Dietary patterns for healthier cognitive ageing

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Thesis

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The idea is
to die young
as late
as possible

Ashley Montagu
(1905-1999)
American anthropologist

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Chapter 1

General introduction

Ageing

It is well known that the world population is ageing. By the year 2050, 20% of the world population will be aged 60 years and above [1]. American and European people aged 65 years between 2000-2015 had a life expectancy of 20 years [2]. Moreover, as assessed in 2014, chronic diseases start to occur at 61.4 years for men and 61.8 years for women in the EU [3]. Thus, not only the population is ageing, but also the number of people living with chronic diseases is increasing.

Cognitive decline during ageing

As part of the ageing process the brain ages as well, leading to a decline in cognitive functioning (**figure 1**). There is convincing evidence demonstrating that age-related cognitive decline starts from the late 20's and continues throughout the lifespan [4]. A progressive – and usually chronic - decline in memory, language, problem-solving and other cognitive skills that affects a person's ability to perform everyday activities leads to mild cognitive impairment (MCI) and may progress to dementia [5]. Dementia is caused by a variety of brain illnesses, of which Alzheimer's Disease (AD), vascular dementia, dementia with Lewy bodies, and frontotemporal dementia are the most common. Dementia results in personal suffering, demands on the family for caregiving, and eventually leads to the need for institutionalized care. In 2015, 9.4 American and 10.5 million European people were living with dementia and this number is estimated to increase to 131.5 billion worldwide by 2050 [6]. Unfortunately, despite this increase in the number of people living with dementia, studies on novel drugs to treat dementia have been negative [7]. Thus, there is an urgent need to find modifiable strategies to postpone the onset of cognitive decline and dementia.
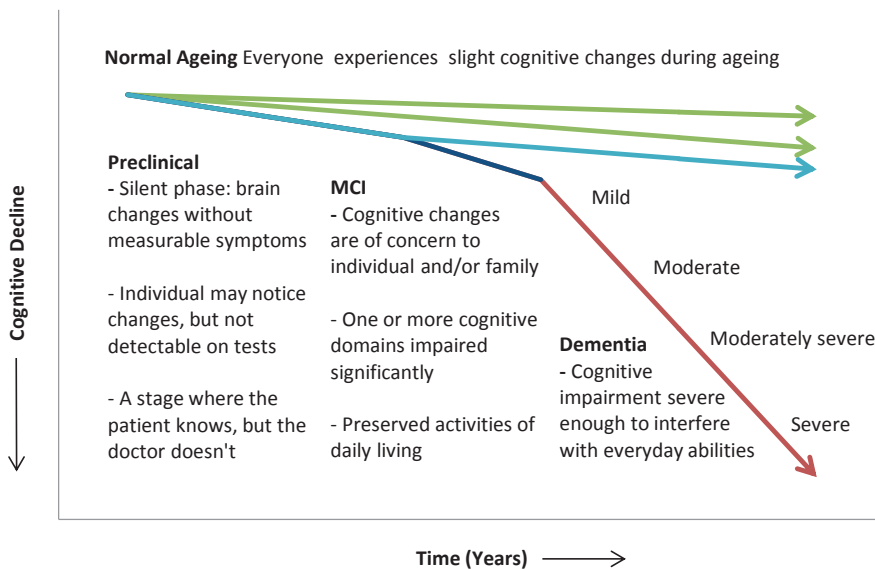

Figure 1. Progression from normal ageing to Alzheimer's disease or another dementia (adapted from [8]).

Risk factors for cognitive decline

There are many risk factors for cognitive decline comprising non-modifiable risk factors and modifiable risk factors. Non-modifiable risk factors include age [9], female sex [9], family history [10, 11] and genetic susceptibility (e.g., the presence of 1 or 2 copies of the apolipoprotein E (APOE) Ɛ4 allele) [9, 12]. Modifiable risk factors can be divided into cardiovascular risk factors (diabetes, mid-life obesity, mid-life hypertension, and hyperlipidaemia), lifestyle risk factors (current smoking, physical activity, diet, alcohol, cognitive training, and social engagement), and other risk factors (education, brain injury, depression and sleep) [13]. There is strong evidence for an increased risk of cognitive decline for brain injury, mid-life obesity and mid-life hypertension, current smoking and diabetes (**figure 2**).
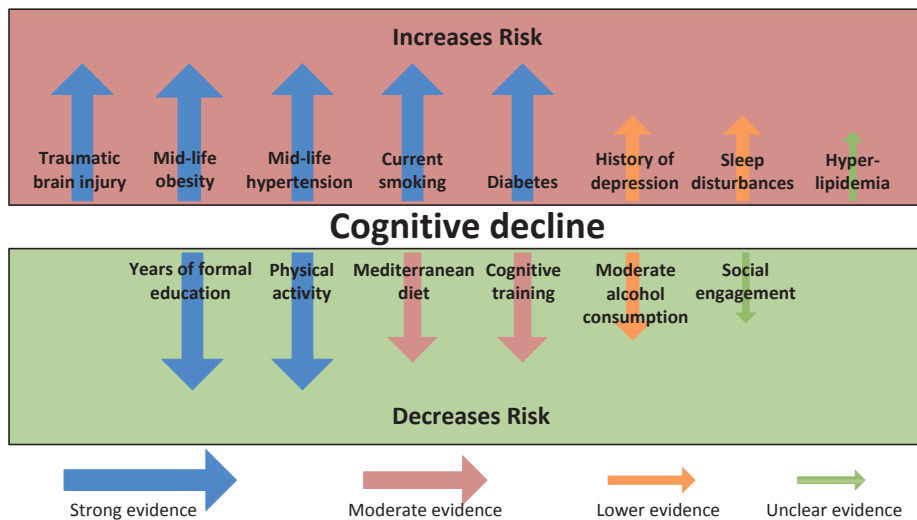
Figure 2. Strength of evidence on risk factors for cognitive decline (adapted from [13])

Nutrition and mechanisms in cognitive functioning

Many of the risk factors described above can be modified by diet. To date, numerous studies have been conducted demonstrating a possible link between the intake of specific nutrients and foods on cognitive functioning. Examples include omega-3 fatty acids, vitamin D, vitamin B6, vitamin B12, folate, flavonoids, and vitamin E, but also foods, such as fish, nuts and seeds, and fruits and vegetables [14]. Omega-3 fatty acids have anti-inflammatory [15] and cardiovascular protective effects [16] and may therefore reduce the risk of atherothrombotic complications such as stroke [17] and subsequent cognitive decline. Furthermore, omega-3 fatty acids may improve the composition of cell membranes and therefore stimulate the development and regeneration of nerve cells [18]. Vitamin D plays a role in the prevention of neurological, autoimmune, and psychiatric diseases [19]. Folate, vitamin B6, and vitamin B12 are required for proper DNA methylation and to prevent the accumulation of homocysteine. Homocysteine is an intermediary amino acid that has been associated with cardiovascular disease and stroke, as well as cognitive decline [14]. Flavonoids and vitamin E have been proposed to have significant antioxidant and anti-inflammatory effects [14]. However, evidence from observational and intervention studies provide mixed results for the role of single nutrients on cognitive functioning.

Dietary patterns; a more powerful approach to study nutrition

Because people do not consume single nutrients but rather a combination of nutrients and foods within a complete diet, the research field has shifted towards studying dietary patterns. Studying dietary patterns allows one to combine the synergistic effects of single nutrients [20-22]. Thus, studying dietary patterns could be a more powerful approach and also more relevant to the general public to unravel the role of diets for a healthier cognitive ageing.

For dietary patterns, roughly three main biological mechanisms have been proposed by which diet could potentially protect against dementia risk and cognitive decline, relating to: 1) the vascular system (e.g., aortic stiffness [23] or microvascular function [24]), 2) oxidative stress (e.g., free radicals and brain protection or damage [25], or loss of neuronal homeostasis and neurodegenerative diseases development [26]) and 3) attenuation of the inflammatory pathway (e.g., microglial priming [27, 28].

Dietary patterns have frequently been studied in relation to, for example, cardiovascular health, but studying the association between dietary patterns and cognitive functioning is relatively new and less frequently done. Especially intervention studies are lacking. As a result, the most effective dietary pattern that impacts brain health has not yet been found. Dietary patterns of interest include the *Mediterranean* diet [29], the *Healthy Diet Indicator* (HDI) [30], the *Dietary Approaches to Stop Hypertension* (DASH) diet [31], the *Mediterranean-Dietary Approach to Systolic Hypertension diet intervention for neurodegenerative delay* (MIND) diet [32], and the *NUtrients and AGEing* (NU-AGE) diet [33]. All these dietary patterns are based on prior knowledge – so called *a priori* or knowledge based dietary patterns – as opposed to *a posteriori* (data driven) dietary patterns. *A priori* dietary patterns can be based on the observation of a healthy dietary pattern within a population, for example the Mediterranean diet. Alternatively, *a priori* dietary patterns can be based on dietary recommendations (NU-AGE diet), or components that have been associated with certain health outcomes, such as the HDI, the DASH diet and the MIND diet. **Table 1** provides an overview of dietary components included in each of the dietary patterns.

Mediterranean Diet

The Mediterranean diet is an example of a healthful dietary pattern observed within a specific population as it is a collection of eating habits traditionally followed by people living in the Mediterranean. This diet is characterized by a high intake of olive oil, fruit, vegetables and legumes, a moderate intake of fish and red wine during meals [34] (**table 1**). The Mediterranean diet is one of the dietary patterns that is most frequently studied in relation to cognitive functioning.

At the start of this thesis in 2011, there were very few publications about the Mediterranean diet and cognitive decline or dementia. Nicolas Scarmeas was one of the first researchers reporting a reduced risk of AD among American older adults with the highest adherence to Mediterranean diet in a prospective study [35] and a case-control study [36]. In the same cohort, Scarmeas demonstrated a reduced risk of (MCI) with higher adherence to the Mediterranean diet [37]. A slower rate of cognitive decline was reported among another population of French older adults with the highest adherence to the Mediterranean diet [38] and the risk of incidence MCI or dementia was reduced in subjects with a high Mediterranean diet score in an Australian cohort [39].

Table 1. Overview of dietary patterns and their components used in this thesis

Components	Mediterranean[a]	HDI[b]	DASH[c]	MIND[d]	NU-AGE
Grains	Non-refined grains, >4/d		Whole grains + (2.1)	Whole grains, ≥3/d	Whole grains, 4-6/d
Fruits	Fruits, >3/d	Fruits and vegetables ≥400g/d	Fruits + (3.4)	Berries, ≥2/wk	Fruits, ≥2/d
Vegetables	Vegetables >4/d Potatoes, >2/d		Vegetables + (4.8)	Green leafy vegetables, ≥6/wk Other vegetables, ≥1/wk	Vegetables, ≥300g/d Legumes, ≥200g/wk
Nuts	Legumes, nuts and beans, , >6/wk		Nuts and legumes + (1.0)	Nuts, ≥5/wk Beans, >3meals/wk	Nuts, ≥2/wk
Dairy	Full-fat dairy, ≤10/wk		Low-fat dairy + (1.5)	Cheese, <1/wk	Low-fat dairy, 500ml/d Low-fat cheese, 30g/d
Meat	Red meat, ≤1/wk Poultry, ≤3/wk		Red and processed meat – (0.5)	Red meat and products, <4 meals/wk Poultry (not fried), ≥2 meals/wk	Low-fat meat and poultry, 4/wk
Fish	Fish, >6/wk			Fish (not fried), ≥1	Fish, 2/wk Eggs, 2-4/wk
Protein	Protein 10-15 en%	Protein 10-15 en%			
Fat	Olive oil, ≥1/d	Saturated fatty acids <10 en% Cholesterol <300mg/d PUFA 6-10 en%	MUFA plus PUFA, mg/d + (29.4mg)	Olive oil, primary source Butter, margarine, <1tbs/d Fast fried foods, <1/wk	Olive oil, ≥20g/d
Sweets		Mono-and disaccharides <10en%	Sweetened beverages – (0.1)	Pastries and sweets, <5/wk	Sweets, limited use
Fibre	Dietary fibre ≥25g/d	Dietary fibre ≥25g/d			
Sodium		Sodium, mg/d – (2168)	Sodium, mg/d – (2168)		Sodium, <2000mg/d
Alcohol	Alcohol, 1-300ml/d			Wine, 1 glass/d	Men: ≤20g, women: ≤10g
Fluid					≥1500ml/d
Vitamin D					10 ug/d (supplement)
Score	0-55, 0-9	0-70	0-45	0-15	0-160

Abbreviations: d; day; wk; week; m; meal, tbs; tablespoon

[a] Mediterranean example from Panagiotakos [40]. Frequently scoring methods for the MeDi include quintile ranks and scores ranging from 1-3, 4-6 and 7-9 when 9 MeDi components are being used.

[b] The HDI score was based on the WHO guidelines for a healthy diet [30, 41], including seven items for which information was available in all studied cohorts, applying an adapted continuous scoring system [42]

[c] An alternate DASH score was used, based on the previously described DASH score [43], and uniquely adding MUFA and PUFA to the original DASH diet. The scoring of the DASH diet was based on quintile ranks according to the population's intake distribution. The plus sign indicates adequacy components, the minus sign indicates components to limit. Values in brackets are mean intake values in the fifth quintile.

[d] MIND diet adapted from Morris et al.[44]

Since then, the interest on the association between the Mediterranean diet and cognitive functioning has increased enormously. Dozens of publications were added, of which the majority of the observational studies demonstrated an association between higher adherence to the Mediterranean diet and cognitive function [45, 46], cognitive decline [47-49], AD [50], or (mild) cognitive impairment [46, 50, 51], and a few reported no association [52-54]. Only one intervention study has been conducted to date, reporting less cognitive decline among participants following the Mediterranean compared to a control group [55]. Another trial reported no association [56], but it was argued that this could be due to an enormous time gap between dietary assessment (1994-1996) and cognitive assessment (2007-2009) [57]. A review including studies up to January 2012 concluded that higher adherence to the Mediterranean diet was associated with better cognitive function, lower rates of cognitive decline and a reduced risk of AD in nine out of 12 studies whereas results for MCI were inconsistent [58]. A meta-analysis up to October 2012 including eight studies reported a lower risk of cognitive impairment for those with higher adherence to the Mediterranean diet [59] and another meta-analysis up to November 2012 including five studies on MCI or AD with a follow-up of at least one year reported a reduced risk of developing MCI and AD and a reduced risk for progressing from MCI to AD [60]. At last, the most recent review based on 14 different cohorts concluded that the role of a Mediterranean diet is not consistent across the many cohorts [61]. Given all this evidence, the Mediterranean diet seems a promising diet to postpone cognitive decline, however, results are mainly based on observational studies and not consistent yet.

Healthy Diet Indicator

The Healthy Diet Indicator (HDI) [30] has been identified as another interesting dietary pattern to impact cognitive functioning. This indicator captures a more general healthy dietary pattern, mainly based on nutrients instead of foods, which is more globally applicable (**table 1**). The HDI is a tool to classify a healthy diet according to the guidelines of the World Health Organization published in 1990 [62], including high intakes of fruits, vegetables and fibre and limited intakes of saturated fatty acids, sodium and sugars. These guidelines were developed to reduce chronic diseases, such as hypertension. As hypertension has been shown to impact cognitive functioning [63-65], it has been hypothesized that the HDI could also reduce cognitive decline. The HDI has been cross-sectionally investigated in relation to cognitive functioning in 1998 and 2001 [66, 67], showing a tendency towards a lower prevalence of cognitive impairment with greater adherence to the HDI. Since then, the HDI has been updated [42] based on the latest scientific knowledge and lessons learned from implementing national intervention strategies to reduce the burden of chronic diseases [41]. The updated HDI has recently been investigated in relation to cardiovascular mortality demonstrating an inverse association with greater HDI adherence [42, 68-70]. However, the updated HDI has not yet been related to cognitive functioning. As cardiovascular health could be one strategy to impact cognitive functioning, the updated HDI seems to be a potential dietary pattern to contribute to healthier cognitive ageing.

Dietary Approach to Stop Hypertension diet

A dietary pattern that has increasingly received attention is the Dietary Approach to Stop Hypertension (DASH) diet. The DASH diet specifies high intakes of fruits, vegetables, low-fat dairy products, whole grains, poultry, fish, and nuts and low intakes of fats, red meat, sweets and sugar-containing beverages [31, 71] (**table 1**), and has been shown to lower blood pressure [31, 72]. It has been hypothesized that the DASH diet could impact cognitive functioning, as high blood pressure is associated with higher risk of MCI [73], vascular dementia [74], and neurocognitive deficits [63]. Studies demonstrating an association between the DASH diet and cognitive functioning are limited. Two observational studies reported a slower cognitive decline rate with greater DASH adherence after 4.7 years [75] and 11 years of follow-up [76]. To date, there is only one trial on the effect of the DASH diet on cognitive functioning, demonstrating that there was an improvement in psychomotor speed compared with the usual diet control among sedentary and overweight/obese individuals with prehypertension and hypertension [77].

Mediterranean-DASH Intervention for Neurodegenerative Delay diet

A more recently defined dietary pattern is the *Mediterranean-dietary approach to stop hypertension (DASH) diet Intervention for Neurodegenerative Delay* (MIND) diet that specifically captures dietary components shown to be neuroprotective [32]. This diet has been developed based on the most recent knowledge and evidence from mostly observational studies targeting brain health in humans and animal experiments. The MIND diet uniquely specifies the consumption of green leafy vegetables and berries (**table 1**). To date, two observational studies have been conducted demonstrating less cognitive decline [32] and a lower risk of AD [44] for those with the highest MIND diet scores [32, 44].

NU-AGE diet

Given the many observational studies, but very limited number of intervention studies on healthful dietary patterns and cognitive functioning, there is a need for well-designed randomized dietary intervention studies. The NU-AGE (*NUtrients and AGEing*) dietary intervention study aimed to improve healthy ageing of a European population by means of a diet based on nutritional recommendations for the ageing population (**table 1**). This approach was taken because dietary intakes of adults aged 60 years and older are often below what is recommended. The prevalence of inadequate dietary intakes in European elderly was above 20% for vitamin D, folic acid, calcium, selenium and iodine [78]. With respect to malnutrition as assessed with the Mini Nutritional Assessment tool, 3% of people with mild to moderate AD were malnourished [79], up to 50% of patients with severe AD [80]. The NU-AGE study aimed at developing a dietary pattern that does not only meet the dietary habits and preferences of older adults but also uniquely targets the dietary recommendations of the ageing population.

Aim and outline of this thesis

As outlined above, it is important to find dietary strategies for healthier cognitive ageing. There is quite some observational evidence demonstrating a link between diet and cognitive functioning. However, evidence from randomized controlled trials on the effect of a healthful diet on cognitive functioning is lacking. Moreover, it is not clear which dietary pattern is most suitable to impact cognitive functioning in an ageing population. The main aim of the thesis was to study the association of healthful dietary patterns with cognitive functioning. In addition, nutrient intake inadequacies were assessed and the potential to change dietary intake in older adults aged 65 years and older was studied. This thesis will add evidence to the link between dietary patterns and cognitive functioning by describing results from a wide range of studies, including a literature review, observational studies and an intervention study.

Chapter 2 presents a cross-sectional description of current dietary intake in a Dutch population of adults aged 65 years and older. **Chapter 3** includes a review about the current evidence on the association between dietary patterns, including the Mediterranean diet, the Dietary Approaches to Stop Hypertension (DASH) diet, and the Healthy Diet Indicator (HDI), with cognitive function and decline. In the following three chapters the HDI (**chapter 4**), the DASH diet (**chapter 5**) and the *Mediterranean-DASH Intervention for Neurodegenerative Delay* (MIND) diet (**chapter 6**) are prospectively studied in relation to cognitive function and decline within a large population including over 16,000 American women aged 70 years and older. The last chapters of this thesis cover the NU-AGE (NUtrients and AGEing) dietary intervention study. **Chapter 7** provides an overview and the design of the NU-AGE dietary intervention study with the aim to improve dietary intakes of a European population aged 65 years and older for healthier ageing. **Chapter 8** presents results of analyses to study if dietary intake of this European population of older adults shifts more towards their dietary recommendations. Additionally this paper describes the development and application of the NU-AGE diet index. The effects of the changes in the dietary intakes within the NU-AGE intervention on cognitive functioning are described in **chapter 9**. At last, **chapter 10** gives an overall reflection on the studies that are presented in this thesis.

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Chapter 2

Conventional foods, followed by dietary supplements and fortified foods, are the key sources of vitamin D,vitamin B6 and selenium intake in Dutch participants of the NU-AGE study

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Abstract

With aging, energy needs decrease, necessitating a more nutrient dense diet in order to meet nutritional needs. To bridge this gap, the use of nutrient dense foods, fortified foods and dietary supplements can be important. This observational study aims to describe current micronutrient intakes of Dutch elderly and to identify the contribution of nutrient dense foods, fortified foods and dietary supplements to the intake of micronutrients that are often inadequately consumed in Dutch elderly. Data of 245 Dutch volunteers from the NU-AGE study aged 65-80 years were used. Dietary intake was assessed by means of 7-day food records and dietary supplement use was recorded with an additional questionnaire. Information on fortified foods was obtained from the Dutch Food Composition Table 2011. Nutrient density of foods was evaluated using the Nutrient Rich Food 9.3 score. The percentages of participants not meeting their average requirement was high for vitamin D (99%), selenium (41%) and vitamin B6 (54%) based on conventional foods, and also when taking into account fortified foods (98%, 41%, 27%, respectively) and vitamin and mineral supplements (87%, 36%, 20%, respectively). Conventional foods were the main source of vitamin D, vitamin B6 and selenium intake (42%, 45%, 82% respectively), followed by vitamin and mineral supplements (41%, 44% and 18%) and fortified foods (17%, 11% and 1%). Foods with the highest nutrient density contributed most to total vitamin B6 intake only. In order to optimize nutrient intakes of elderly, combinations of natural food sources, fortified foods and dietary supplements should be considered.

Introduction

Similarly to several other developed regions of the world, the European population is aging rapidly. It is expected that approximately 30 percent of the European population will be 65 years or older by the year 2050 [1]. With aging, energy needs decrease while micronutrient requirements remain or increase, necessitating a more nutrient dense diet in order to meet nutritional needs [2]. Inadequate nutrient intake may cause chronic metabolic disruption, including mitochondrial decay, resulting in acceleration of various degenerative diseases [3]. The prevalence of malnutrition and under nutrition is relatively high in both institutionalized (40-65%), and non-institutionalized elderly (5-10%) [2]. In Europe, more than 20% of the people aged 65 years and older have inadequate intakes of vitamin D, folate, calcium, selenium and iodine [4], and intakes of vitamin D, calcium, selenium, magnesium, thiamine, riboflavin are of possible public health concern [5].

These findings are in line with EURRECA outcomes for priority nutrients. EURRECA was an EU 6th Framework Programme funded Network of Excellence, aimed to align the micronutrient recommendations among Europe. EURRECA identified 10 priority micronutrients for elderly (vitamin D, folate, vitamin B12, vitamin C, iron, calcium, zinc, selenium, iodine and copper) based on heterogeneity in current recommendations, amount of new evidence and importance for public health [4].

Sources of micronutrients include conventional foods, fortified dietary sources and vitamin and mineral supplements. The intake of micronutrients can be limited resulting in nutrient inadequacies or deficiencies in the population. Over the last few decades, the consumption of nutrient-rich conventional foods (e.g., whole grains, vegetables and low-fat dairy products) has been partially shifted towards the consumption of nutrient-poor, but, at the same time, energy-dense foods (i.e., food with a high content of added sugar or solid fats)(6). This shift in food consumption patterns could be considered as another important factor of the observed insufficient dietary intakes of micronutrients.

Widespread nutrient intake shortfalls and associated deficiencies can be prevented or improved by means of nutritional advice on consumption patterns, but also by using fortified foods [7]. Fortification is the process of adding nutrients or non-nutrient bioactive components to food [7]. Food fortification could be considered a public health strategy to enhance nutrient intakes of a population. Additionally, dietary supplements can effectively counteract inadequate nutrient intakes and its consequences [3]. Besides estimating inadequate nutrient intakes, it is also important to take into account the possibility of excessive nutrient intakes as the availability of food fortification and dietary supplement intake increases [8].

This study aimed to describe current dietary intakes compared to nutritional requirements in Dutch elderly who were part of a cohort study and to explore which component - e.g. conventional foods, fortified foods or dietary supplements - is the main contributor to total dietary intakes of elderly. Because not much is known on how to classify conventional foods consumed by elderly based on their nutrient content, this study additionally aimed to explore if a concept of nutrient density can be used when studying the contribution of nutrient dense foods to total dietary intakes.

We hypothesized that by knowing how conventional foods, fortified foods or dietary supplements differ in their contribution to total dietary intakes, dietary advices can be targeted towards bridging the gap between dietary intakes and nutritional needs in elderly. Therefore, the objectives of this present study were: 1) to estimate dietary intakes from conventional foods, fortified foods and dietary supplements, 2) to compare these intakes to nutritional requirements of the elderly, and 3) to explore the use of a nutrient density score in this respect.

Methods

Study design and population

The present study was commissioned by the Nutrient Intake Optimization Task Force of the European Branch of the International Life Sciences Institute (ILSI Europe). Baseline data from Dutch NU-AGE participants were used. The NU-AGE study is a dietary intervention study among volunteers aged 65 years and older living in the Netherlands, the UK, Italy, Poland and France [9]. that focuses on the effect of nutrition on inflammageing, i.e. chronic low-grade inflammation, and its consequences on the aging process [10]. The NU-AGE dietary intervention study is a one-year randomized, single blind, controlled, parallel trial consisting of a control group and a diet group. The dietary intervention is specifically adapted to the nutritional needs of elderly and is described in detail elsewhere [9].

A total of 252 apparently healthy and independently living men and women, aged 65-80 years were recruited from the Dutch city of Wageningen and surroundings. Exclusion criteria were overt diseases, history of severe heart disease, organ failure, insulin dependent diabetes mellitus, chronic use of corticosteroids or recent use of antibiotics, undernourishment (BMI <18.5 kg/m2), and frailty. A screening questionnaire was used to verify current health, medical history and medication use. The presence of frailty was assessed with a test described by Fried et al.[11] The NU-AGE study has been approved by the Medical Ethical Committee from Wageningen University (NL37818.081.11) and all participants provided written informed consent. The baseline examinations started in April 2012 and continued until March 2013. Participants were visited by trained dieticians once to complete dietary and supplement intake data. Participants completed a questionnaire to collect information on lifestyle, social and economic status, food preferences and a supplement questionnaire. In the present study, we excluded participants who had not completed the supplement questionnaire (n=7), resulting in a population for analysis of 245 participants.

Dietary intake assessment

Micronutrient intake from foods

Usual dietary intake was assessed by means of estimated food records [12] that were kept for seven consecutive days. Participants received both verbal and written instructions to keep pre-structured food records and were trained in advance on the description of foods, portion sizes and food preparation methods in order to obtain complete and accurate food records. The food record included eight meal occasions per day: before breakfast, breakfast, morning snacks, lunch, afternoon snacks, evening meal, evening snacks, night snacks. Portion sizes were reported in household measures, based on pictures or measured in grams or millilitres. The consumed foods were coded according to standardized coding procedures at the Department of Nutrition and Health from Wageningen University and converted to energy and micronutrient intake (mean per day) by use of the Dutch food composition table Nederlands Voedingsstoffenbestand (NEVO) 2011 [13]. This table was also used to create food groups and to classify foods as fortified if at least one micronutrient was added to the food, for example, fortified spreads, milk drinks and juices. No distinction was made between mandatory and voluntary fortified foods. Foods without any fortification were classified as conventional foods.

Micronutrient intake from dietary supplements

Participants completed an additional questionnaire at home in which they indicated the use of pre-defined dietary supplements, i.e. multivitamin multimineral (MVMM), iron, vitamin D, vitamin B-complex, and folic acid supplements. For each supplement, the frequency was recorded in times per day, week or month. Additionally, the amount of tablets or drops, type, brand and name were recorded for each supplement. The reported brand and name of the supplements was used to further specify the type of supplements (e.g., vitamin B12 supplement vs. vitamin B complex supplement). The nutrient content of the supplements was based on the product label information obtained from the manufacturer or corresponding website. The average daily nutrient intake from supplements was calculated per participant by multiplying the frequency by the average amount of tablets per day times the nutrient content per supplement and added to the average daily nutrient intake from foods.

Micronutrient intake from nutrient dense foods

Another way to look at sources of micronutrients is to study foods with a high nutrient density [14]. Nutrient density can be defined as the ratio of the nutrient composition of a food to the nutrient requirements [15], although at the moment, there is no consensus on how best to define nutrient density [16]. One of the possible indices to define nutrient density is the Nutrient Rich Food (NRF) 9.3 score as proposed by Drewnowski [15]. This score is able to distinguish between foods and diets that are lower energy dense and more nutrient-rich [17] and it can provide a clear measure of the nutrient density of overall diets [18]. Furthermore, it has been shown to explain the highest percentage of variation in the Healthy Eating Index (HEI) [19] and it has been shown to correlate very well with the Dutch Healthy Diet Indicator [20], a diet score to rank individuals according to their adherence to the Dutch dietary guidelines for a healthy diet. These features make the NRF9.3 a suitable tool to study the contribution of naturally nutrient dense foods to adequate nutrient intake levels. The NRF9.3 score contains nine nutrients to encourage (NR9; protein, dietary fibre, vitamin A, vitamin C, vitamin E, calcium, magnesium, iron and potassium) and three nutrients to limit (LIM3; saturated fat, added sugar and sodium). Because only limited data on added sugar were available, total sugar was used instead.

1) $NR9_{100kcal}$	$\sum_{I\,=\,1-9}(Nutrient_i/RDV_i)*100$	$Nutrient_i$: content of beneficial nutrients protein, dietary fibre, vitamin A, vitamin C, vitamin E, calcium, magnesium, iron and potassium in 100-kcal edible portion; RDV_i: recommended daily values for $nutrient_i$
2) LIM3	$\sum_{I\,=\,1-9}(Nutrient_i/MDV_i)*100$	$Nutrient_i$: content of limiting nutrients saturated fat, total sugar and sodium in 100-kcal edible portion; MDV_i: maximum daily values for $nutrient_i$
3) $NRF9.3_{100kcal}$	$NR9_{100kcal} - LIM3_{100kcal}$	Difference between sums

To calculate the nutrient density of foods, firstly for each food item, the nutrient contents were calculated per 100 kcal resulting in a nutrient content per food item in 100-kcal edible portion. Secondly, for each participant, the nutrient content of all consumed food items were divided by the corresponding Recommended Daily Values (RDVs) [21-23] or Maximum Daily Values (MDV) [24] (**table 1**) and multiplied by 100, resulting in a nutrient score for each nutrient included in the algorithms below. These nutrient scores were capped at 100% avoiding the possibility of a food item with high amounts of a single nutrient to contain more than 100 percent of the RDV.

Table 1. Recommended (RDV) and Maximum (MDV) Daily Values for selected nutrients used in the NRF9.3 score.

Nutrient	RDV [a, b]	MDV [a, b]
Nutrient-rich (NR) components		
Protein (g)	57 [23]	
Dietary fibre (g)	25 [22]	
Vitamin A (mcg)	800 [21]	
Vitamin C (mg)	80 [21]	
Vitamin E (mg)	12 [21]	
Calcium (mg)	800 [21]	
Iron (mg)	14 [21]	
Magnesium (mg)	375 [21]	
Potassium (mg)	2000 [21]	
Nutrients to limit (LIM)		
Saturated fat (g)		20 [24]
Sugar (g)		90 [24]
Sodium (mg)		2400 [24]

[a] Based on an intake of 2000 kcal per day.
[b] Recommended daily allowances as set by the European Union [21], the labelling reference intakes values as set by the European Food Safety Authority [22] were used. Abbreviations: NR, nutrient rich; LIM, limiting nutrient; RDV, recommended Daily Values; MDV, Maximum Daily Values.

Table 2. Nutrient-rich scores (NR9), Limited nutrient scores (LIM3) and Nutrient Rich Food index scores (NRF9.3) per 100 kcal on food group level of consumed foods (n=1,107 foods)[a]

Food group[b]	No. food items	NR9[c]	LIM3[c]	NRF9.3[c]
Vegetables	130	249.8	17.2	232.6
Legumes	8	83.0	4.7	78.3
Fruits	74	88.7	23.4	65.3
Eggs	7	79.7	15.5	64.2
Alcoholic and non-alcoholic drinks	69	52.3	6.7	50.8
Potatoes	19	53.1	3.6	49.5
Fish	57	60.5	18.6	41.9
Soups	21	97.3	58.8	38.5
Milk and milk products	87	59.7	24.8	34.9
Mixed dishes	36	49.4	16.3	33.1
Meat, meat products and poultry	157	55.7	23.4	32.3
Nuts, seeds and snacks	60	37.4	13.1	24.3
Cereal products and binding agents	38	25.7	2.8	22.9
Savoury sandwich filling	6	33.8	11.5	22.3
Cheese	48	55.8	36.1	19.7
Bread	73	31.2	11.4	19.8
Fats, oils, savoury sauces	61	29.7	22.8	6.9
Pastry, cake and biscuits	83	19.0	19.9	-0.9
Sugar, confectionary, sweets	65	17.8	25.5	-7.7
Soy and vegetarian products	8	46.7	91.4	-44.7

[a] For Dutch elderly participants in NU-AGE study (*n*=245).
[b] Food groups are based on the Dutch food composition table '*Nederlands Voedingsstoffenbestand*' (NEVO) 2011 [13].
[c] Values are expressed as means per 100 kcal of a food group of consumed foods (*n*=1,107 foods). Abbreviations: NR(F), nutrient rich (food); LIM, limiting nutrient.

Thirdly, the NRF9.3 scores were calculated by subtracting the LIM3 scores from the NRF9 scores. **Table 2** lists the mean NR9, LIM3 and NRF9.3 scores per food group of 1,107 consumed food items. Foods with a higher NRF9.3 score have a higher nutrient density per 100 kcal and were considered more nutrient dense than foods with a low NRF9.3 score. To distinguish between conventional foods with a low, middle and high nutrient density, food items were ranked upon their NRF9.3 score and divided into tertiles of nutrient density; 1) Low (NRF9.3 scores -116.7 (e.g. bouillon, gravy) to 9.3 (e.g., nougat, gingerbread), 2) Middle (NRF9.3 scores 9.4 (e.g., rusk, refined bread) to 42.5 (e.g., medium-fat cheese, soup with vegetables), and 3) High (NRF9.3 scores 42.5 (e.g. low-fat chocolate milk, low-fat minced meat) to 574.8 (e.g. canned or fresh spinach, turnip greens).

Contribution of conventional foods, fortified foods and dietary supplements
The relative contribution of nutrient dense conventional foods, fortified foods and dietary supplements to the total nutrient intake was calculated as the proportion of intakes from the different components at the group level and were expressed as percentages. For those micronutrients for which intake was inadequate in more than 20% of the study population, we also calculated the contribution of specific food groups to total intake. This was done by taking the ratio of the nutrient content per food group to the total nutrient intake.

Comparison to Dietary Reference Values
Intakes of nutrients identified to require special attention in elderly according to EURRECA [25] and a recent systematic review on inadequate intakes among community-dwelling elderly in Western countries [5], i.e. vitamin D, folate, vitamin B6, vitamin B12, selenium, iron, calcium, magnesium, zinc, iodine and copper, were compared to their dietary reference values. The Average Requirement (AR) from the European Food Safety Authority [26] was used as reference value for the comparison of most nutrients, as these were also the basis for calculating the NRF9.3 score. The AR represents the average daily nutrient intake level that meets the requirement of half of healthy individuals within a group. For vitamin D, we used the AR from the Health Council of the Netherlands [27], as it was the most up to date at the moment of the study [28]. To assess adequacy of individual nutrient intakes, the mean individual intakes were compared with the AR. The prevalence of inadequate intakes was calculated using the AR cut-point method by taking the proportion of participants with a mean intake below the AR. For magnesium, for which no European AR was set, a quantitative approach was used to estimate the prevalence of inadequacy i.e., a low estimated prevalence if mean population intake was well above the Adequate Intake (AI). The prevalence of usual intakes above the tolerable Upper intake Level (UL) was based on comparisons of mean population intakes with European UL's [29]. For all comparisons, European gender and age-specific ARs and ULs were used.

Statistical analyses
Total nutrient intakes from foods and dietary supplements were calculated as means and standard deviations. Baseline characteristics of participants were compared between men and women using ANOVA for continuous variables and the χ^2 statistics for categorical variables. All statistical analyses were performed using SAS software version 9.2 (SAS institute, Inc, Cary, NC).

Results

General characteristics of the study population are presented in **table 3**. The population's mean age was 70.9 ± 4.0 years. Compared to women, men tended to have a higher BMI, were slightly higher educated, were more likely to have ever smoked, had higher absolute energy, protein, fat, carbohydrate and micronutrient intakes. Macronutrient intakes expressed as percentage of total energy intake were similar between men and women. Of the total population, 43% used dietary supplements, of which MVMMs were most frequently used (24%), followed by vitamin D supplements (10%). Compared to men, the percentage of supplement users was higher in women (57% vs. 26%), particularly for vitamin D supplements (16% vs. 2%).

Table 3. Baseline characteristics[a, b]

Characteristic	Total population (*n*=245)	Men (*n*=111)	Women (*n*=134)
Age, years	70.9 ± 4.0	70.9 ± 4.3	71.0 ± 3.9
BMI, kg/m^2*	26.0 ± 3.6	26.7 ± 3.6	25.5 ± 3.6
Education, years*	12.2 ± 3.8	12.7 ± 3.8	11.7 ± 3.6
Smoking status, *n* (% of total)*			
Never	126 (50)	44 (39)	82 (59)
Former	118 (47)	65 (57)	53 (38)
Current	8 (3)	4 (4)	4 (3)
Macronutrient intakes			
Energy, kcal*	1912 ± 418	2100 ± 476	1756 ± 280
Protein, gram	76.3 ± 16.0	82.5 ± 17.0	71.2 ± 13.1
Protein, EN%	16.1 ± 2.4	15.9 ± 2.2	16.3 ± 2.5
Fat, gram*	73.1 ± 19.9	78.7 ± 23.0	68.4 ± 15.5
Fat, EN%*	34.3 ± 5.0	33.5 ± 4.5	35.0 ± 5.3
Carbohydrates, gram*	202 ± 54.4	222 ± 61.8	185 ± 40.2
Carbohydrates, EN%	42.1 ± 5.9	42.2 ± 5.8	42.0 ± 6.0
Micronutrient intakes			
Iron, mg*	10.9 ± 2.48	11.6 ± 2.80	10.31 ± 2.00
Magnesium, mg*	335 ± 78.1	354 ± 93.5	320 ± 58.7
Zinc, mg*	9.91 ± 2.32	10.7 ± 2.55	9.29 ± 1.91
Iodine, mg*	164 ± 58.6	179 ± 49.9	151 ± 62.4
Calcium, mg	969 ± 310	980 ± 361	960 ± 261
Copper, mg*	1.18 ± 0.32	1.24 ± 0.38	1.14 ± 0.26
Selenium, ug*	44.5 ± 12.8	47.29 ± 12.63	42.11 ± 12.47
Vitamin B6, ug*	1.63 ± 0.52	1.77 ± 0.62	1.51 ± 0.38
Vitamin B12, mg*	5.00 ± 2.68	5.39 ± 3.06	4.68 ± 2.27
Folate, ug	256 ± 71.5	259 ± 78.11	253 ± 65.65
Vitamin D, ug*	3.56 ± 2.09	4.09 ± 2.51	3.12 ± 1.55
Use of dietary supplements			
Any supplements, *n* (% of total)*	106 (43)	29 (26)	77 (57)
Multivitamin multimineral, *n* (% of total)	59 (24)	22 (20)	37 (28)
Vitamin D, *n* (% of total)	24 (10)	2 (2)	22 (16)
Calcium, *n* (% of total)*	6 (2)	0 (0)	8 (6)
Calcium/Vitamin D complex, *n* (% of total)	13 (5)	3 (3)	10 (8)
Vitamin B12, *n* (% of total)	1 (<1)	0 (0)	1 (1)
Vitamin B-complex, *n* (% of total)	7 (3)	0 (0)	7 (5)
Folic acid, *n* (% of total)	6 (2)	0 (0)	6 (5)
Iron, *n* (% of total)*	1 (<1)	1 (1)	0 (0)

[a] For Dutch elderly participants in NU-AGE study (*n*=245).
[b] Values are presented as means ± SD per day, number (percentage for EN% per day and amount of people (% of total)).
* Statistical significant differences between men and women (p < 0.05). Abbreviations: BMI, body mass index; kcal, kilocalories; EN%, energy percentage.

Based on the intake of conventional foods only, 99% of participants did not meet their average requirement of vitamin D. Based on the intake of conventional foods plus fortified foods still 98% did not meet their AR. Taking into account foods plus the intake from vitamin and mineral supplements, 87% did not meet their AR, as shown in **table 4**. For selenium and vitamin B6, based on the intake conventional foods, 41% and 54% did not meet their AR. These percentages of inadequate intakes decreased to 36% and 20% when taking into account the intake from fortified foods and vitamin and mineral supplements. Other nutrients studied showed no or smaller percentages of inadequate intakes when including conventional foods, fortified foods and dietary supplements (folate 17%, copper 8%, iodine 7%, calcium 6%, zinc 5%, vitamin B12 2%, and 0% for iron and magnesium. No nutrient intakes reached levels above the UL.

Table 4. Usual nutrient intakes from conventional foods, fortified foods and dietary supplements compared to Dietary Reference Values[a, b]

Nutrient	Intake[c]	AR	% participants with intakes < AR	UL	% participants with intakes > UL
Vitamin D (ug/day)		10 ug [27]		100 ug [29]	
Conventional foods	2.49 ± 1.91		99.2 %		
Fortified foods	1.06 ± 0.95				
All foods	3.56 ± 3.09		98.4 %		0 %
Supplements	2.47 ± 6.19				
Foods+supplements	6.04 ± 6.59		86.5 %		0 %
Selenium (ug/day)		40 ug [26]		300 ug [29]	
Conventional foods	44.2 ± 12.8		41.2 %		
Fortified foods	0.22 ± 0.53				
All foods	44.5 ± 12.8		41.2 %		0 %
Supplements	9.47±31.38				
Foods+supplements	53.9 ± 33.8		35.9 %		0.4 %
Vitamin B6 (ug/day)		1.3 mg [26]		25 mg [29]	
Conventional foods	1.34 ± 0.32		53.5 %		
Fortified foods	0.29 ± 0.39				
All foods	1.63 ± 0.52		26.5 %		0 %
Supplements	1.21 ± 5.71				
Foods+supplements	2.83 ± 5.73		20.0 %		0.8 %
Folate (ug/day)		200 ug [26]		1000 ug [29]	
Conventional foods	252.7 ± 70.7		24.1 %		
Fortified foods	3.29 ± 9.32				
All foods	256.0 ± 71.5		22.9 %		0 %
Supplements	60.38±155.78				
Foods+supplements	316.7±170.5		16.7 %		1.2 %
Copper (mg/day)		0.8 mg [26]		5 mg [29]	
Conventional foods	1.17 ± 0.32		8.9 %		
Fortified foods	0.01 ± 0.03				
All foods	1.18 ± 0.32		7.8 %		0 %
Supplements	0.18 ± 0.65				
Foods+supplements	1.36 ± 0.72		7.8 %		0.8 %
Iodine (mg/day)		100 ug [26]		600 ug [29]	
Conventional foods	162.7 ± 58.5		8.6 %		0.4 %
Fortified foods	0.97 ± 3.04				
All foods	163.7 ± 58.6		7.8 %		0 %
Supplements	15.06 ± 55.96				
Foods+supplements	178.7 ± 81.2		6.5 %		0.8 %
Calcium (mg/day)		550 mg [26]		2500 mg [29]	
Conventional foods	936.4 ± 304.4		7.8 %		
Fortified foods	32.7 ± 69.9				
All foods	969.2 ± 309.9		6.1 %		0 %
Supplements	56.54±196.24				
Foods+supplements	1026.3±370.8		5.7 %		0.4 %

Nutrient	Intake[c]	AR	% participants with intakes < AR	UL	% participants with intakes > UL
Zinc (mg/day)		7.5mg men, 5.5mg women [26]		45mg [29]	
Conventional foods	9.84 ± 2.34		5.3 %		
Fortified foods	0.07 ± 0.16				
All foods	9.91 ± 2.32		4.9 %		0 %
Supplements	1.74 ± 5.96				
Foods+supplements	11.7 ± 6.2		4.5 %		0.4 %
Vitamin B12 (mg/day)		2.0 ug [26]		N/A[d]	
Conventional foods	4.75 ± 2.67		4.1 %		
Fortified foods	0.25 ± 0.32				
All foods	5.00 ± 2.68		3.3 %		0 %
Supplements	4.53 ± 19.92				
Foods+supplements	9.53 ± 20.1		2.0 %		N/A
Iron (mg/day)		7ug men, 6ug women [26]		75 mg [40]	
Conventional foods	10.7 ± 2.4		1.2 %		
Fortified foods	0.21 ± 0.89				
All foods	10.9 ± 2.5		1.2 %		0 %
Supplements	1.50 ± 5.79				
Foods+supplements	12.4 ± 6.1		0 %		0.4 %
Magnesium (mg/day)		150-500 mg [26]		250 mg [26]e	
Conventional foods	329.8 ± 76.3		0.4 %		
Fortified foods	5.40 ± 14.38				
All foods	335.2 ± 78.1		0 %		0.0 %
Supplements	39.55 ± 55.96				
Foods+supplements	375.5 ± 251.8		0 %		0.0 %

[a] For Dutch elderly participants in NU-AGE study (*n*=245)
[b] Recommended daily allowances as set by European Union [21], the labelling reference intakes values as set by the European Food Safety Authority [22-24], average requirements and tolerable upper intake levels as set by the Health Council of the Netherlands [27,40] and the European Union were used [26,29,41].
[c] Values are expressed as means ± SD
[d] For vitamin B12 no UL of safe intakes was established
[e] The UL for magnesium does not include magnesium normally present in foods and beverages
Abbreviations: AR, average requirement; UL, upper intake level

The contributions of dietary supplements, fortified foods and conventional foods to total nutrient intakes are presented in **figure 1**. Almost half of the total vitamin D, vitamin B6 and more than half of total selenium intake came from conventional foods (42%, 45%, 82%, respectively), followed by dietary supplements (41% , 44%, 18%, respectively) and fortified foods (17%, 11%, 1%, respectively).

When comparing the contribution of low, middle and high nutrient dense foods to total nutrient intakes, high nutrient dense foods contributed most to the total vitamin B6 intake (23%), followed by the middle nutrient dense group (19%) (**figure 1**). For vitamin D and selenium, the middle NRF9.3 group contributed most to total vitamin D and selenium intake (18% and 39%). When comparing the contribution of the different nutrient density groups to the nutrient intake from conventional foods only, half of this intake came from the highest NRF9.3 group for vitamin B6 (50%) while half of the intakes from vitamin D and selenium came from the middle NRF9.3 group (44% and 47%). For all nutrients, the lowest NRF9.3 group contributed least to both total nutrient intake as well as the nutrient intake from conventional foods only.

Of the food groups, fish contributed most to vitamin D intake, followed by eggs, meat (products) and poultry (**figure 2**). Fish, meat (products) and poultry, and bread contributed most to the intake of selenium. For vitamin B6, meat (products) and poultry, followed by potatoes, and (non-) alcoholic drinks contributed most to the intake.
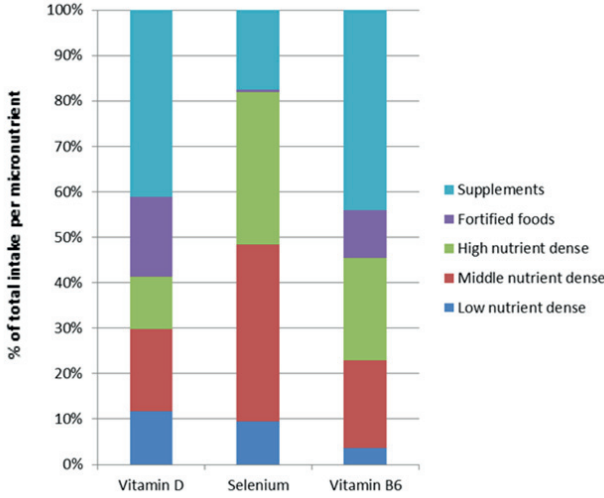
Figure 1. Contribution of dietary supplements, fortified foods and conventional foods categorized into nutrient density scores to the total nutrient intake[a, b]
[a] For Dutch elderly participants in NU-AGE study (*n*=245).
[b] Values are presented as percentage of total intake per micronutrient

Figure 2. Food groups sorted by their NRF9.3 score (high NRF9.3 on the left to low NRF9.3 on the right) and their relative contribution to nutrient intakes (vitamin D, vitamin B6 and selenium)[a, b]
[a] For Dutch elderly participants in NU-AGE study (*n*=245)
[b] Values are presented as percentage of total intake of each micronutrient

Discussion

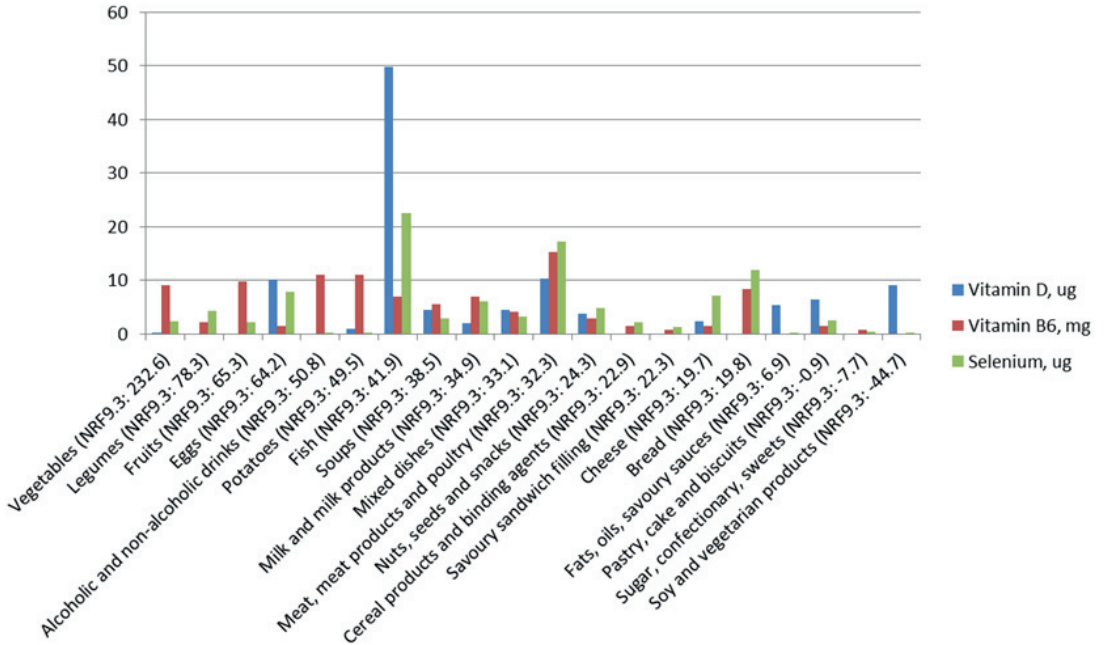In this cross-sectional analysis of 245 Dutch elderly persons based on the consumption of conventional foods, the percentage of inadequate intake was high for vitamin D and moderate for vitamin B6 and selenium. Including the consumption of fortified foods and dietary supplements substantially lowered the percentage of nutrient inadequacy for vitamin B6, but not for vitamin D and selenium without affecting risk of excessive intakes. High nutrient dense foods contributed most to total vitamin B6 intake, whereas middle nutrient dense foods contributed most to total vitamin D and selenium intake.

Our finding of a high percentage of inadequate vitamin D intake is in line with other dietary intake surveys among Dutch (the Dutch National Food Consumption Survey 2010-2012, DNFCS 2010-2012) and Irish elderly, showing percentages of inadequate vitamin D intake ranging from 81% to 100% [30,31] also when including fortified foods and dietary supplements. For selenium, the mean prevalence of inadequate intake in European elderly was above 20%, which mirrored our study (36%) [4]. The low prevalence of inadequate intakes observed for zinc, vitamin B12, and iron is in line with previous Dutch [31] and European findings [31]; showing a mean prevalence of inadequacy below 11% for these nutrients. In contrast, the proportion of elderly with inadequate vitamin B6 intakes was reported as being below 15% in the Netherlands in a previous study [31], compared to 20% in the present study. Additionally, a higher prevalence of inadequate intakes in European elderly were observed for copper (11-20%), folate, calcium, and iodine (all above 20%) [4]. As in the present study, the European survey did not find substantial percentages of excessive intakes of vitamin D, selenium and vitamin B6 [32]. The observed natural main sources of vitamin D (e.g., fish, eggs, and meat), and fortified foods are in line with other studies in Europe [33], as well as the main sources of selenium (e.g., fish, meat and cereals [31,32] and vitamin B6 (e.g., meat, potatoes) [31].

The difference in the prevalence of inadequate intakes between the present study and previous European studies could be explained by differences in study populations and methodological differences. First, the NU-AGE study aimed to recruit independently living, apparently healthy elderly, which resulted in a generally healthy (50% never smoked), and highly educated (12.2 years of education) study population. This could have resulted in better dietary intakes and lower percentages of inadequate intakes compared to other community-dwelling Dutch [31] and European populations [4]. Second, supplement intake or food fortification was not taken into account in the European study [4], possibly overestimating the true percentages of inadequate intakes compared to the present study population. Third, different dietary requirements have been used as cut-off values to define nutrient adequacy. For example, for calcium, the European study used guidelines from the Institute of Medicine (IOM) for North American populations (EAR 800 mg) [28] compared to the EAR from the European Commission (EC) for European populations used in the present study (EAR 550 mg) [26]. If we would have used the IOM guidelines, the prevalence of inadequacy for calcium would have been higher, namely 64% instead of 8% in the current study.

With respect to the relative contribution of fortified foods to total nutrient intakes, our findings are comparable to the DNFCS 2010-2012, showing the smallest contribution for selenium and a highest contribution for vitamin D and vitamin B6 (between 15-8% in DNFCS 2010-2012 [31] and 19% and 12% in the current study, respectively). In contrast, the Irish ingest three times as much vitamin D from fortified foods compared to participants in the present study [30]. This difference in vitamin D intake from fortified foods in the present study compared to the Irish study might be due to the fact that fortification with vitamin D is mandatory in Ireland, but not in the Netherlands [33].

To the best of our knowledge, this is the first study to estimate the contribution of high, medium and low nutrient dense foods to total nutrient intakes. With this approach, we aimed to identify which kind of foods substantially contribute to total nutrient intakes and which foods should be encouraged in elderly people to improve their nutritional intakes to meet nutritional requirements. Using the NRF9.3 score as developed by Drewnowski et al. [15], the current study has shown that the contribution of nutrient dense foods varied between the nutrients investigated. A first explanation could be that we have divided the continuum of NRF9.3 scores in tertiles, based on the distribution of consumed foods. As the positioning of foods categories along the spectrum is arbitrary, this could have led to a ranking of food categories rather than a continuous scale [34]. A second explanation could be related to the fact the NRF 9.3 does not take into account the actual frequency and amount consumption of a food. Although legumes do have a high NRF 9.3 score, they do not substantially contribute to the total nutrient intake as their actual intake is relatively low. At last, the NRF9.3 algorithms include nutrients that are not specifically of interest for the elderly, resulting in poorly fitting nutrient algorithms for this study population [34]. In order to increase the applicability of the NRF9.3 score to diets of the elderly, it might be worthwhile to adapt the NRF9.3 score to nutrients of concern for older populations [15], such as by including vitamin D, vitamin B12, folate and vitamin B6 and other priority micronutrients as proposed by EURRECA. In addition, more detailed nutritional information on current available fortified foods should be collected to optimize estimation of micronutrient intakes via fortified foods; also the list of fortified foods should be updated regularly as new products may be fortified in the future or products may be reformulated.

A strength of the present study includes the extensive information of vitamin intakes from both foods and vitamin and mineral supplements. The food records that were used are based on actual intake. In contrast to food frequency questionnaires (FFQs) and 24-h recalls, the use of food records has the advantage in older populations in that it minimizes reliance on memory, as food intake was recorded by participants at the time the foods were consumed [35]. After completion of the food records, they were extensively reviewed and checked for completeness by trained research dieticians. This method, in combination with information from the NEVO 2011 [13], allowed us to estimate comprehensively nutrient intakes from conventional foods as well as from fortified foods. Because of the detailed information solicited with the extensive supplement questionnaire (e.g., name, brand, frequency, number of tablets, and label information), we were able to collect the specific nutrient contents of all the predefined dietary supplements used.

A limitation of the present study is the generalizability of our study population, because the participants of the NU-AGE study had to be willing to change their dietary habits for the dietary intervention, and as such, it is very likely that particularly health-conscious people were included. Additionally, our sample mainly consisted of highly-educated, non-Hispanic Whites, former smokers, and normal/underweight, which have all been shown to be related to a higher use of dietary supplements [36]. When extrapolating our findings, it should be taken into consideration that results of our study population probably underestimate the prevalence of inadequate micronutrient intakes in the general population, and overestimate the contribution of supplements to micronutrient intake. This further highlights the importance of addressing nutrition inadequacy in the general population and of encouraging the consumption of nutrient-dense and/or fortified foods. Another limitation of the present study concerns the estimation of nutrient adequacy based on dietary intake alone. Taking into account micronutrient bioavailability, bio-accessibility and micronutrient status would better reflect the nutrient adequacy, also capturing different changes in nutrient status for fortified foods or supplement intakes. Some supplements taken separately from food, for example,

may result in a rapid increase in plasma concentrations of the supplemented nutrient, whereas consuming a food fortified with the same nutrient may have a more gradual effect on blood concentrations because of the presence of the food matrix, as has been shown for zinc [37]. For other micronutrients, such as folate, changes in serum values could be higher in food fortification than through supplementation [38].

Because the aim of food fortification is to beneficially impact population health, both the total additional intake and the quality of the nutrient or nutrients supplied must be taken into account, not just the intake of the vehicle (fortified food) alone [7]. Future studies investigating the contribution of dietary supplements, nutrient dense foods and fortified foods to total nutrient intakes should consider the bioavailability and bio-accessibility and include status markers indicating adequacy of intakes, as defined using the EURRECA guidelines [39].

To conclude, the intake of vitamin D was inadequate in the vast majority of this Dutch elderly population, and nutrient intakes of vitamin B6 and selenium are of concern. The inadequacy of vitamin D could be confirmed via biomarkers of vitamin D status. Conventional foods were the main source of vitamin D, B6 and selenium intake, followed by vitamin and mineral supplements and fortified foods. Although fortified foods and vitamin and mineral supplements importantly contributed to the total intake of vitamin D and B6 intake, the use of these components did not necessarily lead to adequate intakes of nutrients that are of concern for older populations. Foods with the highest nutrient density contributed the most to total vitamin B6 intake only. In order to optimize nutrient intakes of elderly, combinations of conventional foods, fortified foods and dietary supplements need to be considered.

Acknowledgments

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Chapter 3

Dietary patterns, cognitive decline and dementia: a systematic review

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Abstract

Nutrition is an important modifiable risk factor that plays a role in the strategy to prevent or delay onset of dementia. Research on nutritional effects has till now mainly focused on the role of individual nutrients and bioactive components. However, the evidence for combined effects, such as multi-nutrient approaches or a healthy dietary pattern such as the Mediterranean diet is growing. These approaches incorporate the complexity of the diet and possible interaction and synergy between nutrients. Over the last few years dietary patterns are increasingly investigated to better understand the link between diet, cognitive decline, and dementia. In this systematic review we provide an overview of the literature on human studies up to May 2014 that examined the role of dietary patterns (both a priori as well as a posteriori derived) in relation to cognitive decline or dementia. The results suggest that better adherence to a Mediterranean diet is associated with less cognitive decline, dementia or AD, as shown by four out of six cross-sectional studies, six out of 12 longitudinal studies, one trial and three meta-analyses. Other Healthy dietary patterns, both a priori (e.g. HDI, HEI and PNNS-GS) and a posteriori derived (e.g. factor analysis, cluster analysis and RRR), have shown to be associated with reduced cognitive decline and/or a reduced risk of dementia as shown by all six cross-sectional studies and six out of eight longitudinal studies. To come to more targeted and detailed guidelines to prevent or postpone cognitive decline more conclusive evidence is needed.

Introduction

It has been estimated that worldwide 44 million people lived with dementia in 2013. With the ageing of the population and with an estimated 7.7 million new cases per year this number doubles every 20 years and will reach 135 million dementia patients by 2050 [1]. The impact of dementia worldwide and the public health importance has been described by the World Health Organization (WHO) and Alzheimer's Disease International (ADI) [2]. They propose to make dementia a global health priority, which underlines the importance to find strategies to prevent dementia. Since there is currently still no effective treatment to modify the course of dementia, prevention is an urgent priority, both to reduce incidence and to slow down progression. Important risk factors need to be further identified and in particular the factors that can be modified, such as lifestyle factors. In this systematic review we focus on the risk factor nutrition, for which promising indications exist that it can contribute in reducing the risk of developing dementia. Over the last years the attention is shifting from the role of single nutrients or foods to the role of dietary patterns, such as the promising association of the Mediterranean diet with cognitive decline and dementia. A dietary pattern approach better reflects the complexity of the diet and our daily eating behavior [3-6]. Multiple reviews and meta-analyses have been written in the last years summarizing the evidence of a substantial number of studies investigating the influence of a Mediterranean diet on cognitive decline and dementia [7-13]. Besides the Mediterranean diet, there are several other knowledge based (*a priori*) dietary patterns, such as the Healthy Diet Index (HDI), Healthy Eating Index-2005 (HEI-2005) and empirically (*a posteriori*) derived dietary patterns (e.g. using factor analysis or principal component analysis) that could be associated to cognitive decline and dementia. So far, there has only been one review summarizing the literature on different dietary patterns and cognitive aging until 2011 [14]. Therefore, the reviews describing studies on the Mediterranean diet and cognitive decline can be updated with several new studies that have been published since that review.

The aim of the present systematic review is to summarize and evaluate available evidence from studies investigating dietary patterns, both *a priori* and *a posteriori*, in relation to cognitive decline and dementia in older adults and elderly people. Although underlying biological mechanisms will be touched upon briefly, this review is not intended to provide an extended description of mechanisms underlying the association between dietary patterns and cognitive performance. Instead, our specific goals are 1) to summarize papers that have studied the Mediterranean diet 2) to summarize papers that have studied other dietary patterns 3) to critically evaluate and summarize all evidence emerging from these studies on associations between dietary patterns and cognitive performance and/or dementia.

Methods

Search strategy

The role of a healthy diet (Mediterranean diet, dietary patterns) in the development of cognitive decline and dementia has been the subject of several recent systematic reviews which were the starting point of the current review. Medline databases and the Cochrane database were searched up to May 2014 for additional, recently published studies. The search strategies used text words and relevant indexing (MeSH terms) to capture studies investigating the association between healthy diet (Mediterranean diet, dietary patterns) with cognitive decline and dementia. When no systematic

reviews were found, narrative reviews were used and both checked and updated using a combination of MeSH and text-based terms.

(*"Diet, Mediterranean"[Mesh] OR "Mediterranean diet" OR ("Mediterranean" AND "Diet")) OR "Dietary pattern" AND (("memory"[MeSH Terms] OR "memory"[All Fields]) OR ("cognition"[MeSH Terms] OR "cognition"[All Fields]) OR cognitive[All Fields] (("alzheimer disease"[MeSH Terms] OR "alzheimer"[All Fields] AND "disease"[All Fields]) OR ("alzheimer disease"[All Fields] OR "alzheimer"[All Fields]) OR ("dementia"[MeSH Terms] OR "dementia"[All Fields])) AND "humans"[MeSH Terms])*

We only included studies performed in older adults and elderly people and of which full papers were published.

Study selection process

Our search strategy resulted in 65 hits on studies, reviews and meta-analyses on diet and cognition and an additional search via reference lists of reviews and meta-analysis resulted in 33 more studies. This resulted in a total of 98 studies, of which 36 studies were selected based on full abstracts and papers. In total, we found 26 studies on the Mediterranean diet and cognitive function or dementia and 15 on other dietary patterns in relation to cognitive function (**figure 1**). Five studies performed analyses on both the Mediterranean diet and other patterns [15-19]. Study selection, data extraction, and quality assessment were performed independently by two reviewers.
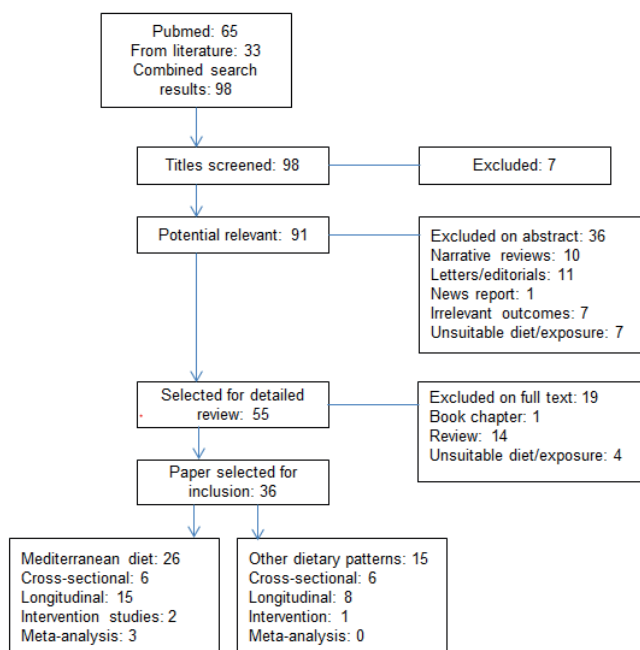
Figure 1. Flowchart of selection process resulting in 36 studies included in the review, of which five studies performed analyses on both the Mediterranean diet and other dietary patterns

Results

Specification and potential underlying mechanisms

In **table 1** characteristics of frequently reported *a priori [20-25]* and *a posteriori* [3, 7, 26-28] dietary patterns are shown. The majority of *a priori* dietary patterns consist of adequacy components - such as fruits, vegetables, cereals, fatty fish and dairy - and limitation components - such as total fat, saturated fatty acids, cholesterol and sodium. In an *a posteriori* data driven approach data are reduced into dietary patterns either based upon differences in intakes between subjects or upon inter-correlations between dietary items [29]. Usually this results in dietary patterns consisting of combinations of high or low loadings of similar components as defined by the *a priori* methods. It has been proposed that these components in combination could impact on several biological mechanisms that may explain how certain healthy dietary patterns can exert their effects on cognitive health and decline. Prevailing mechanisms which are believed to play a role in the pathogenesis of age-related diseases, including cognitive impairment and AD, are oxidative stress, inflammation and vascular risk factors, mechanisms that are ideal targets for nutritional intervention with dietary patterns, such as the Mediterranean diet, that are abundant in antioxidants and monounsaturated fatty acids and have a balanced ratio of essential n-6:n-3 fatty acids. Based on the multiple plausible biological mechanisms, there is a strong theoretical basis that the intake and status of these nutrients may affect the known mechanisms for neurodegeneration.

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Table 1. Characterization of most investigated *a priori* and *a posteriori* defined dietary patterns in relation to neurodegeneration

Dietary patterns	Characterization
A priori (hypothesis driven approach)	
Mediterranean diet (MeDi) [25]	Based on traditional eating habits in Crete, South Italy and other Mediterranean countries
	• High in fruits, vegetables, cereals and legumes
	• Low in saturated fats, olive oil main fat source
	• Moderate in fish
	• Low to moderate in dairy products
	• Low in red meat and meat products
	• Moderate in alcohol (wine)
Healthy Diet Indicator (HDI) [21]	Based on the World Health Organization recommendations for the prevention of chronic diseases
	• Saturated fatty acids ≤10en%
	• Polyunsaturated fatty acids 3-7en%
	• Protein 10-15en%
	• Complex carbohydrates 50-70en%
	• Dietary fiber 27-40 g/day
	• Fruits and vegetables >400 g/day
	• Pulses, nuts and seeds >30 g/day
	• Oligo-/mono- and disaccharides ≤10en%
	• Cholesterol ≤300 mg/day
Healthy Eating Index (HEI) [23]	Based on the food patterns found in MyPyramid and is a sum of 10 individual components:
	• Adequacy of total whole fruits, vegetables, dark green and orange vegetables and legumes, total grains, whole grains, milk, meat and beans, oil
	• Low intakes of saturated fat, sodium and calories from solid fats, alcoholic beverages and added sugars
Recommended Food score (RFS) [22]	Based on the Dietary Guidelines for Americans and calculated as the sum of 23 items that are consumed at least once a week.
	1) Apples or pears, 2) Oranges, 3) Cantaloupe, 4) Orange or grapefruit juice, 5) Grapefruit, 6) Other fruit juices, 7) Dried beans, 8) Tomatoes, 9) Broccoli, 10), Spinach, 11), Mustard, turnip, or collard greens, 12) Carrots or mixed vegetables with carrots, 13) Green salad, 14) Sweet potatoes, yams, 15) Other potatoes, 16) Baked or stewed chicken or turkey, 17) Baked or broiled fish, 18) Dark breads like whole wheat, rye or pumpernickel, 19) Cornbread, tortillas, and grits, 20) High fibre cereals, such as bran, granola, or shredded wheat, 21) Cooked cereals, 22) 2% Milk and beverages with 2% milk, 23) 1% or skim milk
Dietary Approaches to Stop Hypertension (DASH) Trial [24]	Based on in intakes of nutrients hypothesized to alter blood pressure levels
	• Rich in fruits and vegetables
	• Rich in low-fat dairy food
	• Reduced amounts of saturated fat, total fat, and cholesterol
French National Nutrition and Health Program (Program National Nutrition Santé) Guideline Score (PNNS-GS) [20]	Based on French National Nutrition and Health Program recommendations to improve the health status of the general population
	• Fruits and vegetables at least 5/day
	• Bread, cereals, potatoes, and legumes, at each meal
	• Choose whole-grain food and whole-grain bread more often
	• Milk and dairy products 3/d
	• Meat and poultry, seafood, eggs 1-2/d
	• Seafood at least twice per week
	• Limit consumption of added fats
	• Favor fats of vegetable origin
	• Drink water as desired
	• Limit sweetened beverages
	• Limit salt consumption
	• At least 30 min of brisk walking or equivalent/day of physical activity
A posteriori (exploratory approach)	
Cluster analysis [3, 7, 27]	Classification technique, which aggregates subjects with similar defined variables such as dietary pattern and the energy contribution of each food group
Principal Component Analysis (PCA) [7, 15]	Common approach of factor analysis do define dietary patterns, aggregates highly correlated food items to identify underlying dietary patterns
Reduced Rank Regression [7, 26, 28]	Mix of an exploratory and hypothesis-driven approach, involves the element of a priori approach to derive dietary patterns

Abbreviations: en%, percent of energy intake

Mediterranean diet

The role of the Mediterranean diet on cognitive decline and dementia risk has only recently been systematically reviewed by Lourida et al in 2013 [8]. This review included literature published until January 2012. In addition, Alzheimer's Disease International published a report on the available evidence on this subject in the beginning of 2014 [30]. However, because this area of research is developing rapidly the results of these two reviews can already be updated by adding at least 10 new studies on the Mediterranean diet and cognition and dementia. We have found a total of six cross-sectional studies, 15 prospective studies and one intervention trial. The characteristics of these studies are summarized in **table 2**.

Observational evidence

Four out of the six cross-sectional studies showed an inverse association of the Mediterranean diet with cognitive functioning [16, 19, 31] or Alzheimer's Disease (AD) [31, 32] in American, Puerto Rican, and Australian older adults and elderly people. One cross-sectional study in Greek elderly observed a protective association with one unit increase in Mediterranean diet in men (OR 0.88; 95%CI 0.80-0.98), but contrarily a suggestion for an increased risk of cognitive impairment in women (OR 1.11; 95%CI 1.00-1.22) [33]. A study in Hong Kong did not find associations in either men or women (OR$_{T3vsT1}$ 0.89; 95%CI 0.56-1.41 and 1.02; 95%CI 0.75-1.41) [15].

Of the 12 prospective studies on adherence to the Mediterranean diet and better cognitive performance, six studies observed a beneficial association after 3 to 7.6 years of follow-up [17, 18, 34-37]. Of these studies five were performed in the USA [17, 18, 35-37] and one in France [34]. All five studies performed in the USA showed marginally, but significant associations. The French study showed that a one point increase in the Mediterranean diet was associated with fewer MMSE errors, but only marginally significant across categories of the Mediterranean diet (β$_{T3vsT1}$ -0.02, p-value 0.06). In the other six studies, of which three were performed in the USA [38-40] and the others in France [41], Australia [42] and Greece [43], the association was beneficial, but not significant after 2-3 years of follow-up [38-43].

With respect to dementia and AD four out of six studies showed a reduced risk of AD with better adherence to the Mediterranean diet after 3-5.4 years of follow-up in US populations (HR 0.91; 95%CI 0.83-0.98 [44], HR 0.87; 95%CI 0.78-0.97 [45], HR 0.60; 95%CI 0.42-0.97) [46], and a lower risk of developing AD after MCI (HR 0.71; 95%CI 0.53-0.95) [36]. Two studies, performed in a France [34] and US population [38], did not find associations.

Trial evidence

The only trial up until now investigating the effect of a Mediterranean type diet either rich in olive oil or rich in nuts has been performed in 522 participants with a high cardiovascular risk profile. After 6.5 years of intervention, participants in both types of the Mediterranean diet had higher cognitive performance compared to the control group (adjusted differences for MSSE +0.62; 95%CI +0.18 to +1.05, p-value 0.005 and +0.57 (95%CI +0.11 to +1.03), p-value 0.015) [47]. In a smaller subgroup (n=285) of this same trial only the Mediterranean diet with olive oil showed better results for five out of 16 specific cognitive tests and mild cognitive impairment (OR 0.34; 95%CI 0.12-0.97) [48].

Table 2. Characteristics of included studies on Mediterranean diet, cognitive decline and dementia

Author, year, country, study name	Population (sample size, mean age)	Follow-up	Exposure/ Intervention measure	Outcome measure	Effect measure
Cross-sectional studies					
Samieri et al., 2013 [16] USA, NHS	n=10,670 59y	-	FFQ, A-MeDi score	Cognitive decline (TICS), mental health (SF-36)	No significant association between the A-MeDi and mental health or cognitive impairment (OR$_{Q5vsQ1}$ 1.12; 95%CI 1.01-1.20, p-trend <0.001 and OR 0.97; 95%CI 0.95-1.00, p-trend 0.020, resp.)
Chan et al., 2013 [15] Hong Kong	n=3,670 71.8y	-	FFQ, MeDi score	Cognitive function (CSI-D)	No significant association between the MeDi and cognitive function in either men or women (OR$_{T3vsT1}$ 0.89; 95%CI 0.56-1.41, p-trend 0.882 and OR 1.02; 95%CI 0.75-1.41, p-trend 0.952, resp.).
Ye et al., 2013 [19] Puerto Rico, BPRHS	n=1,269 57.3y	-	FFQ, MeDi score	Cognitive function and cognitive impairment (MMSE)	A significant association between a higher MeDi score higher MMSE scores (p-trend 0.012) and a lower risk of cognitive impairment (OR 0.80; 95%CI 0.80-0.94, p-value <0.001)
Katsiardanis et al., 2013 [33] Greece, Velestino Study	n=557 >65y		157-item FFQ, MeDi score	Cognitive impairment (MMSE)	Significant lower risk of cognitive impairment in men per one unit increase in adherence to the MeDi (OR 0.88; 95%CI 0.80-0.98, p-value 0.02) but a higher risk in women (OR 1.11; 95%CI 1.00-1.22, p-value 0.04)
Gardener et al., 2012 [31] Australia, AIBL study	n=970 >60y	-	74-item CCVFFQ, MeDi score	MCI (MMSE), AD (DSM-IV, NINCDS-ADRDA)	Each unit increase in MeDi score was significantly associated with a reduced risk of MCI or AD (OR 0.87; 95%CI 0.75-1.00, p-value <0.05 and OR 0.81; 95%CI 0.71-0.92, p-value <0.01, respectively).
Scarmeas et al., 2006 [32] USA, WHICAP	n=1,984 76.3y	nested case control	61-item FFQ, MeDi score	Prevalent Alzheimer's disease (NINCDS-ADRDA)	Better adherence to the MeDi was significantly associated with lower risk of AD (OR 0.76; 95%CI 0.67-0.87, p-value <0.01, OR$_{T3vsT1}$ 0.32; 95%CI 0.17-0.59, p-trend <0.001)
Longitudinal studies					
Wengreen et al., 2013 [18], USA, CCMS	n=3,831 74.1y	11y	142-item FFQ, MeDi score	Cognitive impairment (3MS)	Better adherence to MeDiet was significantly associated with higher 3MS scores (MeDiet$_{Q5vsQ1}$ 0.94±0.29, p-trend 0.0022)
Tsivgoulis et al., 2013 [37], USA, REGARDS study	n=17,478 64.6y	4y	FFQ, MeDi score	Incident cognitive impairment (SIS)	Higher adherence to MeDiet was significantly associated with a lower likelihood of ICI (OR 0.87; 95%CI 0.76-1.00), especially in non-diabetic participants (OR 0.81; 95%CI 0.70-0.94, p-value 0.0066), but not in diabetic participants (OR 1.27; 95%CI 0.95-1.71, p-value 0.1063)
Samieri et al., 2013 [35] USA, NHS	n=16,058 74.3y	6y	116-item FFQ, A-MeDi score	Cognitive status and cognitive decline (TICS), verbal memory, global cognition	Highest adherence to MeDi was significantly associated with cognitive status at older ages (adjusted mean differences in z-scores Q5vsQ1 were 0.06; 95%CI 0.01-0.11, p-trend 0.004 for TICS, 0.05; 95%CI 0.01-0.08, p-trend 0.002 for Global score and 0.06; 95%CI 0,03-0.10, p-trend <0.001 for Verbal memory score), but not with cognitive decline (0.004; 95%CI -0.011-0.019, p-trend 0.31), -0.001; 95%CI -0.010-0.007, p-trend 0.84, -0.001; 95%CI -0.011-0.010, p-trend 0.70)

Samieri et al., 2013 [39] USA, Women's Health Study	n=6,174 72y	2y	131-item FFQ, A-MeDi score	Cognitive decline (TICS), global cognition, verbal memory	No significant associations between higher A-MeDi scores and mean differences in averaged measures of global cognition and verbal memory (Q5vsQ1 0.02; 95%CI -0.03-0.06, p-trend 0.63 and 0.03; 95%CI -0.02-0.07, p-trend 0.44, respectively), neither over time (p for quintiles medians-x-time interaction = 0.26 for global score and 0.40 for score and cognitive decline)
Kesse-Guyot et al., 2013 [41] France, SU.VI.MAX	n=3,083 65.4y	13y	Repeated 24h records, MeDi score and MSDPS	Cognitive function; episodic and lexical-semantic memory, mental flexibility	No significant association between higher adherence to MeDi and MSDPS and cognitive scores, except for a lower phonemic fluency (-1.00; 95%CI -1.85,-0.15, p-value 0.048) with decreasing MSDPS and lower backward digit with decreasing MDS (-0.64; 95%CI -1.60-0.32, p-value 0.03)
Vercambre et al., 2012 [40] USA, WACS	n=2,504 72.3y	5.4y	116-item FFQ, MeDi score	Cognitive decline (TICS), verbal memory, category fluency score	No significant association between higher adherence to MeDi and adjusted mean differences in annual rates of cognitive decline (T3vsT1 0.00; 95%CI -0.02-0.01, p-value 0.88 for global cognition, -0.03; 95%CI -0.11-0.05, p-value 0.53 for TICS, 0.00; 95%CI -0.02-0.02, p-value 0.97 for verbal memory and -0.03; 95%CI -0.14-0.08, p-value 0.64 for category fluency)
Cherbuin et al., 2012 [42] Australia, PATH	n=1,528 62.5y	4y	215-item FFQ, MeDi score	Mild cognitive impairment, cognitive decline, any MCD (ICC, CDR)	No significant association between the MeDiet and transition from normal aging to MCI, CDR 0.5 and any-MCD (OR 1.41; 95%CI (0.95-2.10), OR 1.18; 95%CI 0.88-1.57 and OR 1.20; 95%CI 0.98-1.47, respectively)
Tangney et al., 2011 [17] USA, CHAP	n=3,790 75.4y	7.6y	139-item FFQ, MeDi score	Global cognitive function (MMSE, EBMT, SDMT)	Higher MeDi scores were significantly associated with better global cognitive scores at baseline (β 0.0070; SEE 0.0022, p-value 0.0013) and with slower rates of decline over time (β 0.0014; SEE 0.0004, p-value 0.0004)
Roberts et al., 2010 [38] USA, MCSA	n=1,233 79.6y	2.2y	128-item FFQ, MeDi score	Mild cognitive impairment (CDR)	A high MeDi score was not statistically associated with risk of incident MCI or dementia (HR_{T3vsT1} 0.75; 95%CI 0.46-1.21, p-value 0.24)
Gu et al., 2010 [45] USA, WHICAP	n=1,219 76.7y	3.8y	61-item SFFQ, MeDi score	Alzheimer's disease (NINCDS-ADRDA)	Better adherence to MeDi was borderline significantly associated with lower risk for AD in fully adjusted model (HR 0.87; 95%CI 0.78-0.97, p-value 0.01 and HR_{T3vsT1} 0.68; 95%CI 0.42-1.08, p-trend 0.06)
Scarmeas et al., 2009 [36] USA, WHICAP	n=1,393 76.9y	4.5y	FFQ, MeDi score	Mild cognitive impairment (DSM-III-R), Alzheimer's disease (NINCDS-ADRDA)	Better adherence to MeDi was significantly associated with a lower risk of MCI (HR 0.85; 95%CI 0.72-1.00, p-trend 0.05, HR_{T3vsT1} 0.72; 95%CI 0.52-1.00, p-value 0.05) and a lower risk of developing AD after MCI (HR 0.71; 95%CI 0.53-0.95, p-trend 0.02, HR_{T3vsT1} 0.52; 95%CI 0.30-0.91, p-value 0.02)
Scarmeas et al., 2009 [46] USA, WHICAP	n=1,880 77.2y	5.4y	61-item FFQ, MeDi score	Alzheimer's disease (NINCDS-ADRDA)	Better MeDi adherence was significantly associated with lower AD risk (HR_{T3vsT1} 0.60; 95%CI 0.42-0.97, p-trend 0.008)

Feart et al., 2009 [34] France, 3C study	n=1,410 75.9y	5y	FFQ and 24-HR, MeDi score	Cognitive performance, dementia risk and Alzheimer's disease risk (MMSE, DSM-III-R)	A one point increase in the MeDi was significantly associated with fewer MMSE errors (β -0.006, p-value 0.04), and borderline significant across categories of MeDi (β$_{T3vsT1}$ -0.02, p-value 0.06). There was no significant association with dementia risk and AD risk (HR 1.06; 95%CI 0.92-1.21, p-value 0.43, HR$_{T3vsT1}$ 1.12; 95%CI 0.60-2.10, p-value 0.72 and HR 1.00; 95%CI 0.85-1.19, p-value 0.96, HR$_{T3vsT1}$ 0.86; 95%CI 0.39-1.88, p-value 0.71, respectively)
Psaltopoulou et al., 2008 [43] Greece, EPIC	n=732 >60y	8y	150-food FFQ, MeDi score	Cognitive decline, MMSE	No significant association per one unit increase in MeDi and MSSE (β 0.05; 95%CI -0.09 – 0.19, p-value 0.485)
Scarmeas et al., 2006 [44] USA, WHICAP	n=2,258 77.2y	4y	61-item SFFQ, MeDi score	Alzheimer's disease (NINCDS-ADRDA)	Better adherence to MeDi was associated with lower risk for AD (HR 0.91; 95%CI 0.83-0.98, p-value 0.015, HR$_{T3vsT1}$ 0.60; 95% CI 0.42-0.87, p-trend 0.007)
Randomized controlled trials					
Martinez et al. 2013 [47], [48] Spain, PREDIMED-Navarra	n=522 [47] 74.6y	6.5y	137-item FFQ. MedDiet intervention 3 arms: · MeDi+olive oil · MeDi + nuts · Low-fat diet	Cognitive performance (MMSE, CDT)	Participants in the MeDi + olive oil and the MeDi + nuts group showed better cognitive performance compared to the control group for MMSE and CDT (adjusted differences: 0.62; 95%CI 0.18-1.05, p-value 0.005 and 0.57; 95%CI 0.11-1.03, p-value 0.015 for MMSE, 0.51; 95%CI 0.20-0.82, p-value 0.001 and 0.33; 95%CI 0.003-0.67, p-value 0.048 for CDT)
Martinez et al. 2013 [47], [48] Spain, PREDIMED-Navarra	n=268 [48] 74.1yr	6.5y	137-item FFQ. MedDiet intervention 3 arms: · MeDi+olive oil · MeDi + nuts Low-fat diet	Cognitive performance, MCI	MeDi with olive oil was related to better cognitive performance (for 5/16 tests) and lower MCI (OR 0.34; 95%CI 0.12-0.97), compared to control group MeDi with nuts was not related to better cognitive performance or MCI (OR 0.56; 95%CI 0.22-1.43)

Abbreviations: 3C, Three-City; 3MS, Modified Mini-Mental State Examination; 24-HR, 24-hour dietary recall; AD, Alzheimer's Disease; AIBL, Australian Imaging, Biomarkers and Lifestyle Study of Ageing cohort; A-MeDi, alternate MeDi; BPRHS, Boston Puerto Rican Health Study; CCMS, Cache County Memory Study; CDR, Clinical Dementia Rating; CDT, Clock Drawing Test; CHAP, Chicago Health and Aging Project; CSI-D, Community Screening Instrument for Dementia; DSM, Diagnostic and Statistical Manual of Mental Disorders; EBMT, East Boston Memory Test; EPIC, European Prospective Investigation into Cancer and Nutrition; FFQ, Food Frequency Questionnaire; ICC, International Consensus Criteria; MCD, Mild Cognitive Disorder; MCI, Mild Cognitive Impairment; MCSA, Mayo Clinic Study of Aging: MeDi, Mediterranean Diet; MMSE, Mini Mental State Examination; MSDPS, Mediterranean Style Dietary Pattern Score; NHS, Nurses' Health Study; NINCDS-ADRDA, National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer Disease and Related Disorders Association; PATH, Personality and Total Health Through Life Project; PREDIMED, PREvencion con DIeta MEDiterranea; REGARDS, Reasons for Geographic and Racial Differences in Stroke; SDMT, Symbol Digit Modalities Test; SEE, Standard Error Estimate; SF-36, Medical Outcomes Short-Form 36 Health Survey; SIS, Six-item Screener; SU.VI.MAX, Supplementation en VItamines et Mineraux Anti-oXydants; TICS, Telephone Interview for Cognitive Status; WACS, Women's Antioxidant Cardiovascular Study; WHICAP, Washington Heights-Inwood Columbia Aging Project

Meta-analyses

An updated systematic review and meta-analysis from 2010 pooled data of prospective cohort studies on adherence to the Mediterranean diet and risk of AD, Parkinson's disease, cognitive decline, dementia and mild cognitive impairment and showed an inverse association between a 2-point increase of adherence to the Mediterranean diet and neurodegenerative diseases (RR 0.87; 95%CI 0.81-0.94, I^2 0%, p-value 0.73 [12]; **table 3**).

Another meta-analysis [9] pooled data of 2 case-control studies, 5 longitudinal studies and 5 cross-sectional studies on moderate and high adherence to the Mediterranean diet and risk of cognitive impairment, which resulted in inverse associations (RR 0.79; 95%CI 0.67-0.94, I^2 28.3% and RR 0.60; 95%CI 0.43-0.83, I^2 76.4%, p-value0.000).

A more recent systematic review and meta-analysis by Singh et al. [11] analysed data of prospective cohort studies with at least 1 year follow-up on the Mediterranean diet and cognitive outcomes (MCI or AD). Based on two longitudinal studies performed in the US, there was an association with high adherence to the Mediterranean diet (HR_{T3vsT1} 0.73; 95%CI 0.56-0.96, I^2 0%), but there was no association with moderate adherence compared to poor adherence (HR_{T2vsT1} 0.82 (95%CI 0.64-1.05, I^2 0%), nor per one unit increase in the Mediterranean diet score (HR 0.95; 95%CI 0.84-1.08, I^2 33%). Pooled analyses of two other longitudinal studies showed an inverse association between adherence to the Mediterranean diet (both continuous and categorical) and risk of AD among cognitively normal individuals (HR 0.92 (95%CI 0.85-0.99), I^2 0% and HR_{T3vsT1} 0.64 (95%CI 0.46-0.89), I^2 0%).

When combining all data this resulted in an inverse association between a better adherence to the Mediterranean diet and cognitive impairment (HR 0.92 (95%CI 0.88-0.97), I^2 0%, HR_{T2vsT1} 0.80 (95%CI 0.67-0.95), I^2 0%, HR_{T3vsT1} 0.67 (95%CI 0.55-0.81), I^2 0%).

Table 3. Characteristics of included reviews and meta-analyses on Mediterranean diet, cognitive decline and dementia

Reference	Studies included	Update until	Exposure	Outcomes measure	Effect
Singh et al. 2014 [11]	Five prospective cohort studies with at least 1y follow-up	Nov. 2012	MeDi score	From normal to mild cognitive impairment, from normal to Alzheimer's disease, cognitive impairment	Better adherence to MeDi was associated with a lower risk of MCI (HR_{T3vsT1} 0.73; 95%CI 0.56-0.96, I^2 0%, HR 0.98; 95%CI 0.84-1.08, I^2 33%), AD (HR 0.92; 95%CI 0.85-0.99, HR_{T2vsT1} 0.87; 95%CI 0.66-1.14, HR_{T3vsT1} 0.64; 95%CI 0.46-0.89) and cognitive impairment (HR 0.92; 95%CI 0.88-0.97, HR_{T2vsT1} 0.80; 95%CI 0.67-0.95, HR_{T3vsT1} 0.67; 95%CI 0.55-0.81, I^2 0%)
Psaltopoulou et al. 2013 [9]	9 studies; case-control, longitudinal, cross-sectional	Oct 31st 2012	MeDi score	Cognitive impairment, depression	Both a moderate and a high adherence to MeDi were associated with a reduced risk of cognitive impairment and depression (RR 0.79; 95%CI 0.67-0.94, I^2 28.3%, p-value 0.19 vs. RR 0.60; 95%CI 0.43-0.83, I^2 76.4%, p-value 0.00 and RR 0.77; 95%CI 0.62-0.95, I^2 54.4% vs. RR 0.68; 95%CI 0.54-0.86, I^2 53.4%, respectively).
Sofi et al. 2010 [12]	Five prospective cohort studies	June 2010	MeDi score	Neurodegenerative diseases (cognitive decline, risk of dementia, MCI, AD Parkinson's disease)	Per 2-point increase of adherence to the MeDi score the risk of incidence of neurodegenerative diseases decreased (RR 0.87; 95%CI 0.81-0.94).

3

Other dietary patterns

Fewer studies have been performed on other dietary patterns than the Mediterranean diet. So far, there has only been one review summarizing the literature of studies on different dietary patterns and cognitive aging until 2011 and this review included a total of 13 studies [14]. We have found a total of 16 studies of which eight cross-sectional studies, seven prospective studies and one trial investigating *a priori* derived dietary patterns and *a posteriori* derived dietary patterns in relation to cognitive decline (**table 4**).

Observational evidence

Of the six cross-sectional studies, four used *a priori* knowledge to study dietary patterns. Better adherence to the Healthy Diet Indicator was associated with a lower prevalence of cognitive deficit (OR 0.85; 95%CI 0.77-0.93[50]) and a reduced risk of cognitive impairment (OR 0.75; 95%CI 0.58-0.97 in an Italian cohort, but not in a Dutch cohort (OR 0.81; 95%CI 0.63-1.04[51]). Higher adherence to the Healthy Eating Index of 2005 (HEI-2005) was associated with a lower risk of cognitive impairment in a Puerto Rican population (OR 0.86; 95%CI 0.74-0.99[19]), but this was not found for higher adherence to the alternative HEI of 2010 in a US population (OR_{Q5vsQ1} 0.99; 95%CI 0.97-1.01 [16]). Two studies used an empirical approach such as Principal Component Analysis [15] or cluster analysis [49]. One study observed less errors on the MMSE with better adherence to a healthy dietary pattern in both men and women (respectively, β -0.11; 95%CI -0.22 to -0.0004 and β -0.13; 95%CI -0.22 to -0.04 [49]). The other study found a beneficial association only in Chinese women with a higher 'Vegetables-Fruits' and 'Snacks-Drinks-Milk products' pattern score and cognitive function, but not in men [15].

Of the eight prospective studies, five studies used *a priori* defined dietary scores [17, 18, 52, 55, 56] and three studies used data driven approaches [26, 53, 54]. Results from studies using *a priori* diet scores showed mixed results with higher cognitive function scores for the Dietary Approaches to Stop Hypertension (DASH) diet (3MS score $DASH_{Q5vsQ1}$ 0.97±0.29) [18] and less cognitive declines for the Recommended Food Score (RFS) after 11 years of follow-up (3MS score RFS_{Q4vsQ1} 1.79) [56], while there was no association between the HEI-2005 (β 0.00002, p-value 0.214) [17] and the C-HEI (β 0.00008, p-value 0.852) [55] and cognitive decline after respectively 7.6 and 3 years of follow-up. A better adherence to the French guidelines (PNNS-GS) was associated with better cognitive function, as measured with a great number of specific cognitive function tests [52]. The other three of the seven studies used data driven approaches showing consistent beneficial associations between dietary patterns and risk of dementia [53], cognitive function [54] and AD [26, 53]. Two studies used Reduced Rank Regression of which one study observed a typical 'Japanese' pattern and the other study reported a 'Healthy' pattern. After 15 years of follow-up the 'Japanese' pattern was association with a reduced risk of dementia (HR 0.66; 95%CI 0.46-0.95), AD (HR 0.65; 95%CI 0.40-1.06) and vascular dementia (HR 0.45; 95%CI 0.22-0.91[53]). The 'Healthy' pattern was strongly associated with a lower AD risk (HR_{T3vsT1} 0.62; 95%CI 0.43-0.89) after 3.9 years of follow-up [26]. The other study used factor analysis and reported higher cognitive function scores in the 'Healthy' pattern compared to a 'Traditional' pattern (50.1±0.7 vs 48.9±0.7, p-trend 0.001) after 13 years of follow-up [54].

Trial evidence

The single trial that has been performed observed better scores on one out of nine cognitive function tests (psychomotor speed; Cohen's D = 0.440, p-value 0.036) after 4-months intervention with the DASH diet compared to the usual diet control group in 124 overweight adults with high blood pressure [57].

Table 4. Characteristics of included studies on dietary patterns other than Mediterranean diet, cognitive decline and dementia

Author, year, country, study name	Population (sample size, mean age)	Follow-up	Exposure/ Intervention measure	Outcome measure	Effect measure
Cross-sectional studies					
Ye et al., 2013 y [19] Puerto Rico, BPRHS	n=1,269 57.3y	-	FFQ, HEI-2005	Cognitive function and cognitive impairment (MMSE)	A higher HEI-2005 score was significant associated with a higher MMSE score (p-trend 0.011) and lower risk of cognitive impairment (OR$_{10points}$ 0.86; 95%CI 0.74-0.99, p-value 0.033)
Chan et al., 2013 [15] Hong Kong	n=1,926 71.8y	-	FFQ, Factor analysis, 3 patterns; 'Vegetables-Fruits', 'Snacks-Drinks-Milk products', 'Meat-Fish'	Cognitive function (CSI-D)	A higher 'Vegetables-Fruits' and 'Snacks-Drinks-Milk products' pattern score was significantly associated with a reduced risk of cognitive impairment in women (OR$_{Q4vsQ1}$ 0.73; 95%CI 0.54-1.00, p-trend 0.018 and OR$_{Q4vsQ1}$ 0.65; 95%CI 0.47-0.90, p-trend 0.003, respectively), but not in men.
Samieri et al., 2013 [16] USA, NHS	n=10,670 59y	-	FFQ, AHEI-2010	Cognitive decline, (TICS), mental health (SF-36)	The AHEI-2010 was significantly associated with greater likelihood of no major limitations in mental health (OR$_{Q5vsQ1}$ 1.31; 95%CI 1.05-1.22, p-trend <0.001) and marginally associated with no cognitive impairment (OR$_{Q5vsQ1}$ 0.99; 95%CI 0.97-1.01, p-trend 0.09)
Samieri et al., 2008 [49] France, 3C	n=1,724 76.0y	-	FFQ, Cluster analysis, five patterns; 'Small eaters', 'Biscuits and Snacking', 'Healthy', 'Charcuterie, Meat and Alcohol', 'Pasta eaters'	Cognitive function (MMSE)	Better adherence to the 'Healthy' dietary pattern was significantly associated with less errors in MMSE (β -0.11; CI -0.22 to -0.004 in men and β -0.13; CI -0.22 to -0.04 in women)
Corrêa Leite et al., 2001 [50] Pavia, Italy	n=1,651 76.6y	-	180-item FFQ, HDI	Cognitive deficit (NPT)	Better adherence to the HDI was associated with a lower prevalence of cognitive deficit (OR 0.85; 95%CI 0.77-0.93)
Huijbregts et al., 1998 [51] Seven Countries Study	n=1,049 76.1y	-	Cross-check dietary history, HDI	Cognitive impairment (MMSE)	Better adherence to the HDI has a protective effect on cognitive impairment, though not consistent over all cohorts (Zutphen OR 0.81; 95%CI 0.63-1.04, Italian OR 0.75; 95%CI 0.58-0.97)
Longitudinal studies					
Wengreen et al., 2013 [18] USA, CCMS	n=3,831 74.1y	11y	142-item FFQ, DASH score	Cognitive impairment, 3MS	Better adherence to DASH diet was significantly associated with higher 3MS scores (DASH$_{Q5vsQ1}$ 0.97±0.29 points, p-trend 0.0001)
Kesse-Guyot et al., 2011 [52] France, SU.VI.MAX	n=2,135 65.5y	12y	Repeated 24-HR, PNNS-GS scores	Verbal memory, executive functioning	Better adherence to nutritional recommendations was significantly associated with the verbal memory factor (β 0.41; 95%CI 0.17-0.64), whereas no association was shown with the executive functioning factor
Ozawa et al., 2013 [53] Japan,	n=1,006 68.5y	15y	70-item FFQ, RRR, 7 patterns; only 'Japanese'	Dementia risk, Alzheimer's disease,	Better adherence to the 'Japanese' pattern was associated with a reduced risk of dementia (all cause

Hisayama Study			presented	Vascular dementia (HDS, HDSR, MMSE)	dementia HR 0.66; 95%CI 0.46-0.95, AD HR 0.65; 95%CI 0.40-1.06 and VaD HR 0.45; 95%CI 0.22-0.91)
Kesse-Guyot et al., 2012, [54] France, SU.VI.MAX	n=3,054 65.4y	13y	Repeated 24h dietary records, Factor analysis, 2 patterns; 'Healthy', 'Traditional'	Global cognitive function, verbal memory, executive functioning	Significant higher cognitive function scores were found with better adherence to the 'Healthy' pattern vs. 'Traditional' pattern (adjusted means ±SD 50.1±0.7 vs 48.9±0.7, p-trend 0.001 for global cognitive function, 49.7±0.4 vs. 48.7±0.4, p-trend 0.01 for verbal memory)
Shatenstein et al., 2012 [55] Canada, NuAge	n=1,488 74.2y	3y	78-item FFQ, C-HEI	Cognitive decline (3MS)	There was no significant association between global diet quality (total C-HEI/100) and cognitive decline after 3-years follow-up (β0.00008, p-value 0.852)
Tangney et al., 2011 [17] USA, CHAP	n=3,790 75.4y	7.6y	139-item FFQ, HEI-2005	Cognitive function (MMSE)	HEI-2005 was neither associated with better global cognitive score at baseline, nor with changes in global cognitive score at follow-up (β-0.0011±0.001, p-value 0.236 and β 0.00002±0.0002, p-value 0.214, respectively)
Gu et al., 2010 [26] USA, WHICAP	n=2,148 77.2y	3.9y	FFQ, RRR, 7 patterns; DP1-DP7	Alzheimer's disease (DSM)	Better adherence to the 'Healthy' pattern was significantly associated with a lower AD risk (HR$_{T3vsT1}$ 0.62; 95%CI 0.43-0.89)
Wengreen et al., 2009 [56] USA, Cache County	n=3,634 74.7y	11y	142-item FFQ, RFS versus non-RFS	Cognitive decline, 3MS	Better adherence to RFS at baseline was associated with less decline in 3MS scores after 11 years of follow-up (RFS$_{T3vsT1}$ 1.79 point, p-value 0.0013)
Randomized controlled trials					
Smith et al., 2010 [57] USA, ENCORE	n=124 52.3y	4m	DASH diet 3-arms: - DASH Alone - DASH Weight Management - Usual Care	Cognitive functioning	DASH diet alone resulted in better psychomotor speed (Cohen's D=0.440; p-value 0.036) compared to control group in subjects with high blood pressure.

Abbreviations: 3C, Three-City; 3MS, Modified Mini-Mental State Examination; 24-HR, 24-hour dietary recall; AD, Alzheimer's Disease; AHEI-2010, Alternative Healthy Eating Index-2010; BPRHS, Boston Puerto Rican Health Study; CCMS, Cache County Memory Study; CHAP, Chicago Health and Aging Project; CHEI, Canadian Healthy Eating Index; CSI-D, Community Screening Instrument for Dementia; DASH, Dietary Approaches to Stop Hypertension; DSM, Diagnostic and Statistical Manual of Mental Disorders; ENCORE, Exercise and Nutrition Interventions for Cardiovascular Health Study; FFQ, Food Frequency Questionnaire; HDI, Healthy Diet Indicator; HDS, Hasegawa Dementia Scale; HDSR, Hasegawa Dementia Scale-Revised; HEI, Healthy Eating Index, MMSE, Mini-Mental State Examination; NHS, Nurses' Health Study; NPT, neuropsychological test; NuAge, Longitudinal Study on Nutrition and Successful Aging; PNNS-GS, Program National Nutrition Santé Guideline Score; RFS, Recommended Food Score; RRR, Reduced Rank Regression; SF-36, Medical Outcomes Short-Form 36 Health Survey; SU.VI.MAX, Supplementation en VItamines et Mineraux Anti-oXydants; TICS, Telephone Interview for Cognitive Status; VaD, vascular dementia; WHICAP, Washington Heights-Inwood Columbia Aging Project

Discussion

We reviewed the current evidence from observational studies and intervention trials investigating healthy dietary patterns in relation to cognitive decline and dementia. Overall, the results of all types of dietary pattern approaches suggest that better adherence to a healthy dietary pattern is associated with less cognitive decline and/or a lower risk of dementia. However, most studies are observational and evidence from intervention trials is limited to two trials, of which one investigated the effect of the Mediterranean diet [47, 48] and one the effect of the DASH diet [57]. There are several different methodological factors between the studies. This heterogeneity hinders comparison between studies; therefore the most important points are discussed below.

Both *a priori* and *a posteriori* approaches to define dietary patterns [3, 7, 58] have been used in studies included in this review and each method has its strengths and limitations. A limitation of *a priori* indexes is that they are based on current scientific knowledge on what a healthy diet comprises. Evolutions in knowledge should be considered each time the index is applied, which also changes the index over time [59]. Also few cues about how to weight food groups or guidelines have been proposed. A limitation of both *a priori* and *a posteriori* approaches is that complex correlations of food matrixes are not taken into account, neither are all components specifically related to cognitive outcomes. Indexes described in this review are mainly based on improving overall health status [20-23, 25] or blood pressure [60], rather than improving cognitive health specifically. A limitation of *a posteriori* methods is the limited comparability and reproducibility in other study samples because dietary patterns are based on food behavior in specific study samples [61]. Another limitation of *a posteriori* methods is that good skills in multidimensional statistical methods are required to select the best components of which the choice is subjective [62].

Another subjective choice, which could be confusing, is the naming of the dietary patterns. Therefore, for studies performed in different populations, the food consumption characterizing the pattern should be described clearly. Populations studied were almost all Western populations, mostly from the US and the Mediterranean countries in Europe and a few studies have been performed in Australia, China or Japan. According to Solfrizzi et al the components of the Mediterranean diet in Western countries could be different from the traditional Mediterranean diet, in particular for the high intakes of olive oil and regular consumption of wine with meals [63]. In general, a healthy dietary pattern comprises a diet high in fruits, vegetables, other plant-derived products, and fish and lower intakes of meat, saturated fats, and added refined sugar. We found no clear differences in associations between healthy dietary patterns and cognitive decline and dementia across countries. Also Singh and colleagues did not find any heterogeneity in their analysis, however, this could be due to the fact that they included three US studies and one French study [11].

Dietary intake can be assessed with different methods such as food records, 24 hour recalls or food frequency questionnaires (FFQ), of which the latter has been mostly used in the studies included in the current review. Different intake methods not only limit comparisons between studies, but also impacts on the number of variables to be used for dietary pattern analyses. This could affect both the number of derived dietary patterns as well as the dietary patterns itself [62]. It has been shown in studies using principle component analyses that this could lead to attenuated disease odds [62, 64].

The outcome measures that were included in our review ranged from cognitive performance in cross-sectional studies to cognitive decline and risk of AD or dementia in longitudinal studies. There are many differences in the way cognitive outcomes have been measured and reported. This makes comparison between studies more difficult and limits comparison of studies in a meta-

analysis, which would provide a more quantitative understanding of the relation between dietary patters and cognitive impairment. Length of follow-up time of longitudinal studies ranged from 2 years to 15 years. To capture changes in cognitive functioning several years of follow-up are needed, but how long exactly is sufficient and what the best period to start follow-up would be is not clear. It is nowadays suggested to already start at middle-age.

Some of the inconsistency in findings may be explained by the general considerations that should be taken into account when interpreting results of observational studies, such as residual confounding, possible over-adjustment and the fact that different covariates were included across the studies. Another important issue with observational studies is that they do not allow causal inference. This can be overcome with well-designed intervention studies. However, the only trial on the effect of the Mediterranean diet and cognitive decline did not measure cognition at baseline but only at follow-up, which limits the possibility to really study cause-effect relations.

Another point to take into account when interpreting results could be effect modification by sex as suggested by findings from Chan and colleagues who found that higher intakes of 'vegetables-fruit' and 'snacks-drinks-milk' patterns were associated with reduced risk of cognitive impairment in women, but no association was observed in men [15]. In contrast, a Greek study reported an increased risk of cognitive impairment with better adherence to the Mediterranean diet in women, but a reduced risk in men [33]. As eating behavior may differ between men and women it should be taken into account that dietary patterns could have been derived for men and women separately. Unfortunately, none of the included studies have examined dietary patterns by gender. These possible sex-specific differences merit further investigation and clarification.

The results suggest that better adherence to a Mediterranean diet is associated with less cognitive decline, dementia or AD, as shown by four out of six cross-sectional studies, six out of 12 longitudinal studies, one trial and three meta-analyses. Other Healthy dietary patterns, both *a priori* (e.g. HDI, HEI and PNNS-GS) and *a posteriori* derived (e.g. factor analysis, cluster analysis and RRR), have shown to be associated with reduced cognitive decline and/or a reduced risk of dementia as shown by all seven cross-sectional studies and five out of seven longitudinal studies.

Investigating whole diet approaches instead of individual nutrients is an attractive strategy, because combined effects may yield larger results since effects of individual nutrients may be small. Furthermore, a whole diet approach is more comparable to dietary intake in daily life. A dietary index specifically aimed at improving cognitive performance would be desirable. To further advance this field of research, more intervention trials of sufficient sample size investigating what type of dietary pattern is favorable with respect to prevention of cognitive decline are recommended. In this respect findings of the ongoing NU-AGE dietary intervention study, in which the effect of a 1-year healthful diet on cognitive performance is investigated, are to be awaited [65]. Furthermore, more observational studies starting at middle-aged adults and with a sufficient duration of at least >10-15 years of follow-up are warranted. Those studies should, if possible, take into account the methodological issues as pointed out above and should aim for more homogeneity in for example cognitive outcomes and composition of dietary patterns to facilitate comparison between studies. Also, the suggestion for differences in associations between men and women needs further investigation. If effects of certain dietary approaches will be proven, it will be a challenging task to change people's dietary habits, but it is important to take up the challenge now in order to provide (pre-) dementia patients some perspective of treatment or delay of the disease process and to come to clear recommendations in the future for a cost-effective, safe, and sustainable solution. This is of special importance, since there are currently no curative treatments for this disease.

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Chapter 4

Association of adherence to a healthy diet with cognitive decline in European and American older adults: a meta-analysis within the CHANCES consortium

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Abstract

Aim: To examine the association between a healthy diet, assessed by the Healthy Diet Indicator (HDI), and cognitive decline in older adults.

Methods: Data from 21,837 participants aged ≥55 years from 3 cohorts (Survey in Europe on Nutrition and the Elderly, a Concerted Action [SENECA], Rotterdam Study [RS], Nurses' Health Study [NHS]) were analyzed. HDI scores were based on intakes of saturated fatty acids, polyunsaturated fatty acids, mono- and disaccharides, protein, cholesterol, fruits and vegetables, and fiber. The Telephone Interview for Cognitive Status in NHS and Mini-Mental State Examination in RS and SENECA were used to assess cognitive function from multiple repeated measures. Using multivariable-adjusted, mixed linear regression, mean differences in annual rates of cognitive decline by HDI quintiles were estimated.

Results: Multivariable-adjusted differences in rates in the highest versus the lowest HDI quintile were 0.01 (95% CI –0.01, 0.02) in NHS, 0.00 (95% CI –0.02, 0.01) in RS, and 0.00 (95% CI –0.05, 0.05) in SENECA with a pooled estimate of 0.00 (95% CI –0.01, 0.01), I2 = 0%.

Conclusions: A higher HDI score was not related to reduced rates of cognitive decline in European and American older adults.

Introduction

The world's population aged over 60 years is predicted to double from 11% to 22% between 2000 and 2050 [1]. This demographic shift is likely to further increase the prevalence of age-related diseases and disabilities in the near future. In 2013, there were 44.4 million people with dementia worldwide and this number will increase to an estimated 135.5 million in 2050 [2]. Identifying modifiable risk factors for cognitive decline as a precursor of dementia is likely to be an important strategy for delaying the onset, and reducing the number of people with dementia [3]; a healthy diet is hypothesized to reduce risk [4].

A common approach to explore the impact of nutrition is studying dietary patterns comprising combinations of nutrients and foods. A frequently studied dietary pattern is the Mediterranean Diet, which is rich in fruits and vegetables and unsaturated fatty acids. Greater adherence to the Mediterranean Diet has been associated with a lower rate of cognitive decline in a number of observational and intervention studies [5]. However, there is a need to jointly study information from multiple studies to establish clear associations between a healthful dietary pattern, cognitive function and cognitive decline.

Recommending dietary patterns at an international level requires the operationalization of globally applicable dietary guidelines. Therefore, the 1990 World Health Organization (WHO) guidelines for a healthy diet [6] were translated into the Healthy Diet Indicator (HDI) [7, 8]. These guidelines were developed to reduce chronic diseases, such as hypertension. As hypertension has been shown to impact cognitive function [9-11], it has been hypothesized that the HDI could reduce cognitive function decline.

The HDI based on initial WHO recommendations has been associated with a lower prevalence of cognitive impairment [12, 13], however, the association between updated WHO guidelines and cognitive decline has not been quantified. We therefore prospectively examined the association between baseline HDI and cognitive decline at older age among 21,837 men and women from Europe and the United States (US) by conducting a meta-analysis of individual participant data from three population-based cohorts involved in the Consortium on Health and Ageing: Network of Cohorts in Europe and the United States (CHANCES) [14]. We hypothesized that a higher HDI score would be related to less cognitive decline.

Materials and methods

Data assessment and harmonization

The aim of CHANCES is to combine prospective cohort studies to produce, improve, and clarify the evidence on the distribution and risk factors of chronic diseases in the elderly and on their socioeconomic impact (www.chancesfp7.eu). Data standardization and harmonization procedures were largely based upon the experience from the MORGAM project and previous experiences of project partners [15]. Data assessment procedures included examination of availability and comparability of cohort data, questionnaires and measurement procedures used in the individual cohorts, and methods for collection of data on health outcomes [14, 16]. For the present study, CHANCES cohorts were selected with harmonized variables on dietary intake, cognitive function and covariates according to predefined rules.

Study design and population

We included participants aged ≥55 years from three cohorts, namely the cognitive sub-study of the Nurses' Health Study (NHS) from the United States (US) [17]; the Rotterdam Study (RS) from the Netherlands [18]; and the Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA) Study from Europe (Belgium, Denmark, France, Italy, The Netherlands, Portugal, Spain, Switzerland, and Poland) [19].

NHS began in 1976, with 121,700 female registered nurses aged 30-55 years [17]. During 1995–2001, women aged ≥70 years were invited to participate in a telephone-based study of cognitive function. For the first interview, 93% of eligible women participated (n=19,415). Follow-up assessments were performed up to 3 times at 2-year intervals. RS began baseline measures between 1990 and 1993 in 7,983 men and women aged ≥55 years [20]. The first follow-up examination took place between 1993 and 1994 in 6,315 participants (follow-up 88%) and continued in 1997, 1999 and 2001. The total SENECA population consisted of 2,585 European men and women aged 70-75 years at inclusion in 1988, and 124 participants were additionally added in a second wave in 1993. Follow-up measures were performed in 1993 and 1998 [19]. In all cohorts, the collaborative research procedures were in accordance with the ethical standards of the responsible institutional or regional committees on human experimentation and informed consent was obtained from all participants.

Dietary assessment

Information on dietary intake was obtained with a validated 116-item semi quantitative Food Frequency Questionnaire (FFQ) in NHS in 1994 and 1998 [17] and a validated 170-item FFQ in RS at baseline (1990-1993) [20]. In SENECA, dietary intake was assessed in 1988 (n=2,585) and 1993 (n=1,301) by means of a dietary history method including a three-day food record and a frequency checklist of foods [19]. Participants were interviewed by a dietician about their usual food consumption per day during the past month. Food intake estimations were converted into nutrient intakes by multiplying the consumption of each food by its nutrient content, using the US Department of Agriculture database in NHS and the Dutch food composition table (NEVO) [21] in RS and SENECA. The FFQs and dietary history method provided information allowing to estimate usual dietary intake per day during a specified period of time.

Healthy Diet Indicator

We used dietary intake immediately preceding the first cognitive assessment (ranging from zero to three years across the three cohorts) to estimate daily energy intake and to assess adherence to the updated WHO dietary guidelines in 2003 [8]. From 15 dietary items listed in the guidelines, seven items from which information was available across all cohorts were included in the HDI. This resulted in a HDI including saturated fatty acids, polyunsaturated fatty acids (PUFAs), mono- and disaccharides, protein, cholesterol, fruits and vegetables, and dietary fiber. Not included in the score were the intake of n-3 PUFAs, n-6 PUFAs, trans fatty acids, and sodium due to unavailability of data across cohorts. Furthermore, as suggested before [7], we excluded total fat and total carbohydrates from the HDI score calculation to avoid duplicating weights for these 2 components by the component factors and excluded non-starch polysaccharide as it also overlapped with the recommendation for total dietary fibre. We also excluded MUFAs, because the WHO guideline does not clearly specify the recommended intake of MUFAs in contrast to other fats. We applied a recently developed continuous HDI scoring system [22] with scores ranging from 0-10 per dietary component to provide greater variation between individuals and to overcome the use of definite cut-

offs [22]. Intakes below the lower cut-off were assigned 0 points and intakes above the upper cut-off were assigned 10 points. Within a given range of intakes for each component, the range was divided into 10 and points were given in proportion to the distance from the 0 point cut-off. The scoring criteria, as well as the median component scores by cohort are shown in **table 1**. Total HDI scores were divided into sex- and cohort-specific quintiles based on study population intake distributions.

Assessment of cognitive function

In NHS, the Telephone Interview of Cognitive Status (TICS) [23], a telephone adaptation of the Mini-Mental State Examination (MMSE) was administered first at baseline (1995-2001), then at approximately two-year intervals, with up to three repeated measures. The TICS contains measures of orientation, immediate verbal recall, registration, opposites, current events, serial subtraction, counting and other elements and assesses global cognitive performance, with scores ranging from 0 to 41.

The MMSE [24] assessed global cognitive function in RS in 1990, 1993, 1997, 1999 and 2001 and in SENECA in 1993 and 1999. The MMSE includes questions on orientation to time and place, registration, attention and calculation, recall, language and visual construction, resulting in a score from 0 to 30. A correlation of 0.94 between TICS and MMSE and a high test–retest reliability for TICS ($r = 0.97$) was reported [23]. Higher TICS or MMSE scores indicate better cognitive performance. We calculated z-scores at each time point using cohort-specific distributions of scores at first cognitive assessment allowing comparability of findings across cohorts.

Other variables

Demographic, health and lifestyle information were obtained from self-administered questionnaires. Height and weight were measured at baseline in RS and SENECA and self-reported in NHS [25]. Physical activity was assessed by validated questionnaires for elderly by estimating mean energy expended per week in NHS (in metabolic equivalent-hours, METs) and as being vigorously physically active in SENECA and RS. In RS, physical activity was assessed six years after baseline as no baseline measure was available. Prevalence of diabetes mellitus, myocardial infarction, high blood pressure, depression, and hypercholesterolemia was obtained by questionnaires. In SENECA, hypercholesterolemia was estimated on use of dyslipidemia medications.

Population for analysis

We excluded participants with incomplete dietary intake data (n=2,144 NHS, n=2 RS, n=16 SENECA), without at least one cognitive assessment (n=1,771 RS, n=1,676 SENECA), and missing data for physical activity (n=111 NHS, n=1,769 RS), resulting in a total population of 21,837 participants (17,160 NHS, 3,660 RS, and 1,017 SENECA).

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Table 1. Scoring criteria of the Healthy Diet Indicator components based on the WHO's 2003 guidelines for a healthy diet and mean component intakes by cohort and sex

HDI component[a]	0 points	0 - 10 points[e]	10 points	NHS	RS		SENECA	
				women	men	women	men	women
Saturated fatty acids, en%[b,c]	>15	≥10-≤15	0-<10	9.3 (2.8)	14.9 (3.1)	14.7 (3.3)	14.3 (4.4)	14.8 (4.4)
Mono- and disaccharides, en%[b,c,d]	>30	≥10-≤30	0-<10	27.3 (7.3)	21.6 (6.2)	22.7 (5.9)	19.1 (6.5)	20.3 (7.1)
Cholesterol, mg/day[c]	>400	≥300-≤400	0-<300	181.5 (88.4)	259.1 (88.8)	215.5 (69.2)	314.9 (132.2)	272.0 (121.9)
Polyunsaturated fatty acids, en%[b,c]	>10	0-<6	≥6-≤10	5.6 (1.8)	7.6 (2.9)	6.9 (2.8)	6.2 (3.9)	6.2 (3.2)
Protein, en%[b]	>20	0-<10 or >15-≤20	≥10-≤15	17.0 (3.2)	17.0 (2.9)	17.9 (3.2)	15.1 (2.7)	15.1 (3.2)
Total dietary fibre, g/day	0	0-<25	≥25	5.4 (2.2)	18.3 (5.3)	16.2 (4.5)	22.2 (7.7)	18.5 (6.6)
Fruits and vegetables, g/day[b]	0	0-<400	≥400	400.8 (206.6)	429.0 (170.3)	461.8 (180.1)	563.3 (257.5)	497.3 (213.5)

Abbreviations: HDI, Healthy Diet Indicator; WHO, World Health Organization; NHS, Nurses' Health Study; RS, Rotterdam Study; en%, energy percent. Values are mean (SD)

[a] Standard in accordance with WHO guidelines. The joint WHO Food and Agriculture Organization of the United Nations guidelines of 2003 do not clearly indicate fiber cutoff values. Fulfillment of the fruit and vegetable recommendation and consumption of whole grains should sum to 20 g of non-starch polysaccharides, which equals approximately 25 g of dietary fiber. Total fat, and total carbohydrates were excluded to avoid overlap with other components of the score

[b] Calculated without energy from alcohol.

[c] The cutoff value at which a participant would score 0 points was based on the 85th percentile of the population's intake distribution. Calculation of points for dietary intake between the upper limit and the standard intake for maximum number of points: 10-(intake- recommendation upper limit)*(10/standard upper limit-recommendation upper limit)).

[d] Mono- and disaccharides were studied instead of free sugars

[e] The range was divided into 10 and then points were given in proportion to the distance from the 0 point cut-off.

Statistical analyses

Means and standard deviations were calculated for normally distributed continuous variables and numbers and percentages were calculated for categorical variables.

In the primary approach, we modelled trajectories of repeated cognitive measures using linear mixed models [26], with follow-up time from baseline as the time metameter. The linear model included an intercept representing the baseline level of cognitive score and a slope representing annual cognitive change as well as a random intercept and random slope accounting for inter-individual variability. Linear trends across quintiles of HDI score were examined using a continuous variable in which participants in a given HDI quintile were assigned the median value.

In a secondary approach, all repeated measures of cognitive function were averaged to create an outcome representing long-term cognitive status. Averaging repeated measures of cognition attenuates variability in each single cognitive assessment, which may be helpful when cognition is measured over a relatively short follow-up period with a modest decline over time in healthy, educated participants in NHS, RS and SENECA [17, 27]. Mean differences in cognitive status across quintiles of HDI score were modelled using linear regression.

Adjustments were made for confounding factors that have been related to both dietary intake and cognitive function: age, sex, education (low, middle, high) (model 1), and employment history (employed, housekeeper, unemployed/retired), BMI (<22, 22-25, ≥25-30, ≥30 kg/m^2), smoking status (never, former, current), energy intake (cohort specific quintiles), alcohol intake (<1, 1-14.9, ≥15 g/day), physical activity (yes/no vigorous exercise in RS and SENECA, quintiles of METS in NHS) and depression (yes/no) (model 2). Vascular conditions (history of diabetes (yes/no), myocardial infarction (yes/no), high blood pressure (yes/no), and hypercholesterolemia (yes/no)) were tested as mediators by adding them to the full model (model 3). In the linear regression model studying long-term cognitive status, a study center variable was added for SENECA to adjust for differences in baseline MMSE score between study centers. For BMI, 4.4% was missing in NHS; for employment, data were missing for 3.7% in RS; in SENECA data were missing for education (5.9%) and depression (15%), thus a specific missing category was created for these 4 variables. For all other covariates, participants with missing information were <1% of the sample and were assigned to the reference group. In subgroup analyses, we repeated our primary analyses while stratifying by sex (not applicable in NHS), baseline cognitive function (worst 10% versus best 90%), age (median split), BMI (<25 versus ≥25 kg/m^2) and having any major cardiovascular risk factor (high blood pressure, hypercholesterolemia, myocardial infarction). As a sensitivity analysis, we determined the potential impact on our estimates of a learning effect when participants are administered the same cognitive tests multiple times by averaging the first two cognitive assessment within NHS and RS to derive a more robust baseline measure variable as first cognitive assessment and then repeating our analysis. Subsequently, we summarized the multivariable-adjusted mean differences in slopes of the fifth quintile versus first quintile per cohort by random-effects pooling by using DerSimonian and Laird random-effects models [28], accounting for differences in sample size and the possibility of statistical heterogeneity among the studies. Between-study heterogeneity was assessed via the I^2 statistic [29], expressing the percentage of variation attributable to between-study heterogeneity. All statistical analyses were carried out using SAS, version 9.3, software (SAS Institute Inc., Cary, North Carolina, USA). For random-effects meta-analyses, we used the *metaphor* package in R, version 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria). *P*-values < 0.05 were considered significant.

Results

General characteristics
Median HDI scores were 44.6 (range 13.3-62.5) in NHS, 45.5 (range 14.4-62.9) in RS, and 47.9 (range 20.6-69.9) in SENECA. At the first cognitive assessment, mean age of participants was 74.2 (2.3) years in NHS, 65.7 (7.3) in RS, and 78.0 (2.8) in SENECA. Across all cohorts, participants with a higher HDI score were more likely to be physically active, higher educated, never smokers, normal weight, to have higher energy intakes, and a history of myocardial infarction and to be less likely to have a history of diabetes (**table 2**).

Relation between HDI score and cognitive decline
A higher HDI score was not associated with cognitive decline in the basic adjusted model (adjusted mean differences in rates between extreme quintiles = 0.005 (95%CI -0.005,0.016), *P*-trend=0.17 in NHS, 0.001 (95%CI -0.014,0.015), *P*-trend=0.69 in RS and 0.008 (95%CI -0.041,0.052), *P*-trend=0.49 in SENECA), nor in the multivariable-adjusted model (0.005, [95%CI -0.006,0.016]), *P*-trend=0.20 in NHS, -0.002 (95%CI -0.016,0.013), *P*-trend=0.98 in RS and 0.002 (95%CI -0.047,0.052), *P*-trend=0.52 in SENECA (**table 3**)). Pooled analyses showed no association between highest HDI scores and cognitive decline (pooled multivariable-adjusted mean difference = 0.002 (95%CI: -0.006,0.011), I^2=0%), (**figure 1**). Adding mediators to the model (type 2 diabetes mellitus, myocardial infarction, hypertension and hypercholesterolemia) did not affect the estimates (adjusted mean differences in rates between extreme quintiles 0.006 (95%CI -0.005,0.017), *P*-trend=0.15 in NHS, -0.002 (95%CI -0.017,0.012), *P*-trend=0.98 in RS, and -0.001 (95%CI -0.049,0.048), *P*-trend=0.56 in SENECA, respectively). Subgroup analyses by sex, baseline cognitive function, age, and BMI did not show differences by strata or statistical interaction. Among those with a history of cardiovascular risk factors there was less cognitive decline with the highest HDI score in SENECA (*P*-trend=0.03, *P*-interaction=0.03, **table 4**). Furthermore, in sensitivity analyses to address practice effects, in the Rotterdam Study and NHS where >2 repeated measures were available, we used models where the average of the first two cognitive assessments was considered as the new baseline from which cognitive change was evaluated; results did not differ from the main analyses (data not shown).

Relation between the HDI score and cognitive status
The HDI score was not associated with cognitive status in NHS, RS and SENECA in the basic adjusted model (*P*-trend=0.28 in NHS, 0.19 in RS, and 0.34 in SENECA), nor in the multivariable-adjusted model (*P*-trend=0.87 in NHS, 0.27 in RS, and 0.25 in SENECA), **table 5**.

Table 2. Baseline characteristics of participants in the Nurses' Health Study (n=17,160), Rotterdam Study (n=3,660) and SENECA study (n=1,017) by extreme cohort- and sex-specific quintiles of HDI score[a]

	NHS		RS		SENECA	
	Quintile 1	Quintile 5	Quintile 1	Quintile 5	Quintile 1	Quintile 5
	35.7	52.7	36.4	53.2	38.1	57.1
	(32.6-37.7)	(51.4-54.4)	(33.8-38.2)	(51.9-55.2)	(35.4-40.2)	(55.3-59.23)
	n=3,422	n=3,431	n=731	n=731	n=202	n=203
Age	74.1 (2.3)	74.4 (2.4)	66.3 (7.4)	65.6 (6.3)	77.8 (2.8)	78.0 (2.8)
Men (%)	N/A	N/A	40.8	40.8	49.0	48.8
Educational level (%)						
Low	78	77	34	29	53	49
Middle	16	16	57	60	30	33
High	6	6	8	11	10	10
BMI (%)						
≤21, kg/m^2	19	22	8	11	9	12
22-24, kg/m^2	24	27	28	34	22	20
25-29, kg/m^2	33	34	50	44	43	44
≥30, kg/m^2	20	14	15	12	20	14
Physical activity[b]	13.8 (18.7)	18.3 (22.1)	83%	89%	8%	9%
Employment (%)						
Employed	5	4	79	78	75	78
Homemaker	7	7	11	9	17	12
Other/retired	88	89	10	13	9	10
Smoking (%)						
Never	44	48	30	36	55	60
Former	44	46	39	46	30	30
Current	12	6	31	18	15	10
History of disease (%)						
Myocardial infarction	6	5	9	11	24	32
Hypertension	56	55	36	37	32	37
Hypercholesterolemia	60	67	2	3	11	6
Diabetes	11	9	9	6	16	13
Depression	10	9	9	9	19	19
Dietary variables						
Energy intake, kcal	1518 (572)	1806 (497)	2007 (609)	2053 (414)	1897 (618)	2071 (607)
Alcohol, g	5.3 (10.4)	4.6 (8.6)	12.1 (15.8)	7.46 (10.6)	12.6 (18.1)	10.7 (8.9)
HDI components						
Saturated fatty acids	11.7 (3.2)	8.1 (1.7)	16.9 (3.1)	13.1 (2.5)	16.6 (4.4)	12.2 (3.6)
Polyunsaturated fatty acids	5.5 (2.3)	6.3 (1.4)	6.5 (3.6)	7.7 (1.7)	6.3 (4.1)	6.4 (2.2)
Mono- and disaccharides	26.2 (7.8)	26.2 (6.1)	21.5 (6.1)	23.2 (6.0)	20.8 (7.3)	17.9 (6.3)
Protein	18.5 (3.5)	15.0 (1.9)	18.6 (3.3)	16.2 (2.4)	16.9 (3.9)	13.9 (1.7)
Cholesterol, g	221 (121)	166 (68)	285 (105)	204 (55)	351 (144)	239 (78.5)
Fruit and vegetables, g	286 (172)	505 (205)	395 (193)	500 (149)	465 (246)	604 (255)
Dietary fiber, g	4.1 (1.9)	6.8 (2.3)	14.6 (4.7)	20.1 (4.4)	17.0 (6.9)	23.9 (7.5)
MMSE/TICS	33.8 (2.7)	33.8 (2.7)	27.9 (1.5)	28.1 (1.6)	26.9 (2.7)	26.3 (2.7)

Abbreviations: HDI, Healthy Diet Indicator; NHS, Nurses' Health Study; RS, Rotterdam Study; MET-hours=metabolic equivalent-hours, PUFAs, polyunsaturated fatty acids.
[a] Values are mean (SD) or percentage (%)
[b] Physical activity in NHS represented in MET-hours, in RS and SENECA as % vigorous physical activity.
HDI scores [median (IQR)] are provided in brackets for each quintile.
Dietary variables represent mean intakes per day expressed in energy percentages, unless otherwise noted.

Table 3. Multivariable-adjusted mean differences in annual rates of cognitive change by cohort- and sex-specific quintiles of baseline HDI score[a]

	Model 1[b]	Model 2[c]
NHS (*n* = 17,160)		
Quintile 1	Ref	Ref
Quintile 2	-0.004 (-0.014, 0.006)	-0.004 (-0.014, 0.007)
Quintile 3	0.013 (0.003, 0.023)	0.012 (0.002, 0.023)
Quintile 4	0.002 (-0.008, 0.012)	0.002 (-0.009, 0.012)
Quintile 5	0.005 (-0.005, 0.016)	0.005 (-0.006, 0.016)
p-trend	*0.17*	*0.20*
RS (*n* = 3,660)		
Quintile 1	Ref	Ref
Quintile 2	0.000 (-0.014, 0.015)	0.000 (-0.015, 0.014)
Quintile 3	0.012 (-0.003, 0.026)	0.010 (-0.004, 0.025)
Quintile 4	0.004 (-0.011, 0.018)	0.002 (-0.013, 0.016)
Quintile 5	0.001 (-0.014, 0.015)	-0.002 (-0.016, 0.013)
p-trend	*0.69*	*0.98*
SENECA (*n* = 1,017)		
Quintile 1	Ref	Ref
Quintile 2	-0.015 (-0.063, 0.034)	-0.029 (-0.079, 0.019)
Quintile 3	0.022 (-0.028, 0.071)	0.017 (-0.033, 0.066)
Quintile 4	0.006 (-0.043, 0.055)	0.000 (-0.049, 0.049)
Quintile 5	0.008 (-0.041, 0.057)	0.002 (-0.047**,** 0.052)
p-trend	*0.49*	*0.52*

Abbreviations: HDI, Healthy Diet Indicator; NHS, Nurses' Health Study; RS, Rotterdam Study; Ref, reference
[a] Values are mean differences (95% confidence interval).
[b] Adjusted for age, gender, education
[c] Additionally adjusted for employment status, BMI, smoking status, energy intake, alcohol intake, physical activity, and depression

Table 4. Multivariable-adjusted mean differences in annual rates of cognitive change by cohort- and sex-specific quintiles of baseline HDI score by cardiovascular risk factor history in SENECA[a,b]

	No cardiovascular risk factor	Cardiovascular risk factor
SENECA	*n* = 495	*n* = 522
Quintile 1	Ref	Ref
Quintile 2	-0.077 (-0.149, -0.005)	0.022 (-0.045, 0.087)
Quintile 3	-0.053 (-0.128, 0.021)	0.076 (0.010, 0.142)
Quintile 4	-0.061 (-0.132, 0.010)	0.076 (0.009, 0.143)
Quintile 5	-0.048 (-0.122, 0.027)	0.056 (-0.009, 0.121)
p-trend	0.23	0.03
p-interaction		0.03

Abbreviations: HDI, Healthy Diet Indicator; Ref, reference. Cardiovascular risk factor or disease included high blood pressure, hypercholesterolemia or myocardial infarction.
[a] Values are mean differences (95% confidence interval).
[b] Adjusted for age, education, study centre, employment status, BMI, smoking status, energy intake, alcohol intake, physical activity and depression.
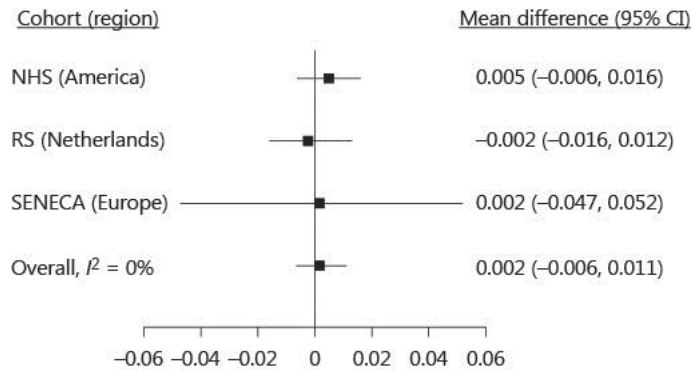
Figure 1. Cohort- and sex-specific and pooled mean differences in the annual rate of cognitive change in relation to highest vs. lowest quintile of adherence to the WHO guidelines and cognitive decline.

Adjusted for age, gender (TS and SENECA), education, employment status, BMI, smoking status, calorie intake, alcohol intake, physical activity, and depression in the Consortium on Health and Ageing: Network of Cohorts in Europe and the United States (CHANCES), 1988–2011. Cohorts are ordered according to cohort size, beginning with the largest cohort. I^2 is expressed as the percentage of total variability caused by heterogeneity. All data were obtained from CHANCES (www.chancesfp7.eu). Bars indicate 95% confidence intervals (CIs). NHS, Nurses' Health Study; RS, Rotterdam Study; SENECA, Survey in Europe on Nutrition and the Elderly, a Concerted Action.

Table 5. Multivariable-adjusted mean differences in average cognitive status by cohort- and sex-specific quintiles of baseline HDI score[a]

	Model 1[b]	Model 2[c]
NHS (*n* = 16,807)		
Quintile 1	Ref	Ref
Quintile 2	0.00 (-0.04, 0.05)	-0.02 (-0.06, 0.03)
Quintile 3	0.04 (-0.01, 0.08)	0.01 (-0.03, 0.06)
Quintile 4	0.00 (-0.04, 0.05)	-0.02 (-0.07, 0.02)
Quintile 5	0.03 (-0.02, 0.07)	0.00 (-0.04, 0.04)
p-trend	*0.28*	*0.87*
RS (*n* = 3,660)		
Quintile 1	Ref	Ref
Quintile 2	0.02 (-0.08, 0.12)	0.01 (-0.09, 0.11)
Quintile 3	0.06 (-0.04, 0.16)	0.04 (-0.06, 0.14)
Quintile 4	0.06 (-0.04, 0.15)	0.04 (-0.06, 0.14)
Quintile 5	0.06 (-0.04, 0.15)	0.06 (-0.04, 0.15)
p-trend	0.19	0.27
SENECA (*n* = 1,017)		
Quintile 1	Ref	Ref
Quintile 2	0.00 (-0.19, 0.19)	-0.02 (-0.21, 0.18)
Quintile 3	0.25 (0.06, 0.45)	0.25 (0.05, 0.44)
Quintile 4	0.01 (-0.19, 0.20)	-0.01 (-0.20, 0.19)
Quintile 5	-0.12 (-0.31, 0.08)	-0.14 (-0.34, 0.06)
p-trend	0.34	0.25

Abbreviations: HDI, Healthy Diet Indicator; NHS, Nurses' Health Study; RS, Rotterdam Study
[a] Variables are mean differences (95% confidence interval).
[b] Adjusted for age, gender, education, study centre (SENECA only)
[c] Additionally adjusted for employment status, BMI, smoking status, energy intake, alcohol intake, physical activity, and depression

Discussion

In the present consortium study, including 21,837 older men and women from Europe and the US, we found that a healthier diet adhering to the most recent WHO guidelines was not associated with a slower rate of cognitive decline, nor with cognitive status at older ages, which is in contrast to the findings between other dietary patterns [30-32] and cognition.

As the HDI is developed to prevent chronic diseases, we hypothesized that it could also impact cognitive function. Previously, two cross-sectional studies assessed the association between a higher HDI score and cognitive impairment and reported a lower prevalence of cognitive impairment in 1,049 Italian older men (odds ratio 0.75 (95%CI 0.58-0.97) [13]) and in a group of 1,651 Italian older men and women (odds ratio 0.85 (95%CI 0.77-0.93) [12]). Our study, using a much larger population, a prospective design, and using the updated HDI guidelines, did not confirm previous findings.

The lack of significant associations could be a result of low variability in cognitive function as measured by the MMSE and TICS due to the ceiling effects of these tests. Future studies on cognitive functioning should preferably include tests that are able to measure areas of cognition most affected by common dementing illnesses, such as memory, attention, language and visuospatial abilities [33]. However, in our cohorts, we have observed associations between major risk factors and cognitive change based on TICS [34-38]. Two previous studies examining the initial HDI guidelines used the MMSE [13] and an extended and validated version of the MMSE, namely the 0-70 point neuropsychological test (NPT) [12], to assess cognitive function. Although these tests are comparable to the tests that we used, the outcome was differently defined. We studied cognitive function and cognitive decline, whereas the previous studies evaluated cognitive impairment based on predefined cut-offs. This latter approach does not allow a distinction between initial level and change [33]. Furthermore, as our cohorts included relatively healthy, well-educated participants compared to the other populations [12, 13], it is possible that our participants reported healthy diets at baseline with an overall good cognitive functioning, limiting the ability to detect an association between the HDI and cognitive decline. Nonetheless, we had extremely high ability to identify even modest associations given our very large sample size. At last, previous studies used the initial HDI including pulses and nuts, which were not part of the updated HDI. While we didn't include nuts and pulses from the original HDI, the nutrients that would be neuroprotective from these foods, such as MUFAs, PUFAs, protein and fiber, would have been captured by the updated HDI, making it less likely that the omission of these two foods would explain the null findings.

Another point to consider is that due to the limited amount of dietary information across the three cohort studies we included seven out of ten HDI components. However, we aimed to increase variability within the HDI score by using the recently developed continuous calculation system based on seven dietary components (range 0-70 points) as proposed by Jankovic et.al. [22]. It has been shown that using seven components rather than ten HDI components (where instead of polyunsaturated fat in the 7 component HDI model, n-3 PUFAs, n-6 PUFAs are used in addition to trans fatty acids and sodium) resulted in less heterogeneity when studying the HDI in multiple cohort studies [22]. To test the effect of using seven HDI components only compared to using ten HDI components, we studied ten HDI components in NHS and found similar null findings, indicating this probably does not explain our null-findings.

Other dietary patterns that have been shown to be related to cognitive function and decline in European and American observational studies in older adults include the Mediterranean diet [17, 39-46], the Dietary Approaches to Stop Hypertension (DASH) diet [42, 44, 47] and the Healthy Eating Index (HEI) [45, 48]. Dietary intervention studies with the Mediterranean diet [30, 31] and the DASH diet [32] confirmed these findings. These food based scores include high intakes of vegetables, fruits, legumes, whole grains, nuts, fish, monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), moderate amounts of low-fat dairy products and alcohol and low amounts of red and processed meats, and the MUFA-to-saturated fats ratio.

More recently, dietary components linked to neuroprotection and cognitive function have been summarized into one dietary pattern score, namely the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet [49]. The MIND diet is a hybrid of the Mediterranean-DASH diets and includes high intakes of green leafy vegetables, other vegetables, nuts, berries, beans, whole grains, fish, poultry, olive oil, and wine and low amounts of red meats, butter and stick margarine, cheese, pastries and sweets, and fried/fast food. This diet has been related to slower cognitive ageing in a single study of 960 older adults (mean age 81.4 years) of the Memory and Aging Project [49]. As the HDI does not include the majority of these components [8], nor specific components shown to be associated with cognitive function, such as green leafy vegetables [36, 50] and berries [34] it could be that the HDI was well-designed to reduce risk of chronic diseases in general, but not cognitive function. On the contrary, the inclusion of very specific food items limits the possibility to jointly study effect estimates of multiple studies and to target multiple diseases at once by means of one dietary pattern, as does the HDI.

In stratified analyses, we observed less cognitive function decline with higher HDI scores in participants reporting a history of cardiovascular risk factors in SENECA. As the HDI has been developed to prevent chronic diseases, including hypertension, and as hypertension has been associated with a worse cognitive function [9-11], it was hypothesized that this high risk group for cognitive decline would benefit from a healthy diet as the HDI. Biologically, the blood pressure lowering effect of the HDI reduces thickening of the vessel wall, leading to less vascular narrowing, resulting in less thickening of the media and atheromatous plaques within larger cerebral arteries. This could result in a lower risk of rupture of these plaques which could cause complete occlusion of arteries and infarction of surrounding tissues [51].

The availability of harmonized dietary intake variables is a major strength of the present study. Furthermore, the use of harmonized outcome variables and covariates reduces heterogeneity. Additionally, four HDI components are expressed as energy percentage, increasing the comparability of diet between different cohorts using different dietary assessment methods. Another advantage is the relatively long follow-up time of cognitive function with multiple repeated measures (NHS and SENECA 6 years, RS 10 years), which could have been sufficient to measure changes in cognitive function, as other studies were able to measure changes in cognitive function after 4 to 5 years of follow-up [39, 52]. Another strength is that we were able to study changes in cognitive function over time, allowing us to use all available data of all cohorts using a comparable measure across cohorts. Finally, studying dietary intake prior to the cognitive assessments including many years of follow-up minimized possible reverse causation.

It may be considered a limitation that we used one dietary intake assessment, assuming stable diets over time. Additional analyses confirmed that dietary intakes were similar over multiple time points in NHS and SENECA (data not shown). Furthermore, FFQ's rely on participants' estimates of food intake which can lead to misclassification of dietary pattern adherence. This is a common limitation of studies of diet and our results from SENECA using a dietary history method did not result in different conclusions. Lastly, although we have tried to differentiate between a healthy lifestyle and a healthy diet by extensively adjusting for risk factors for cognitive decline, residual confounding remains possible due to unmeasured or imprecisely measured covariates.

We demonstrated that greater adherence to the WHO dietary guidelines for a healthy diet was not associated with reduced rates of cognitive decline in European and American older adults.

Acknowledgements

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Chapter 5

The Dietary Approaches to Stop Hypertension diet, cognitive function, and cognitive decline in American older women

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Abstract

Objectives: To examine the association between long-term adherence to the Dietary Approaches to Stop Hypertension (DASH) diet with cognitive function and decline in older American women.

Setting: The Nurses' Health Study (NHS), a cohort of registered nurses residing in 11 U.S. states.

Participants: A total of 16,144 women from NHS, aged ≥70 years, who underwent cognitive testing a total of 4 times by telephone from 1995-2001 (baseline), with multiple dietary assessments between 1984 and the first cognitive exam. DASH adherence for each individual was based on scoring of intakes of nine nutrient or food components.

Measurements: Long-term DASH adherence was calculated as the average DASH adherence score from up to five repeated measures of diet. Primary outcomes were cognitive function calculated as the average scores of the four repeated measures, as well as cognitive change of the Telephone Interview for Cognitive Status (TICS) score and composite scores of global cognition and verbal memory.

Results: Greater adherence to long-term DASH score was associated with better average cognitive function, irrespective of apolipoprotein E (APOE) ε4 allele status (multivariable-adjusted differences in mean z-scores between extreme DASH quintiles = 0.04 (95% CI 0.01-0.07), p-trend=0.009 for global cognition; 0.04 (95%CI 0.01-0.07), p-trend=0.002 for verbal memory, and 0.16 (95%CI 0.03–0.29), p-trend=0.03 for TICS, p-interaction >0.24). These differences were equivalent to being one year younger in age. Adherence to the DASH score was not associated with change in cognitive function over six years.

Conclusion: Our findings in the largest cohort on dietary patterns and cognitive function to date indicate that long-term adherence to the DASH diet is important to maintain cognitive function at older ages.

Introduction

With the aging population, it is important to find modifiable risk factors to prevent cognitive decline and reduce the number of people with dementia [1]. One hypothesized factor is nutrition, and specifically dietary patterns in which combinations of nutrients and foods are studied simultaneously [2].

A frequently studied dietary pattern is the Mediterranean Diet, which has been associated with a lower rate of cognitive decline [3]. Another dietary pattern of interest is the Dietary Approaches to Stop Hypertension (DASH) diet. The DASH diet specifies higher intakes of fruits, vegetables, low-fat dairy products, whole grains, poultry, fish, and nuts and low intakes of fats, red meat, sweets and sugar-containing beverages [4]. This diet has been shown to lower blood pressure in pre-hypertensive and hypertensive adults [5]. It has been hypothesized that the DASH diet could impact cognitive function, as high blood pressure is associated with higher risk mild cognitive impairment [6], vascular dementia [7], and neurocognitive deficits [8]. A few observational studies have evaluated the DASH diet in relation to cognitive function and reported that the DASH diet was associated with a better cognitive function in elderly men and women (*n*=3,831) in the United States (U.S.) over an 11-year period [9], and with less cognitive decline among elderly American men and women (*n*=826) after 4.1 years of follow-up [10]. A dietary intervention study (*n*=124) demonstrated that four months on the DASH diet improved psychomotor speed in hypertensive American adults [11].

We examined the association of long-term DASH diet adherence to cognitive function and 6-year cognitive decline among 16,144 women.

Materials and methods

Study population

The Nurses' Health Study (NHS) began in 1976, with 121,700 female, registered nurses, aged 30-55 years, residing in 11 U.S. states. Participants completed a mailed questionnaire about their health and lifestyle. Follow-up questionnaires are sent every two years; follow-up of the cohort remains ~90% to date. In 1984, participants completed a 116-item semi-quantitative food frequency questionnaire (FFQ) [12] and similar FFQs were sent in 1986 and every four years thereafter. During 1995–2001, women who were ≥70 years old and free of stroke were invited to participate in their first telephone-based study of cognitive function. For the first interview, 93% of eligible women participated (n=19,415). Follow-up assessments were performed three times at two-year intervals until 2008, with a high participation rate (>90% among those remaining alive at each follow-up point). The study was approved by the Institutional Review Board of Brigham and Women's Hospital (Boston, MA).

In the present study, we excluded those who had not completed at least one of the two initial FFQs in 1984 or 1986 (n=2,892), without at least one complete cognition battery at follow-up (n=334), had no data for physical activity (n=36), or no information on energy intake (n=9), resulting in a total population for analysis of 16,144 participants.

Dietary assessment and DASH diet score

For each item of the FFQ, responses ranged from 'never or <1 time/month' to '≥6 times/day'; a standardized portion size was specified for each food item to estimate quantities consumed daily. Food intake estimations were converted into nutrient intakes by multiplying the frequency of consumption of each food by its nutrient content by using the U.S. Department of Agriculture database.

In this study, we used the 1984, 1986, 1990, 1994 and 1998 FFQs to estimate daily energy intake and to construct, at each of these time points, an alternate DASH 9-point score [13]. This score enhances the original DASH score by Appel et al. [4]. and is based on 8 criteria: high intake of fruits, vegetables, nuts and legumes, whole grains, low-fat dairy products, and low intake of sodium, red and processed meats and sweetened beverages [13]. As the DASH diet also aims for lower amounts of saturated fat and cholesterol with higher levels of unsaturated fatty acids, the component monounsaturated fatty acids (MUFA) plus polyunsaturated fatty acids (PUFA) has been added to the original score, resulting in a total of 9 components. For each of the components, we classified women into quintiles according to their intake ranking. Component scores were the women's quintile ranking; one point was given for the lowest quintile of intake and five points for the highest intake. The scoring was reversed for components to limit (sodium, red and processed meat and sweetened beverages). Long-term dietary habits are likely most relevant because cognitive decline develops over many years [14]. Therefore, long-term DASH score was computed as the mean of up to five DASH scores from all dietary assessments from 1984 (or 1986, if 1984 FFQ was missing) to the questionnaire immediately preceding the first cognitive exam. Total long-term DASH scores were divided into quintiles based on the study population distribution.

Cognitive assessment

Cognitive testing was performed by trained interviewers using validated telephone interviews. The cognitive battery included: 1) the Telephone Interview for Cognitive Status (TICS) [15]; 2) immediate; and 3) delayed recalls of the East Boston Memory test (EBMT) [16]; 4) delayed recall of the TICS 10-word list; 5) category fluency; and 6) digit span-backward. In the initial cognitive interview, we administered only the TICS and gradually added the five other tests. Thus, the sample size slightly differs across tests, but the participation rate remained identical for all tests.

The TICS [15] (range 0–41 points) is a telephone adaptation of the Mini-Mental State Examination [17], which assesses global cognitive performance. The immediate and delayed recalls of the EBMT[16] (range 0–12 points) assess verbal (episodic) memory. The delayed recall of the TICS 10-word list (range 0–10 points) also assesses verbal memory. A category fluency test assesses language and executive function [18] by scoring performance on naming as many animals as possible in one minute. Finally, the digit-span backward test (range 0–12 points), in which participants repeat backwards and increasingly long series of digits, assesses working memory and attention.

The primary outcomes were the TICS and composite scores of global cognition and verbal memory. A global cognition score was computed as the mean of Z-scores of all six cognitive tests in the cognitive battery. A verbal memory score was calculated as the mean of Z-scores of the four tests assessing verbal memory (i.e., immediate and delayed recalls of both the EBMT and the TICS 10-word list). We calculated Z-scores at each time point using the distribution of scores at the first cognitive assessment.

Other variables

Socio-demographic, lifestyle and health-related information were obtained from the questionnaires. Using cumulative and updated information from 1976, covariates were determined at the time of the first cognitive interview, except physical activity and energy intake, for which values were averaged across multiple assessments over time, similar to diet. Physical activity was assessed in 1986, 1988 and 1992, and every 2 years thereafter by estimating mean energy expended per week (in metabolic equivalent-hours, mets). In a randomly selected subsample of 5,822 participants, data on apolipoprotein E (APOE) ε4 polymorphisms was available from cheek cell specimens (n=3,469) and genome wide association studies (n=2,353).

Statistical analyses

Relation between long-term DASH adherence, cognitive function and decline.

Statistical analyses utilized two complementary approaches. As primary analyses, we averaged the four repeated measures of cognitive function to create an outcome representing average cognitive function at older ages and modelled the association of long-term DASH score with average cognitive status using linear regression models. Averaging repeated measures of cognition was relevant to our data, as it attenuates variability in each single cognitive assessment, which may be helpful when cognition is measured over a relatively short follow-up period in largely healthy, educated participants [19]. In another approach, we modelled trajectories of the four repeated cognitive scores using linear mixed models [20]. The linear mixed models included an intercept that represented the level of cognitive score at baseline and a slope that represented the annual change in scores over time as well as a random intercept and random slope to account for inter-individual variability. We examined linear trends across quintiles using a continuous variable in which participants in a given category were assigned the median value of the long-term DASH score.

Adjustments were made for possible confounding factors age and education (registered nurse, bachelor, master or doctorate) (crude, model 1), and subsequently for long-term energy intake (quintiles) and physical activity (MET-hours/week, quintiles), body mass index (BMI, kg/m^2, ≤21, 22-24, 25-29, ≥30), smoking status (never, former, current), alcohol intake (<1, 1-15, ≥15 g), history of depression (yes/no), multivitamin use (yes/no), and cardiovascular risk factors / disease (history of diabetes (yes/no), hypertension (yes/no), hypercholesterolemia (yes/no), and myocardial infarction (yes/no)) (Model 2). Estimates did not change after adjusting for *ApoE* ε4 within the subsample, therefore, *ApoE* ε4 was not included in the multivariable adjusted model. Because for BMI and multivitamin use, data was missing for >4% of the sample (4.4 and 7.1%, respectively); a specific missing category was created for these two variables. For other covariates, participants with missing data were <1% and were assigned to the reference group. Effect modification was tested adding an interaction term for DASH quintiles with age (median), high blood pressure (yes/no), and presence of the *ApoE* ε4 allele (yes/no, within the *ApoE* ε4 subsample).

Relative contribution of DASH-components and cognitive function.

To examine the relative importance of individual DASH components, we recalculated separate long-term DASH scores excluding one DASH component at a time while including this specific component as covariate in the model to adjust for this specific component (as quintiles of intake).

All statistical analyses were carried out using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). A two-sided *p*-value of <0.05 was considered significant.

Results

General characteristics

At first cognitive assessment, the mean age of participants was 74.3±2.3 years, and the mean TICS score was 33.8±2.7 points (maximum=41). The median long-term DASH score was 28.0 points (range 12-41) with a large variation in intake of the individual DASH components across all quintiles. Women with higher long-term DASH adherence had lower BMI, greater physical activity, higher education (**table 1**), and better scores for global cognition, verbal memory and TICS (**table 2**). They were less likely to report a history of myocardial infarction and to be current smokers. They were more likely to use a multivitamin supplement and slightly more likely to have a history of hypercholesterolemia.

Table 1. Baseline characteristics of the participants in the Nurses' Health Study by quintiles of long-term adherence to the DASH score (n=16,144)

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
	23.0	25.8	28.0	29.8	32.3
	(12.0-24.7)	(24.8-26.8)	(27.0-28.8)	(29.0-30.8)	(31.0-41.0)
	n =3,176	*n* =3,107	*n* =3,325	*n* =3,184	*n* =3,345
Age	74.0 ± 2.3	74.2 ± 2.3	74.3 ± 2.3	74.4 ± 2.3	74.5 ± 2.3
BMI					
≤21, kg/m^2	666 (21.0)	586 (18.9)	597 (18.0)	617 (19.4)	724 (21.6)
22-24, kg/m^2	752 (23.7)	755 (24.3)	893 (26.9)	805 (25.3)	921 (27.5)
25-29, kg/m^2	1,056 (33.2)	1,082 (34.8)	1,107 (33.3)	1,098 (34.5)	1,028 (30.7)
≥30, kg/m^2	566 (17.8)	543 (17.5)	585 (17.6)	536 (16.8)	514 (15.4)
Long-term physical activity	12.4 ± 12.7	14.9 ± 13.9	17.4 ± 16.0	18.9 ± 15.5	22.3 ± 19.0
Educational level					
Registered nurse	2,672 (84.1)	2,494 (80.3)	2,621 (78.8)	2,385 (74.9)	2,332 (69.7)
Bachelor	373 (11.7)	448 (14.4)	503 (15.1)	577 (18.1)	732 (21.9)
Master or doctorate	131 (4.1)	165 (5.3)	201 (6.0)	222 (7.0)	281 (8.4)
Smoking					
Never	1,366 (43.0)	1,385 (44.6)	1,547 (46.5)	1,533 (48.1)	1,685 (50.4)
Former	1,350 (42.5)	1,434 (46.2)	1,573 (47.3)	1,471 (46.2)	1,538 (46.0)
Current	460 (14.5)	288 (9.3)	205 (6.2)	180 (5.7)	122 (3.6)
Disease history					
Myocardial infarction	215 (6.8)	203 (6.5)	191 (5.7)	186 (5.8)	179 (5.4)
Hypertension	1,770 (55.7)	1,706 (54.9)	1,855 (55.8)	1,777 (55.8)	1,828 (54.6)
Hypercholesterolemia	1,997 (62.9)	2,033 (65.4)	2,179 (65.5)	2,130 (66.9)	2,210 (66.1)
Diabetes	315 (9.9)	326 (10.5)	335 (10.1)	316 (9.9)	329 (9.8)
Depression	290 (9.1)	313 (10.1)	309 (9.3)	315 (9.9)	297 (8.9)
Multivitamin use	1,603 (50.5)	1,761 (56.7)	2,027 (61.0)	1,985 (62.3)	2,235 (66.8)
Long-term dietary variables (servings per day unless otherwise noted)					
Daily energy intake, kcal	1,640 ± 432	1,635 ± 436	1,698 ± 433	1,746 ± 420	1,800 ± 401
Alcohol, g/day	7.2 ± 11.5	5.9 ± 9.1	5.4 ± 8.2	5.0 ± 7.5	4.5 ± 6.9
DASH components					
Fruit	1.8 ± 1.0	2.3 ± 1.0	2.6 ± 1.0	3.0 ± 1.2	3.4 ± 1.2
Vegetables	2.4 ± 1.0	3.0 ± 1.2	3.5 ± 1.3	4.0 ± 1.5	4.8 ± 1.7
Nuts and legumes	0.5 ± 0.3	0.6 ± 0.4	0.7 ± 0.4	0.8 ± 0.4	1.0 ± 0.5
Sodium, mg	2,093 ± 660	2,064 ± 659	2,132 ± 661	2,158 ± 648	2,168 ± 631
Red and processed meat	0.9 ± 0.4	0.8 ± 0.4	0.7 ± 0.4	0.6 ± 0.4	0.5 ± 0.3
Whole grains	0.8 ± 0.6	1.1 ± 0.7	1.4 ± 0.8	1.7 ± 0.9	2.1 ± 1.0
Sweetened beverages	0.4 ± 0.5	0.3 ± 0.3	0.2 ± 0.3	0.1 ± 0.2	0.1 ± 0.2
Low-fat dairy	0.5 ± 0.6	0.8 ± 0.7	1.0 ± 0.7	1.2 ± 0.8	1.5 ± 0.8
MUFA+PUFA, mg	32.3 ± 5.0	31.6 ± 4.9	31.0 ± 5.0	30.2 ± 5.1	29.4 ± 5.5
DASH score	22.4 ± 1.9	25.8 ± 0.6	27.9 ± 0.6	29.8 ± 0.6	32.7 ± 1.5

Values are means ± SD or number (%)
All dietary variables are in servings per day, unless otherwise indicated

Table 2. Baseline cognitive function and average cognitive function of the participants in the Nurses' Health Study by quintiles of long-term adherence to the DASH score (n=16,144)

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
Animal naming score	16.6 ± 4.7	16.9 ± 4.6	17.0 ± 4.6	17.2 ± 4.6	17.2 ± 4.8
Digit backward test	6.6 ± 2.3	6.7 ± 2.4	6.7 ± 2.4	6.8 ± 2.4	6.8 ± 2.5
East Boston memory test					
Immediate recall	9.4 ± 1.7	9.5 ± 1.7	9.4 ± 1.7	9.4 ± 1.9	9.4 ± 1.7
Delayed recall	9.0 ± 2.0	9.0 ± 2.0	8.9 ± 2.0	8.9 ± 1.9	8.9 ± 2.1
TICS word list					
Immediate recall	4.5 ± 1.6	4.6 ± 1.7	4.6 ± 1.7	4.7 ± 1.7	4.7 ± 1.8
Delayed recall	2.2 ± 1.9	2.3 ± 1.9	2.3 ± 2.0	2.4 ± 1.9	2.4 ± 2.1
TICS score, total	33.6 ± 2.7	33.8 ± 2.6	33.8 ± 2.7	33.8 ± 2.8	33.9 ± 2.8
Composite scores					
Global cognition	-0.04 ± 0.60	-0.00 ± 0.59	-0.01 ± 0.61	0.01 ± 0.61	0.02 ± 0.62
Verbal memory	-0.04 ± 0.68	-0.01 ± 0.68	-0.01 ± 0.69	-0.00 ± 0.69	0.01 ± 0.71
Average cognitive function					
Global cognition	-0.08 ± 0.59	-0.05 ± 0.60	-0.05 ± 0.60	-0.03 ± 0.62	-0.02 ± 0.64
Verbal memory	-0.03 ± 0.63	-0.01 ± 0.66	-0.02 ± 0.70	0.01 ± 0.67	0.02 ± 0.69
TICS	33.5 ± 2.6	33.6 ± 2.5	33.6 ± 2.6	33.6 ± 2.7	33.7 ± 2.6

Values are means ± SD

Average cognitive function

Greater adherence to the long-term DASH score was linearly related to better average cognitive function, comprising the mean of the four repeated measures of cognitive function, in both the crude and the multivariable-adjusted model (mean differences in global cognition, verbal memory and TICS scores between the top and bottom quintiles of the DASH score in the fully adjusted model were 0.04, p-trend=0.009, 0.04, p-trend=0.002 and 0.16, p-trend=0.03 in model 2, **table 3**).

There was no interaction between long-term DASH score and age (p-interaction=0.67, 0.80, 0.36 for global cognition, verbal memory and TICS, respectively), high blood pressure (p-interaction=0.85, 0.66, 0.53 for global cognition, verbal memory and TICS, respectively), or *ApoE ε4* status (p-interaction=0.35, 0.32, 0.24 for global cognition, verbal memory and TICS, respectively). The association between long-term DASH score and average cognitive function was not mediated by high blood pressure as the effect estimates remained exactly the same when taking out high blood pressure from the multivariable-adjusted model (data not shown).

Cognitive decline over time

After 4.1 years, long-term DASH score was not associated with change over the four repeated measures of global cognition, verbal memory, and TICS, in neither the crude, nor in the multivariable-adjusted model (p-trend=0.51, 0.68, and 0.98, for global cognition, verbal memory and TICS, respectively in model 2) (**table 4**).

DASH-components and average cognitive function.

Taking out the vegetables or the nuts plus legumes component of the total long-term DASH score attenuated the associations between long-term DASH score and average cognitive function (p-trends across the DASH score categories of excluding the vegetables component were 0.27, 0.96, 0.09, and 0.18, 0.19, and 0.04 while excluding the nuts plus legumes component, for global cognition, verbal memory and TICS, respectively).

Table 3. Multivariable-adjusted mean differences in average cognitive function over the follow-up period by quintiles of long-term DASH score in 16,144 older women[a]

	Model 1[b]	p	Model 2[c]	p
Global cognitive score				
Quintile 1	Ref		Ref	
Quintile 2	0.03 (0.00,0.06)	0.07	0.02 (-0.01,0.05)	0.20
Quintile 3	0.03 (0.00,0.06)	0.03	0.01 (-0.02,0.04)	0.40
Quintile 4	0.06 (0.03,0.09)	<0.01	0.03 (0.00,0.06)	0.02
Quintile 5	0.06 (0.03,0.09)	<0.01	0.04 (0.01,0.07)	0.01
p-trend	<0.001		0.009	
Verbal memory score				
Quintile 1	Ref		Ref	
Quintile 2	0.02 (-0.01,0.06)	0.14	0.02 (-0.01,0.05)	0.29
Quintile 3	0.02 (-0.01,0.05)	0.19	0.00 (-0.03,0.04)	0.82
Quintile 4	0.05 (0.02,0.08)	<0.01	0.03 (0.00,0.07)	0.05
Quintile 5	0.06 (0.03,0.09)	<0.01	0.04 (0.01,0.07)	0.01
p-trend	<0.001		0.002	
TICS score				
Quintile 1	Ref		Ref	
Quintile 2	0.13 (0.01,0.26)	0.04	0.10 (-0.03,0.22)	0.12
Quintile 3	0.16 (0.03,0.28)	0.01	0.08 (-0.05,0.20)	0.24
Quintile 4	0.18 (0.05,0.30)	<0.01	0.09 (-0.04,0.22)	0.17
Quintile 5	0.27 (0.14,0.39)	<0.01	0.16 (0.03,0.29)	0.02
p-trend	<0.001		0.03	

[a] Values are mean (95% Confidence Interval).
[b] Adjusted for age and education.
[c] Adjusted for age, education, physical activity, calorie intake, alcohol intake, smoking status, depression, multivitamin use, BMI, and history of, high blood pressure, hypercholesterolemia, myocardial infarction, and diabetes mellitus.

Table 4. Multivariable-adjusted mean differences in slopes of cognitive change by quintiles of long-term DASH score in 16,144 older women[a]

	Model 1[b]	p	Model 2[c]	p
Global cognitive score				
Quintile 1	Ref		Ref	
Quintile 2	0.000 (-0.008,0.008)	0.96	-0.002 (-0.010,0.006)	0.64
Quintile 3	0.001 (-0.007,0.008)	0.87	-0.002 (-0.010,0.006)	0.29
Quintile 4	-0.001 (-0.009,0.006)	0.74	-0.004 (-0.012,0.004)	0.62
Quintile 5	0.002 (-0.006,0.010)	0.64	-0.002 (-0.010,0.006)	0.68
p-trend	0.77		0.51	
Verbal memory score				
Quintile 1	Ref		Ref	
Quintile 2	-0.003 (-0.013,0.006)	0.52	-0.005 (-0.014,0.005)	0.31
Quintile 3	-0.002 (-0.011,0.007)	0.66	-0.004 (-0.014,0.005)	0.36
Quintile 4	-0.002 (-0.011,0.007)	0.68	-0.005 (-0.014,0.005)	0.35
Quintile 5	0.001 (-0.008,0.010)	0.83	-0.003 (-0.012,0.007)	0.61
p-trend	0.77		0.68	
TICS score				
Quintile 1	Ref		Ref	
Quintile 2	-0.006 (-0.038,0.027)	0.73	-0.011 (-0.043,0.022)	0.52
Quintile 3	-0.002 (-0.034,0.029)	0.89	-0.009 (-0.041,0.023)	0.57
Quintile 4	-0.001 (-0.033,0.031)	0.89	-0.007 (-0.039,0.026)	0.69
Quintile 5	0.006 (-0.025,0.038)	0.69	-0.001 (-0.034,0.032)	0.94
p-trend	0.89		0.98	

[a] Values are mean (95% Confidence Interval).
[b] Adjusted for age and education.
[c] Adjusted for age, education, physical activity, calorie intake, alcohol intake, smoking status, multivitamin use, BMI, and history of depression, high blood pressure, hypercholesterolemia, myocardial infarction, and diabetes mellitus.

Discussion

In this large longitudinal study, long-term adherence to the DASH diet was associated with a higher average global cognitive function, verbal memory and TICS score at older ages. To put these results into perspective, the effect estimates we found for long-term DASH adherence were cognitively equivalent to ~1 year of aging. No associations were observed between long-term DASH adherence and cognitive change after 4.1 years.

We found that long-term greater adherence to the DASH diet was associated with better average TICS score, global cognitive function and verbal memory, which is a strong predictor of Alzheimer's Disease (AD) [21]. Previous longitudinal studies showed that greater adherence to a DASH diet was associated with better global cognitive function in the Cache County Study (3MS score quintile 5 versus quintile 1 = 0.97, p-trend<0.001)[9] and with a slower rate of cognitive decline in the Memory and Aging Project (0.007, p-trend=0.003 per unit increase in DASH score) [10]. The only trial on the effect of the DASH diet on cognition did not study global cognition, verbal memory or TICS, but showed better psychomotor speed after 4 months on the DASH diet, compared to control group (Cohen's D = 0.440, p = 0.036) [11].

Other dietary patterns emphasizing foods and nutrients similar to those in our DASH score (e.g., fruits, vegetables, whole grains), have been associated with less cognitive decline, such as the extensively investigated (alternate) Mediterranean diet [9, 22-25], the alternate Healthy Eating Index 2005 and 2010 [25], and the Healthy Diet Indicator [26]. In previous studies investigating the effect of single components, interactions between the remaining diet score components have been ignored. We studied the relative contribution of individual components to the DASH score to the association with average cognitive function. Using this approach, excluding vegetables and nuts plus legumes attenuated the association, indicating that these two components are the most important contributors to the overall DASH score in relation to average cognitive function. Other studies have demonstrated that nuts [19, 27] and nuts plus legumes [9] are associated with better average cognitive status, ignoring the relative contribution of these components. Future research should further explore the relative instead of the absolute contribution of components in the context of diet pattern scores.

Biologically, the DASH diet could be linked with cognitive function by influencing blood pressure, thereby lowering vascular dementia risk and corresponding cognitive decline. The DASH diet lowered blood pressure in an 8-week randomized controlled trial (RCT) in middle-aged normotensive American men and women [4] and in a 6-month multi-centre RCT in middle-aged pre-hypertensive or hypertensive American men and women [28]. Given this blood pressure-lowering effect, and the fact that blood pressure is an important risk factor for cognitive decline and late-life dementia [29], there is a clear basis for hypothesizing that the DASH diet may be inversely associated with cognitive outcomes.

Genetically, carrying one or more copies of the *ApoE-ε4* allele is associated with an increased AD risk and cognitive function decline [30-33]. This supports the importance of finding strategies to prevent cognitive decline especially among *ApoE-ε4* carriers. However, we did not observe an interaction between the DASH diet and *ApoE-ε4* status. Other studies investigating the interaction between dietary patterns and cognitive function have shown inconsistent findings, with studies reporting better cognitive function among *non-carriers* [34, 35], others showing relations in *carriers* [36], and studies finding no interaction [35, 37, 38]. Overall, no consistent conclusion on the role of the *ApoE4* ε4 allele with diet and cognitive function can be drawn and this requires further research.

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We did not find an association between the long-term DASH diet and cognitive change over time. Possibly our participants had higher education than participants in the Cache County Study [9] and Memory and Aging Project [10], with relatively slower cognitive decline over time. This could have resulted in a more narrow distribution of average cognitive function and cognitive change, reducing the magnitude of association in our study. Participants in our study were followed for a relatively short period, compared to the Cache County Study (11 years) [9]. Additionally, participants in the DASH-trial were obese, younger, and had above-normal blood pressure, compared to our relatively healthy older population [11]. At last, our modest association may be due to lower long-term adherence to the DASH diet compared to participants of the DASH-trial who received controlled feeding for exact DASH diet compliance, resulting in a vegetable intake of 5.7 [5] compared to 4.8 servings/day in the highest long-term DASH score quintile in our study.

Several potential limitations of our study should be discussed. First, the FFQ was not designed to provide precise measurements of some dietary components, such as sodium. However, it is a hallmark of the DASH-Sodium trial; thus, using the quintile approach would be least prone to misclassification. Using the actual cut-off approach for number of servings weakened our results, likely owing to greater misclassification. Hence, we chose to award points for adhering to what the DASH diet emphasizes rather than precise compliance to the DASH recommendations in number of servings. Although these population-specific cut-offs limits generalizability to other populations, this approach addresses the focus of the DASH diet and allows for adequate variation in adherence to examine any association with cognitive function. Second, as this was an observational study, residual confounding might influence analyses of average cognition due to factors that persist over time (e.g., education) and affect the cognitive level a person achieves more than a person's cognitive trajectory in later life. However, we carefully adjusted for many potentially confounding variables. These adjustments did not meaningfully change our results for average cognition, suggesting that residual confounding would be unlikely to completely explain our findings. Third, cognitive function was assessed with use of telephone assessment, possibly causing some misclassification of cognitive function. A validation study, however, has shown that telephone-based cognitive batteries perform well compared with in-person interviews ($\rho=0.81$ comparing the two models) [39]. At last, as participants of this cohort are cognitively relatively healthy, middle-aged nurses, we expect a limited generalizability of our results to American women, and men.

The long follow-up allowed us to assess long-term associations between the DASH score and cognitive function. By averaging repeated measures of diet to obtain a better assessment of long-term overall diet we accounted for within-person change in intake and reduced measurement error, as previously documented by our group [40]. Measurement error in cognitive function assessments has also been limited by averaging all repeated measures into a more robust measure of average cognitive function at older ages. The prospective nature reduces the probability of recall bias and selection bias. In addition, a high follow-up rate reduced potential loss of follow-up bias. Nevertheless, our results need to be replicated in other populations.

Conclusion
Our findings in the largest cohort on dietary patterns and cognitive function to date indicate that long-term adherence to the DASH diet is important to maintain global cognitive function, verbal memory and TICS scores at older ages. Future studies should focus on participants at risk of poorer cognitive function, to elucidate the role of healthy diets on cognitive decline over time.

Acknowledgements

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Chapter 6

Association of long-term adherence to the MIND diet with cognitive function and cognitive decline in American women

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Abstract

Objectives: There is increasing attention for dietary patterns as a potential strategy to prevent cognitive decline. We examined the association between adherence to a recently developed Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet with cognitive function and cognitive decline, taking into account the interaction between the apolipoprotein E ε4 genotype and the MIND diet.

Design: Population-based prospective cohort study. Participants: A total of 16,058 older women aged 70 and over from the Nurses' Health Study.

Measurements: Dietary intake was assessed five times between 1984 and 1998 with a 116-item Food Frequency Questionnaire. The MIND score includes ten brain-healthy foods and five unhealthy foods. Cognition was assessed four times by telephone from 1995 to 2001 with the Telephone Interview for Cognitive Status (TICS) and by calculating composite scores of verbal memory and global cognition. Linear regression modelling and linear mixed modelling were used to examine the associations of adherence to the MIND diet with average cognitive function and cognitive change over six years, respectively.

Results: Greater long-term adherence to the MIND diet was associated with a better verbal memory score (multivariable-adjusted mean differences between extreme MIND quintiles=0.04 (95%CI 0.01-0.07), p-trend=0.006), but not with cognitive decline over 6 years in global cognition, verbal memory or TICS.

Conclusion: Long-term adherence to the MIND diet was moderately associated with better verbal memory in later life. Future studies should address this association within populations at greater risk of cognitive decline.

Introduction

The proportion of the world's population over 60 years is expected to double from 11% in 2010 to 22% by 2050 [1]. With population ageing, there will be an increase in the prevalence of age-related diseases and disabilities, such as dementia [2]. As, to date, there is no effective treatment for dementia [3], it is important to identify disease-modifying risk factors to prevent cognitive decline. One of these factors could be nutrition. Especially studying dietary patterns as a potential strategy to prevent cognitive decline has increasingly received attention in recent years. The Mediterranean diet (MeDi) is a frequently studied dietary pattern which has been shown to be associated with a slower rate of cognitive decline [4]. A less studied dietary pattern in relation to cognition is the Dietary Approaches to Stop Hypertension (DASH) diet. So far, three observational studies and one intervention study have demonstrated that higher adherence to the DASH diet was associated with less cognitive decline [5-8]. However, the MeDi and DASH diets do not include specific dietary components that optimize brain health.

Recently, a hybrid of these two dietary patterns has been developed by Morris et al. [9], namely the Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet. Similar to the MeDi and DASH diets, the MIND diet emphasizes natural plant-based foods and limited intakes of animal and high saturated fat foods. Uniquely, it also specifies the consumption of berries and green leafy vegetables [9], which have been demonstrated to have antioxidant and anti-inflammatory properties and inhibit β-amyloid deposition and neurotoxic death [10]. Greater adherence to the MIND diet was associated with slower rates of cognitive decline in the only study conducted to date [10].

Besides lifestyle factors, the *apolipoprotein E ε4* (*ApoE ε4*) allele has been shown to be a major genetic risk factor for more rapid cognitive decline and earlier onset of Alzheimer's Disease (AD) [11, 12]. The few human studies that have investigated an interaction between dietary patterns and cognitive function showed inconsistent findings [13-17]. Overall, the contribution of the *ApoE ε4* allele in the association between dietary patterns and cognitive function is unclear.

We evaluated the association between long-term adherence to the MIND diet with cognitive function and cognitive decline during 6 years in 16,058 women from the Nurses' Health Study. Additionally, we examined the interaction between the *ApoE ε4* genotype and the MIND diet in relation to cognitive function.

Methods

Study population

The Nurses' Health Study (NHS) began in 1976, with 121,700 female registered nurses aged 30-55 years and resided in 11 U.S. states. Participants completed a mailed questionnaire about their health and lifestyle. Follow-up questionnaires are sent every two years. To date, follow-up of the cohort remains ~90%. In 1984, participants completed a 116-item semi-quantitative food frequency questionnaire (FFQ) and similar FFQs were sent in 1986 and every 4 years thereafter [18]. During 1995–2001, women who were ≥70 years old and free of stroke were invited to participate in the first telephone-based study of cognitive function. A total of 19,415 eligible women completed the first interview (93%). Follow-up assessments were performed three times at two-year intervals until 2008, with a participation rate of >90% among those who remained alive and could be contacted at

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each follow-up point. The study was approved by the Institutional Review Board of Brigham and Women's Hospital (Boston, MA) and all participants consented.

In the present study, we excluded those who had not completed at least one of the two initial FFQs in 1984 and 1986 (n=2,990), without at least one complete cognition battery at follow-up (n=331), or had no data for physical activity (n=36), resulting in a total population for analysis of 16,058 participants.

Dietary assessment and MIND diet score

For each item of the FFQ, possible responses ranged from 'never or <1 time/month' to '≥6 times/day', and specific standardized portion sizes. Food intake estimations were converted into nutrient intakes by multiplying the frequency of consumption of each food by its nutrient content by using the U.S. Department of Agriculture database and other sources of updated supplementary information.

In this study, we used the 1984, 1986, 1990, 1994 and 1998 FFQs to estimate daily energy intake and to construct a MIND diet score at each of these time points using the methodology described by Morris et al. [9]. The MIND score is based on 10 brain-healthy foods groups: namely, higher intake of green leafy vegetables (spinach, lettuce, kale), other vegetables, berries (blueberries, strawberries), nuts, whole grains, fish, beans, poultry, limited intake of wine (red and white wine), and use of olive oil as primary source of fat; and 5 unhealthy foods: namely, lower intakes of butter and margarine, cheese, red meat and products, fast fried foods, and pastries and sweets [9] (**table 1**). For healthy components, we assigned 0, 0.5 or 1 point for higher intakes, and for unhealthy components the scoring was reversed (total score 0 to 15 points). As long-term dietary habits are likely most relevant because cognitive decline develops over many years [19], long-term MIND score was computed as the mean of up to five MIND scores from all dietary assessments from 1984 (or 1986, if 1984 FFQ was missing) to the questionnaire immediately preceding the first cognitive interview. Total MIND scores were divided into quintiles based on the study population distribution.

Table 1. Components and scoring of the MIND diet within the Nurses' Health Study

MIND component	Score		
	0	0.5	1
Green leafy vegetables	≤2 servings/week	>2 to <6/week	≥6 servings/week
Other vegetables	<5 servings/week	5 to <7/week	≥1 serving/day
Berries	<1 serving/week	1-2/week	≥2 servings/week
Nuts	<1/month	1/month to <5/week	≥5 servings/week
Olive Oil	Not primary oil		Primary oil used
Butter, margarine	>2 table spoon/day	1-2 table spoon/day	<1 table spoon/day
Cheese	≥7 servings/week	≥1-<7/week	<1 serving/week
Whole grains	<1 serving/day	≥1-<3/day	≥3 servings/day
Fish (not fried)	Rarely	1-3/month	≥1 meals/week
Beans	<1 meal/week	1-3/week	>3 meals/week
Poultry (not fried)	<1 meal/week	≥1-<2/week	≥2 meals/week
Red meat and products	>6 meals/week	≥4-≤6/week	<4 meals/week
Fast fried foods	≥4 times/week	1-<4/week	<1 time/week
Pastries and sweets	≥7 servings/week	≥5-<7/week	<5 servings/week
Wine	>1 glass/day or never	1/month to 6/week	1 glass/day
Total score	0	7.5	15

Cognitive assessment

Cognitive testing was performed by trained interviewers using validated telephone interviews. The cognitive battery included: 1) the Telephone Interview for Cognitive Status (TICS) [20]; 2) immediate; and 3) delayed recalls of the East Boston Memory test (EBMT) [21]; 4) delayed recall of the TICS 10-word list; 5) category fluency; and 6) digit span-backward test. The sample size slightly differs across tests as in the initial stage of the first cognitive interview only the TICS was administered and gradually five other tests were added. The participation rate remained identical for all tests.

The TICS [20] (0–41 points) is a telephone adaptation of the Mini-Mental State Examination [22] and assesses overall cognitive performance. The EBMT [21] (0–12 points) and delayed recall of the TICS 10-word list (0–10 points) assesses verbal (episodic) memory. The category fluency test assesses language and executive function [23]; performance is based on naming as many animals as possible in one minute. Finally, the digit-span backward test (0–12 points) assesses working memory and attention, where participants repeat backwards an increasingly long series of digits.

The primary outcomes were the TICS and composite scores of global cognition and verbal memory. A global cognitive score was computed as the mean of z-scores of all six cognitive tests. A verbal memory score was calculated as the mean of z-scores of four tests assessing verbal memory (i.e., immediate and delayed recalls of both the EBMT and the TICS 10-word list). We calculated z-scores at each follow-up using means and standard deviations of scores at the first cognitive assessment.

Other variables

Socio-demographic, lifestyle and health-related information were obtained from questionnaires. Using cumulative and updated information from 1976, covariates were determined at the time of the first cognitive exam. For physical activity and energy intake values were averaged across multiple assessments over time, similar to diet. Physical activity was assessed in 1986, 1988 and 1992, and every 2 years thereafter by estimating mean energy expended per week (in metabolic equivalent-hours, Met-hrs). In a randomly selected subsample (n=5,822), data on *ApoE* polymorphisms was available from cheek cell specimens (n=3,469) and genome wide association studies (n=2,353).

Statistical analyses

Long-term MIND adherence, cognitive function and decline

Statistical analyses consisted of two complementary approaches. As primary analyses, we averaged the four repeated measures of cognitive function to create an outcome representing overall cognitive status at older ages. Averaging repeated measures of cognition was relevant to our data, as it attenuates variability in each single cognitive assessment, which may be helpful when cognition is measured over a relatively short follow-up period in largely healthy, educated participants, as described in previous studies [24, 25]. The association of long-term MIND score with cognitive status was modelled using linear regression models.

As secondary approach, we modelled trajectories of the four repeated cognitive scores using linear mixed models [26]. The linear mixed models included an intercept representing the level of cognitive score at baseline and a slope representing the annual change in scores over time, as well as a random intercept and random slope to account for inter-individual variability.

Adjustments were made for confounding factors age and education (registered nurse, bachelor, master or doctorate) (Model 1), and additionally for long-term energy intake (quintiles) and physical activity (Met-hrs/week, quintiles), body mass index (BMI, ≤21, 22-24, 25-29, ≥30 kg/m^2),

smoking status (never, former, current), alcohol intake (<1, 1-15, ≥15 g/d), history of depression (yes/no), multivitamin use (yes/no), and cardiovascular risk factors (history of diabetes, hypertension, hypercholesterolemia, and/or myocardial infarction (yes/no) (Model 2). Because for BMI and multivitamin use data was missing for >4% of the sample (4.4 and 7.1%, respectively); a missing category was created. For other covariates, participants with missing data were <1% of the sample and were assigned to the reference group. Effect modification was tested by adding an interaction term for MIND score quintiles with age (median split), BMI (<25/≥25 kg/m^2), high blood pressure (yes/no), hypercholesterolemia (yes/no), myocardial infarction (yes/no). In a subset of 5,822 participants, a variable indicating the product of the number of *ApoE* ε4 alleles (0,1,2) was used to test for effect modification by *ApoE* ε4 genotype.

MIND-components and cognitive function
To examine the relative importance of the individual MIND components, we recalculated separate MIND scores by excluding one MIND component at a time while including this component as covariate (in quintiles of intake).

All statistical analyses were carried out using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). A two-sided *p*-value of <0.05 was considered significant. We examined linear trends across quintiles of the MIND score using a continuous variable in which participants in a given category were assigned the median value.

Results

General characteristics
At the first cognitive assessment, the mean age of participants was 74.3 ± 2.3 years (mean ± SD), and the mean TICS score was 33.8 ± 2.7 points. The median MIND score was 6.4 points (range 2.6–11.0) with a large variation in intake of individual MIND components across quintiles of the average MIND score of 4.4 dietary assessments over 12.9 years (**table 2**). Women with greater long-term MIND adherence were more likely to have a lower BMI, to be more physically active, to be higher educated, to use a multivitamin supplement, to report a history of hypercholesterolemia and were less likely to be current smokers and to report a history of depression.

Average cognitive function
Greater long-term adherence to the MIND score was linearly related to significantly higher verbal memory scores, comprising the mean of the 4 repeated measures of cognitive function, in both the age and education adjusted model and the multivariable-adjusted model (p-trend <0.0001 for model 1, 0.006 for model 2), but not with a better global cognition or TICS score (**table 3**). The mean difference in mean verbal memory between the top and bottom quintiles of the MIND score in the fully adjusted model was 0.04 standard units.

Table 2. Baseline characteristics of the participants in the Nurses' Health Study by quintiles of long-term adherence to the MIND score (n=16,058)[a]

	Quintile 1 4.9 (2.6-5.4) n = 3,339	Quintile 2 5.8 (5.5-6.1) n = 3,259	Quintile 3 6.4 (6.1-6.7) n = 2,962	Quintile 4 7.0 (6.7-7.3) n = 3,261	Quintile 5 7.9 (7.4-11.0) n = 3,237
Age at first cognitive exam	74.3 ± 2.3	74.2 ± 2.3	74.3 ± 2.3	74.3 ± 2.4	74.3 ± 2.3
BMI at first cognitive exam					
≤21, kg/m^2	709 (21.2)	612 (18.8)	561 (18.9)	602 (18.5)	686 (21.2)
22-24, kg/m^2	780 (23.4)	771 (23.7)	829 (28.0)	866 (26.6)	860 (26.6)
25-29, kg/m^2	1,138 (34.1)	1,090 (33.4)	955 (32.2)	1,127 (34.6)	1,041 (32.2)
≥30, kg/m^2	592 (17.7)	647 (19.9)	488 (16.5)	526 (16.1)	476 (14.7)
Long-term physical activity	12.5 ± 12.6	15.0 ± 13.8	17.2 ± 14.7	18.7 ± 15.9	23.1 ± 1 9.8
Educational level					
Registered nurse	2,785 (83.4)	2,589 (79.4)	2,325 (78.5)	2,445 (75.0)	2,304 (71.2)
Bachelor	403 (12.1)	505 (15.5)	459 (15.5)	588 (18.0)	657 (20.3)
Master or doctorate	151 (4.5)	165 (5.1)	178 (6.0)	228 (7.0)	276 (8.5)
Smoking					
Never	1,582 (47.4)	1,554 (47.7)	1,360 (45.9)	1,512 (46.4)	1,466 (45.3)
Former	1,376 (41.2)	1,408 (43.2)	1,377 (46.5)	1,552 (47.6)	1,627 (50.3)
Current	381 (11.4)	297 (9.1)	225 (7.6)	197 (6.0)	144 (4.4)
History of:					
Myocardial infarction	214 (6.4)	197 (6.0)	180 (6.1)	196 (6.0)	182 (5.6)
Hypertension	1,860 (55.7)	1,810 (55.5)	1,634 (55.2)	1,829 (56.1)	1,757 (54.3)
Hypercholesterolemia	2,107 (63.1)	2,122 (65.1)	1,942 (65.6)	2,179 (66.8)	2,152 (66.5)
Diabetes	331 (9.9)	336 (10.3)	329 (11.1)	326 (10.0)	289 (8.9)
Depression	346 (10.4)	315 (9.7)	273 (9.2)	305 (9.4)	271 (8.4)
Multivitamin use	1,736 (54.5)	1,888 (58.8)	1,925 (58.7)	1,983 (61.6)	2,026 (64.1)
Long-term dietary variables (servings per day unless otherwise noted)					
Energy intake, kcal/day	1,646 ± 440	1,686 ± 428	1,694 ± 421	1,712 ± 409	1,783 ± 420
Alcohol, g/day	4.6 ± 8.8	5.2 ± 8.9	5.8 ± 9.0	6.1 ± 8.8	6.3 ± 8.4
MIND components					
Green leafy vegetables[b]	3.4 ± 5.2	5.0 ± 6.7	6.5 ± 8.6	7.4 ± 8.6	9.6 ± 10.1
Other vegetables	2.0 ± 1.8	2.6 ± 2.3	3.1 ± 2.9	3.4 ± 2.9	4.2 ± 3.7
Berries[b]	0.8 ± 2.7	1.3 ± 3.5	1.6 ± 4.1	1.8 ± 4.0	2.4 ± 4.7
Nuts[b]	1.5 ± 2.6	2.1 ± 3.9	2.6 ± 4.7	2.6 ± 4.8	3.4 ± 6.1
Primarily vegetable oil	0.2 ± 0.3	0.3 ± 0.4	0.3 ± 0.4	0.4 ± 0.5	0.6 ± 0.5
Butter, margarine	1.4 ± 1.0	1.2 ± 0.9	1.1 ± 0.9	1.0 ± 0.8	0.8 ± 0.8
Cheese[b]	5.9 ± 7.9	5.4 ± 7.2	5.5 ± 7.6	5.3 ± 7.3	5.2 ± 7.6
Whole grains	1.0 ± 1.2	1.4 ± 1.6	1.7 ± 2.1	1.8 ± 2.1	2.4 ± 2.6
Fish (not fried)[b]	1.1 ± 2.8	1.8 ± 3.8	2.3 ± 4.8	2.6 ± 5.0	3.3 ± 5.2
Beans[b]	0.6 ± 2.2	1.2 ± 3.4	1.5 ± 4.1	1.8 ± 4.6	2.3 ± 5.4
Poultry (not fried)[b]	2.3 ± 3.8	3.0 ± 4.7	3.5 ± 4.8	3.7 ± 4.8	4.3 ± 5.2
Red meat and products[b]	6.7 ± 6.0	6.3 ± 6.9	6.1 ± 7.6	5.8 ± 8.8	5.4 ± 9.6
Fast fried foods[b]	8.0 ± 1.6	8.0 ± 1.7	8.2 ± 1.7	8.3 ± 1.7	8.3 ± 1.9
Pastries and sweets[b]	15.1 ± 15.0	14.3 ± 17.8	14.2 ± 21.5	13.0 ± 22.2	13.0 ± 27.4
Wine	0.1 ± 0.4	0.2 ± 0.6	0.3 ± 0.6	0.3 ± 0.6	0.3 ± 0.7

Abbreviation: MIND= Mediterranean-DASH Intervention for Neurodegenerative Delay
[a] Values are means ± SD or number (%)
[b] Servings per week

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Table 3. Multivariable-adjusted mean differences[a] in cognition averaged over the follow-up period across quintiles of the MIND score in 16,058 older women participating in the Nurses' Health Study

	Model 1[b]	Model 2[c]
Global cognitive score		
Quintile 1	Ref	Ref
Quintile 2	0.02 (-0.01,0.05)	0.00 (-0.02,0.03)
Quintile 3	0.02 (-0.01,0.05)	0.00 (-0.03,0.03)
Quintile 4	0.02 (-0.01,0.05)	0.00 (-0.03,0.03)
Quintile 5	0.03 (0.00,0.06)	0.00 (-0.03,0.03)
p-trend	0.03	0.88
Verbal memory score		
Quintile 1	Ref	Ref
Quintile 2	0.02 (-0.01,0.06)	0.01 (-0.02,0.05)
Quintile 3	0.03 (0.00,0.07)	0.02 (-0.01,0.05)
Quintile 4	0.04 (0.01,0.08)	0.02 (-0.01,0.06)
Quintile 5	0.06 (0.03,0.09)	0.04 (0.01,0.07)
p-trend	<0.0001	0.02
TICS score		
Quintile 1	Ref	Ref
Quintile 2	-0.01 (-0.13,0.11)	-0.05 (-0.18,0.07)
Quintile 3	0.06 (-0.07,0.19)	0.00 (-0.13,0.12)
Quintile 4	0.07 (-0.05,0.19)	-0.02 (-0.14,0.11)
Quintile 5	0.02 (-0.10,0.14)	-0.09 (-0.21,0.04)
p-trend	0.44	0.31

Abbreviations: MIND= Mediterranean-DASH Intervention for Neurodegenerative Delay; TICS= Telephone interview of cognitive status
[a] Values are mean difference in score (95% confidence interval)
[b] Model 1: adjusted for age and education
[c] Model 2: additionally adjusted physical activity, calorie intake, alcohol intake, smoking status, multivitamin use, BMI, depression, and history of high blood pressure, hypercholesterolemia, myocardial infarction, and diabetes mellitus

The associations did not change when additionally adjusting for family history of dementia and socio-economic variables such as income, job, and marital status (data not shown). There was no interaction between adherence to the MIND diet and age, BMI, high blood pressure or *ApoE ε4* status for global cognition, verbal memory or TICS (p-interaction age: 0.45, 0.70, 0.82, BMI: 0.63, 0.61, 0.69, high blood pressure: 0.90, 0.70, 0.85, *ApoE ε4* status: 0.38, 0.40, 0.27 for global cognition, verbal memory and TICS, respectively). The association between the MIND diet and cognitive function was not mediated by high blood pressure as the effect estimates remained exactly the same when taking out high blood pressure from the multivariable adjusted model (data not shown).

Cognitive decline over time

Long-term adherence to the MIND score was not significantly associated with change over time in the global cognitive score, verbal memory score, or the TICS score, in either the age and education adjusted models, or in the multivariable-adjusted model (p-trend 0.95, 0.98, and 0.73 for global cognition, verbal memory and TICS, respectively) (**table 4**).

Table 4. Multivariable-adjusted mean differences[a] in slopes of cognitive change by quintiles of long-term MIND-score in 16,058 older women

	Model 1[b]	Model 2[c]
Global cognitive score		
Quintile 1	Ref	Ref
Quintile 2	0.002 (-0.005,0.010)	0.001 (-0.007,0.009)
Quintile 3	-0.002 (-0.010,0.006)	-0.004 (-0.011,0.004)
Quintile 4	0.000 (-0.009,0.008)	-0.002 (-0.010,0.006)
Quintile 5	0.004 (-0.003,0.012)	0.001 (-0.007,0.009)
p-trend	0.44	0.95
Verbal memory score		
Quintile 1	Ref	Ref
Quintile 2	0.002 (-0.007,0.011)	0.000 (-0.009,0.009)
Quintile 3	-0.005 (-0.015,0.004)	-0.007 (-0.017,0.002)
Quintile 4	-0.001 (-0.010,0.008)	-0.003 (-0.013,0.006)
Quintile 5	0.005 (-0.004,0.015)	0.002 (-0.008,0.011)
p-trend	0.44	0.98
TICS score		
Quintile 1	Ref	Ref
Quintile 2	0.017 (-0.015,0.048)	0.014 (-0.018,0.045)
Quintile 3	0.007 (-0.025,0.039)	0.003 (-0.030,0.035)
Quintile 4	-0.007 (-0.039,0.024)	-0.011 (-0.043,0.020)
Quintile 5	0.011 (-0.021,0.042)	0.004 (-0.028,0.036)
p-trend	0.95	0.73

Abbreviations: MIND= Mediterranean-DASH Intervention for Neurodegenerative Delay; TICS= Telephone interview of cognitive status
[a] Values are mean difference in score (95% confidence interval)
[b] Model 1: adjusted for age and education
[c] Model 2: additionally adjusted for physical activity, calorie intake, alcohol intake, smoking status, multivitamin use, BMI, depression and history of high blood pressure, hypercholesterolemia, myocardial infarction, and diabetes mellitus.

MIND-components and cognitive function

Excluding the component 'Butter and margarine' and 'Pastries and sweets' attenuated the association between the MIND score and average verbal memory (adjusted mean differences between extreme quintiles of intake were 0.02 (95%CI -0.02,0.05) for 'Butter and margarine' and 0.03 (95%CI -0.01,0.06) for 'Pastries and sweets, p-trends across categories of intake = 0.14 for both components), demonstrating that these components were the most important contributors to the association, as part of the MIND diet.

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Discussion

In this large observational study in 16,058 older women, we observed that greater long-term adherence to the MIND diet was associated with a better verbal memory score, irrespective of *ApoE ε4* status. We did not find evidence of an association between MIND diet adherence with global cognition or the TICS score, nor with cognitive change over time in any of the domains.

To put our results into perspective, we observed that each increase of one year of age of participants in this study population was associated with a mean difference of -0.05 standard units for verbal memory score. To provide a more clinical interpretation of the findings, this means that the effect estimate we found for MIND adherence was cognitively equivalent to being approximately one year younger.

The Memory and Aging Project (MAP), an open prospective cohort study in 960 older persons, demonstrated that the MIND diet was associated with slower decline in the global cognitive score and also in each of the five cognitive domains studied. The difference in rate of decline for highest adherence to the MIND diet compared to the lowest adherence was equivalent to that seen in women who were 7.5 years apart in age [10]. To date, there have been no other studies on the MIND diet and cognitive function. A possible explanation for the difference in the strength of associations as reported in the MAP study and the present study could be related to different population characteristics of the two cohorts. Compared to NHS participants, MAP study participants were on average ten years older than NHS participants, had a lower education level, mainly lived in retirement houses, were more likely to have ever smoked and more likely to have reported high blood pressure, diabetes and myocardial infarction. This would suggest that MAP participants were at higher risk of poor cognitive function and faster cognitive decline compared to NHS participants, resulting in a stronger association between the MIND diet and cognitive decline over time. Another explanation may relate to a difference in the cohort design, specifically the number and timing of dietary and cognitive assessments. First, the MAP study used 19 cognitive tests ten times over a period of nine years to compute a global measure of cognitive function and five cognitive domains, whereas the NHS used six cognitive tests four times during six years to construct a global measure of cognitive function and three cognitive domains. It could be that the MAP study was better able to detect differences in cognitive change with the more extensive cognitive assessment battery over a longer period of follow-up. Second, in the NHS study, we used all dietary assessments prior to the first cognitive assessment to create a measure of long-term dietary intake capturing changes in dietary intake over time, whereas the MAP study used the first dietary assessment to study cognitive change from that point forward. Hence, it could be that MAP study participants with good cognitive function at baseline may have reported healthier dietary intakes, which could have led to less decline in cognitive function at follow-up. On the other hand, those with poor cognitive function at baseline probably reported worse dietary intakes, resulting in a stronger association between the MIND diet and cognitive decline. These two issues could also explain why we were not able to find any association with cognitive change over time.

We additionally examined the relative contribution of individual components when excluding one component from the MIND score at a time, while including it as a covariate into the regression models. Using this approach, we observed that butter plus margarine and pastries plus sweets were the main components as part of the total MIND diet driving the association between the MIND diet

and verbal memory within the NHS population. So far, no other studies have taken into account the relative contribution of these components to cognitive function. Future research should further explore the relative contribution of components of dietary patterns to better understand underlying mechanisms.

There are several biological mechanisms by which the MIND diet could impact cognitive function. First, by keeping the intake low for items such as butter and margarine or pastries and sweets, the consumption of saturated fats and trans-fatty acids would be kept low, resulting in a better fat composition of the diet that can improve the blood-brain barrier function and decrease Aβ aggregation [27]. Second, in an Alzheimer's disease mouse model, higher intakes of long-chain n-3 fatty acids from fish has been shown to reduce Aβ formation and oxidative damage and increase synaptic proteins and dendritic spine density [28, 29]. Finally, the components of berries and green leafy vegetables provide high amounts of vitamin E, flavonoids, folate and carotenoids, which, in rodents, have been shown to have antioxidant and anti-inflammatory properties [30, 31] and to inhibit Aβ deposition [30, 32-35]. Thus, there is a solid biological basis that the MIND diet could impact brain health.

Genetically, carrying one or more copies of the *Apo*E ε4 is associated with an increased risk of Alzheimer's disease (AD) and cognitive function decline [11, 36]. Observational studies have demonstrated the largest cognitive decline among homozygous and an intermediate cognitive decline in heterozygous *ApoE* ε4 carriers after three years [37] and a more rapid decline in all cognitive domains in those carrying at least one *ApoE* ε4 allele [12]. This evidence supports the importance of finding strategies to prevent cognitive decline especially among *ApoE ε4* carriers. However, we were not able to demonstrate an interaction between the MIND diet and *ApoE* ε4 status. Additionally, adding the *ApoE* ε4 genotype to our fully adjusted model did not result in different or stronger associations. Other studies investigating an interaction between different dietary patterns and cognitive function have shown inconsistent findings, with studies reporting better cognitive function among non-carriers [13, 14], one study reporting an association among carriers [15], and three studies presenting no interaction [14, 16, 17]. Overall, no consistent conclusion on the role of the *ApoE4* ε4 allele between diet and cognitive function can be drawn and this requires further research.

This study has several limitations to consider. First, the FFQ did not specifically ask if olive oil was the primary source of fat, as this is not commonly consumed in this US population. As a best estimate, we chose to study the use of primarily vegetable oil instead of butter or margarine. Second, cognitive function was assessed by telephone assessment, possibly leading to some misclassification of cognitive function. A validation study, however, has shown that telephone-based cognitive battery performed well compared with detailed, in-person interviews (ρ = 0.81 comparing the two modes of assessment) [38]. Third, participants were relatively healthy and highly educated, limiting the generalizability of the results to other populations. It could be that a healthy diet is not so beneficial in the NHS population, whereas it may be more beneficial in the general population with more risk factors for poorer health and a lower educational level. Finally, we cannot exclude the possibility of residual confounding by unmeasured variables within this cohort, although we have adjusted for many potential confounders.

The relatively long follow-up allowed us to assess long-term associations between the MIND score and cognitive function. By averaging repeated measures of diet to obtain an assessment of long-term overall diet, we accounted for within-person change in intake and reduced measurement error (as previously documented by our group [39]). The same approach has been taken to limit measurement error in cognitive function assessments, by averaging all repeated measures into a more robust measure of cognitive function at older ages. The prospective nature of this analysis reduces the probability of recall bias and selection bias. In addition, a high follow-up rate reduced potential bias due to loss of follow-up. Nevertheless, our results need to be replicated in other populations at risk of poorer health to clarify the relevance of this association within populations at risk of cognitive decline.

To summarize, long-term adherence to the MIND diet, with high intakes of berries, green leafy vegetables and low intakes of pastries and sweets, was associated with a better verbal memory in later life, but not with global cognitive function, TICS score or cognitive decline.

Acknowledgements

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Chapter 7

A parallel randomized trial on the effect of a healthful diet on inflammageing and its consequences in European elderly people: design of the NU-AGE dietary intervention study

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Abstract

Background: The proportion of European elderly is expected to increase to 22% in 2050. Specific individual dietary components may reduce disease risk and improve quality of life in elderly. Combining dietary components may modulate many processes involved in ageing and so it is likely that a whole diet approach might have greater impact on age-related decline than individual components. This intervention studies the effects of a whole diet on health and quality of life in European elderly.

Methods: The NU-AGE study is a randomized one year intervention in 1250 apparently healthy, independently living European subjects aged 65 to 80 years. Subjects are randomised into either the diet group or the control group. Participants in the diet group received dietary advice aimed at meeting the nutritional requirement of the ageing population. Special attention was paid to nutrients that may be inadequate or limiting in diets of elderly.

Discussion: The NU-AGE study is the first dietary intervention investigating the effect of a whole diet and providing targeted nutritional recommendations for optimal health and quality of life in apparently healthy European elderly. Results of this intervention will provide evidence on the effect of a whole diet on the prevention of age related decline.

Introduction

The number of people aged 65 years and older is continuously increasing. By the year 2050 the proportion of elderly in Europe will be approximately 22% [1]. This demographic shift will most probably increase age-related diseases and disabilities; therefore it is of critical importance to find strategies that decrease morbidity by slowing down the ageing process and thereby increasing the number of European elderly in good health.

The ageing process is currently defined as a dynamic process involving the continual adaptation of the body to life-long exposure to internal and external stressors, conceptualised as the 'remodelling theory of ageing'[2]. It is a complex process involving interactions between environmental, stochastic and genetic-epigenetic variables. One of the basic molecular mechanisms of ageing is the development of a chronic, low-grade inflammatory status, also referred to as 'inflammageing' [3-5]. Inflammageing plays a role in the onset of age-related chronic diseases, such as atherosclerosis and type 2 diabetes mellitus, and geriatric conditions, such as sarcopenia, frailty and disability [3-5]. Of all variables involved in inflammageing, diet and lifestyle appear to be the most modifiable. Dietary patterns of the elderly can be inappropriate [6-11] and intake of vitamin D, folate, iron, vitamin B12 and calcium [12] are often below what is recommended.

Results from randomized controlled trials, meta-analyses and reviews have shown that certain foods and nutrients can reduce the risk of disease and improve the quality of life of the elderly. Monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), olive oil or oily fish have been shown to have beneficial effects on blood pressure, inflammatory processes and inflammatory bowel diseases, cognitive function, mood and depression [13-15]. Whole grains and some cereals positively influenced diabetes, BMI, cardiovascular disease and blood lipids [16-20]. To reduce the development of sarcopenia, meat and protein have shown to be important [21]. Other components frequently studied, such as fruits, vegetables, vitamin C, vitamin D, vitamin B12 and folate, improved nutritional status and markers of inflammation, beneficially affected type 2 diabetes mellitus [22-24], risk of falls [25], and were shown to improve bone health and reduce fractures [26-29].

Manipulating dietary patterns can be a more powerful approach than single nutrients or foods because these may have synergistic or antagonistic effects when consumed in combination [30, 31]. Many studies have shown that altering dietary patterns is more effective than effects of single dietary components on reducing disease risk [32-37]. A frequently studied dietary pattern is the Mediterranean diet. Observational and intervention studies have shown that the Mediterranean diet increases survival among older people [35, 37] and in patients with a history of myocardial infarction [33], reduces incidence of cardiovascular events in patients at risk [38], improves anthropometric and metabolic parameters in subjects aged 51-59 years [36], influences cardiovascular risk factors [34] and lowers risk of cognitive function decline in older individuals [32].

Besides the impact on survival [33], anthropometric and metabolic parameters [36], cardiovascular risk factors [34] and cognitive function [32], a whole diet approach might also have impact on the ageing process and inflammatory parameters [39]. However, apart from one study showing that components of the Mediterranean-type food pattern influence serum inflammatory markers [40] there appear to be no studies on the effects of a whole diet on the ageing process in an

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elderly population. Therefore NU-AGE focuses on nutrition as a major modulator of inflammageing and other age-related functional outcomes.

The NU-AGE dietary intervention will investigate whether a newly designed, personally tailored, whole diet, designed to meet the nutritional needs of people over 65 years of age can counteract or slow down the decline of function at the level of different organs and systems which occurs with age. This approach will allow an evaluation of the whole-organism response using a systems biology approach. It will consider several tissues and systems as a functional network instead of assessing the single tissue and organ responses separately.

Materials and methods

Study design

The NU-AGE project involves a consortium of 30 partners from 17 European countries. A detailed description of this project has been described in the paper of Santoro et al. in this special issue. The NU-AGE dietary intervention comprises a one-year, randomized, parallel trial with two arms – control and intervention - carried out in five European study centres (Clermont Ferrand in France, Bologna in Italy, Wageningen in the Netherlands, Warsaw in Poland and Norwich in the United Kingdom). Each centre aimed to recruit 250 subjects to end up with a combined total of 1250 subjects. Inclusion and exclusion criteria of eligible participants can be found in **table 1**.

Table 1. Inclusion and exclusion criteria for participants of the NU-AGE dietary intervention study

Inclusion criteria	Exclusion criteria
Men and women	Overt disease such as cancer or dementia
Aged 65 to 79 years	History of severe heart disease
Free of disease compromising 2–year survival	Unstable organ failure
Free and independent living	Organ failure necessitating a special diet
Competent to make own decisions	Diabetes Mellitus Type I
	Diabetes Mellitus Type 2 with insulin therapy
	Chronic use of corticosteroid medication
	Recent use of antibiotics
	Change in habitual medication use
	Malnutrition
	Presence of frailty

All participants signed informed consent prior to their inclusion in the study. Recruitment started in April 2012 and will continue until October 2013. We used screening questionnaires to verify that none of the exclusion criteria were present. In case of recent use of corticosteroids or antibiotics or change in use of habitual medication we postponed inclusion for 1, 2 or 3 months, respectively. Presence of frailty was assessed with a test described by Fried et al. [41] combining measures of height, weight, BMI, hand grip strength, gait speed, depression and physical activity. Participants were randomly allocated to the intervention or control group to a 1:1 ratio after stratification by gender, age (65-72 or 73-79 years), frailty status (pre-frail or non-frail) and BMI (<25 or ≥25 kg/m^2). Randomization was performed by entering the described variables of a subject into a program that automatically randomly allocates and

generates a unique ID-code. Participants were informed about their intervention group after the randomisation. Only technicians performing the laboratory analyses were blinded to the group assignment. All eligible participants were invited for baseline measurements at the research centre for a blood withdrawal, urine and faeces collection, anthropometric measurements, completion of a general questionnaire, DEXA-scan, physical performance and cognitive function tests. All measures were taken by trained research assistants. Trained dieticians/research nutritionists assessed dietary intake. After 4 and 8 months all participants were contacted by telephone to complete a follow-up questionnaire. After one year intervention all measurements were repeated (**table 2**). The NU-AGE study is registered with clinicialtrials.gov since December 21st 2012, NCT01754012. The study protocol is approved by the South-East 6 Person Protection Committee (France), Independent Ethics Committee of the S. Orsola-Malpighi Hospital Bologna (Italy), the Wageningen University Medical Ethics Committee (Netherlands), the National Research Ethics Committee - East of England (UK) and the Bioethics Committee of the Polish National Food and Nutrition Institute (Poland).

Table 2. Measurements over time (months)

Measurements	Timeline (months)			
	0	4	8	12
Blood	X			X
Urine	X			X
Faeces	X			X
Anthropometric measurements	X			X
DEXA-scan	X			X
Physical activity	X			X
Physical performance	X			X
General questionnaire	X			X
Follow-up questionnaire		X	X	X
Dietary intake	X	X[a]	X[a]	X

[a] Dietary intake at 4 month and month 8 is assessed only in participants in the diet group

Dietary intake assessment

Average usual food and nutrient intake of all participants at baseline and after one year follow-up was estimated from 7-day food records. These take into account the dietary pattern, infrequently consumed foods that can be important sources of specific nutrients, and portion sizes [42-44]. Within two weeks after baseline measurements records were collected by means of a paper-based self-administered pre-formatted food diary. This diary includes 8 meal occasions (before breakfast, breakfast, morning snacks, lunch, afternoon snacks, evening meal, evening snacks, night snacks) referring to the current day. Portion sizes of frequently consumed foods were estimated with real-life models. Other portion sizes were estimated in household measures, based on pictures or measured in gram or millilitres. Participants received oral and written instructions about the level of detail required to describe foods and amounts consumed, including the name of food, preparation methods, recipes for mixed foods and portion sizes [45]. Whenever possible, a trained dietician/research nutritionist visited the participants at their homes (in the Netherlands and the UK) to review the records and to check frequently used household measures; otherwise this meeting took place at the research unit (in France, Italy and Poland). If participants did not prepare the meals themselves, the one in charge of meal preparation was consulted. Dieticians/research nutritionists were familiar with basic rules of communication and

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interviewing, composition of local menus and standard recipes and encoding or entering of data. The consumed foods were coded according to standardized coding procedures and translated into nutrients by use of local food composition tables (NEVO 2011 in The Netherlands, WISP in The UK, INRAN and IEO in Italy, NFNI in Poland and CIQUAL French food composition table in France).

Dietary intervention

All participants had an interview with a trained dietician/research nutritionist to complete the 7-day food records. At the end of the interview participants randomised into the control group received only a leaflet with general national dietary guidelines that are freely available in France, Italy, the Netherlands, Poland and the United Kingdom [46-50]. Participants randomised to the diet group received individually tailored dietary advice aiming to meet on average the nutritional requirements for the elderly. The advice was based on FBDG's and recommended daily allowances (RDA's) from the countries of the 5 participating study centres [46, 48, 51-56], on the modified MyPyramid for Older Adults [57, 58], European Commission [59] and Institute of Medicine [60] to ensure no risk of inadequate or excessive intakes in the diet group. Adequacy components of the NU-AGE diet included consumption of 4-6 servings of whole grain products per day (25g of bread or 50g breakfast cereal/serving); 2 servings of fresh fruits per day (including fresh fruit juices, dried fruits and frozen fruits); 3 servings of vegetables per day (100g of salad or cooked vegetables/serving and 2 servings of legumes per week (100g/serving); up to 500g low-fat calcium-enriched dairy per day (of which 30g of low-fat cheese); 2 servings of fish or seafood per week (125g/serving, at least one serving of fatty fish); 4 servings of lean meat or poultry per week (125g/serving); 2 or more servings nuts per week (20g/serving); 3 servings of potatoes (50g/serving), or 1 serving of whole-grain rice, or 1 serving pasta per day (80g/serving) of which at least 2 times per week whole grain rice or pasta; 2-4 eggs per week; total oils and fat consumption of 50g per day (of which at least 20g olive oil and a maximum of 30g margarine rich in MUFA and PUFA); alcohol consumption of maximum 2 glasses per day for men and 1 glass per day for women; 1500 ml of fluids. Components of the NU-AGE diet that were restricted included salt where intake was reduced to 5g per day (eliminate or limit adding salt and ready-made meals) and sweets and sweet drinks (e.g., sweet pastries, cookies, carbonated and/or sugared beverages).

The dietary advice was based on individual education using the Motivational Interview technique taking into account the Stages of changes theory model and goal setting [61]. To improve compliance to the NU-AGE diet this individually-tailored dietary advice, either given face-to-face or by telephone, was administered nine times during one year and supported by mail or e-mail (**table 3**). A trained dietician/research nutritionist adapted the dietary advice to personal situations and dietary preferences, habits and beliefs.

Table 3. Frequency of dietary advice

	Timeline (months)												
	0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Control	DA				T				T				DA
Intervention	DA	T/E	E	T/E	FR/V	T/E	E	T/E	FR/V	T/E	E	T/E	DA

DA = Dietary Assessment (7-day food records), including leaflet containing general healthy eating guidelines for the control group and motivational interview fostering the NU-AGE diet for the diet group. E = e-mail with recipes, daily menus; T = Telephone contact for motivational interview; FR/V = 3-day food records prior to study centre Visit to verify compliance by the dietician/research nutritionist and for motivational interview.

To standardise the dietary counselling each dietician/research nutritionist followed the same process (**table 4**). The dietary counselling covered a total of fifteen dietary goals, including the vitamin D supplement. During the first meeting (month 0) the dietician/research nutritionist and participant agreed on 5 dietary goals to work on. After one month, the participant could choose 5 other dietary goals to work on until month 3. At month 3, the dietician/research nutritionist called the participant to evaluate the first 10 goals and to implement the last set of 5 dietary goals. To measure compliance participants were asked to keep 3-day food records (3d-FR's) before month 4. Based on the 3d-FR, months 5, 6 and 7 were used to optimise compliance to the NU-AGE diet. Compliance was measured again in month 8 with 3d-FR's. Months 9, 10 and 11 were used to motivate the participant to continue the NU-AGE diet. In between, additional information was sent out on topics that were considered important to achieve the NU-AGE FBDG's (e.g. fish consumption, food labelling, fruit and vegetable consumption, variation in foods, protein intake). Finally, at month 12 habitual intake and compliance was measured by means of 7-day food records.

Meeting NU-AGE's FBDG's by means of a normal diet can be a challenge in terms of financial resources and food availability. Therefore, participants in the diet group received commercially available foods meeting the criteria of the NU-AGE dietary guidelines. Most products - cheese, wholegrain pasta, olive oil and margarine - were provided for free and to all study centres to obtain diets as similar as possible across all five study centres. Italy additionally provided vegetable soup to their participants. If a product could not be included in the diet, a similar product was chosen in consultation with the dietician/research nutritionist. Each participant in the diet group received their first allocation of products – sufficient for the first four months – at the first meeting with the dietician/research nutritionist. During the research unit visit after 4 and 8 months the participants received a new allocation of products.

It is well-known that achieving an adequate vitamin D intake from diet alone is merely impossible [53, 62] because vitamin D is only present in a limited number of foods and the vitamin requirement for elderly is set at a high level. To ensure the NU-AGE diet reached the recommended vitamin D intake of 15-20 µg, participants in the diet group received a vitamin D supplement of 10 µg produced by MCO Health, the Netherlands. Each participant in the diet group received a bottle containing vitamin D supplements for 4 months during the first meeting with the dietician/research nutritionist. After 4 and 8 months during the research unit visits another bottle of vitamin D was provided to the participants in the diet group. The compliance was evaluated by counting the remaining vitamin D capsules, which were returned simultaneously with the 3d-FR at month 4 and 8.

Compliance in all participants was enhanced through newsletters, contact via e-mail and with regular telephone calls - bimonthly for participants in the diet group and once per 4 months for participants in the control group.

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Table 4. Structure and main topics per contact moment

Month	Occasion	Topic
0	Home visit or Research Unit visit	Assess dietary intake (7–day estimated food records)
		Agree on first set of five dietary goals (vitamin D supplement should at least be included)
		Hand out information of the first set of 5 dietary goals and hand out vitamin D supplement plus products
1	Telephone	Evaluate first set of five dietary goals and agree on second set of five dietary goals for the next month
	E-mail/post	Send out tips and/or recipes for second set of five dietary goals
2	E-mail/post	Send out additional information of the first two sets of five dietary goals and about **fish consumption**
3	Telephone	Evaluate second set of five dietary goals and agree on third set of five dietary goals
		Explain keeping the 3d-FR and make an appointment for the Research Unit visit in month 4
	E-mail/post	Send out information regarding the third set of dietary goals and the 3d-FR plus explanation
4	Research Unit visit	Receive bottle with remaining vitamin D tablets and go through the 3d-FR.
		Evaluate compliance to the dietary goals and agree on goals that need extra attention.
		Hand out the vitamin D supplement and foods for the next 4 months plus info about goals that were not met yet.
		Hand out additional information regarding **food labelling, recipes, variation**
5	Telephone	Evaluate goals that needed more attention and determine which goals need extra information/attention
	E-mail/post	Send out appropriate info sheet belonging to goals that need extra attention/ were not obtained.
6	E-mail/post	Send out additional information regarding **fruit and vegetable consumption**
7	Telephone	Evaluate goals that needed more attention and determine which goals need extra information/attention
		Explain keeping the 3d-FR and make an appointment for the Research Unit visit in month 8
	E-mail/post	Send out additional information regarding goals that were not met yet and the 3d-FR plus explanation
8	Research Unit visit	Receive bottle with remaining vitamin D tablets and go through the 3d-FR.
		Evaluate compliance to the dietary goals and agree on goals that need extra attention.
		Hand out the vitamin D supplement and foods for the next 4 months plus info about goals that were not met yet.
9	Telephone	Evaluate goals that needed more attention and determine which goals need extra attention during the last months
	E-mail/post	Send out appropriate info sheet belonging to goals that need extra attention/ were not obtained.
10	E-mail/post	Send out additional information regarding **protein intake**
11	Telephone	Evaluate goals that were not met yet and agree on goals that need extra attention in the last month
		Explain keeping the 7d-FR and make appointment for the Research Unit visit in month 12
	E-mail/post	Send out the 7d-FR plus explanation
12	Home visit or Research Unit visit	Assess dietary intake (7-day Estimated Food Record)

Topics in bold are additional information regarded as important for compliance to NU-AGE FBDG's

Standardisation and training

In order to perform all measurements in the same standardized way across the five study centres, all questionnaires, procedures and tests were practiced and discussed during a training session prior to the start of the intervention. These training sessions took place in December 2011 in Warsaw and in January 2012 in Wageningen. From each study centre at least one research assistant was required to attend this training. Based on these training sessions standard operating procedures were developed and standardized across all research centres.

Outcome measures

Several health outcomes were measured to investigate whether adhering to the NU-AGE diet impacts on age-related diseases and functional decline, and thereby contribute to improved health and quality of life in the ageing population in Europe.

　　　The primary outcome measure of the intervention is inflammatory status measured as plasma levels of C-reactive protein (hsCRP). Secondary measures of inflammatory status include plasma levels of IL-1Beta, IL-12, INF gamma, IL-6, sIL-6R, IL-1RA, TNFalpha, IL-17, IL-8, IL-10 and TGF-beta1.

　　　Additionally, a limited number of basic parameters for describing the function of several domains, e.g. metabolism, bone, heart, muscle, intestine, cognition, mood, anthropometry were measured before and after dietary intervention. These parameters include measures of insulin sensitivity, liver function status, hormonal function status, bone health, cardiovascular health, cognitive status, mental health, quality of life, physical functioning, digestive health, anthropometry and measures of nutritional status. Also genetic analysis will be performed on DNA to characterize volunteers for polymorphisms involved in nutrition, ageing and inflammageing, in order to explain the individual variability of the effect of dietary intervention. Further details on the outcome measurements can be found in the paper of Santoro et al. in this special issue.

Discussion

To the best of our knowledge, the NU-AGE study is the first dietary intervention study investigating the effect of a whole diet targeted on the nutritional requirements of the elderly with respect to optimal health and quality of life in an apparently healthy elderly European population. Currently, dietary advice specifically adapted to the elderly population is not standard included in public health care practices in Europe. Therefore this intervention is an excellent opportunity to investigate the effect of a whole diet approach on the prevention of ageing related decline in a European ageing population across cultural differences. Positive findings from this study might prevent age-related decline and so facilitate longer healthy lives in the elderly. Implementation of structured dietary advice in health care of the elderly might therefore contribute to a reduction in health-care related costs.

　　　Adequate dietary intakes of individual macro- and micronutrients and food groups have been shown to positively influence several organ systems and diseases, [13, 15, 16, 18, 19, 21, 26, 63] but until now, there are not many studies investigating the effect of a whole dietary intervention on inflammageing and overall age-related decline at the cellular, organ and whole body level. There have been studies showing positive effects of certain dietary patterns, such as the Mediterranean diet, on

cardiovascular health [64-66], metabolic syndrome [67, 68], survival [37] and mortality [35]. However, none of these studies investigated health in such a broad way as the NU-AGE study. Therefore the broad range of outcomes measured in the NU-AGE study is unique, and will clarify the potential synergistic effects of many different food constituents, and the interaction between the different organ systems.

We expected the effect of a whole dietary intervention to be most effective and efficient in generally healthy elderly. We therefore only included elderly people without major medical conditions and with a maximum frailty score of one [41]. As a consequence, findings from this study in generally healthy subjects cannot be generalized to the total elderly population which includes less healthy and more fragile elderly. Another reason that results cannot be generalised is the fact that potential participants were informed in advance that there was a chance that they would have to change their dietary intake depending on the randomisation. This could have contributed to the inclusion of health-conscious individuals who have an above average interest in diet and health.

Participants in the diet group were given frequent dietary advice, foods and a vitamin supplement aiming to meet the nutritional requirements of the elderly. Because it is practically not possible to provide participants in the control group with placebo advice and placebo foods they were only given a leaflet with general dietary guidelines instead. As a consequence the diet group does not only differ in dietary intake and advice but also in received attention compared to the control group. This could result in a better rating of health in this group, rather than an improvement in their diet. In order to reduce this bias to some extent participants in both control and diet group were assessed every 4 months and fulfilled a follow-up questionnaire to check for health status, unexpected events and treatment changes.

The major strength of this study is its whole diet approach specifically adapted to target the nutritional requirements for the elderly with a strong Mediterranean Diet influence. This allows us to study the synergy of multiple nutrients and adequacy of single nutrients. Although nutritional requirements for elderly people in all five participating countries have been merged into NU-AGE dietary guidelines flexibility was allowed for country specific food based dietary habits.

Other strengths include the use of Motivational Interviewing in the dietary counselling. Motivational Interviewing focuses on exploring and resolving ambivalence and centres on motivational processes within the individual that facilitate change [61]. Quarterly administration of a motivational interview has been shown to improve adherence to a Mediterranean-type diet [66]. By administering this interview nine times during one year we strived for an optimal compliance with the dietary advice.

The provision of foods and a vitamin supplement were also used to improve compliance. It has been shown that free provision of foods is effective in improving dietary habits [66, 69, 70]. Both the counselling and free provision of foods most likely will result to the best possible strategy to promote dietary change in participants in the diet group.

The one year duration of the dietary intervention is long enough for participants in the diet group to change their dietary habits over time, and avoids any seasonal-dependent changes in outcome measures. A nutrition education trial of 15 weeks and a primary prevention trial of 12 months have both been shown to be effective in improving dietary intakes [36, 66]. Within six months and one year respectively, it would also be possible to measure changes in health outcomes such as a reduction in systolic blood pressure [71] and change in reduction of bone mineral density [72]. Within even five

weeks of consuming a Mediterranean Diet it has shown to be possible to reduce inflammatory status (CRP) with 26.1% in men with metabolic syndrome [73].

The use of 7-day food records instead of a dietary history or 24-hour recall is another strength of this study. Keeping 7-day food records does not rely on the memory of the elderly participants thus minimising the possible recall bias [45]. Also keeping 7-day food records helps participants to become aware of what they eat and represents a useful tool to encourage participants in the diet group to change dietary habits.

The NU-AGE dietary intervention study is the biggest randomized trial ever conducted on the effect of a whole diet targeting the nutritional requirements of elderly and counteracting inflammageing, age related decline and contributing to increase the healthy lifespan in the elderly

Acknowledgments

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Chapter 8

Changes in dietary intake and adherence to the NU-AGE diet after a one-year dietary intervention among European older adults

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Abstract

With the ageing population, it is important to find modifiable strategies that slow down the ageing process and its consequences. One proposed strategy to postpone the development of age-related chronic diseases is improving nutrition. We evaluated if Mediterranean-like diet could be effective to shift dietary intake of older adults towards a more healthful diet. Apparently healthy older adults aged 65-80 years, living in Italy, the UK, the Netherlands, Poland, and France were randomly assigned to an intervention group or control group. The intervention group followed one year of dietary intervention on a Mediterranean-like diet uniquely designed to target dietary recommendations for the ageing population (NU-AGE diet). The NU-AGE diet specified the consumption of 15 food groups and relative portions plus the use of a vitamin D supplement. During one year, participants in the intervention group received counselling and individually tailored dietary advice, food products (e.g., whole grain pasta, extra-virgin olive oil, margarine high in monounsaturated and polyunsaturated fatty acids, low fat cheese) and a vitamin D supplement. Participants in the control group only received a leaflet with general national dietary guidelines. At baseline and after one-year follow-up dietary intake was assessed by means of 7-day food records. In total, 1,296 participants were randomized and 1,141 participants completed the intervention (571 intervention, 570 control). After one-year follow-up, the intervention group improved mean intake of 13 out of 16 dietary recommendations of the NU-AGE diet ($p < 0.05$) with the greatest magnitude of improvement observed for the consumption of wholegrains, (79%), legumes (70%) and nuts (49%) with a significant increase of the total NU-AGE index, compared to the control group (mean change in NU-AGE index 21.3 ± 15.9, $p < 0.01$). In conclusion, the NU-AGE dietary intervention, based on dietary recommendations for older adults may be a feasible strategy to improve dietary intake in an ageing European population.

Introduction

With the ageing population [1], it is important to find modifiable strategies that slow down the ageing process and its consequences in order to increase the number of years in good health in European elderly.

The ageing process can be described as a chronic low-grade inflammatory process, which is referred to as inflammageing. Inflammageing impacts many organ systems and domains and plays a role in the onset of age-related chronic diseases [2-4]. One proposed strategy to postpone the development of age-related chronic diseases is improving nutrition. Evidence shows, that especially monounsaturated fatty acids, polyunsaturated fatty acids [5, 6], protein [7], vitamin D [8], vitamin B12 [9], folate [10], and anti-oxidants [11] can impact age-related health outcomes.

At the same time, it is known that with increasing age, elderly people are at higher risk of low nutrient intakes [12]. At the food group level, diets of elderly are lacking in multiple food groups, such as fruit, vegetables, and fish [13]. On the other hand diets of elderly are exceeding the requirements for saturated fatty acids [13]. Manipulating dietary patterns can be a more powerful approach than changing single nutrients or foods, because these may have synergistic or antagonistic effects when consumed in combination [14, 15]. Many studies have shown that altering dietary patterns is more effective than altering single nutrients on reducing disease risk [16-21].

To bridge the gap between current dietary intakes and a healthful pattern in an ageing population, the NU-AGE study was started. The NU-AGE study aimed to improve health of the ageing population by means of a dietary intervention specifically targeting the dietary recommendations of older adults [22].

The aim of the current study was to evaluate if the NU-AGE dietary intervention is effective to shift the dietary intake of apparently healthy elderly subjects more towards the NU-AGE guidelines for a healthful diet. Additionally, we evaluated if adherence to the NU-AGE guidelines is comparable at five European sites involved within the NU-AGE study.

Materials and methods

Study design

This study was performed using baseline and follow-up data of the NU-AGE dietary intervention study. The NU-AGE study is a one-year, randomized, parallel trial to investigate whether a newly designed, personally tailored diet, designed to target dietary recommendations of people over 65 years of age can counteract or slow down the decline of function at the level of different organs and systems. The study was carried out in five European study centres (Bologna in Italy, Norwich in the UK, Wageningen in the Netherlands, Warsaw in Poland, and Clermont-Ferrand in France). Recruitment started in in April 2012 and finished in January 2014 including 1,296 apparently healthy European men and women aged 65-80 years. The rationale and design of this intervention study are described in detail elsewhere [22, 23]. In short, participants completed questionnaires about their health and lifestyle and a 7-day food record to obtain information about their dietary intake. Tests were conducted to measure a wide range of health outcomes. All questionnaires and measurements were repeated after one year. The study protocol was approved by local medical ethics committees at all study sites and the NU-AGE study is registered with clinicialtrials.gov since December 21st 2012, NCT01754012.

In the present study, we excluded those who had not completed both 7-day food records at baseline and follow-up (n=145), those with missing data on vitamin D supplement use (n=3), incomplete diet data (n=4) and those with an energy intake below 500 kcal (n=0) or exceeding 3500 kcal (n=3)[32], resulting in a total population for analysis of 1,141 participants (**figure 1**) of which 241 participants were from Italy, 252 from the UK, 241 from the Netherlands, 220 from Poland, and 187 from France.
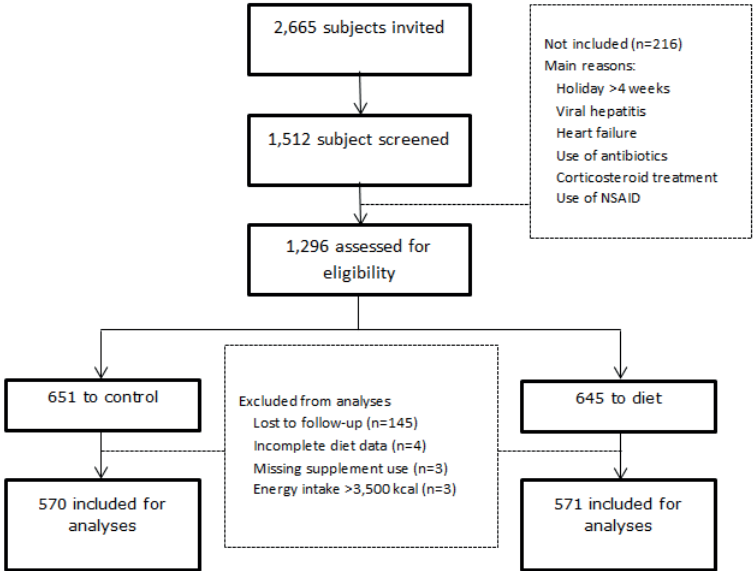

Figure 1. Flow chart of recruitment process NU-AGE participants

Dietary intervention

Participants randomized into the intervention group received monthly counselling from a trained dietician/research nutritionist and individually tailored dietary advice aiming to meet the NU-AGE Food Based Dietary Guidelines (FBDG's) (**table 1**). To achieve changes in dietary intake by means of intrinsic motivation the dietary advice was based on the Motivational Interview technique [24] and the Stages of Changes model [25]. Dietary advice was provided nine times during one year, either face-to-face or by telephone [22] using standardized procedures. To sustain compliance to the NU-AGE FBDG's, participants in all five study centres received commercially available foods meeting the NU-AGE dietary guidelines, namely low-fat and low-salt cheese, wholegrain pasta, olive oil and margarine rich in monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). Italy additionally provided vegetable soup to their participants. Additionally, participants received a vitamin D supplement, because it is not possible to achieve an adequate vitamin D intake from diet alone [26, 27]. Participants in the control group received a leaflet with national dietary guidelines that are generally available in Italy [28], the United Kingdom, the Netherlands [29], Poland [30] and France [31].

Table 1. Components and scoring of the NU-AGE diet index

Component	Servings	Minimum score (0)	Range (1-10)	Maximum score (10)	Upper range (10-1)
			Scoring		
Wholemeal bread, wholegrain pasta and rice	4-6 servings/day (140-210g/day) Rice/pasta 2x80 g/week (23g/day)	max	1-163g	163-233 g	233-max
Fruits[a]	2 servings/day (240 gram)	0g	0-240g	≥240	
Vegetables	300 gram/day	0g	0-300g	≥300g	
Legumes	200 gram/week (29g/day)	0g	0-200g	≥200g	
Low-fat dairy	500 ml /day	0g	0-500g	≥500g	
Low-fat cheese	30 gram/day	0g	0-30g	≥30g	
Fish	2 times 125g/week (36g/day)	0g	0-36g	≥36g	
Low-fat meat and poultry	4 times 125g/week (71g/day)	Max	0-71g	71-125	125-max
Nuts[b]	2 times 20g/week (6g/day)	0g	0-6g	>6g	
Eggs	2-4/week (14-26g/day)	0g	0-14g	>14g	
Olive oil	20 gram/day	0ml	0-20ml	≥20ml	
Alcohol	2 for men 1 for women	>10g women >20g men		≤10 women ≤20 men	
Fluid	1.5 litre/day	<1000ml	1000-1500ml	>1500ml	
Salt	5 grams/day (1200 mg sodium)	≥85th	0-1500 mg	1500-2000mg	2000-85th
Sweets	Limited use	≥85th		0	0-85th
Vitamin D	Use supplement	No		Yes	

[a] Fruit: maximum 1 glass of fresh fruit juices (120 ml) can be counted as one portion of fruit.
[b] Nuts: includes salted and unsalted nuts

Dietary assessment

Dietary intake was estimated by means of 7-day food records completed by the participants. During a one hour interview with dieticians the food record was checked to obtain more detailed information about types of foods, preparation methods and recipes and to estimate portion sizes by using real-life models and pictures. Consumed foods were coded according to standardized coding procedures and translated into nutrients by use of local food composition tables (NEVO 2011 in The Netherlands [33], WISP in The UK, INRAN and IEO in Italy, NFNI in Poland and CIQUAL French food composition table in France). Data on supplement use are obtained by means of a self-reported supplement questionnaire and checked by a trained dietician/research nutritionist.

Other variables

Data on smoking status (current, former), educational level (years), employment (defined as 1) employed, 2) retired, or 3) other, and medical history (prevalence of hypertension [yes/no], heart disease [yes/no], diabetes mellitus type II [yes/no], hypercholesterolemia [yes/no], neurological diseases [yes/no], osteoporosis [yes/no]) were obtained by means of questionnaires. Frailty status (non-frail/pre-frail) was assessed using the frailty criteria from Fried et al. [34]. Height was measured

with a stadiometer to the nearest 0.1 cm. Weight was measured to the nearest 0.1 kg with a calibrated scale while wearing light clothes. Body Mass Index (BMI) was calculated as weight/height^2. All measures were taken by trained research assistants.

NU-AGE index

Food based dietary guidelines for the elderly population
The NU-AGE index is meant to reflect adherence to guidance based on nutrient reference values and food based dietary guidelines for elderly from Italy [28], the UK [35], the Netherlands [26, 36-39], Poland [30], and France [40], on the modified MyPyramid for Older Adults [41, 42], and nutrient requirements from the European Community [43], and from the Institute of Medicine [44]. These recommendations were jointly integrated into NU-AGE food based dietary guidelines in order to inform participants on a food level on what they should consume (**table 1**). The NU-AGE index comprises recommendations of minimum amounts to consume for fruits, vegetables, legumes, low-fat dairy, low-fat cheese, fish, low-fat meat and poultry, nuts, olive oil, fluids, and vitamin D (from a supplement). Two recommendations give a definition of minimum and maximum intake frequencies for whole grains and eggs and three recommendations refer to components to limit (alcohol, salt and sweets).

Definition of food groups
Specific NU-AGE food groups were created based on the underlying principles of the NU-AGE diet (e.g. low-fat, low-sodium, high MUFA and PUFA, high fibre), using country-specific food grouping systems as starting point (e.g. INRAN from Italy, WISP from the UK, NEVO from the Netherlands, DIETA-5 from Poland, and SUVIMAX from France). To reach consensus on the NU-AGE food groups, expert meetings with trained dieticians/research nutritionists from all five study centres were held. All individually consumed foods were grouped into NU-AGE food groups in each country, resulting in comparable food groups across countries.

Choice of cut-offs and scoring
A continuous scoring system was created to assign participants a score according to their level of adherence to each of the 16 NU-AGE components. This continuous scoring system allows us to better observe changes in dietary intake compared to a dichotomous scoring system [45]. Cut-off values for each NU-AGE component were based on the NU-AGE food based dietary guidelines. For the adequacy components (whole grains, fruits, vegetables, legumes, low-fat dairy, low-fat cheese, fish, low-fat meat and poultry, nuts, olive oil, fluids) a score ranging from 0 to 10 could be obtained for greater intakes of a specific food component. For moderation components (alcohol, sodium and sweets) participants with lower intakes received 10 points, ranging to 0 points for participants with greater intakes. For several components an maximum level of intake was established based on the country-specific population's intake distribution (i.e., 85th pct. for sodium and sweets, 100th pct. for whole grains and low-fat meat). In total, the NU-AGE index ranged from 0 to 160 points, ranking participants according to their compliance to the NU-AGE diet. Components and their cut-off values are shown in **table 1**.

Statistical analyses

Population characteristics are reported as mean and standard deviation (SD), or percentages (number). To compare nutrient intake changes from baseline to follow-up and differences in changes between intervention groups, a t-test was used, and the chi-squared test was used for vitamin D supplement use. The magnitude of differences in change in dietary intake was calculated as the percentage in change from baseline intake in the diet group. Two categories of adherence to the NU-AGE guidelines at follow-up were established; the first if the intake fell below the NU-AGE guideline and the second if the intake was equal or greater than the guideline.

All statistical analyses were carried out using SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA). A two-sided *p*-value of <0.05 was considered significant.

Results

The mean age of NU-AGE participants was 71.0±4.0 years, with a BMI of 26.7±3.9, and 45% were men (**table 2**). History of hypertension was most frequently reported in both control and intervention group (39% and 42% respectively) and 18% of the control group were pre-frail compared to 24% in the intervention group.

Table 2. Baseline characteristics of the NU-AGE participants by intervention group (n=1,141)

	Control (n=570)	Intervention (n=571)
Men	262 (46.0)	248 (43.3)
Age, years	71.1±3.9	70.7±4.0
Current occupation[a]		
Employed	19 (3.4)	24 (4.2)
Retired	540 (94.7)	541 (94.7)
Other	11 (2.1)	6 (1.1)
Education, years	12.6±3.6	12.7±3.6
BMI, kg/m^2	26.7±3.7	26.7±4.1
Physical activity, pase score	135.1±57.4	132.5±54.9
Multivitamin use, yes	67 (11.8)	86 (15.1)
Nutrient intake		
Energy, Kcal	1899±448	1854±432
Carbohydrates, en%	46.9±7.9	47.3±7.2
Protein, en%	16.3 ± 2.4	16.5±2.8
Fat, en%	34.3±5.4	34.1±5.5
Current smokers	21 (7.9)	26 (9.7)
Pre-frail	101 (17.8)	137 (24.0)
Prevalence of diseases		
Hypertension	222 (39.0)	242 (42.4)
Heart disease	97 (17.0)	94 (16.5)
Diabetes Mellitus type II	26 (4.6)	29 (5.1)
Hypercholesterolemia	142 (24.9)	154 (27.0)
Neurological disease	12 (2.1)	17 (3.0)
Osteoporosis	59 (10.4)	70 (12.3)

Values are mean SD or number (percentage), en% = energy percent
[a] Current occupation was defined as 1) employed (including part-time, 2) full-time and self-employed),
3) retired (including fully retired and semi-retired), or 4) other (including disability pension and other)

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Changes in dietary intake by intervention group
Baseline mean dietary intake did not differ between the two treatment groups (e.g., NU-AGE index was 82.6±16.5 in both the control and intervention group (range 36-132), except for low-fat dairy (155±155g versus 173±163g, respectively, **table 3**).
A significant increase in the NU-AGE diet score was found in the intervention group compared to the control group (mean change 21.3g (±15.9), p<0.01, (**table 3**). Dietary intake of thirteen out of sixteen NU-AGE guidelines improved in the intervention group compared to the control group (p <0.05), except for low-fat meat and poultry (p=0.53), eggs (p=0.16) and sodium (p=0.34). Major differences in changes expressed as percentages of dietary intake in the intervention group compared to the control group were observed for whole grains (79%), legumes (70%), nuts (49%), low-fat cheese (46%), fish (42%), low-fat dairy (30%), and olive oil (29%) (**figure 2**).

Adherence to the NU-AGE guidelines by intervention group
After one year intervention, the number of NU-AGE guidelines to which participants in the intervention group completely adhered to was 6.2±1.9 (range 1-12) compared to 4.5±1.7 out of 16 guidelines in the control group. The highest percentage adherence in both control and intervention group was obtained for fluid (90% and 94%, respectively), followed by alcohol (77% and 81%, respectively) and eggs (54% and 67%, respectively) (**figure 3**). The largest differences in percentage adherence to the guidelines between the intervention and control group was observed for vitamin D (69%), fish (18%) and nuts (16%). For low-fat meat and poultry the intervention group had a lower adherence at follow-up compared to the control group, possible demonstrating an overall reduction in meat intake.

Changes in dietary intake by country
At baseline, the mean NU-AGE index of participants was 84.6±15.3 in Italy, 80.8±14.0 in the UK, 74.5±15.6 in the Netherlands, 85.4±15.9 in Poland and 89.8±14.4 in France (*data not shown*). Within each country, baseline dietary intake for the control and intervention group were similar (**table 4**), whereas between countries baseline dietary intakes were different (e.g. wholegrains 30.8±42.9g in Italy vs. 74.2±61.1g in Poland, vegetables 165±83.9g in the Netherlands vs. 282±170g in Poland, low-fat dairy 74.0±93.1g in Poland vs. 264±162g in the UK, olive oil 16.8±8.5g in Italy vs. 2.4±3.5g in the Netherlands). Overall, the NU-AGE diet score significantly improved in intervention diet group compared to the control group in all countries (p<0.01). Of all study centres, French participants in the intervention group changed 12 out of 16 NU-AGE guidelines, followed by the Netherlands (11 out of 16), Italy and Poland (8 out of 16), and the UK (6 out of 16).

Table 3. Changes in dietary intake of NU-AGE diet components in 1,141 participants in the NU-AGE study

NU-AGE components	Control group (n=570)				intervention group (n=571)				Difference	p
	Baseline	Follow-up	Change	p	Baseline	Follow-up	Change	p		
Whole grains	54.4±53.9	62.6±60.7	8.1±57.4	0.02	55.7±58.3	107.2±66.4	51.5±62.5	<0.01	43.3±64.7	<0.01
Fruits	260.0±158.7	255.7±154.0	-4.3±156.3	0.64	248.2±140.2	268.2±140.0	20.1±140.1	0.02	24.4±127.3	<0.01
Vegetables	221.4±120.7	213.2±125.7	-8.2±123.2	0.26	214.5±110.8	234.2±103.7	19.7±107.3	<0.01	27.9±110.6	<0.01
Legumes	11.1±20.0	10.8±19.2	-0.3±19.6	0.77	10.4±20.9	17.6±21.9	7.2±21.5	<0.01	7.5±25.6	<0.01
Low-fat dairy	155.0±154.6	158.2±177.5	3.2±166.4	0.74	173.0±163.2	230.3±174.4	57.3±168.9	<0.01	54.1±131.8	<0.01
Low-fat cheese	11.9±21.9	11.6±19.6	-0.39±20.8	0.75	12.7±26.6	17.6±24.9	4.9±25.8	<0.01	5.3±23.1	<0.01
Fish	28.4±29.3	24.9±23.2	-3.5±26.4	0.02	28.4±25.3	37.1±28.1	8.7±26.7	<0.01	12.3±32.4	<0.01
Low-fat meat and poultry	41.2±33.4	40.5±31.4	-0.71±32.4	0.71	40.5±31.6	38.5±27.9	-2.0±29.8	0.26	-1.3±34.7	0.53
Nuts	6.4±12.8	6.1±9.9	-0.31±11.4	0.65	5.7±10.7	8.3±9.4	2.5±10.1	<0.01	2.8±11.1	<0.01
Eggs	17.5±17.0	17.3±17.2	-0.13±17.1	0.90	17.3±17.6	18.7±13.9	1.4±15.9	0.13	1.5±18.4	0.16
Olive oil	9.4±9.9	8.9±9.9	-0.39±9.9	0.50	9.1±9.2	11.3±8.9	2.2±9.1	<0.01	2.6±8.0	<0.01
Alcohol	9.6±10.9	8.9±10.9	-0.66±10.9	0.31	8.8±10.3	7.2±9.1	-1.6±9.7	<0.01	-0.92±6.6	0.02
Fluid	2311±654	2282±651	-28.8±652	0.46	2329±671	2482±657	154±664	<0.01	182±469	<0.01
Sodium	2628±1365	2455±965	-173±1182	<0.01	2554±1500	2309±946	-245±1254	<0.01	-72.1±1271	0.34
Sweets	84.1±113.7	76.3±102.3	-7.8±108.1	0.22	87.2±103.5	63.6±82.8	-23.6±93.7	<0.01	15.8±91.7	<0.01
Vitamin D, n (%)	81 (14.2)	138 (24.2)	75 (10)	<0.01	84 (14.7)	529 (92.6)	445 (77.9)	<0.01	370 (67.9)	<0.01
NU-AGE index	82.6±16.5	84.6±16.1	1.9±16.3	0.05	82.6±15.3	105.7±17.6	23.1±16.2	<0.01	21.3±15.9	<0.01

Values are mean values and standard deviations, unless otherwise indicated.
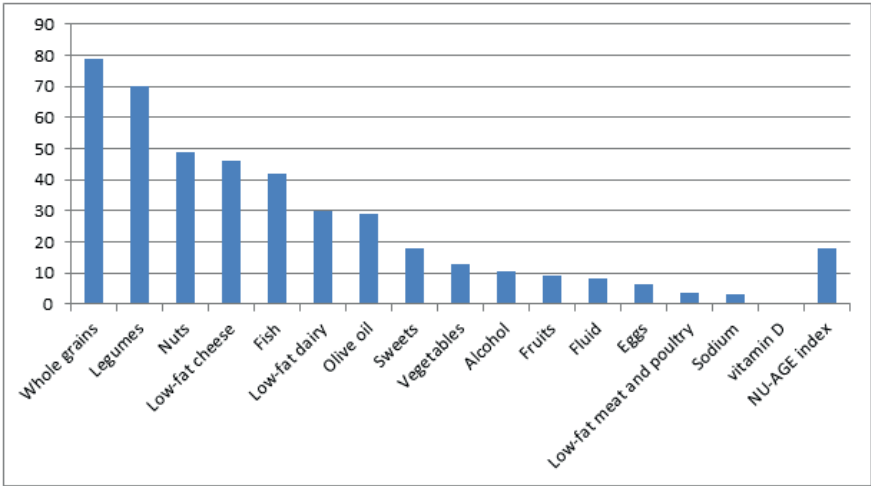
8

Figure 2. Percentage of differences in change from baseline to follow-up in the intervention group compared to the control group
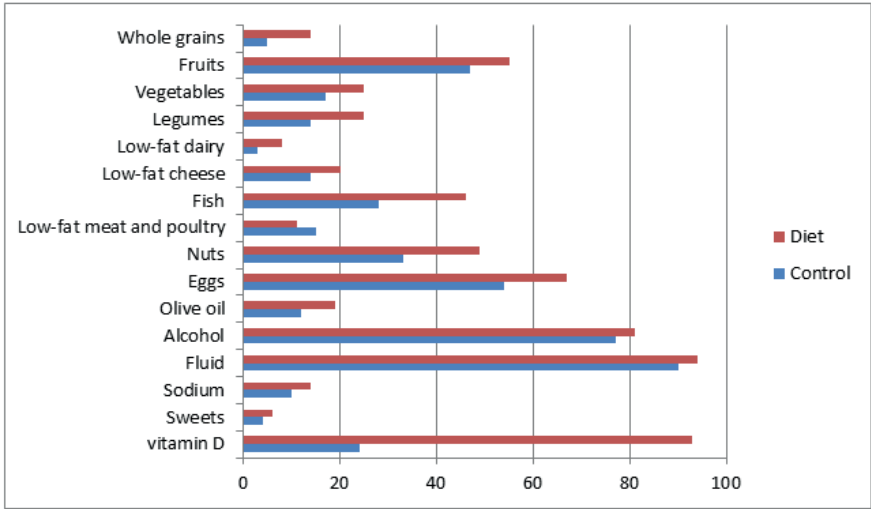

Figure 3. Percentage of participants in the control and intervention group meeting the NU-AGE guidelines

Table 4. Mean baseline and changes in dietary intake of NU-AGE diet components by intervention group stratified by study centre (n=1,141)

NU-AGE Components	Group	Italy (n=114 control, 127 diet)		UK (n=126 control, 126 diet)		Netherlands (n=124 control, 117 diet)		Poland (n=106 control, 114 diet)		France (n=100 control, 87 diet)	
		Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
Wholegrains	Control	30.8±42.9	0.52±39.4	60.3±41.1	23.5±54.4[2]	71.4±61.7	12.7±60.2	74.2±61.1	2.2±64.4	31.8±42.4	-1.7±41.9
	Diet	23.1±32.3	60.4±41.5[2,b]	61.9±46.4	34.2±54.5[2]	82.5±64.2	34.1±67.2[2,b]	78.9±70.0	38.3±73.2[2,b]	28.2±42.3	104±54.1[2,b]
Fruits	Control	293±177	18.3±182	259±137	-2.2±136	234±146	6.8±145	228±159	-24.4±152	290±167	-25.4±153
	Diet	278±149	14.0±144	259±139	2.4±140	235±127	43.2±129[2,a]	198±121	9.5±129[a]	273±151	37.2±142[b]
Vegetables	Control	216±113	-6.7±112	208±91.6	15.0±97.2	165±83.9	-6.9±80.9	282±170	-17.6±183	249±101	-30.9±97.6[1]
	Diet	216±101	23.2±96.3[a]	218±105	10.1±106	162±77.9	25.5±78.1[2,b]	261±146	11.3±137	217±87.6	31.6±84.2[2,b]
Legumes	Control	16.5±23.1	-2.3±21.9	17.4±22.4	2.2±20.9	3.8±12.6	1.5±12.1	4.8±14.1	0.3±18.0	12.9±21.3	-4.2±19.6
	Diet	8.7±10.9	2.7±13.2[b]	19.9±25.9	3.9±25.4	3.4±11.9	6.5±17.2[2,a]	10.2±29.5	8.4±26.1[1,a]	8.6±15.1	17.9±18.1[2,b]
Low-fat dairy	Control	89.7±103	1.1±108	264±162	31.2±175	196±166	8.0±177	74.0±93.1	-20.9±110	127±138	-10.0±134
	Diet	87.6±93.7	45.8±99.9[2,b]	278±165	31.4±168	256±183	40.9±183[a]	96.5±102	61.3±1312[2,b]	134±140	128±156[2,b]
Low-fat cheese	Control	0.17±1.0	0.18±2.15	2.2±6.5	1.3±8.2	9.6±21.6	-0.6±19.2	30.2±32.9	-4.6±30.6	21.3±15.9	1.5±17.3
	Diet	1.46±4.87	0.93±5.27	2.76±6.65	1.42±7.5	6.5±10.6	7.7±11.9[2,b]	38.8±46.8	10.0±41.6[a]	17.5±15.4	5.0±14.3[1]
Fish	Control	29.9±28.8	-2.2±26.3	24.7±23.5	5.2±22.3	22.3±26.2	-3.0±25.5	31.4±40.7	-8.9±33.4	35.8±23.3	-11.0±22.2[2]
	Diet	27.1±23.1	17.1±27.5[2,b]	29.5±24.3	2.3±23.4[a]	23.9±24.9	5.3±22.9[a]	31.0±31.2	12.2±33.1[2,b]	31.0±21.2	12.5±21.5[2,b]
Low-fat meat & poultry	Control	49.8±35.9	-2.6±34.8	33.7±26.6	-0.83±26.3	32.5±25.4	-0.7±24.8	33.3±36.2	-0.36±33.4	60.3±34.1	1.3±33.4
	Diet	51.4±28.9	-2.1±27.4	33.4±31.8	-1.7±28.6	31.2±24.3	-1.66±23.3	34.2±32.3	-3.7±31.6	56.1±33.2	0.45±29.4
Nuts	Control	8.1±14.4	-0.14±12.6	6.1±11.8	-0.19±11.2	6.3±11.6	0.2±10.8	4.1±8.4	0.9±8.2	7.6±16.7	-2.7±13.8
	Diet	4.3±10.7	0.00±9.2	5.3±8.3	4.1±8.7[2,b]	6.8±11.0	3.6±11.1[1,a]	4.6±8.2	3.3±8.6[2,a]	8.4±14.9	1.5±12.3[a]
Eggs	Control	7.3±8.7	1.7±10.2	16.6±15.5	-0.64±15.2	16.8±17.3	0.86±17.4	30.7±20.3	-2.8±21.0	16.9±12.9	-0.1±13.5
	Diet	7.8±7.7	1.5±7.9	17.2±15.4	6.7±15.1[2,b]	14.8±15.2	2.9±14.4	28.4±22.9	-4.9±19.2	20.2±17.7	0.0±15.3
Olive oil	Control	16.8±8.5	1.0±8.3	2.85±2.97	-0.16±2.73	2.4±3.5	0.19±3.6	11.9±10.7	0.13±11.9	14.9±10.8	-3.6±9.6[2]
	Diet	17.1±6.7	1.2±6.6	2.5±2.5	2.0±3.9[2,b]	3.2±3.9	1.9±4.5[2,b]	10.4±8.9	2.1±8.7	13.2±11.8	4.2±10.2[2,b]
Alcohol	Control	8.1±9.9	-1.1±9.6	10.9±10.8	-1.4±10.7	13.4±12.5	0.0±12.8	3.9±6.4	-0.7±6.1	10.6±11.7	-0.1±11.7
	Diet	9.0±9.9	-2.6±8.8[1]	9.6±10.1	-1.6±9.4	11.9±11.2	0.8±11.5	3.7±5.8	-1.7±4.9[1]	9.5±12.1	-3.1±10.4[b]
Fluid	Control	1756±542	64.9±552	2565±623	-80.5±633	2438±614	-71.7±607	2405±605	-68.9±603	2367±553	25.3±595
	Diet	1901±588	144±584[1]	2721±732	66.7±719[b]	2356±561	116±574[b]	2451±593	127±580[b]	2189±529	378±546[2,b]
Salt	Control	1731±545	-3.2±543	2376±879	-162±801	2435±670	-175±677[1]	3495±1118	-68.5±1110	3289±2282	-489±1711[1]
	Diet	1809±698	-173±615[1,a]	2357±679	-295±669[2]	2323±618	-54.5±577	3523±1143	-122±1144	2968±2988	-695±2166[1]
Sweets	Control	43.6±51.2	2.3±56.7	137±166	-11.7±156	83.6±93.5	-16.8±82.4	96.3±111	-10.6±97.7	51.11±77.9	0.4±91.8
	Diet	55.5±67.7	-16.6±61.7[1,a]	132±129	-49.8±107[2,b]	79.5±102	-5.3±106	104±113	-21.6±102	56.4±58.2	-23.2±51.6[2,a]
Vitamin D	Control	25 (21.9)	17 (14.9)[1]	12 (9.5)	7 (5.6)	15 (12)	8 (6.6)	22 (20.8)	17 (16.0)[2]	7 (7.0)	8 (8.0)
	Diet	22 (17.3)	83 (65.4)[2,b]	11 (8.7)	108 (85.7)[2,b]	16 (13.7)	88 (75.2)[2,b]	22 (19.3)	83 (72.7)[2,b]	13 (14.9)	68 (78.2)[2,b]
NU-AGE index	Control	86.9±14.9	2.5±14.7	79.9±15.2	6.7±15.5[2]	72.9±15.8	3.6±16.6	84.7±17.2	0.53±16.5	91.2±12.9	-5.4±13.4[2]
	Diet	82.5±15.4	21.2±14.4[2,b]	81.5±12.7	20.8±13.9[2,b]	76.1±15.4	20.3±15.5[2,b]	86.1±14.8	21.4±15.2[2,b]	88.2±15.8	35.6±16.0[2,b]

[1,2] Significant change from baseline to follow-up ([1] p<0.05, [2] p<0.01)

[a,b] Significant difference in change in the diet group compared to the control group ([a] p<0.05, [b] p<0.01)

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Discussion

The NU-AGE study is a one-year randomized dietary intervention with a healthful diet targeting the dietary recommendations of the ageing population versus a control diet to reduce inflammageing, optimise health and quality of life in European older adults. In the current study we demonstrated that following a one-year dietary intervention, comprising dietary counselling, provision of free foods and a vitamin D supplement, the dietary intake of an elderly European population shifts more towards the NU-AGE guidelines for a healthful diet.

Changes in in dietary intake in elderly populations has been reported in previous studies. For example, a dietary intervention study of a personalized nutrition program consisting of home visits, phone calls and newsletters observed that after 6 months of intervention in participants aged 70 years and above, their fruit, vegetables and dairy intake improved [46]. A one-year trial demonstrated favourable changes in dietary intakes for olive oil, nuts, vegetables, legumes, fruits, meat and pastries, cakes and sweets in persons aged 55 to 80 years of age [47].

However, none of these studies investigated a dietary pattern that was specifically developed to target the dietary recommendations for the ageing population. To date, dietary recommendations for the elderly population to improve healthy ageing at a global level are lacking. Our study, investigating a diet that was specifically designed at targeting the dietary recommendations of elderly people could fill this knowledge gap. Besides incorporating the dietary recommendations, also nutrients related to various ageing related health outcomes and nutrients involved in inflammageing have been taken into account when designing the NU-AGE diet, including monounsaturated fatty acids, polyunsaturated fatty acids, olive oil or oily fish (inflammatory processes [5] and inflammatory bowel diseases [48]), protein (sarcopenia [7]), vitamin D (falling [8]), vitamin B12 (cognitive impairment [9]), vitamin C and fruit (CRP [11]), and whole grains (type 2 diabetes [49], cardiovascular disease [50], BMI [51], and blood lipids [52]). Thus, a solid basis of evidence was used to develop the NU-AGE diet for healthier ageing.

The NU-AGE diet seems to be feasible to implement in the increasingly ageing population. As demonstrated in the present study, following one year of intervention, participants in the intervention group had a 21 point higher NU-AGE diet score compared to the control group. Compared to a trial studying the Mediterranean diet in which 2 points (out of a 14) change has been achieved resulting in a reduction of cardiovascular risk factors, e.g., decreased blood pressure, blood glucose levels, and cholesterol-HDL cholesterol ratio and increased HDL cholesterol levels [18], the effects of the changes in dietary intake within the NU-AGE dietary intervention seem promising to improve health at a European level.

An important strength of the dietary intervention is the approach to change the entire dietary pattern, rather than single foods or nutrients. Changing an entire dietary pattern has been shown to be the preferred method as it is easier to change a dietary pattern rather than focussing on specific nutrients [53]. Moreover, we were able to achieve changes in dietary intake by making use of dietary counselling, the provision of free foods and a vitamin D supplement only. It has been demonstrated by a review that the provision of free foods is effective to improve adherence [54]. The use of Motivational Interviewing [24] in the dietary counselling could be the key of success in this intervention. A systematic review and meta-analysis has demonstrated that interventions incorporating behaviour change techniques are effective ingredients of interventions to change dietary intakes [55]. The provision of free foods and a vitamin D supplement were also used to improve compliance. It has been shown that free provision of foods is effective in improving dietary habits [56, 57]. Thus, with relatively less effort, without making use of a controlled feeding trial, the

NU-AGE intervention seemed to be successful in making older adults change their dietary habits - even after only one year of follow-up. Additionally, the construction of the NU-AGE diet index is another strength of this study, as this enables us to rank participants according to their adherence to the NU-AGE diet. This approach will allow us to study the effects of the changes in dietary intake on health outcomes using an intention to treat analysis (efficacy), but also by using a per protocol analysis (efficiency). Furthermore, to make sure that all outcome measurements were conducted in the same standardized way across the five study centres, all questionnaires, procedures and tests were practiced and discussed during training sessions prior to the start of the intervention period. At last, the use of standardized and harmonized food based dietary guidelines across five study centres in Europe enabled us to provide a comparable dietary advice and to implement the same dietary principles at a large scale.

Our study has limitations. Firstly, the use of country specific food composition databases and nutrient calculation systems could have resulted in differences in intake between the countries caused by a different food composition database rather than a real-life difference in intake. We have improved the comparability in calculations by organizing training sessions in which all research dieticians of each research centre participated. Secondly, we had losses to follow-up - primarily pre-frail participants in the control group - likely because participants did not receive food incentives and their frailty status could have limited their ability to comply to the intervention. Finally, participants were apparently healthy, well-educated, which limits the generalizability of our findings to the general elderly population.

Our results show that the NU-AGE diet, based on dietary recommendations of older adults, is feasible to achieve favourable changes in dietary intake in an elderly population. Evidence on the effects from this diet on health outcomes will contribute to the development of European-wide dietary guidelines for healthier ageing across Europe.

8

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Chapter 9

Effect of the NU-AGE dietary intervention on cognitive decline in Dutch older adults

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Abstract

The Mediterranean diet may affect cognitive functioning in older adults, but results are inconsistent. The present study aimed to examine the effect of the NU-AGE diet - a Mediterranean-like dietary pattern for older adults - on cognitive functioning. Between April 2012 and March 2013, a total of 252 independently living non-frail and pre-frail participants from the Netherlands (mean age 71.0±4.0 years) were enrolled into the NU-AGE parallel, randomized dietary intervention study. Participants were randomly assigned to the NU-AGE diet group (n=123) or to the control diet group (n=129). The primary outcome was one-year change in global cognition and in four cognitive domains as measured through a comprehensive neuropsychological test battery. Data were analysed using mixed-effects linear regression models for the effect of the NU-AGE intervention on cognitive change over time. Comparing the intervention with the control group, there was no effect of the intervention on cognitive functioning. Estimated mean change in global cognition z-score at 1 year was 0.33 (95%CI 0.21,0.45) in the intervention group and 0.42 (95%CI 0.29,0.54) in the control group, with a mean difference of -0.10 (-0.45,0.25, p group × time = 0.56). Additionally, the intervention was not related to change over time for any of the cognitive domains. The results of this study suggest that a one year intervention with the NU-AGE diet did not improve cognitive functioning in Dutch older adults aged 65 years and older.

Introduction

Life expectancy continues to rise due to technical and medical developments, which results in a shift towards an ageing population. The proportion of adults aged over 60 years will be more than 20% of the world population by 2050 [1]. This increase in older adults will result in a higher prevalence of age-related diseases and disabilities, such as the occurrence of dementia [2]. In 2015, 46.8 million people were living with dementia and it is estimated that this number will increase to 74.7 million in 2030 and 131.5 million in 2050 [3].Therefore, the delay of onset of dementia or cognitive impairment is a major public health priority [4].

It has been estimated that a third of all Alzheimer's disease (AD) cases might be attributable to modifiable risk factors, including hypertension, obesity and diabetes [5]. Nutritional components might play a role in the prevention of cognitive decline by impacting these modifiable risk factors. A combination of nutrients might have even stronger beneficial effects on cognitive health, due to interactive or cumulative effects. Therefore, the interest in the relationship between dietary patterns and cognitive health has increased.

One of the dietary patterns which is of great interest is the Mediterranean diet, characterized by a high consumption of plant based foods including vegetables and fruits and low consumption of red meat and sugary or high-fat food products [6]. Two meta-analyses on longitudinal studies reported that a higher adherence to the Mediterranean diet is associated with a reduced risk of Mild Cognitive Impairment (MCI) and AD [7, 8]. One randomized trial reported that a Mediterranean diet supplemented with nuts or olive oil was associated with improved cognitive function among older Spanish participants [9]. However, data from additional randomized controlled trials are lacking.

Additionally, to date, there is no study investigating the effect of a diet that is based on dietary recommendations for the ageing population. The NU-AGE dietary intervention study investigates the effect of a Mediterranean-like dietary pattern specifically adjusted to the dietary recommendations of elderly people on health outcomes. The present study aimed to study the effect of the NU-AGE intervention on cognitive change over one year in Dutch adults aged 65-80.

Materials and methods

Study design and participants

The NU-AGE study is a randomized one-year dietary intervention study conducted in five European countries: Italy, the United Kingdom (UK), the Netherlands, France, and Poland [10, 11]. For the present analyses we used data from the Dutch cohort. The design of the study has been extensively described elsewhere [10]. In short, between April 2012 and March 2013 a total of 252 apparently healthy non-frail and pre-frail older adults aged 65-80 years were recruited in the surroundings of Wageningen, the Netherlands. At baseline and after one year follow-up participants completed questionnaires, and a cognitive assessment and physical examination were conducted. Dietary intake was assessed by means of a 7-day food record at baseline and follow-up. All foods consumed during the day, including their portion sizes, were reported. Written informed consent was obtained from all participants. The study protocol was approved by the Medical Ethics Committee of Wageningen University and the NU-AGE study is registered at clinicialtrials.gov since December 21st 2012, NCT01754012.

Dietary intervention

Participants were randomized to either the intervention or control group. The intervention group received individual dietary counselling including motivational interviewing nine times during one year to improve adherence to the NU-AGE diet. The NU-AGE diet is based on nutrient reference values and food based dietary guidelines for elderly from Italy [12], the UK [13], the Netherlands [14-18], Poland [19], and France [20], on the modified MyPyramid for Older Adults [21, 22], and nutrient requirements from the European Community [23], and the Institute of Medicine [24]. Specific products – e.g. low-salt low-fat cheese, whole grain pasta, margarine, olive oil and a vitamin D supplement (10 µg) – were provided for free to the intervention group to facilitate adherence to the diet. The control group received a leaflet with general nutritional guidelines.

Cognitive functioning

Cognitive functioning was measured at baseline and follow-up by trained research assistants following standardized procedures. The comprehensive neuropsychological test battery included the Consortium to Establish a Registry for Alzheimer Disease (CERAD) [25] test battery (Verbal Fluency Category [26], Boston Naming Test [27], Constructional Praxis [28], Word List Memory [25], Word List Delayed Recall [25], and Word List Recognition [25]), plus five additional tests; 1) Babcock Story Recall [29], 2) Trail Making Test A (TMT-A) [30], 3) Trail Making Test B (TMT-B) [30], 4) Number Cancellation [31], and 5) Pattern Comparison [32].

Covariates

Information on age, sex, educational level (years), smoking status (non-smoker vs. current smoker), medical history (hypertension, hypercholesterolemia, diabetes mellitus type 2 with no insulin therapy, myocardial infarction, and heart disease) was collected using questionnaires. A history of either myocardial infarction or heart disease was evaluated as having a history of cardiovascular disease (CVD). Body weight, height, subjective cognitive complaints (range 0-10), Mini-Mental State Examination (MMSE, range 0-30) [33], and frailty status (non-frail or pre-frail [34], were examined by trained research assistants following standardized procedures. Physical activity was measured using a validated version of the Physical Activity Scale for Elderly [35]. Energy intake was derived from the means of the 7-day food records. Depressive symptoms were categorized as having two or more points on the Geriatric Depression Scale [36], to differentiate subjects with no and a few depressive symptoms.

Statistical analyses

Data are expressed as mean±SD for continuous variables, or as number (percentage) for categorical variables. Baseline characteristics were compared between treatment groups using analysis of variances (ANOVA) for continuous and Pearson $\chi 2$ or Fisher's exact test for categorical variables.

Z-scores for each cognitive test at each time point were standardized to the baseline mean and standard deviation. Higher Z-scores represent a higher cognitive test outcome. Z-scores for TMT-A and TMT-B were reversed, as for these tests higher test outcomes represented lower cognitive functioning. An interference measure for TMT-A was calculated (TMT-B/TMT-A), to control for the effect of motor speed on performance [37]. CERAD Z-score was calculated based on the sum of individual tests. Z-scores for cognitive tests were clustered into compound scores for four cognitive domains, namely 1) episodic memory, 2) executive functioning, 3) information processing speed and 4) visuospatial ability:

Episodic memory = $Z_{\text{WordList Immediate}} + Z_{\text{WordList Delayed Recall}} + Z_{\text{WordList Recognition}} + Z_{\text{Babcock Story Recall immediate}} + Z_{\text{Babcock Story Recall delayed}}$) / 5

Executive functioning = $Z_{\text{VerbalFluencyCategory}} + -Z_{\text{TrailMakingTest B/A}}$ / 2

Information processing speed = $-Z_{\text{TrailMakingTestA}} + Z_{\text{NumberCancellation}} + Z_{\text{PatternComparison}}$ / 3

Visuospatial ability = $Z_{\text{ConstructionalPraxis}} + Z_{\text{ContructionalPraxis recall}}$ / 2

Tests included in the four cognitive domains are specified in **table 1**.

Table 1. Cognitive domains and included tests in the NU-AGE study

Domain	Test	Description	Scoring
Episodic memory			
	Word list memory	Recall of 10 presented words in 3 sessions	0-30
	Word list delayed recall	Delayed recall of the 10 presented words in the Word list memory test	0-10
	Word list recognition	Recognition of the 10 presented words in the Word list memory test in a list of 20 words	0-20
	Babcock story immediate recall	Recall of a brief story	0-21
	Babcock story delayed recall	Delayed recall of the brief story in Babcock story recall	0-21
Executive functioning			
	Verbal fluency category	Mention as many words of a category in 60 seconds	0 - ∞
	Trail Making Test B / A	B: Draw lines sequentially, connecting 13 numbers and letters in order 1 A 2 B etc., as fast as possible A: Draw lines sequentially connecting 25 encircled numbers, as fast as possible Outcome ratio of TMT B and TMT A	0-300 seconds
Information processing speed			
	Trail Making Test A	A: Draw lines sequentially connecting 25 encircled numbers, as fast as possible	0-300 seconds
	Number cancellation	Cross out all number '4' from a list of numbers, as fast as possible	0-30
	Pattern comparison	Same/different decisions about pairs of patterns needs to be made, as fast as possible in 2 sessions	0-60
Visuospatial ability			
	Constructional praxis	Drawing of presented figures	0-11
	Constructional praxis recall	Recall of drawn figures on blank paper	0-14

Mixed-effects linear regression models were used to study the effect of the NU-AGE intervention on cognitive change over time as a function of intervention, time and intervention × time interaction. This analysis was based on an intention-to-treat approach in which all randomized participants were included in the analysis. Sensitivity analyses were performed by excluding all participants with missing values for cognitive outcomes and stratified analyses by sex and age (median) were performed. Stratified analyses were relevant because beneficial effects of Mediterranean-like dietary patterns were reported in women [9], and in subjects with a higher age [16], while no effects were reported in men [19] and in subjects with a lower age [18]. Two-sided p-values <0.05 were considered significant for all tests. All statistical analyses were performed using SAS version 9.3.

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Results

Between April 2012 and March 2013, 350 individuals were screened and 252 were randomly assigned to the intervention group (n=123) or control group (n=129), **figure 1**. A total of 244 (97%) participants completed the follow-up assessments. The intervention was completed in March 2014. The analyses included 250 participants (99% of enrolled participants). Dropout rates were similar in the intervention (4 [3%] participants) and control (4 [3%] participants) groups. Main reasons for drop-out were difficulties adhering to the intervention (n=1), health related (n=3), and difficulties in arranging participation (n=3). One participant in the control group died during the study.
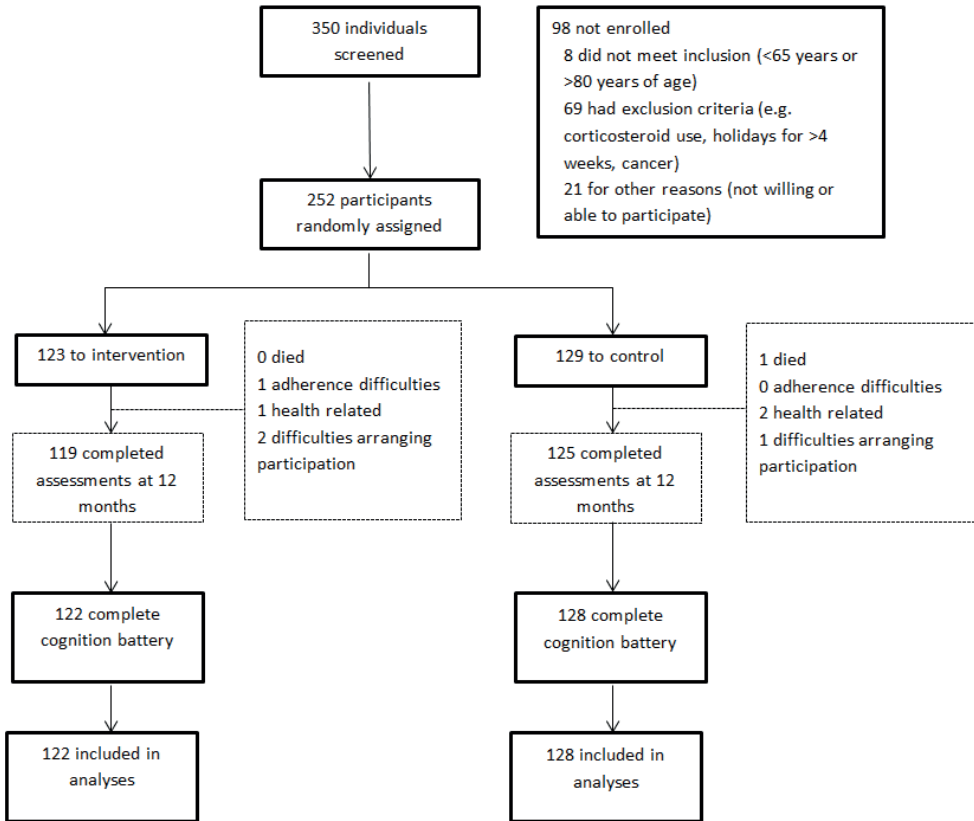

Figure 1. Flow chart of Dutch NU-AGE participants

The mean age of the population was 71.0 (±4.1) years and MMSE score was 27.7±1.8. Compared to the control group those in the intervention group were more likely to be women (57% vs. 54%), were higher educated (12.8 vs. 11.8% years), had a lower BMI (25.4±3.7 vs. 26.5±3.5 kg/m^2) and had a higher prevalence of depressive symptoms (26.2% vs. 19.5%; **table 2**).

Table 2. Baseline characteristics of Dutch participants in the NU-AGE study

	Intervention group (n=122)	Control group (n=128)
Demographic characteristics		
Age at baseline, years	70.9 ± 4.2	71.0 ± 3.9
Women	70 (57.0)	69 (54.0)
Education, years	12.8 ± 3.7	11.8 ± 3.6
Vascular factors		
Systolic blood pressure, mmHg	130.0 ± 19.1	136.3 ± 18.0
Diastolic blood pressure, mmHg	69.4 ± 9.5	71.0 ± 10.3
Body mass index, kg/m^{2a}	25.4 ± 3.7	26.5 ± 3.5
Lifestyle factors		
Physical activity, met	137.1 ± 51.7	137.9 ± 55.5
Pre-frail	26 (21.3)	28 (21.9)
Current smokers	6 (4.9)	2 (1.6)
Energy intake, kcal	1902 ± 369	1911 ± 432
Self-reported medical disorders		
Hypertension	38 (31.1)	44 (34.4)
Hypercholesterolemia	28 (23)	34 (26.6)
Diabetes Mellitus, type II	3 (2.5)	6 (4.8)
Cardiovacular disease	17 (14.0)	15 (11.7)
Depressive symptoms	32 (26.2)	25 (19.5)
Cognition		
MMSE (0-30)	27.8 ± 1.8	27.6 ± 1.8
SCC (0-10)	2.9 ± 1.8	2.9 ± 1.9

Data are mean ± SD, or n (%)
MMSE = Mini-Mental State Examination; SCC = Subjective Cognitive Complaints
[a] p-value <0.05

Estimated mean change in CERAD total z-score at one year was 0.33 (95%CI 0.21; 0.45) in the intervention group and 0.42 (95%CI 0.29; 0.54) in the control group. The mean difference between groups (group × time interaction) in change of cognitive function per year was -0.10 (95%CI -0.45; 0.25), p=0.56; **table 3.** There was no significant intervention effect over time for the cognitive domains episodic memory (p=0.56), executive function (p=0.58), information processing speed (p=0.75), and visuospatial ability (p=0.30).

The results remained unchanged when adjusting for potential confounding variables and while excluding subjects with missing data in cognitive outcomes (n=5). No interaction was observed for sex, age or energy intake (*data not shown*).

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Table 3. Baseline cognitive test scores and one-year change by study group

	Intervention			Control			Mean difference	p group x time[a]
	Baseline	Change	p[a]	Baseline	Change	p[a]		
CERAD z-score	0.00±1.02	0.33 (0.21;0.45)	<0.01	-0.00±0.98	0.42 (0.29;0.54)	<0.01	-0.10 (-0.45;0.25)	0.56
Episodic memory								
Word list memory	19.24±3.76	1.66 (1.13;2.19)	<0.01	19.29±3.40	1.63 (1.04;2.22)	<0.01	-0.04 (-1.34;1.25)	0.95
Word List Recall	6.69±1.82	0.67 (0.42;0.92)	<0.01	6.76±1.73	0.73 (0.44;1.01)	<0.01	-0.06 (-0.69;0.57)	0.86
Word list recognition	19.35±1.01	0.13 (-0.20;0.46)	0.44	19.37±1.11	0.02 (-0.37;0.41)	0.93	0.09 (-0.49;0.69)	0.76
Babcock story recall	7.84±3.09	-0.61 (-1.18;-0.03)	<0.01	8.09±2.78	-1.28 (-1.80;-0.75)	<0.01	0.65 (-0.37;1.67)	0.21
Babcock story delayed recall	11.01±4.07	-1.02 (-1.71;-0.33)	<0.01	11.14±3.47	-1.61 (-2.27;-0.95)	<0.01	0.52 (-0.75;1.79)	0.42
Excecutive functioning								
Verbal fluency	22.58±5.49	1.09 (0.25;1.94)	0.01	22.70±5.88	0.92 (0.02;1.83)	0.05	0.17 (-1.82;2.16)	0.87
TMT B/A[b]	2.43±0.87	-0.37 (-0.52;-0.22)	<0.01	2.34±0.78	-0.28 (-0.43;-0.13)	<0.01	-0.08 (-0.34;0.17)	0.54
Information processing speed								
TMT A[b]	39.02±10.41	1.24 (-0.30;2.78)	0.12	40.97±12.64	0.06 (-1.93;2.04)	0.96	0.98 (-2.92;4.88)	0.62
Number cancellation	19.09±3.37	-0.11 (-0.74;0.52)	0.73	19.34±3.79	0.25 (-0.35;0.86)	0.41	-0.40 (-1.76;0.95)	0.56
Pattern comparison	27.43±4.96	1.69 (0.99;2.41)	<0.01	27.45±5.01	1.46 (0.74;2.19)	<0.01	0.15 (-1.64;1.93)	0.87
Visuospatial ability								
Constructional praxis	9.98±1.07	-0.44 (-0.67;-0.21)	<0.01	9.84±1.33	-0.23 (-0.46;-0.00)	0.05	-0.23 (-0.67;0.21)	0.29
Constructional praxis recall	8.50±2.32	0.00 (-0.39;0.39)	0.99	8.25±2.26	0.25 (-0.17;0.66)	0.25	-0.19 (-0.56;0.17)	0.29
Domain specific Z-scores								
Episodic memory	-0.02±0.76	0.09 (-0.00;0.19)	0.05	0.02±0.72	-0.00 (-0.11;0.10)	0.96	0.08 (-0.19;0.35)	0.56
Executive function	-0.04±0.81	0.33 (0.21;0.44)	<0.01	0.04±0.76	0.25 (0.12;0.37)	<0.01	0.07 (-0.18;0.33)	0.58
Information processing speed	0.02±0.72	0.07 (-0.02;0.17)	0.13	-0.01±0.82	0.12 (0.02;0.21)	0.02	-0.04 (-0.32;0.23)	0.75
Visuospatial ability	0.06±0.76	-0.18 (-0.33;-0.04)	0.01	-0.05±0.90	-0.05 (-0.19;0.09)	0.52	-0.16 (-0.47;0.15)	0.30

CERAD = Consortium to Establish a Registry for Alzheimer Disease; TMT = Trail Making Test

[a] p-value by means of mixed-effects linear regression models

[b] Lower scores indicate improvement

Scores on episodic memory, executive function, information processing speed and visuospatial ability are mean values of z-scores of the cognitive tests included in each cognitive outcome, with higher scores suggesting better performance

Discussion

We investigated the effect of the one-year NU-AGE dietary intervention on cognitive functioning in 252 non-frail and pre-frail Dutch older adults. Compared to the control diet, the NU-AGE intervention did not improve global cognition or the cognitive domains episodic memory, executive function, information processing speed, and visuospatial ability. To the best of our knowledge, this is the first dietary intervention study investigating the effect of a diet specifically targeting dietary recommendations for the elderly population on cognitive functioning in non-frail and pre-frail older adults aged 65-80 years of age.

Other dietary patterns that have been investigated in relation to cognitive functioning include the Mediterranean diet and the DASH diet. The effect of a Mediterranean diet has been investigated in a dietary intervention study with three arms (Mediterranean diet supplemented with mixed nuts (30 g/d), or extra virgin olive oil (1 L/week), or control) in 447 cognitively healthy volunteers. Compared to the control group, the Mediterranean diet plus olive oil group had a better RAVLT test score (p=0.049) and memory composite score (p=0.04), and participants in the Mediterranean diet plus nuts group had a better frontal cognition composite score and global cognition score [9]. The less frequently studied DASH diet has been shown to result in a better psychomotor speed after four months intervention in 124 participants with elevated blood pressure randomized to the DASH diet compared to a control group [38].

The most recent developed dietary pattern specifically capturing dietary components shown to be neuroprotective is the 'Mediterranean-dietary approach to stop hypertension diet Intervention for Neurodegenerative Delay' (MIND) diet. The MIND diet was prospectively associated with slower decline in global cognitive score; participants with highest adherence to the MIND diet were cognitively 7.5 years younger in age compared to those with the lowest adherence after 4.7 years of follow-up [39].

However, dietary intervention studies on the effect of a dietary pattern specifically targeting nutritional recommendations and dietary habits of the ageing population on cognitive functioning are scarce. Very recently, a two-year multi-domain intervention including diet, exercise, cognitive training and vascular risk monitoring, or a control group (general health advice) was conducted [40]. The intervention group received dietary advice based on the Finnish Nutrition Recommendations in three individual sessions and seven to nine group sessions. In addition they received a physical exercise program, cognitive training and metabolic and vascular risk factors were managed. After two years of intervention, mean change in neuropsychological test battery (NTB) z-score was 0.20±0.51 in the intervention group and 0.16±0.51 in the control group with a between group difference in change per year of 0.022 (95% CI 0.002-0.042, p = 0.030). It was concluded that this multi-domain approach could improve or maintain cognitive function in at-risk elderly people [41]. Thus, a multi-domain approach could be more effective in achieving beneficial changes in cognitive functioning than targeting dietary recommendations only as in the current intervention study.

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To investigate the effect of the NU-AGE intervention on cognitive functioning, we included an extensive cognitive test battery of 14 sensitive neuropsychological tests covering the domains most affected by aging, which is a major strength of the current study. As recommended by previous research [42], we have clustered data of the individual neuropsychological tests by standardizing values to z-scores in order to construct more robust cognitive domain scores. All tests were conducted by trained research assistants in a valid and standardized way following a strict protocol.

Moreover, randomization was used to obtain two equally balanced intervention groups. However, despite the randomization participants in the intervention group were more likely to be women (i.e. 57% vs. 54%) and had a lower BMI compared to the control group (i.e. 25.4±3.7 vs. 26.5±3.5 kg/m^2). Though, we have performed multivariate adjusted linear mixed model regression analyses including these and other factors and the results did not change. Importantly, with respect to the exposure and outcome variables participants in both groups were comparable at baseline.

Furthermore, we were able to conduct stratified analyses to explore if the intervention would be effective in the oldest participants. This was relevant as studies in older populations (e.g., mean age 81.5 years) demonstrated a relation of the DASH and MIND diet with cognitive decline [8, 43]. Our stratified analyses by age, however, did not result in differences between age groups (*data not shown*).

At last, adherence to the NU-AGE guidelines did substantially improve over time in the intervention group compared to the control group and it was concluded that the NU-AGE dietary intervention was feasible to make elderly people change their dietary intakes more towards their nutritional needs (*Berendsen 2017, in preparation, Chapter 8 in this thesis*).

Our relatively short follow-up time is a limitation of our study and could be an explanation for our null-findings. It has been recommended to conduct trials with a duration of at least 18 months to study effects on cognitive functioning [44]. However, it was expected that the follow-up period would be sufficient to impact cognitive functioning in our population including a proportion of older adults at greater risk of cognitive function decline (i.e. the pre-frail). Another explanation for our null finding of the NU-AGE diet could be the lack of power, as the sample size was not calculated to detect an effect on cognitive functioning. The required sample size has been calculated to be at least 600 participants per intervention group to detect a 50% difference change in the Neuropsychological Test Battery score between two intervention groups over two years [45]. The effect of the NU-AGE diet on cognitive functioning will be further investigated in the total NU-AGE sample including over 1,250 participants.

Summarizing, this study did not find an effect of the NU-AGE intervention on cognitive change over time. Longer-term randomized controlled trials with a sufficient sample size and multiple repeated measures of cognitive function are needed to study the effect of a dietary pattern specifically targeting dietary recommendations, but also incorporating components that have been shown to impact brain health of older adults at risk of cognitive impairment.

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Chapter 10

General discussion

Aim and main findings

The main aim of this thesis was to study the association of healthful dietary patterns with cognitive functioning. In addition, nutrient intake inadequacies were assessed and the potential to change dietary intake in older adults aged 65 years and older was studied. This was done using data of a cross-sectional study, by conducting a literature review, by analysing data of several prospective studies, and by conducting a dietary intervention study.

The findings of these studies are summarized in **table 1.** A high percentage of inadequate intake was observed for the nutrients vitamin D, selenium, and vitamin B6 in Dutch adults aged 65 years and older (**chapter 2**). Of all dietary patterns studied in our systematic literature review we found most evidence for the Mediterranean dietary pattern to be associated with less cognitive decline, dementia or Alzheimer's Disease (AD) risk in older adults, demonstrated by many observational studies and the first intervention study conducted to date (**chapter 3**). Whilst the Healthy Diet Indicator (HDI) was not associated with cognitive decline or functioning at older ages in European and American populations (**chapter 4**), greater adherence to the *Dietary Approach to Stop Hypertension* (DASH) was associated with better cognitive functioning **(chapter 5)**, and the *Mediterranean-DASH Intervention for Neurodegenerative Delay* (MIND) diet with a better verbal memory score at older ages (**chapter 6**). Although the NU-AGE (*NUtrients and AGEing*) diet was specially designed at targeting the dietary recommendations for the ageing population (**chapter 7**) and considering the dietary habits of elderly people (**chapter 8**), it did not result in a better cognitive functioning after one year intervention in a Dutch population aged 65 years and older (**chapter 9**).

Table 1. Summary of main findings

Exposure	Study	Design and study population	Results	Chapter
Nutrients	NU-AGE	Cross-sectional study; 252 Dutch men and women ≥65 years	High prevalence of inadequate dietary intakes for vitamin D (99%), selenium (41%) and vitamin B6 (54%).	2
Dietary patterns	Review	Cross-sectional, prospective, interventions, reviews, meta-analyses	Better adherence to a Mediterranean diet is likely to be associated with less cognitive decline, dementia, or AD.	3
Healthy Diet Indicator (HDI)	NHS, RS, SENECA	Meta-analysis of three cohort studies; 17,160 women ≥70 years, 3,660 men and women ≥55 years, 1,017 men and women ≥70 years	A higher HDI score is not related to reduced rates of cognitive decline in European and American older adults	4
DASH	NHS	Prospective cohort study; 16,144 women ≥70 years	Long-term adherence to the DASH diet is important to maintain cognitive functioning (global cognition, verbal memory and TICS) at older ages	5
MIND	NHS	Prospective cohort study; 16,058 women ≥70 years	Long-term adherence to the MIND diet is moderately associated with better verbal memory in later life	6
Healthful diet	NU-AGE	Design of a one-year randomized dietary intervention study in 1,250 European adults aged 65-80 years	Results of this intervention will provide evidence on the effect of a healthful diet on the prevention of age related decline	7
		One-year randomized dietary intervention study; 1,250 European adults aged 65-80 years	The NU-AGE diet, specifically targeting nutritional recommendations for older adults, is feasible to achieve favourable changes in dietary intake in an ageing European population	8
		One-year randomized dietary intervention study; sub-population of 252 Dutch adults aged 65-80 years	After one year, the NU-AGE diet did not improve cognitive functioning in Dutch older adults aged 65 years and older	9

Abbreviations: AD, Alzheimer's Disease; DASH, Dietary Approach to Stop Hypertension; HDI, Healthy Diet Indicator; MIND, Mediterranean-DASH Intervention for Neurodegenerative Delay; NHS, Nurses' Health Study; NU-AGE, NUtrients and AGEing; RS, Rotterdam Study; TICS, Telephone Interview for Cognitive Status

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From single nutrients to combinations of nutrients

Chapter 2 describes nutrient intake inadequacies for vitamin D, vitamin B6 and selenium in a population of Dutch adults aged 65-80 years. Additionally, to simultaneously study the intake of several nutrients and to rank foods based on their nutrient density, we explored the possibility of applying the Nutrient Rich Food (NRF) 9.3 score [1]. The NRF9.3 score includes nine nutrients to encourage and three nutrients to limit, based on current regulatory frameworks and dietary guidance in the US [2]. The NRF9.3 was of interest as it was demonstrated in an observational study that adults aged 55 years and older with a higher NRF9.3 score had a lower risk of all-cause mortality [3]. As such, we were interested to know if this score could be used in a population of older adults aged 65 years and older to cluster nutrients of concern within foods and to study nutrient intake inadequacy in relation to cognitive outcomes. We confirmed that this score was able to rank foods based on their nutrient density, however, these foods did not necessarily contain high amounts of nutrients that were inadequate in this Dutch population of older adults. Thus, the score at its present form had limited applicability to study combinations of nutrients in older adults in this thesis.

Dietary patterns and cognitive functioning

In **chapters 3 to 9**, methodological considerations specific for the respective chapters have been addressed. The following paragraphs will discuss how the main findings of this thesis fit with the existing literature and overarching methodological considerations are receiving due attention. First, a reflection is given on issues related to dietary patterns and cognitive functioning, followed by a discussion about the study population. Finally, suggestions for further research and public health implications will be presented together with a final conclusion.

Dietary patterns; assessment, construction and evaluation

In this thesis we focussed on dietary patterns derived using so called *a priori* diet scores based on existing knowledge of healthful dietary components, namely the Mediterranean diet (**chapter 3**), the HDI (**chapter 4**), the DASH diet (**chapter 5**), the MIND diet (**chapter 6**), and the NU-AGE diet (**chapter 7-9**). When evaluating associations between dietary patterns and health outcomes, one should take into account the dietary assessment method used, the origin of the included foods and components, the cut-offs and scoring system used, and the applied weighting factors. We will discuss each of these factors in the paragraphs below. **Table 2** provides an overview of all these aspects of the studied dietary patterns in this thesis.

Assessment of dietary intake

Dietary patterns are based on dietary intake data assessed by means of several dietary assessment methods. Food frequency questionnaires (FFQ) have most frequently been used to assess dietary intake to derive the Mediterranean diet (**chapter 3**), the HDI (Rotterdam Study, Nurses' Health Study, **chapter 4**), the DASH diet (**chapter 5**), and the MIND diet (**chapter 6**), **table 2**. A dietary history method and a 7-day food record were used to assess dietary intake to construct the HDI in SENECA (**chapter 4**) and the NU-AGE index, respectively (**chapters 7-9**).

Table 2. Overview of dietary assessment methods, selection of dietary components, cut-off values and scoring systems used to define dietary patterns in this thesis

Dietary pattern	Dietary assessment	Basis of included components	Basis of cut-off values	Scoring
Mediterranean	Mostly FFQ	Healthful dietary pattern in the Mediterranean region	Population's intake distribution	Quintiles, 0-3,4-6,7-9
HDI	FFQ (NHS, RS), dietary history (SENECA)	Recommendations to prevent chronic diseases	Pre-defined & Population's intake distribution	Continuous
DASH	FFQ	Recommendations to lower blood pressure	Population's intake distribution	Quintiles
MIND	FFQ	Literature/human/animal studies on neuroprotection	Pre-defined	0, 0.5, 1
NU-AGE	7-day food record	European dietary guidelines for the ageing population, inflammation	Pre-defined & Population's intake distribution	Continuous

Abbreviations: DASH, Dietary Approach to Stop Hypertension; FFQ, Food Frequency Questionnaire; HDI, Healthy Diet Indicator; MIND, Mediterranean-DASH Intervention for Neurodegenerative Delay; NHS, Nurses' Health Study; NU-AGE, NUtrients and Ageing; RS, Rotterdam Study

All these dietary assessment methods rely on self-report and may be influenced by socially desirable answers, the participant's ability to recall dietary intake (except for the 7-day food record), and to estimate portion sizes correctly, possibly resulting in random and systematic errors [4]. Random errors lead to a correct – but less precise – estimate of the mean dietary pattern score if the sample size is large enough. Systematic under- or overreporting of dietary intake results in a biased mean dietary pattern score which is of concern when comparing the intake of the population to dietary guidelines. However, as systematic errors do not influence the ranking of participants, they do not attenuate diet-disease associations as assessed in this thesis. Differential under- or overreporting on the other hand may influence the ranking of participants [5]. For example, obese people tend to underreport energy intake and overreport fruit intake more than normal weight people do, and sex seems to differentially result in an over- or underestimation of energy intake [6, 7], resulting in a biased mean dietary pattern score. At the same time, obesity is a risk factor for cognitive decline [8] and thus this misclassification could lead to a biased association between dietary patterns and cognitive functioning. Using correction factors (e.g., calculated from summary questions on for example fruit consumption to correct fruit consumption obtained from the sum of questions on individual fruit items) does not necessarily improve estimates of dietary intake, yet [9]. By adding BMI and sex to the multivariable adjusted models, this bias was taken into account to some extent.

Dietary intake data are prone to errors, which will be reflected in dietary patterns. This is important to consider when evaluating study results, especially from observational studies when comparing dietary intakes to dietary guidelines.

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Constructing dietary patterns relevant for cognitive functioning

There are different ways to construct dietary patterns and many decisions have to be taken in doing so, such as which components to include, which cut-offs to use and how to score the components.

Selecting dietary components

When comparing the components of the dietary patterns studied in this thesis (see **table 1, chapter 1)** it should be noted that there are different dietary components included, but also some similar components, such as whole grains, fruits, vegetables, nuts and legumes.

Of all dietary patterns studied, the MIND diet uniquely emphasizes dietary components and servings linked to neuroprotection and dementia prevention (**table 2**, [10]). As there was no evidence for an association between fruit consumption and cognitive decline [11-15] the MIND diet does not specify daily fruit servings - in contrast to the Mediterranean diet and the DASH diet. The MIND diet did include a component for berry consumption [10] as there was evidence for a positive association between intakes of blueberries and strawberries with slower cognitive decline [16], supported by a number of rodent models [17-20]. Additionally, green leafy vegetables were included in the MIND diet based on studies reporting less cognitive decline with a greater consumption of green leafy vegetables [12, 13].

Although the inclusion of such specific components can be considered a unique feature of the MIND diet, this feature introduces a limited applicability of this score in other studies, especially in studies using a FFQ. In a FFQ, individual foods (e.g., berries) are normally clustered into food groups (e.g., fruits) to reduce the total number of foods. This limits the possibility to obtain intake data of those specific foods. Adding these specific and relevant components to optimize brain health to FFQs should be considered in future studies investigating the association between diet and cognitive functioning.

This problem has been overcome within the NU-AGE study by using food records with which it is possible to assess and revisit the intake of an almost infinite number of specific foods within each country, as opposed to a FFQ. Moreover, as the NU-AGE study is an international study, it was necessary to construct food groups that are comparable across the different countries. To achieve a comparable food group classification, we created standardized rules to assign individual foods to food groups in each of the five study centres. Additionally, the underlying concepts (e.g., a nutrient dense diet based on dietary recommendations for older adults, **table 2**) of the NU-AGE diet were taken into account when constructing food groups enabling us to distinguish for example low-fat or high-fat dairy and low-fibre or high-fibre products. This allowed us to study culturally different dietary intakes with similar food groups using an internationally comparable dietary index.

Another advantage of the NU-AGE diet is that it has a wider scope by implementing different cultures from five European countries, as opposed to the Mediterranean diet that is based on a healthful dietary pattern observed in the Mediterranean. Despite the demonstrated health benefits of the Mediterranean diet (e.g., cardiovascular prevention [21, 22], cancer [23], and mortality [22]), strong intrinsic Western socio-cultural values, traditions and norms may hamper the feasibility of adhering to the Mediterranean diet in non-Mediterranean countries [24], such as to the high consumption of olive oil, fish and legumes.

> *When designing a dietary pattern relevant for cognitive functioning, components should be selected that; 1) are related to neuroprotection, 2) can be assessed by means of commonly used dietary assessment methods, and 3) that are feasible to be consumed within the population of study.*

Cut-off values, scoring systems and weighting of dietary components

Cut-off values either represent the required levels of intake to receive the maximum number of points for adequacy components, or the amount of food at which zero points are given in case of maximum levels of intake (e.g., components to limit).

When comparing the cut-off values used for the dietary patterns studied in this thesis it should be noted that two different approaches towards cut-off values have been used, namely pre-defined cut-off values and cut-off values based on the intake distribution in a population (**table 2**). Pre-defined cut-off values are either based on literature or derived from healthy dietary patterns observed in a population (e.g., in the Mediterranean). If no biological references are available for maximum levels of intake, cut-off values based on the intake distribution in a population can be used. For example, to define maximum levels of intake for a component to limit, such as sodium, the 85th percentile of the population's intake distribution can be used.

Both the HDI and the NU-AGE diet score include a combination of cut-off values. Pre-defined cut-off values were based on worldwide (WHO; HDI, **chapter 4**) or European (NU-AGE, **chapters 7-9**) dietary guidelines. The cut-off values for maximum levels of intake were derived from the populations' intake distributions within a country to optimally fit the intake within the study populations and to obtain a maximum variation in scores.

The MIND diet includes pre-defined cut-off values based on literature only. We were able to demonstrate an association between the MIND diet and verbal memory in the Nurses' Health Study, but the effect sizes were smaller compared to results from the Memory and Aging Project (MAP) [25]. The use of predefined cut off values can be considered an advantage as they are comparable among different study populations. However, if these cut-off values do not match dietary intake of another study population, this could result in less variation in dietary exposure causing a weaker association, as observed in the Nurses' Health Study (**chapter 6**).

Dietary components within a dietary pattern can be scored using a dichotomous, continuous or proportional scoring system. A continuous (e.g., HDI, NU-AGE) or proportional scoring system (e.g., DASH; quintiles of intake), results in a larger variation in dietary component scores covering a wide range of adherence and a higher sensitivity and specificity achieving maximum diagnostic accuracy, compared to a dichotomous scoring system [26, 27]. However, proportional scoring of intakes above the maximum level of intake could result in less variation in the component scores. This could have caused a weakened association of the NU-AGE diet with cognitive functioning.

On the contrary, the MIND diet uses a dichotomous-like scoring system assigning participants 0, 0.5, or 1 point depending on the degree of adherence. A dichotomous-like scoring system is easier to apply in clinical practice and to translate the dietary goals to the general public.

A general limitation of all used scoring systems is that they do not take into account that certain food groups might need different cut-offs as not all components show a linear relation with cognitive functioning. As such, the possibility that some associations of dietary components with cognitive functioning could be non-linear (e.g., J-shaped or U-shaped), has not been taken into account.

In the dietary patterns studied, all dietary components received equal weights, assuming that each component is equally important for cognitive functioning. However, it is very likely that some components are more important. Therefore, some authors have suggested applying weighting factors when constructing dietary pattern scores, such as relative risks or odds ratios for specific food

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groups and health outcomes [26, 28, 29]. However, taking this approach would result in different dietary patterns for each possible cognition outcome, limiting the general applicability and comparability of these dietary patterns. Although certain components within a dietary pattern are correlated (e.g., fruits, vegetables and dietary fibre [30]), we did not apply weighting factors in any of the dietary patterns studied. Because of this collinearity, these components indirectly weigh more heavily within the dietary pattern than other components.

Dietary patterns including cut-off values based on the population's intake distribution and a continuous scoring system allow capturing the full range of variation in diet scores relevant when studying diet disease associations. Dietary patterns using pre-defined cut-off values and a dichotomous scoring system are easier to apply and understand for the general public.

Evaluating dietary patterns

As the MIND diet and NU-AGE diet are newly developed dietary patterns their validity needs to be evaluated. There are various aspects to consider, namely 1) *association with nutrient intake*, 2) *content validity*, 3) *construct validity*, and 4) *reliability* [31].

Firstly, the *association with nutrient intakes* among several degrees of adherence can be studied. For the NU-AGE and MIND diet we observed that nutrient intake increased with higher diet scores without increasing energy intake (e.g., for NU-AGE, mean energy intake was ~1,900 kcal across tertiles, PUFA intake was 10.9g, 11,3g and 12.9g, and fibre intake was 19.3g, 21.9g, and 25.8g).

Secondly, *content validity* assesses if the dietary pattern captures all aspects of the specific dietary patterns. Both the MIND diet score and the NU-AGE diet score qualitatively capture the various components relevant for both diets.

Thirdly, *construct validity* includes a check if the dietary pattern score provides the maximum range to discriminate participants according to their adherence. Another part of construct validity is the possibility to distinguish between groups with known differences in dietary quality, e.g., across levels of education. The range of the MIND diet score was 2.6-11 (out of 0-15) in our study (**chapter 6**) and 36-132 (out of 0-160) for the NU-AGE diet score (**chapter 8**), indicating that both indexes are able to measure a wide range of adherence. For the MIND diet score the ability to distinguish between populations with known differences in dietary intake has been confirmed; participants with the lowest MIND scores had lower education, were more likely to be obese and to have diabetes, and reported fewer hours of physical activity and more depressive symptoms [10].

At last, *reliability* evaluates if a dietary pattern score performs the same at different time points and which dietary components have most influence on the overall dietary pattern score. We have calculated the relative contribution of the MIND diet components, demonstrating that some components had more influence on the total MIND diet score (e.g., 'Butter and margarine' and 'Pastries and sweets'). To evaluate the NU-AGE diet score, it could be studied in relation to markers of inflammation, as it was targeted to impact inflammation. Moreover, reliability of both the MIND diet score and the NU-AGE diet score should be tested using different dietary assessment methods.

Although the newly developed MIND diet and NU-AGE diet are based on components relevant for (brain) health and include cut-off values and a scoring system relevant for studying diet disease associations, their validity needs to be confirmed.

Biomarkers to assess dietary pattern adherence

Ideally, adherence to dietary patterns should be quantifiable; therefore, the use of biomarkers to assess dietary pattern intake should be considered. Biomarkers of dietary intake are less prone to misclassification bias in older adults with and without mild cognitive impairment (MCI) compared to conventional methods of dietary assessment, e.g., food frequency questionnaires [32]. One cross-sectional study explored the use of nutrient status to construct nutrient biomarker patterns to examine the relation with psychometric and imaging indices of brain health (Magnetic Resonance Imaging; MRI) in dementia free women [33]. Two nutrient biomarker patterns (one high in plasma vitamins B (B1, B2, B6, folate, and B12), C, D, and E, and another high in plasma omega-3 fatty acids) were associated with more favourable cognitive and brain MRI measures. In contrast, a third pattern (high in trans-fat) was associated with less favourable cognitive functioning and less total cerebral brain volume. Despite this interesting finding, it remains questionable if dietary patterns can be captured by nutrient biomarker pattern analysis, as nutrient status only does not cover the large diversity of food within a dietary pattern. Additionally, currently only a limited number of validated biomarkers is available [34].

When creating dietary patterns many decisions need to be taken based on the aim of the study and – preferably – the inclusion of biomarkers should be considered to improve and validate estimates of dietary pattern exposure.

Cognitive functioning; assessment and challenges

Cognitive functioning can be measured by means of a broad range of neuropsychological tests. These tests capture different cognitive processes that are important to maintain activities in daily life. Studies described in this thesis include commonly used and validated paper-pencil tests, but also telephone based methods to assess cognitive functioning. An overview of included tests is given in **table 3,** thereby indicating in which chapters they have been used. Roughly, these tests can be divided into screening tools as a global measure of cognitive function and tests that are able to assess specific cognitive domains.

Screening tools for cognitive functioning

In large prospective observational studies it is common to use the Mini Mental State Examination (MMSE) [35], or its telephone version; the Telephone Interview of Cognitive Status (TICS) [36], as a screening tool for cognitive functioning.

The MMSE and the TICS were used in **chapter 4** to study the association between the Healthy Diet Indicator (HDI) and cognitive functioning in three cohort studies. Although we used harmonized exposure and outcome variables we were not able to demonstrate an association between the HDI and reduced rates of cognitive decline in European and American adults aged 65 years and older. The MIND diet (**chapter 5**), the DASH diet (**chapter 6**) and the NU-AGE diet (**chapter 9**) were also not associated with the TICS or MMSE in this thesis. An important limitation of the MMSE and TICS is that they are both very global tests that have originally been developed to grade cognitive functioning of patients with relatively severe cognitive deficits [36].

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Table 3. Overview of neuropsychological tests and cognitive domains used in this thesis

Cognitive domains and tests	Explanation test	Scoring	Chapter
Screening tools for cognitive functioning			
Mini Mental State Examination (MMSE) [35]	Basic screening tool including a variety of questions and tasks	0-30 points	4
Telephone Interview for Cognitive Status (TICS) [36]	Telephone adaptation of the MMSE	0-41 points	4, 5, 6
Global cognition[a]			
CERAD [37]	Consortium to Establish a Registry for Alzheimer Disease (CERAD) test including; Verbal Fluency Category, Boston Naming Test, Constructional Praxis, Word List Memory, Word List delayed recall and Word List Recognition. Boston Naming Test: Naming of objects presented as line drawings (0-15) [38]. Other tests are specified below.	Sum of all tests	9
Episodic memory			
Word list memory [37]	Recall of 10 presented words in 3 sessions	0-30 points	9
Word list delayed recall [37]	Delayed recall of the 10 presented words in the Word list memory test	0-10 points	9
Word list recognition [37]	Recognition of the 10 presented words in the Word list memory test in a list of 20 words	0-20 points	9
Babcock story immediate recall [39]	Recall of a brief story	0-21 points	9
Babcock story delayed recall [39]	Delayed recall of the brief story in Babcock story recall	0-21 points	9
Executive functioning			
Verbal fluency category [40]	Mention as many words of a category in 60 seconds	0 - ∞ points	9
Trail making Test B / A [41]	A: Draw lines sequentially connecting 25 encircled numbers, as fast as possible B: Draw lines sequentially connecting 13 numbers and letters in order, as fast as possible	0-300 seconds	9
Information processing speed			
Trail making Test A [41]	Draw lines sequentially connecting 25 encircled numbers, as fast as possible	0-300 seconds	9
Number cancellation [42]	Cross out all number '4' from a list of numbers, as fast as possible	0-30 points	9
Pattern comparison [43]	Same/different decisions about pairs of patterns, as fast as possible in 2 sessions	0-60 points	9
Visuospatial ability			
Constructional praxis [44]	Drawing of presented figures	0-11 points	9
Constructional praxis recall [44]	Recall of drawn figures on blank paper	0-14 points	9
Verbal memory			
East Boston Memory test (EBMT), immediate recall [45, 46]	Recall three sentences	Range 0-12 points	5, 6
East Boston Memory test (EBMT), delayed recall [45, 46]	Delayed recall three sentences	Range 0-12 points	5, 6
TICS 10-word list, immediate recall [36]	Immediate recall of 10 presented words	Range 0-10 points	5, 6
TICS 10-word list, delayed recall [36]	Delayed recall of 10 presented words	Range 0-10 points	5, 6
Language and executive function			
Category fluency [37]	Naming as many unique animals as possible in one minute	Number of animals	5, 6
Working memory and attention			
Digit span-backward [47]	Repeating backwards and increasingly long series of digits	Number of recalls	5, 6

[a] In the Nurses' Health Study, (chapter 5 and 6) global cognition was calculated by averaging z-scores from 1) TICS, 2) immediate and 3) delayed recalls of the East Boston Memory test (EBMT), 4) delayed recall of the TICS 10-word list; 5) category fluency; and 6) digit span-backward test

Thus, a possible explanation for the lack of an association of the HDI, DASH, MIND and NU-AGE diet with the MMSE and TICS is that these tests are not sensitive and specific enough to detect subtle cognitive differences in cognitive domains within generally healthy study participants.

Commonly used screening tools for cognitive functioning are useful to characterize a study population for their level of cognitive functioning, however, these tests are not sensitive and specific enough to detect differences or decline in cognitive functioning in a relatively healthy study population.

Specific cognitive domains

The use of more specific cognitive tests as compared to the MMSE or TICS enables detection of smaller differences in specific cognitive domains, such as verbal memory, information processing speed, and executive functioning. For example, impaired verbal memory is part of the typical memory-dominant dementia syndrome in AD [48], and thus relevant to measure.

In **chapters 5, 6 and 9** we were able to use many neuropsychological tests to construct cognitive domains. Greater adherence to the DASH diet was associated with a better global cognitive function - a composite score based on six cognitive tests - and with better verbal memory at older ages in the Nurses' Health Study (**chapter 5**). This was in line with the few observational studies reporting that the DASH diet was associated with less cognitive decline among elderly American men and women (*n*=826) after 4.1 years of follow-up using 19 cognitive tests combined into a global measure of cognition [49]. A dietary intervention study in hypertensive American adults (*n*=124) demonstrated that four months on the DASH diet improved psychomotor speed [50].

Greater adherence to the MIND diet was moderately associated with a better verbal memory at older ages (**chapter 6**). In a prospective cohort study, the MIND diet was associated with slower decline in global cognitive function (a composite score of 19 cognitive tests) [25] and a lower risk of AD [10] over 4.7 and 4.5 years in 960 and 923 American men and women from the Memory and Aging Project (MAP) study. We were not able to confirm this association with global cognitive function in a sample of 16,058 American women living in the Boston area (**chapter 6**). A possible explanation could be the timing of dietary and cognitive assessment, as cognitive assessment (starting in 1997) preceded dietary assessment (2004-2013) in the MAP study, whereas dietary assessment preceded cognitive assessment in the NHS study, importantly limiting the effect of reversed causation.

Although we used specific cognitive tests within the NU-AGE study as well, we were not able to demonstrate an effect of the NU-AGE diet on any of the cognitive domains in the Dutch NU-AGE cohort (**chapter 9**). This could be due to the relatively short period of follow-up, as it has been proposed that it takes at least 1.5 years to induce and detect changes in cognitive functioning [51]. Another important explanation for our null finding for the NU-AGE diet could be the lack of power, as the sample size was not calculated to detect an effect on cognitive functioning. The required sample size has been calculated to be at least 600 participants per intervention group to detect a 50% difference change in the Neuropsychological Test Battery score between two intervention groups over two years [52]. The effect of the NU-AGE diet on cognitive functioning will be further investigated in the total NU-AGE sample including ~1,250 participants.

As outlined above, the use of specific cognitive tests allows to detect small differences within a study population and to study specific cognitive domains. However, the lack of harmonized construction of these domains limits the comparability of findings from different studies. This also

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limited us to study the TICS and MMSE only, instead of including specific cognitive domains as well within the three cohorts used to study the relation of the HDI with cognitive functioning (**chapter 4**). Moreover, the use of many different cognitive tests between studies complicates drawing an overall conclusion based on multiple studies. For example, although four meta-analyses reported an inverse association of the Mediterranean diet with cognitive functioning based on pooled analysis of observational studies [53-56] we concluded in **chapter 3** that better adherence to the Mediterranean diet is likely associated with less cognitive decline, dementia or AD. Our conclusion is more conservative in comparison to the meta-analyses as the results of the studies are mixed, studies reporting an association were mainly conducted in the US, many different measures of global cognition were used, and because evidence from intervention studies is limited.

Standardised neuropsychological test batteries and harmonized rules to construct specific cognitive domains are needed, covering the domains on which effects of the respective intervention can be expected and that are sensitive to ageing.

Biological markers to assess cognitive functioning

Another possibility to study cognitive functioning would be to include biological markers as outcome measures (e.g., biomarkers and imaging techniques). Examples of brain imaging techniques are (fluorodeoxyglucose) positron emission tomography (f)(PET) (to detect tracers of fibrillary amyloid [57]) and MRI [58]. Cerebrospinal fluid (CSF) can be used to measure biomarkers of disease type and progression (e.g., levels of amyloid-β protein ($A\beta_{1-42}$), total tau (tTau) or phosphorylated tau (pTau)$_{181}$ [59]. With these measures early stages of AD can be diagnosed. Few studies have prospectively investigated the association between dietary patterns and these more objective measures of brain health. One cross-sectional study demonstrated that adherence to the Mediterranean diet was associated with a lower white matter hyperintensity volume in 1,090 non-demented participants (mean age 72 years) [60], which is a marker of small vessel damage in the brain. A prospective study reported that lower adherence to the Mediterranean diet was associated with greater 3-year reduction in total brain volume [61]. Higher adherence to the Mediterranean diet was found to be cross-sectionally associated with larger cortical thickness in 672 cognitively normal participants) mean age 79.8) [62], which was not confirmed in a prospective study including 323 older aged 73 to 76 years of age [61]. Less brain atrophy was observed in 674 participants without dementia (mean age 80.1)in a cross-sectional study – with an effect similar to 5 years of ageing [63]. A prospective study in 146 participants (mean age 73 years) reported that adherence to the Mediterranean diet was associated with preserved white matter microstructure, nine years after completion of a FFQ [64].

Novel biomarkers and imaging techniques for early clinical stages of dementia need to be further developed, applied and consensus needs to be reached on which of these biological markers is best to use for specific purposes.

Populations to study

To demonstrate a relation between diet and cognitive functioning, a study population should be selected that is either at risk of suboptimal dietary intake or status, or at risk of cognitive impairment, providing an optimal window of opportunity (**figure 1**).
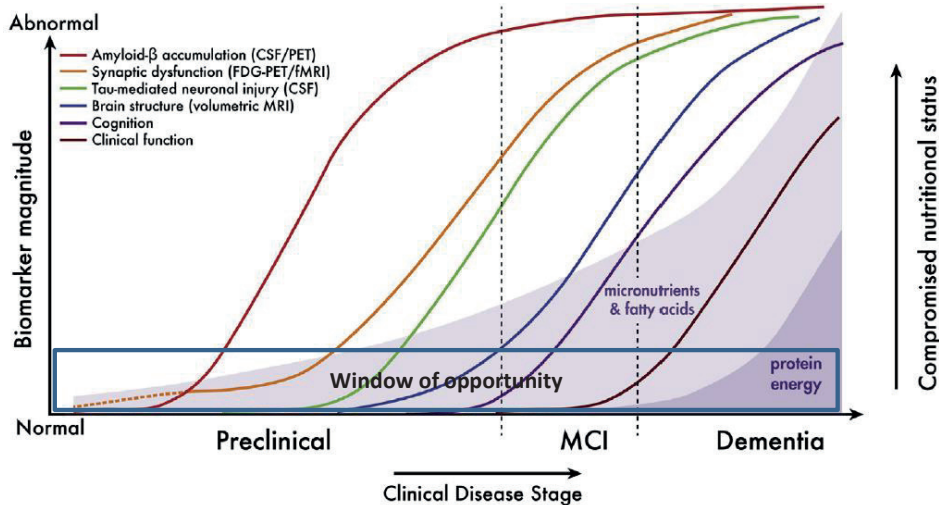

Figure 1. Window of opportunity within a hypothetical model of dynamic biomarkers and nutritional status across the dementia spectrum (adapted from Mi, Nutrition 2013) [65]

Cognitive functioning

The age at which cognitive decline becomes evident remains a subject of debate [66-68]. Although some authors demonstrated that there is no decline before the age of 60 years [69], it has been demonstrated that cognitive decline is already evident in middle age (age 45-49 years) [70]. Prior to the diagnosis of dementia, many years of underlying brain damage have been present [71, 72]. Moreover, it is relevant to consider risk factors of cognitive decline when selecting study participants, e.g., low educational level, obesity and Apolipoprotein E (APOE) genotype, to study cognitive decline or MCI occurrence.

In intervention studies, when targeting populations with preclinical symptoms of cognitive decline, it might be of concern whether the brain damage is reversible. Given this information, there is a general consensus that nutritional intervention studies should aim at prevention rather than treatment of cognitive decline. Thus middle-aged people should be targeted before brain function is too comprised for substantial benefit, thereby focussing on the earliest stages of the disease [71, 73].

Participants in the NU-AGE study (**chapters 7-9**) were aged 65 to 80 years, potentially allowing us to detect changes in cognitive functioning. Besides the follow-up time being relatively short and the fact that the NU-AGE study was not powered to detect an effect on cognitive functioning – as mentioned above – another explanation for our null-finding could be that the NU-AGE participants were apparently healthy individuals at a relatively good cognitive function level limiting the possibility to detect a decline in cognitive functioning within the study period. Although

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intervention studies are considered to be the standard for providing evidence, assessing long-term effects of nutritional factors with intervention studies is considered to be too expensive and difficult [71]. In this respect, prospective studies with long-term follow-up will provide useful information on the role of nutritional compounds on cognitive functioning [71].

An important advantage of prospective studies is the possibility to follow participants for a long period of time. For example, a recent study demonstrated that cumulative burden of cardiovascular risk factors, such as high blood pressure, from childhood (3-18 years) was associated with worse midlife cognitive performance after 31 years of follow-up [74]. Another important advantage of prospective studies is the selection of cohorts with a specific exposure, such as APOE genotype, allowing the comparison of disease development.

Participants within the Nurses' Health Study (NHS) (**chapters 4-6**) comprised relatively healthy adults aged 70 years and older without a major decline in cognitive function over 4.1 years of follow-up. Studying populations that remain at optimal brain health do not provide enough variation in the outcome to detect an association between diet and cognitive functioning. To overcome the limited variation in cognitive functioning all measures were averaged to create a measure of cognitive status at older ages in **chapter 5 and 6**. Despite this method, we demonstrated a smaller effect size of the MIND diet with cognitive functioning in the NHS compared to the Memory and Aging Project study (MAP) (e.g., one year difference in cognitive age in NHS versus 7.5 years in MAP). Compared to NHS, MAP participants were on average ten years older than NHS participants, had a lower educational level, mainly lived in retirement houses, were more likely to have ever smoked and more likely to have reported high blood pressure, diabetes and myocardial infarction demonstrating that MAP participants were at higher risk of poor cognitive function and faster cognitive decline compared to NHS participants.

Thus, ideally, a life course approach would be needed to study changes in cognitive functioning, already starting before the preclinical stages of dementia, e.g., by including a heterogeneous population aged 60 years and older followed for a period of up to 20 to 30 years.

> *When designing intervention or prospective studies, participants at risk, or at a very early stage, of cognitive decline should be included and followed for a sufficient period – ranging from at least 1.5 year in intervention studies to ideally 20 to 30 years for prospective studies.*

Nutrient intake and/or nutrient status

Nutrient intake and/or status in elderly populations is relevant to consider, as with aging, energy needs decrease while nutrient requirements remain or increase, necessitating a more nutrient dense diet [75]. Inadequate nutrient intake may cause chronic metabolic disruption, including mitochondrial decay, resulting in acceleration of various degenerative diseases [76]. The prevalence of inadequate intakes is relatively high in both institutionalized (40-65%), and non-institutionalized older adults (5-10%) [75]. In Europe, over 20% of adults aged 65 years and older have inadequate intakes of vitamin D, folate, calcium, selenium and iodine [77]. Importantly, low nutrient status is highly prevalent in MCI and AD patients. Lower plasma levels of folate and vitamin A, vitamin B12, vitamin C, vitamin E [78], omega-3 fatty acids [79], and selenium [80] were reported in AD patients compared to cognitively healthy controls. In patients with MCI, nutrient status was low for folate [81], vitamin A, vitamin C, vitamin E, lutein, zeaxanthin, α-carotene [82, 83] and DHA [79]. Such

compromised nutritional status may result from alteration in nutrient intake, uptake, metabolism, and utilization [65]. Thus, low nutrient status is a consistent finding during disease progression [65] (**figure 1**).

For intervention studies reporting a null association between a single nutrient and cognitive functioning, the lack of taking baseline nutrient status into account may be a possible explanation for their null findings. Studying apparently healthy individuals with optimal nutrient status has the disadvantage that there is not much room for improvement on the exposure side and thus no effects between nutrients and cognitive outcomes can be detected. Thus, participants who are likely to be sensitive and gain most benefit from the intervention should be included. In the NU-AGE study, plasma and serum values of nutrient have been measured, although not used as an inclusion criterion to specifically select participants at risk of suboptimal nutrient intake or status. As the NU-AGE participants were relatively healthy, it is very well likely that we have included participants with adequate nutrient intake and status, thus resulting in less room for improvement with respect to changes in nutrient intake and status.

Despite the relevance of nutrient intake and/or status in older adults, a major obstacle of long-term intervention studies is the lack of information about history of deficiencies, e.g., whether the deficiency is a chronic state or just a snapshot of temporary physiological state [71]. Conducting prospective studies with a long follow-up period including measures of nutrient intake and/or status could provide insights to this. Additionally, within a study population a large variety in dietary intake should be covered to study combinations of nutrients or dietary patterns in relation to cognitive functioning. Studying a study population with limited variation in dietary intake could hamper the detection of diet-disease associations. For example, we observed a weaker association of the MIND diet with cognitive functioning in the NHS compared to the MAP study, possibly caused by a limited variation in MIND diet score, e.g., total MIND score was 7.4 (range 2.5-12.5 in MAP [10] versus 6.4 (2.6-11.0) in NHS (**chapter 6**).

When selecting participants for intervention studies, low baseline nutrient intake and/or status should be considered as an inclusion criterion. For prospective studies, including a population with a wide range of dietary intake and status allows the detection of diet-cognition associations.

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Further research

The increase in life expectancy and its accompanying decline in cognitive functioning and increased risk of dementia call for research on dietary strategies that impact brain health, especially as effective medicines to prevent or cure dementia are lacking. Based on the current evidence and as outlined above, there is need for both well-designed intervention studies and prospective studies, which should include:

- Dietary assessment methods that are able to assess intake of specific foods relevant for the dietary pattern of interest
- Biomarkers of dietary patterns or cognitive functioning
- Harmonized cognitive test batteries allowing the construction of specific cognitive domains
- Imaging techniques (e.g., MRI or fMRI)
- Sufficient follow-up time, e.g., at least 1.5 years for intervention studies, and ideally up to 20 to 30 years in prospective studies
- Participants either at risk of cognitive decline or with suboptimal nutrient intake and/or status for intervention studies, or a heterogeneous population for prospective studies.

Additionally, to define guidelines for healthier cognitive ageing a holistic approach should be used considering multi-domain exposures. For example, the Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER) study not only focussed on a healthy diet, but also included exercise, cognitive training and social activity, and management of metabolic and vascular risk factors [52] observed promising effects on cognitive functioning [84].

On the other hand, long-term prospective studies simultaneously studying multiple health outcomes should be used to better understand why some people develop age-related diseases while others age more healthily. An example is the Rhineland study, which envisages to include up to 30.000 participants from Bonn in which both mental and physical health are uniquely being assessed over their lifespan - starting at age 30 [85].

Conclusions and public health implications

This thesis described results from a literature review, a cross-sectional study, observational studies and an intervention study on the relation between diet and cognitive functioning. The Mediterranean diet seems to be most consistently associated with less cognitive decline, dementia or AD (**chapter 3**). Although we did not find an association between the Healthy Diet Indicator or the NU-AGE diet with global cognitive function (**chapter 4 & 9**), we demonstrated that participants with the highest adherence to the DASH diet and MIND diet were cognitively one year younger compared to participants with the lowest adherence (**chapter 5 & 6**). Although previous studies showed that dietary intakes of elderly are rather stable [86, 87], **chapter 8** importantly adds to this knowledge, by demonstrating that adults aged 65 years and older are able to change their dietary intakes. This is an encouraging finding, demonstrating that diet is a modifiable *and* feasible risk factor for cognitive decline prevention - even at older ages.

This last chapter demonstrates that there are many aspects to consider when studying dietary patterns in relation to cognitive functioning. This thesis adds scientific evidence to ultimately define the optimal dietary pattern to preserve cognitive functioning, describing results from multiple study designs. Additionally, this thesis does provide important insights into methodological considerations and suggestions for further research. When grading the reported associations in previous studies and studies in this thesis [88], we can conclude that there is probable evidence for a role of the Mediterranean diet on cognitive functioning. For the DASH and MIND diet there is a possible link with cognitive functioning; for the HDI and the NU-AGE diet there is no sufficient evidence yet to conclude that there is a relation with cognitive functioning.

We do call for communication strategies to the general public about the consumption of healthier dietary patterns to not only impact cardiovascular but also potentially impact brain health. We should not only invest in further studies, but also invest in communication to the general public, as many individuals will face progressive cognitive decline and the sooner they can act the more benefit they may have. A first step was the inclusion of dementia as one of the targeted diseases in the updated Dutch food based dietary guidelines [89], and subsequently the 'Wheel of five' (in Dutch: Richtlijnen Schijf van Vijf) [90] has been updated. However, evidence on dementia was included in only one of the guidelines. Therefore, a next step would be the inclusion of findings from further research to be used for nutrition education in the Netherlands.

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Summary

Summary

With ageing of our population and the accompanying increase in the number of people living with dementia, it is important to find modifiable risk factors to postpone the onset of cognitive decline. Diet has been proposed such a modifiable risk factor. To date, numerous studies have been conducted demonstrating a possible role of specific nutrients and foods in cognitive functioning. However, as people do not consume single nutrients, the research field has shifted towards studying dietary patterns in which synergistic effects of single nutrients and/or foods can be studied. The main aim of this thesis was to study the association of healthful dietary patterns with cognitive functioning. In addition, nutrient intake inadequacies were assessed and the potential to change dietary intake in older adults aged 65 years and older was studied.

Chapter 2 describes nutrient intake of 245 Dutch adults aged 65-80 years. We identified the contribution of nutrient dense foods, fortified foods and dietary supplements to the total intake of micronutrients. Nutrient density of foods was evaluated using the Nutrient Rich Food score 9.3. Nutrient intake inadequacies were observed for vitamin D, vitamin B6 and selenium. Conventional foods were the main source of vitamin D, vitamin B6 and selenium intake. Foods with the highest nutrient density contributed most to total vitamin B6 intake. In order to optimize nutrient intakes of elderly, combinations of natural food sources, fortified foods and dietary supplements should be considered.

Chapter 3 provides a systematic review of the literature on human studies up to May 2014 that examined the role of dietary patterns in relation to cognitive decline or dementia. The results demonstrate that better adherence to a Mediterranean diet was associated with less cognitive decline, dementia or Alzheimer's Disease (AD) in four out of six cross-sectional studies, six out of 12 longitudinal studies, one trial and three meta-analyses. Other healthy dietary patterns, among which the Healthy Diet Indicator, have shown to be associated with reduced cognitive decline and/or a reduced risk of dementia in all six cross-sectional studies and six out of eight longitudinal studies. The conclusion of this literature review was that more conclusive evidence is needed to come to more targeted and detailed dietary guidelines to prevent or postpone cognitive decline.

In **chapter 4** the association between the Healthy Diet Indicator (HDI) and cognitive functioning in older adults from three different cohort studies was investigated. The cohorts included in total 21,837 subjects from Europe (SENECA and the Rotterdam Study [RS]) and the Unites States (Nurses' Health Study [NHS]). Cognitive functioning was measured using the Mini-Mental State Examination (MMSE) in SENECA and RS, and the Telephone Interview for Cognitive Status (TICS) in NHS. In all three cohorts, the HDI was not significantly associated with cognitive decline, nor with cognitive function.

In **chapter 5** the association of long-term adherence to the Dietary Approaches to Stop Hypertension (DASH) diet with cognitive function and decline in older American women was examined. A total of 16,144 women from the Nurses' Health Study, aged ≥70 years, who underwent cognitive testing a total of 4 times by telephone from 1995-2001 (baseline), with multiple dietary assessments between 1984 and the first cognitive exam were studied. Greater adherence to long-term DASH score was significantly associated with better average global cognition, verbal memory and TICS score at older ages, but not with cognitive decline.

In **chapter 6**, the same cohort as in chapter 5 was studied to examine the association between long-term adherence to a recently developed Mediterranean-DASH Intervention for Neurodegenerative Delay (MIND) diet with cognitive function and decline. Higher MIND diet scores were associated with better verbal memory at older ages, but not with cognitive decline over 6 years in global cognition, verbal memory or TICS.

Chapter 7 provides an overview of the NU-AGE (NUtrients and AGEing) dietary intervention study. The NU-AGE study is a randomized one-year intervention in 1,250 apparently healthy, independently living European subjects aged 65 to 80 years. Subjects were randomised into either the intervention group or the control group. Participants in the intervention group received dietary advice aimed at meeting the dietary recommendations of the ageing population. At the start of this thesis, the NU-AGE study was the first dietary intervention investigating the effect of a whole diet and providing targeted dietary recommendations for optimal health and quality of life in apparently healthy European elderly.

In **chapter 8**, we evaluated if the NU-AGE intervention could be effective to shift the dietary intake of apparently healthy older adults aged 65-80 years living in Italy, the UK, the Netherlands, Poland, and France towards a more healthful diet. The NU-AGE index was created to assess adherence to the NU-AGE diet. At baseline and after one-year follow-up dietary intake was assessed by means of 7-day food records. In total, 1,296 participants were randomized and 1,145 participants completed the intervention (571 in intervention group, 570 in control group). After one-year follow-up, the intervention group improved mean intake of 13 out of 16 dietary recommendations of the NU-AGE diet (p<0.05) with a significant increase of the total NU-AGE index, compared to the control group (mean change in NU-AGE index 21.3±15.9, p<0.01). The NU-AGE dietary intervention, based on dietary recommendations for older adults, may be a feasible strategy to improve dietary intake in an ageing European population.

Chapter 9 shows the results of the NU-AGE parallel randomized dietary intervention study on cognitive functioning in the Dutch NU-AGE subpopulation, including 252 older adults aged 65-80 years (123 intervention, 129 control). The primary outcome was one-year change in global cognition and in four cognitive domains as measured through a comprehensive neuropsychological test battery. Comparing the intervention with the control group, there was no effect of the intervention on cognitive functioning.

In **chapter 10** the main findings of this thesis were summarized and a reflection on methodological aspects was given. When grading the reported associations in previous studies and studies in this thesis, it was concluded that there is *probable* evidence for a role of the Mediterranean diet on cognitive functioning. For the DASH and MIND diet there is a *possible* link with cognitive functioning; for the HDI and the NU-AGE diet there is *no sufficient* evidence yet to conclude that there is a relation with cognitive functioning. Not only is there a need for well-designed intervention and prospective studies, we also call for communication strategies to the general public about the consumption of healthier diets to not only impact cardiovascular but also potentially impact brain health as many individuals will face progressive cognitive decline in the near future.

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About the author

Curriculum Vitae

Agnes Berendsen was born on May 22nd, 1985 in Arnhem, the Netherlands. After completing secondary school at the 'Candea College' in Duiven in 2001, she started her higher vocational education 'Nutrition and Dietetics' at the HAN, Nijmegen. For her first clinical internship she worked with a private dietician in Eindhoven and Waalre. Agnes completed her second clinical internship at the St. Antonius hospital in Nieuwegein. For her research thesis she went to Umeå University in Sweden, where she studied the dietetic treatment of coeliac disease patients. After graduating as registered dietician, Agnes worked several years as a clinical dietician in the St. Antonius hospital in Nieuwegein, the Mesos Medisch centrum in Utrecht and later on in Rijnstate hospital in Arnhem. Because Agnes was highly interested to learn more about the scientific evidence behind dietary guidelines, she decided to go back to college to study Nutrition and Health in Wageningen in 2008. For her first MSc thesis, she went to the Radboud University Medical Centre to study the association between B-vitamin intake and bladder cancer risk. She completed her second MSc thesis at the division of Human Nutrition, on the association between the use of vitamin supplements and colorectal cancer recurrence. In 2010, she completed her Masters with the specialization Epidemiology and Public Health. After this, Agnes worked on a large randomized trial – the B-PROOF study - at the Division of Human Nutrition. Soon, she was appointed as Educational Developer within EURRECA - a European project. In July 2011, Agnes started her PhD project at the group of Nutritional Epidemiology and Public Health of the Division of Human Nutrition of Wageningen University under supervision of Prof. Lisette de Groot, Prof. Edith Feskens, and Dr. Ondine van de Rest. Her research was part of a large European project entitled; "New dietary strategies addressing the specific needs of elderly population for a healthy ageing in Europe, NU-AGE", funded by the European Commission. During her PhD, she closely collaborated with researchers from the University of East Anglia (the UK), UNIBO in Bologna (Italy), CRNH in Clermont-Ferrand (France), and WULS-SGGW in Warsaw (Poland). In addition, she visited Harvard T.H. Chan School of Public Health, to work with a large observational dataset, resulting in three publications. Furthermore, she was part of an expert panel within the "Nutrient Intake Optimisation" task force from ILSI Europe, resulting in one publication. The results of her PhD project and other collaborations are described in this thesis entitled "Dietary patterns for healthier cognitive ageing". During her PhD Agnes supervised fifteen students working on their BSc and MSc thesis. Besides working on her PhD research, Agnes contributed to an external scientific report of EFSA on the collection and analysis of published scientific information as preparatory work for the setting of Dietary Reference Values for vitamin D. Furthermore, Agnes was responsible for the design and teaching of epidemiology courses for the online Master Nutritional Epidemiology and Public Health. Moreover, she coordinated the kick-off meeting and other meetings for FOODBALL, a JPI HDHL program. At last, she participated in organising a study tour to Australia for 25 PhD students from the division of Human Nutrition. In 2015, Agnes was third prize winner of the Foppe ten Hoor Award during the Dutch Nutritional Science Days. In 2016, she was selected to participate in the 22nd seminar of the European Nutrition Leadership Platform, and she was one of the five price winners of the Writing Competition. Currently, Agnes continues her work as a researcher to identify the most efficient lifestyle pattern for the prevention of type-2 diabetes. Additionally, she will continue her educational tasks for several bachelor and master courses on epidemiology.

List of publications

Peer reviewed publications

1. **Berendsen AM**, Kang JH, van de Rest O, Jankovic N, Kampman E, Kiefte-de Jong JC, et al. Association of Adherence to a Healthy Diet with Cognitive Decline in European and American Older Adults: A Meta-Analysis within the CHANCES Consortium. Dementia and geriatric cognitive disorders. 2017;43(3-4):215-27.

2. **Berendsen AM**, Kang JH, van de Rest O, Feskens EJ, de Groot LC, Grodstein F. The Dietary Approaches to Stop Hypertension Diet, Cognitive Function, and Cognitive Decline in American Older Women. J Am Med Dir Assoc. 2017;18:427-32.

3. **Berendsen AM**, Kang JH, Feskens EJM, de Groot CPGM, Grodstein F, van de Rest O. Association of long-term adherence to the mind diet with cognitive function and cognitive decline in American women. The journal of nutrition, health & aging. 2017:1-8.

4. Vaes AM, Brouwer-Brolsma EM, van der Zwaluw NL, van Wijngaarden JP, **Berendsen AM**, van Schoor N, et al. Food sources of vitamin D and their association with 25-hydroxyvitamin D status in Dutch older adults. The Journal of steroid biochemistry and molecular biology. 2016.

5. Brouwer-Brolsma EM, **Berendsen AM**, Vaes AMM, Dullemeijer C, de Groot CPGM, Feskens EJM. Collection and analysis of published scientific information as preparatory work for the setting of Dietary Reference Values for Vitamin D. . 2016 March 2016. Report No.

6. **Berendsen AM**, van Lieshout LE, van den Heuvel EG, Matthys C, Peter S, de Groot LC. Conventional foods, followed by dietary supplements and fortified foods, are the key sources of vitamin D, vitamin B6, and selenium intake in Dutch participants of the NU-AGE study. Nutrition research. 2016;36:1171-81.

7. **Berendsen AM**, van de Rest O, Haveman-Nies A, de Groot CPGM. Dietary patterns, cognitive decline, and dementia: a systematic review. Adv Nutr. 2015;6(2):154-68.

8. Santoro A, Pini E, Scurti M, Palmas G, **Berendsen AM**, Brzozowska A, et al. Combating inflammaging through a Mediterranean whole diet approach: The NU-AGE project's conceptual framework and design. Mechanisms of ageing and development. 2014;136-137:3-13.

9. **Berendsen AM**, Santoro A, Pini E, Cevenini E, Ostan R, Pietruszka B, et al. Reprint of: A parallel randomized trial on the effect of a healthful diet on inflammageing and its consequences in European elderly people: design of the NU-AGE dietary intervention study. Mechanisms of ageing and development. 2014;136-137:14-21.

Abstracts and presentations

1. **Berendsen AM**, van de Rest O, Haveman-Nies A, de Groot CPGM. Dietary patterns, cognitive decline, and dementia: a systematic review. JNHA. 2015. Barcelona, Spain. Poster presentation.

2. **Berendsen AM**, van de Rest O, de Groot CPGM, Feskens EJM. Dietary patterns and Cognitive Ageing. Annual Dutch Nutritional Science Days. 2012, 2015 (Foppe ten Hoor). Deurne, The Netherlands. Oral presentation.

3. **Berendsen AM**, de Groot CPGM, Feskens EJM. Compliane and changes in dietary intake among elderly people in five EU countries. NU-AGE Final conference. 2015. Brussel, Belgium. Oral presentation.

Overview of completed training activities

Discipline specific activities
Courses
- Nutritional and lifestyle epidemiology, graduate school VLAG, Wageningen, 2011
- Summer school 'Why and How we age', Leyden Academy, Leiden, 2011
- Nutrient Density of Milk, Milk Genomics and Health Benefits of Dairy, VLAG/WIAS/NZO, Wageningen, 2011
- Epigenesis and Epigenetics (2nd edition), VLAG, Wageningen, 2011
- Analysis using R, VLAG/WUR, Wageningen, 2012
- Confounding, VLAG, Wageningen, 2014
- Biomarkers for intake of foods and food groups, WUR, Wageningen, 2014

Conferences and meetings:
- Health Benefits of foods. From emerging science to innovative products, ILSI, Prague, Czech, 2011
- Annual NU-AGE meetings, 2011 (Norwich, UK), 2013 (Granada, Spain), 2014 (Spetses, Greece), 2015 (Cork, Ireland), 2016[1] (Brussels, Belgium)
- NU-AGE Training for recruitment, dietary intervention and measurements[1,3], WULS-SGGW/WUR, Warsaw, Poland, 2011-2012
- Annual Dutch Nutritional Science Days, NWO, Deurne, 2012, 2015[1] (Foppe ten Hoor)
- International conference on Aging & Cognition, Dortmund, Germany, 2013
- International Congress of Nutrition, IUNS, Granada, Spain, 2013
- Gezonde voeding, gezond ouder worden - 25 jaar voedingsonderzoek bij ouderen in Wageningen[2], Wageningen, 2013
- Ageing Research@NL[2], NCHA, Den Haag, 2013
- ILSI expert group meetings, ILSI, Brussels, Belgium, 2014[1], 2016[1]
- Nutrition for the ageing brain, ILSI, Milan, Italy, 2014
- Mix & Match meeting, Alzheimer Nederland, Utrecht, 2015
- International Academy Nutrition & Aging congress[2], Barcelona, Spain, 2015
- Voeding Nederland - Wetenschap en praktijk, Utrecht, 2015
- Challenges in translational research to promote healthy ageing[2], DUSRA/KNAW, Leiden, 2017

General courses
- Scientific Writing Workshop, VLAG, Wageningen, 2011
- Wet- en regelgeving voor klinisch onderzoek, ZGV, Ede, 2011
- Competence Assessment, WGS, Wageningen, 2012
- VLAG PhD week (26th edition), Baarlo, 2012
- Supervising MSc thesis, ESD/WUR, Wageningen, 2012
- Project and Time Management, WGS, Wageningen, 2012
- Stress Identification and management, VLAG, Wageningen, 2013
- Philosophy and Ethics of Food Science and Technology, VLAG, Wageningen, 2013
- Data management, VLAG, Wageningen, 2014
- PhD Workshop carrousel, VLAG, Wageningen, 2014

- European Nutrition Leadership Program, Luxembourg, Luxembourg, 2016
- Teaching an online course, ESD/WUR, Wageningen, 2017

Optional courses and activities
- Preparation PhD research proposal, Wageningen, 2011
- PhD study tour[3], Division of Human Nutrition, Melbourne/Sydney, Australia, 2013
- Epi-research meetings, HNE, Wageningen, 2011-2015
- Methodology Club, HNE, Wageningen, 2011-2015
- Rothman lunches, HNE, Wageningen, 2013-2016

[1] Oral presentation given; [2] Poster presentation given, [3] Member of the organizing committee

Notes

Colophon