloed verhogen, wat een risicofactor is voor cognitieve achteruitgang. In ouderen met een verhoogde concentratie homocysteïne werd daarom onderzocht wat het effect was van dagelijkse suppletie van 500 μg vitamine B_{12} en 400 μg foliumzuur gedurende twee jaar, vergeleken met een placebo op cognitief functioneren (n = 856-2919) (**hoofdstuk 6**). Na twee jaar bleek er geen verschil te zijn in domeinscores (episodisch geheugen, aandacht en werkgeheugen, informatieprocessnelheid, en uitvoerende functies) tussen de B-vitaminegroep en de placebogroep. Wel was er een klein significant verschil op globaal cognitief functioneren, gemeten met de Mini-Mental State Examination (MMSE, p = 0.05). Twee jaar na de start van de interventie zijn er in een subgroep (n = 218) MRI hersenscans gemaakt om volumes van grijze en witte stof en totaal hersenvolume te bepalen (**hoofdstuk 7**). Middels regressieanalyses zijn de associaties onderzocht tussen bloedconcentraties van folaat, homocysteïne en drie markers voor vitamine B_{12} status (serum vitamine B_{12}, methylmalonzuur, en holotranscobalamine) met hersenvolumes. Volledig gecorrigeerde regressiemodellen lieten een marginaal-significante associatie zien tussen plasma homocysteïne en totaal hersenvolume, waarbij de associatie sterker was in de groep die de B-vitaminesuppletie had ontvangen dan in de placebogroep. Serum vitamine B_{12} en holotranscobalamine waren niet geassocieerd met hersenvolumes, terwijl hoge concentraties methylmalonzuur geassocieerd waren met minder hersenvolume in de groep die B-vitaminen had gehad. Hogere folaatconcentraties bleken geassocieerd met een kleiner hersenvolume. Verder had de groep die B-vitaminen had gekregen een kleiner totaal hersenvolume dan de placebogroep, hetgeen mogelijk verklaard kan worden door het leeftijdsverschil van twee jaar tussen de groepen.

De conclusie van dit proefschrift is dat de voedingsinterventies slechts kleine tot geen effecten op cognitief functioneren laten zien in relatief gezonde ouderen. Desalniettemin blijft onderzoek naar risicofactoren voor dementie, inclusief voeding, belangrijk. Dit kan door middel van grote, lange-termijn observationele en interventiestudies, met gevoelige studiepopulaties en vroege biomarkers (bijvoorbeeld met MRI scans of bloedmarkers) voor cognitieve achteruitgang in combinatie met neuropsychologische testen. Op deze manier zal duidelijk worden op welke manier voeding kan bijdragen aan de strijd tegen dementie.

Dankwoord

Yes, het is af! Ik heb met veel plezier aan dit proefschrift gewerkt, maar ik had het zeker niet alleen kunnen doen; dankzij de bijdrage van vele anderen is het geworden zoals het nu is.

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Curriculum vitae

Nikita Laura van der Zwaluw was born on February 25, 1986 in Lelystad, the Netherlands. After moving to Doetinchem and Uden, she finished secondary school (VWO) at the Udens College, Uden in 2004. Nikita then started the study Nutrition and Health at Wageningen University. Her BSc thesis was a literature study about vitamin D and diabetes. During her MSc thesis she investigated the reliability of depression scales, under supervision of Ondine van de Rest. Nikita did her internship at Gothenburg University, Sweden. After graduating in 2009, she started as a research assistant on the ProMuscle project at the Division of Human Nutrition, Wageningen University. In this project, the effects of protein supplementation with and without exercise training on muscle strength, muscle mass, and cognitive performance were investigated. At the end of the project, Nikita started on a new project about the effects of glucose and sucrose on cognitive performance. Next to that, she was involved in the B-PROOF project, a large multicenter intervention trial in which participants received B-vitamin supplementation for two years. By that time, Nikita started her PhD project about the effects of nutrition on cognition, based on the aforementioned projects. The results are presented in this thesis. Currently, Nikita is appointed as an educational employer on the project of developing a distance learning master at the Division of Human Nutrition. Furthermore, she is exploring new job opportunities.

List of publications

Publications in this thesis

Van der Zwaluw NL, Van de Rest O, Kessels RPC, De Groot LCPGM. Effects of a glucose load on cognitive functions in elderly people: A literature review. Nutrition Reviews. In press.

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Educational activities

Description	Location	Year
Discipline specific activities		
Annual meeting NWO nutrition	Deurne, the Netherlands	2011
Annual meeting NWO nutrition	Deurne, the Netherlands	2012
NutriMenthe	Rotterdam, the Netherlands	2011
Netherlands Consortium for Healthy Ageing	The Hague, the Netherlands	2013
Netherlands Consortium for Healthy Ageing, final meeting	The Hague, the Netherlands	2013
The international conference Aging & Cognition	Dortmund, Germany	2013
Alzheimer's Association International Conference	Boston, United States	2013
International Conference on Nutrition and the Brain	Washington, United States	2013
Symposium Nutricia Nutrition and the Brain	Nijmegen, the Netherlands	2013
Geriatriedag	Den Bosch, the Netherlands	2014
Nutrition for the Ageing Brain: Towards Eevidence for an Optimal Diet	Milan, Italy	2014
Course Medical Neuroscience	Coursera, organized by Duke University	2013
General courses		
Good Clinical Practice	Ede, the Netherlands	2011
VLAG PhD week	Baarlo, the Netherlands	2013
Teaching and Supervising Thesis students	Wageningen, the Netherlands	2012
Techniques for writing and presenting a scientific paper	Wageningen, the Netherlands	2012
Project and time management	Wageningen, the Netherlands	2013
How to write a world class paper?	Wageningen, the Netherlands	2011
Optional courses and workshops		
Preparation METC-protocol	Wageningen, the Netherlands	2011
Teambuilding course B-PROOF	Ede, the Netherlands	2011
Final report session TIFN A-1002	Maastricht, the Netherlands	2012
MRI certificate	Ede, the Netherlands	2012
Staff seminars	Wageningen, the Netherlands	2012-2014
PhD study tour	Sydney/Melbourne, Australia	2013
Masterclass Confounding	Wageningen, the Netherlands	2014
25jr ouderenonderzoek	Wageningen, the Netherlands	2013
Symposium Orthica	Amersfoort, the Netherlands	2013

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