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(71) Applicant: **NESTEC S.A.** [CH/CH]; Avenue Nestlé 55, 1800 VEVEY (CH).

(72) Inventors: **BOWMAN, Gene**; Chemin du Boschet 8B, 1025 ST-SULPICE (CH). **HUDRY-LABBE, Julie, Laure**; Chemin de la Caboletàz 11, 1066 EPALINGES (CH). **SCHMITT, Jeroen, Antonius, Johannes**; Chemin du Général Voruz 13, 1510 Moudon (CH). **BOSCHAT, Corina**; Route du Jorat 112, 1000 LAUSANNE (CH).

(74) Agent: **CHAUTARD, Cécile**; Centre de Recherche Nestlé, Case postale 44 Vers-chez-les-Blanc, 1000 LAUSANNE 26 (CH).

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(54) Title: OMEGA-3 FATTY ACID AND VITAMIN D LEVELS TO IDENTIFY AND ATTENUATE COGNITIVE AGING IN INDIVIDUALS

(57) Abstract: A method for identifying pre-disposition to cognitive decline in a subject, the method comprising determining levels of: (a) omega-3 fatty acids, and vitamin D or a metabolite thereof; (b) omega-3 fatty acids, and homocysteine; (c) vitamin D or a metabolite thereof, and homocysteine; or (d) omega-3 fatty acids, vitamin D or a metabolite thereof, and homocysteine, independently in one or more samples obtained from the subject.

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OMEGA-3 FATTY ACID AND VITAMIN D LEVELS TO IDENTIFY AND ATTENUATE COGNITIVE AGING IN INDIVIDUALS

FIELD OF THE INVENTION

The present invention relates to methods for identifying pre-disposition to cognitive decline in subjects, and agents for reducing or preventing cognitive decline, in particular reducing or preventing cognitive decline in subjects identified according to a method of the invention.

BACKGROUND TO THE INVENTION

Population ageing has been a remarkable demographic event. As the growth of the older population has outpaced the total population due to increased longevity, the proportion of older persons relative to the rest of the population has increased considerably due to decreased fertility rates. For example, one in every twelve individuals was at least 60 years of age in 1950, and one in every ten was aged 60 years or older by the end of 2000. By the end of 2050, the number of persons worldwide that are 60 years or over is projected to be one in every five.

Aged or ageing individuals frequently suffer some degree of cognitive impairment, including decline in cognitive function that progresses with age and age-related changes in brain morphology and cerebrovascular function are commonly observed. Cognitive decline has been consistently reported with ageing across a range of cognitive domains including processing speed, attention, episodic memory, spatial ability and executive function. Brain imaging studies have revealed that these normal age-related cognitive declines are associated with decreases in both grey and white matter volume in the brain, with the fronto-striatal system most heavily compromised with ageing. These decreases in cortical volume can be attributed to a number of detrimental cellular processes involved with normal ageing, such as accumulation of damage by free radicals over time leading to oxidative damage, chronic low-grade inflammation, homocysteine (Hcy) accumulation, and decreased mitochondrial efficiency. In addition to direct cellular damage, the brain is also indirectly impaired by insults to micro-vascular structures. It is evident that the pathology of ageing and also dementia involves a complexity of these interacting factors, which are linked together. For example, mitochondrial dysfunction leads to increased oxidative stress, and oxidative stress can trigger inflammation and vascular insults.

Furthermore, cognitive decline is an early predictor for Alzheimer pathology and begins before the onset of dementia. In this context, the cognitive composite score represents a reliable means to assess the cognitive decline preceding dementia. Considerable evidence suggests that maintaining brain health and preventing cognitive decline with advancing age may prevent

or delay development of dementia due to Alzheimer's disease and other age-related neuropathologies.

Nutrition, education, physical exercise and cognitive exercise have been recently demonstrated as possible interventions to prevent cognitive decline with ageing. An abundance of clinical, epidemiological and individual evidence is in favour of individual nutritional factors that reduce dementia risk and age-related neurodegeneration. However, formal trial testing of nutritional interventions has yielded mixed results (Schmitt et al. (2010) Nutrition Reviews 68: S2–S5).

Several long-term studies have failed to observe any cognitive benefits with interventions using combinations of B6, B12 and folate. McMahon et al. (2006) N Engl J Med 354(26): 2764-2772 found no effect on cognition in adults aged 65+ after 2 years consumption of a supplement containing folate (1000 µg), Vitamin B12 (500 µg) and B6 (10 mg). Similarly, Hankey et al. (2013) Stroke 44(8): 2232-2239 found that daily supplementation with folic acid (2000 µg), Vitamin B6 (25 mg) and Vitamin B12 (500 µg) to cognitively unimpaired patients with previous stroke or transient ischemic attack, lowered mean tHcy but had no effect on the incidence of cognitive impairment or cognitive decline, as measured by the Mini Mental State Examination (MMSE), during a median of 2.8 years.

Several short-term studies have also failed to show an effect of the combination of B6, B12 and folate for improving cognitive function. Lewerin et al. (2005) Am J Clin Nutr 81(5): 1155-1162, found that 4 months of supplementation of folic acid (800 µg), Vitamin B12 (500 µg) and Vitamin B6 (3 mg) had no effect on cognition in older adults (median age 76 years).

Accordingly, there remains a significant need for methods of reducing or preventing cognitive decline in subjects. Furthermore, there exists a need for identifying subjects who are pre-disposed to cognitive decline, for example to enable earlier intervention in those subjects to reduce the occurrence and/or extent of cognitive decline.

SUMMARY OF THE INVENTION

The inventors utilised banked bio-specimens originating from the Multi-domain Alzheimer Preventive Trial (MAPT; a study which was designed to assess the effects of an omega 3 supplement, a multi-domain intervention comprised of nutritional counselling, physical exercise and cognitive engagement, or a combination of the supplement and multi-domain intervention, versus a placebo in preventing cognitive decline in 1680 non-demented adults aged 70 and older) to quantify three biomarkers that represent distinct pathways toward cognitive decline and dementia. There biomarkers were: homocysteine as a marker of

disturbed one carbon metabolism; 25-hydroxyvitamin D as a steroid hormone marker of disturbed vitamin D binding protein and receptor activity in the brain; and the omega 3 index indicative of fatty acid metabolism. The inventors found that each of these markers are independent risk factors for cognitive decline and that combined they compound the rate of cognitive decline.

Based on these findings, the inventors developed a "nutritional risk index" (NRI) based on omega-3 fatty acids, homocysteine and vitamin D levels, which identifies adults with distinct trajectories of cognitive decline, independent of age, gender, education, APOE4 genotype and intervention arms. Each point increase in the NRI is associated with more accelerated cognitive decline over 3 years. These data suggest that reducing nutritional risk attributable to low vitamin D3 and erythrocyte omega 3 fatty acids, and/or high homocysteine may reduce or prevent age-related cognitive decline.

Furthermore, the inventors believe that prior nutritional interventions attempting to reduce cognitive decline, dementia risk and age-related neurodegeneration have focused on the administration of nutrients in isolation rather than together intelligently in combination to increase the magnitude of effect through nutrient interaction. Moreover, studies investigating the effects of combined ingredients on cognitive function have used a mixture of constituents that all target the same mechanism (e.g. a mix of folate, and Vitamins B12 and B6 targeting Hcy levels, or a mix of Vitamins C and E targeting oxidative damage), which may be why that evidence is as inconsistent as the single ingredient research. In contrast, the present disclosure relates to a multi-intervention approach whereby each of the interventions targets a different risk factor associated with cognitive decline.

Accordingly, in one aspect the invention provides a method for identifying pre-disposition to cognitive decline in a subject, the method comprising determining levels of:

(a) omega-3 fatty acids, and vitamin D or a metabolite thereof;

(b) omega-3 fatty acids, and homocysteine;

(c) vitamin D or a metabolite thereof, and homocysteine; or

(d) omega-3 fatty acids, vitamin D or a metabolite thereof, and homocysteine,

independently in one or more samples obtained from the subject.

In one embodiment, the method comprises determining levels of omega-3 fatty acids, and vitamin D or a metabolite thereof. In one embodiment, the method comprises determining

levels of omega-3 fatty acids, and homocysteine. In one embodiment, the method comprises determining levels of vitamin D or a metabolite thereof, and homocysteine.

In a preferred embodiment, the method comprises determining levels of omega-3 fatty acids, vitamin D or a metabolite thereof, and homocysteine.

In one embodiment, the method comprises:

(a) determining the level of two or more of omega-3 fatty acids, vitamin D or a metabolite thereof, or homocysteine independently in one or more samples obtained from the subject; and

(b) comparing the levels of the two or more of omega-3 fatty acids, vitamin D or a metabolite thereof, or homocysteine to two or more reference values,

wherein the levels of the two or more of omega-3 fatty acids, vitamin D or a metabolite thereof, or homocysteine compared to the two or more reference values is indicative of pre-disposition to cognitive decline in the subject.

In one embodiment:

(a) a level of omega-3 fatty acids is determined and a decrease in the level of omega-3 fatty acids in the sample from the subject compared to a reference value is indicative of pre-disposition to cognitive decline;

(b) a level of vitamin D or a metabolite thereof is determined and a decrease in the level of vitamin D or metabolite thereof in the sample from the subject compared to a reference value is indicative of pre-disposition to cognitive decline; and/or

(c) a level of homocysteine is determined and an increase in the level of homocysteine in the sample from the subject compared to a reference value is indicative of pre-disposition to cognitive decline.

In one embodiment, the one or more samples are independently selected from the group consisting of a blood sample, plasma sample and serum sample.

In one embodiment, the level of omega-3 fatty acids is determined in a blood sample, preferably an erythrocyte sample. In one embodiment, the level of vitamin D or metabolite thereof is determined in a serum sample. In one embodiment, the level of homocysteine is determined in a plasma sample.

In one embodiment, the omega-3 fatty acid is eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). In a preferred embodiment, the omega-3 fatty acid is erythrocyte membrane EPA and/or erythrocyte membrane DHA.

In one embodiment, the omega-3 fatty acid is EPA. In one embodiment, the omega-3 fatty acid is DHA. In one embodiment, the omega-3 fatty acid is EPA and DHA. In a preferred embodiment, the omega-3 fatty acid is erythrocyte membrane EPA. In a preferred embodiment, the omega-3 fatty acid is erythrocyte membrane DHA. In a particularly preferred embodiment, the omega-3 fatty acid is erythrocyte membrane EPA and erythrocyte membrane DHA.

In one embodiment, the vitamin D or metabolite thereof is vitamin D3, vitamin D2, 25-hydroxyvitamin D3 and/or 25-hydroxyvitamin D2.

In one embodiment, the vitamin D or metabolite thereof is vitamin D3. In one embodiment, the vitamin D or metabolite thereof is vitamin D2. In one embodiment, the vitamin D or metabolite thereof is 25-hydroxyvitamin D3. In one embodiment, the vitamin D or metabolite thereof is 25-hydroxyvitamin D2.

In a preferred embodiment, the vitamin D or metabolite thereof is 25-hydroxyvitamin D. In a preferred embodiment, the vitamin D or metabolite thereof is 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2.

In one embodiment, the omega 3 fatty acid level in the sample is measured using using gas chromatography. In one embodiment, the vitamin D or a metabolite thereof level in the sample is measured using an electrochemiluminescence binding assay. In one embodiment, the homocysteine level in the sample is measured using an enzymatic cycling assay.

In one embodiment, the subject is a human subject.

In one embodiment, the subject is an ageing human subject. In one embodiment, the subject is a human subject of at least 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95 years of age. In a preferred embodiment, the subject is a human subject of 50 years of age or more. In a particularly preferred embodiment, the subject is a human subject of 70 years of age or more.

In one embodiment, the subject does not have dementia.

In one embodiment, the subject has a Clinical Dementia Rating (CDR) of 0.5 at baseline.

In one embodiment, the subject has a risk score in Cardiovascular Risk Factors, Aging and Dementia (CAIDE) of 10 to 15 at baseline.

In one embodiment, the subject is amyloid positive on amyloid PET scans at baseline.

In one embodiment, the subject has a genotype indicating risk of cognitive decline. In one embodiment, the subject is an APOE4 carrier.

In another embodiment, the subject is at risk of dementia determined by one or more risk factors selected from the group consisting of age, vascular risk factors (e.g. hypertension and/or diabetes), APOE4 genotype, amyloid positive (e.g. on amyloid PET scans), presence of white matter lesions, other signs of cerebral small vessel disease (e.g. infarcts and/or lacunes) and depression.

In one embodiment, the method further comprises combining the level of the omega-3 fatty acids, vitamin D or a metabolite thereof, and/or homocysteine with one or more anthropometric measures and/or lifestyle characteristics of the subject. Preferably, the anthropometric measure is selected from the group consisting of gender, weight, height, age and body mass index. Preferably, the lifestyle characteristic is whether the subject is a smoker or a non-smoker.

In one embodiment, the method further comprises combining the level of the omega-3 fatty acids, vitamin D or a metabolite thereof, and/or homocysteine with the gender of the subject.

In one embodiment, the method further comprises combining the level of the omega-3 fatty acids, vitamin D or a metabolite thereof, and/or homocysteine with the age of the subject.

Preferably, the method is an in vitro method.

In another aspect, the invention provides an omega-3 fatty acid for use in reducing or preventing cognitive decline in a subject, wherein the omega-3 fatty acid is administered to the subject simultaneously, sequentially or separately with vitamin D or a metabolite thereof, and/or an agent capable of reducing plasma homocysteine levels.

In one embodiment, the omega-3 fatty acid is administered to the subject simultaneously, sequentially or separately with vitamin D or a metabolite thereof. In one embodiment, the omega-3 fatty acid is administered to the subject simultaneously, sequentially or separately with an agent capable of reducing plasma homocysteine levels. In a preferred embodiment, the omega-3 fatty acid is administered to the subject simultaneously, sequentially or separately with vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels.

In another aspect, the invention provides vitamin D or a metabolite thereof for use in reducing or preventing cognitive decline in a subject, wherein the vitamin D or metabolite thereof is administered to the subject simultaneously, sequentially or separately with an omega-3 fatty acid, and/or an agent capable of reducing plasma homocysteine levels.

In one embodiment, the vitamin D or a metabolite thereof is administered to the subject simultaneously, sequentially or separately with an omega-3 fatty acid. In one embodiment, the vitamin D or a metabolite thereof is administered to the subject simultaneously, sequentially or separately with an agent capable of reducing plasma homocysteine levels. In a preferred embodiment, the vitamin D or a metabolite thereof is administered to the subject simultaneously, sequentially or separately with an omega-3 fatty acid, and an agent capable of reducing plasma homocysteine levels.

In another aspect, the invention provides an agent capable of reducing plasma homocysteine levels for use in reducing or preventing cognitive decline in a subject, wherein the agent capable of reducing plasma homocysteine levels is administered to the subject simultaneously, sequentially or separately with an omega-3 fatty acid, and/or vitamin D or a metabolite thereof.

In one embodiment, the agent capable of reducing plasma homocysteine levels is administered to the subject simultaneously, sequentially or separately with an omega-3 fatty acid. In one embodiment, the agent capable of reducing plasma homocysteine levels is administered to the subject simultaneously, sequentially or separately with vitamin D or a metabolite thereof. In a preferred embodiment, the agent capable of reducing plasma homocysteine levels is administered to the subject simultaneously, sequentially or separately with an omega-3 fatty acid, and vitamin D or a metabolite thereof.

In another aspect, the invention provides a combination of (a) an omega-3 fatty acid; (b) vitamin D or a metabolite thereof; and (c) an agent capable of reducing plasma homocysteine levels for use in reducing or preventing cognitive decline in a subject, wherein (a), (b) and (c) are administered to the subject simultaneously, sequentially or separately.

In another aspect, the invention provides a method for reducing or preventing cognitive decline in a subject comprising administering:

(a) an omega-3 fatty acid, and vitamin D or a metabolite thereof;

(b) an omega-3 fatty acid, and an agent capable of reducing plasma homocysteine levels;

(c) vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels; or

(d) an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels,

to the subject.

In a preferred embodiment, the method comprises administering an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels to the subject.

In one embodiment, the subject is a subject identified as pre-disposed to cognitive decline by a method of the invention.

In one embodiment, the omega-3 fatty acid is eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA).

In one embodiment, the omega-3 fatty acid is EPA. In one embodiment, the omega-3 fatty acid is DHA. In one embodiment, the omega-3 fatty acid is EPA and DHA.

In one embodiment, the vitamin D or metabolite thereof is vitamin D3, vitamin D2, 25-hydroxyvitamin D3 and/or 25-hydroxyvitamin D2.

In one embodiment, the vitamin D or metabolite thereof is vitamin D3. In one embodiment, the vitamin D or metabolite thereof is vitamin D2. In one embodiment, the vitamin D or metabolite thereof is 25-hydroxyvitamin D3. In one embodiment, the vitamin D or metabolite thereof is 25-hydroxyvitamin D2.

In one embodiment, the vitamin D or metabolite thereof is 25-hydroxyvitamin D. In one embodiment, the vitamin D or metabolite thereof is 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2.

In one embodiment, the agent capable of reducing plasma homocysteine levels is vitamin B6 and/or vitamin B9.

In one embodiment, the agent capable of reducing plasma homocysteine levels is vitamin B6. In one embodiment, the agent capable of reducing plasma homocysteine levels is vitamin B9. In one embodiment, the agent capable of reducing plasma homocysteine levels is vitamin B6 and vitamin B9.

In one embodiment, the subject is a human subject.

In one embodiment, the subject is an ageing human subject. In one embodiment, the subject is a human subject of at least 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95 years of age. In a preferred embodiment, the subject is a human subject of 50 years of age or more. In a particularly preferred embodiment, the subject is a human subject of 70 years of age or more.

In one embodiment, the subject does not have dementia.

In one embodiment, the subject has a Clinical Dementia Rating (CDR) of 0.5 at baseline.

In one embodiment, the subject has a risk score in Cardiovascular Risk Factors, Aging and Dementia (CAIDE) of 10 to 15 at baseline.

In one embodiment, the subject is amyloid positive on amyloid PET scans at baseline.

In one embodiment, the subject has a genotype indicating risk of cognitive decline. In one embodiment, the subject is an APOE4 carrier.

In another embodiment, the subject is at risk of dementia determined by one or more risk factors selected from the group consisting of age, vascular risk factors (e.g. hypertension and/or diabetes), APOE4 genotype, amyloid positive (e.g. on amyloid PET scans), presence of white matter lesions, other signs of cerebral small vessel disease (e.g. infarcts and/or lacunes) and depression.

In one embodiment, the administration is a dietary intervention.

In one embodiment, the omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels is orally administered to the subject daily for at least one month.

In one embodiment, the subject is further administered one or more B vitamins selected from the group consisting of Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B5, Vitamin B7 and Vitamin B12.

In a preferred embodiment, the subject is further administered Vitamin B12. In one embodiment, the vitamin B12 is administered at a dosage of 0.1 to 40 times the RDA of vitamin B12 per day, preferably 10 to 40, 10 to 30 or 10 to 25 times the RDA of vitamin B12 per day, more preferably 12 to 21 times the RDA of vitamin B12 per day.

In one embodiment, the omega-3 fatty acid; vitamin D or a metabolite thereof; and/or agent capable of reducing plasma homocysteine levels are administered to the subject simultaneously, sequentially or separately with vitamin B12, wherein the vitamin B12 is

administered at a dosage of 0.1 to 40 times the RDA of vitamin B12 per day, preferably 10 to 40, 10 to 30 or 10 to 25 times the RDA of vitamin B12 per day, more preferably 12 to 21 times the RDA of vitamin B12 per day.

In one embodiment, the subject is further administered one or more antioxidants selected from the group consisting of Vitamin C, Vitamin D, Vitamin E and selenium.

In one embodiment, the use or method of the invention provides an improvement of neuronal fluidity, stimulation of neuronal plasticity and activity, improvement of the anti-inflammatory potential, support or maintenance of cognitive performance, support or maintenance of brain performance, slowing down ageing of the brain, support of an active mind and brain fitness, support or maintenance of a healthy brain, enhancement of memory, enhancement of executive functions, enhancement of attention, maintenance of cognitive health and/or maintenance of brain cellular health.

In one embodiment, the omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels is in the form of a food product, preferably further comprising an ingredient selected from the group consisting of protein, carbohydrate, fat and combinations thereof.

In one embodiment, the omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels is in the form of a pharmaceutical composition further comprising a pharmaceutically acceptable carrier, diluent or excipient.

In another aspect, the invention provides a method of achieving one or more benefits selected from the group consisting of decreasing brain atrophy, increasing or maintaining number of synapses, increasing or maintaining amyloid-β phagocytosis and decreasing neuroinflammation in a subject in need thereof, the method comprising administering:

(a) an omega-3 fatty acid, and vitamin D or a metabolite thereof;

(b) an omega-3 fatty acid, and an agent capable of reducing plasma homocysteine levels;

(c) vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels; or

(d) an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels,

to the subject.

In a preferred embodiment, the method comprises administering an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels to the subject.

In another aspect, the invention provides a method of reducing or preventing dementia in a subject at risk thereof, the method comprising administering: to the subject a therapeutically effective amount of a composition comprising an omega-3 fatty acid, Vitamin B6 and Vitamin B9.

(a) an omega-3 fatty acid, and vitamin D or a metabolite thereof;

(b) an omega-3 fatty acid, and an agent capable of reducing plasma homocysteine levels;

(c) vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels; or

(d) an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels,

to the subject.

In a preferred embodiment, the method comprises administering an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels to the subject.

In one embodiment, the dementia is selected from the group consisting of Alzheimer's disease, vascular dementia, Lewy body dementia, frontotemporal dementia and combinations thereof.

In another aspect, the invention provides a method of improving cognitive ability in a subject, the method comprising administering:

(a) an omega-3 fatty acid, and vitamin D or a metabolite thereof;

(b) an omega-3 fatty acid, and an agent capable of reducing plasma homocysteine levels;

(c) vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels; or

(d) an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels,

to the subject.

In a preferred embodiment, the method comprises administering an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels to the subject.

In one embodiment, the subject does not have dementia.

In another aspect, the invention provides a method of selecting a modification in lifestyle of a subject comprising the steps:

(a) determining whether the subject is pre-disposed to cognitive decline according to the method of the invention; and

(b) selecting a modification in lifestyle capable of preventing or reducing cognitive decline in a subject identified to be in need thereof.

In one embodiment, the method further comprises applying the selected modification in lifestyle to the subject.

In one embodiment, the modification in lifestyle comprises administering:

(a) an omega-3 fatty acid, and vitamin D or a metabolite thereof;

(b) an omega-3 fatty acid, and an agent capable of reducing plasma homocysteine levels;

(c) vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels; or

(d) an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels,

to the subject.

In a preferred embodiment, the method comprises administering an omega-3 fatty acid, vitamin D or a metabolite thereof, and an agent capable of reducing plasma homocysteine levels to the subject.

In another aspect, the invention provides a computer program product comprising computer implementable instructions for causing a programmable computer to determine whether a subject is pre-disposed to cognitive decline according to the method disclosed herein.

In another aspect, the invention provides a computer program product comprising computer implementable instructions for causing a programmable computer to determine whether a subject is pre-disposed to cognitive decline given the levels of the omega-3 fatty acids, vitamin D or a metabolite thereof, and/or homocysteine from the user.

DESCRIPTION OF THE DRAWINGS

Figure 1

Figure 1 shows how rates of cognitive decline differ by Nutritional Risk Index (NRI) scores. Each subject is allocated a score of 0 or 1 depending on whether they meet the following criteria: Serum 25-hydroxyvitamin D ≤ 15 ng/mL = 1, otherwise 0; plasma homocysteine ≥ 18.1 = 1, otherwise 0; RBC omega 3 ≤ 4.82 = 1, otherwise 0. Thus, each subject receives a NRI of 0-3, where NRI = 3 is considered as highest nutritional risk for cognitive decline. Cognitive change is measured over four time points over 3 years.

The “lowh highd higho” line illustrates rates of cognitive decline in subjects with NRI = 0. The “high highd higho”, “lowh lowd higho” and “lowh highd lowo” lines illustrates rates of cognitive decline in subjects with NRI = 1. The “high lowd higho”, “lowh lowd lowo” and “high highd lowo” lines illustrate the rates of cognitive decline in subjects with NRI = 2 and the “high lowd lowo” line illustrates subjects with highest nutritional risk (NRI = 3) and their rates of cognitive decline.

Figure 2

Distribution and probability plots of vitamin D levels (ng/mL) in the subject population.

Figure 3

Distribution and probability plots of homocysteine levels (µmol/L) in the subject population.

DETAILED DESCRIPTION OF THE INVENTION

The terms “comprising”, “comprises” and “comprised of” as used herein are synonymous with “including” or “includes”; or “containing” or “contains”, and are inclusive or open-ended and do not exclude additional, non-recited members, elements or steps. The terms “comprising”, “comprises” and “comprised of” also include the term “consisting of”.

All percentages expressed herein are by weight of the total weight of the composition unless expressed otherwise.

The terms “food,” “food product” and “food composition” as used herein mean a product or composition that is intended for ingestion by an individual, such as a human, and provides at least one nutrient to the individual. The compositions of the present disclosure, including the embodiments described herein, can comprise, consist of or consist essentially of the elements disclosed herein, as well as any additional or optional ingredients, components, or elements described herein or otherwise useful in a diet.

As used herein, an “effective amount” is an amount that prevents a deficiency, treats a disease or medical condition in an individual or, more generally, reduces symptoms, manages progression of the diseases or provides a nutritional, physiological, or medical benefit to the individual. The relative terms “improved,” “increased,” “enhanced” and the like refer to the effects of the composition disclosed herein relative to a composition lacking one or more ingredients and/or having a different amount of one or more ingredients, but otherwise identical.

Cognitive decline and ageing

The terms “cognition” and “cognitive ability” as used herein may mean the intellectual process by which an individual becomes aware of, perceives or comprehends ideas. Cognitive ability embraces the quality of knowing, which includes all aspects of perception, recognition, conception, sensing, thinking, reasoning, remembering and imaging. Loss of cognitive ability is the difficulty in dealing with or reacting to new information or situations. Cognitive decline or impairment may manifest itself in many ways, e.g. short-term memory loss, diminished capacity to learn, diminished rate of learning, diminished attention, diminished motor performance and/or dementia, among other indicia. Non-limiting examples of specific cognitive domains that include abilities that decrease with age are (i) attention: processing speed, and selected and divided attention; (ii) learning and memory: delayed free recall, source memory, prospective memory and episodic memory; (iii) language: verbal fluency, visitation naming and word finding; (iv) visuospatial abilities: visual construction skills; and (v) executive functioning: planning, decision making, reasoning and mental flexibility.

The terms “cognitive ageing” and “age-related cognitive decline” as used herein mean a decline in cognitive ability that progresses with age, for example an elderly age that is increasing, and can include age-related changes in brain morphology and/or cerebrovascular function. Cognitive ageing does not include impaired cognitive ability caused by an underlying condition other than ageing, such as a head injury or depression.

Levels of and improvements in cognition can be readily assessed by the skilled person using any suitable neurological and cognitive tests that are known in the art, including cognitive tests designed to assess speed of information processing, executive function and memory. Suitable example tests include Mini Mental State Examination (MMSE), Cambridge Neuropsychological Test Automated Battery (CANTAB), Alzheimer's Disease Assessment Scale-cognitive test (ADAScog), Wisconsin Card Sorting Test, Verbal and Figural Fluency Test and Trail Making Test, electroencephalography (EEG), magnetoencephalography (MEG), Positron Emission Tomography (PET), Single Photon Emission Computed Tomography (SPECT), Magnetic Resonance Imaging (MRI), functional Magnetic Resonance Imaging (fMRI), computerised tomography and long-term potentiation.

EEG, a measure of electrical activity of the brain, is accomplished by placing electrodes on the scalp at various landmarks and recording greatly amplified brain signals. MEG is similar to EEG in that it measures the magnetic fields that are linked to electrical fields. MEG is used to measure spontaneous brain activity, including synchronous waves in the nervous system.

PET provides a measure of oxygen utilisation and glucose metabolism. In this technique, a radioactive positron-emitting tracer is administered, and tracer uptake by the brain is correlated with brain activity. These tracers emit gamma rays which are detected by sensors surrounding the head, resulting in a 3D map of brain activation. As soon as the tracer is taken up by the brain, the detected radioactivity occurs as a function of regional cerebral blood flow. During activation, an increase in cerebral blood flow and neuronal glucose metabolism can be detected within seconds.

Suitable analysis can also be based on neuropsychiatric testing, clinical examinations and individual complaints of loss of cognitive function (e.g. subjective memory loss).

Cognitive decline may be, for example, interpreted as a statistically significant difference from the baseline performance in a suitable test.

A “non-demented” individual (also referred to herein as a subject “that does not have dementia”) has a Clinical Dementia Rating of up to 0.5. The CDR measures dementia severity and is a global rating of dementia with scores ranging from 0 to 3 (0, 0.5, 1, 2 and 3) rated by a semi-structured subject and informant interview (Hughes et al. (1982) Br. J. Psychiatry 140: 566-72). A clinician synthesises the cognitive and functional abilities based on six domains, including memory, orientation, judgment and problem solving, community affairs, home and hobbies, and personal care. The scale has good inter-rater agreement.

The non-demented individual does not have any of Alzheimer's disease, vascular dementia, Lewy body dementia or frontotemporal dementia. In some embodiments, the non-demented individual is a healthy ageing individual. In other embodiments, the non-demented individual has a phenotype associated with age-related cognitive impairment. For example, when compared to a control individual not having the phenotype, the non-demented individual may have a phenotype that includes one or more of decreased ability to recall, short-term memory loss, decreased learning rate, decreased capacity for learning, decreased problem solving skills, decreased attention span, decreased motor performance or increased confusion.

A non-limiting example of a non-demented individual at risk of cognitive ageing is a human with spontaneous memory complaints, but who nevertheless has a Mini Mental State Examination (MMSE) score of at least 24 and has independence in basic daily activities as shown by an Activities of Daily Living (ADL) score of at least 4. An MMSE score for the present purpose may be e.g. 24 to 30, more preferably 26 to 30.

The MMSE is a very brief, easily administered/executed mental status examination that has proved to be a highly reliable and valid instrument for detecting and tracking the progression of the cognitive impairment associated with neurodegenerative diseases. The MMSE is a fully structured scale that consists of 30 points grouped into seven categories: orientation to place (state, county, town, hospital and floor), orientation to time (year, season, month, day and date), registration (immediately repeating three words), attention and concentration (serially subtracting 7, beginning with 100, or, alternatively, spelling the word world backward), recall (recalling the previously repeated three words), language (naming two items, repeating a phrase, reading aloud and understanding a sentence, writing a sentence and following a three-step command), and visual construction (copying a design) (Folstein et al. (1975) J. Psychiat. Res. 12: 189-198).

The MMSE is scored in terms of the number of correctly completed items; lower scores indicate poorer performance and greater cognitive impairment. The total score ranges from 0 to 30.

The ADL is an informant-based activity of daily living scale widely used measure to assess activities of daily living in people with and without AD. The instrument assesses ability over a wide range of performances. The ADL has shown sensitivity to change among mildly impaired individuals compared to non-impaired controls and can capture functional changes (Galasko et al. (1997) Alzheimer Dis. Assoc. Disord. 11 Suppl. 2: S33-9).

As noted earlier herein, considerable evidence suggests that maintaining brain health and preventing cognitive decline with advancing age may prevent or delay development of

dementia. Therefore, the methods disclosed herein which prevent or reduce cognitive decline or ageing can also ultimately prevent dementia such as Alzheimer's disease. Accordingly, another aspect of the present disclosure is a method of preventing dementia in an individual at risk thereof. The method comprises administering to the individual a therapeutically effective amount of the compositions disclosed herein. The dementia that is prevented can be selected from the group consisting of Alzheimer's disease, vascular dementia, Lewy body dementia, frontotemporal dementia and combinations thereof.

Alzheimer's disease

Alzheimer's disease is caused by atrophy of areas of the brain. Although it is not known what initiates the atrophy, studies have found amyloid plaques, neurofibrillary tangles and acetylcholine imbalances in the brains of Alzheimer's patients. Vascular damage in the brain, which may damage healthy neurons, is also common in Alzheimer's patients.

Alzheimer's disease is a progressive condition that affects multiple brain functions. Early signs of the disease usually include minor memory problems, for example forgetting recent events or the names of places and objects. As the disease progresses, memory problems become more severe and additional symptoms can develop, such as confusion, disorientation, difficulty making decisions, problems with speech and language, and personality changes.

Vascular dementia

Vascular dementia results from reduced blood flow to the brain, which damages brain cells. The reduced blood flow can occur for a number of reasons, including narrowing of the blood vessels in the brain (subcortical vascular dementia), stroke (single-infarct dementia) and numerous small strokes (multi-infarct dementia). The reduced blood flow may additionally be caused by Alzheimer's disease, a combination referred to as mixed dementia.

Early symptoms of vascular dementia include slowness of thought, difficulty with planning, difficulty with language, problems with attention and concentration, and behavioural changes. The symptoms typically worsen in steps, with intervening stable periods of months or years.

Parkinson's disease

Parkinson's disease is a condition in which nerve cells in the substantia nigra become progressively damaged. Nerve cells in this area of the brain produce dopamine, which acts as a messenger between the parts of the brain and nervous system that control body movement. Damage to these nerve cells results in a reduction in the amount of dopamine produced in the brain, which has the effect of reducing function in the part of the brain controlling movement.

Symptoms of the Parkinson's disease include tremors, slow movement, and stiff and inflexible muscles. Parkinson's disease patients may also experience additional symptoms, including depression, constipation, insomnia, anosmia and memory problems.

Determining biomarker levels

The level of the individual biomarker species in the sample may be measured or determined by any suitable method known in the art. For example, mass spectrometry (MS), antibody-based detection methods (e.g. enzyme-linked immunosorbent assay, ELISA), non-antibody protein scaffold-based methods (e.g. fibronectin scaffolds), radioimmunoassays (RIA) or aptamer-based methods may be used. Other spectroscopic methods, chromatographic methods, labelling techniques or quantitative chemical methods may also be used.

Suitable example methods to determine individual biomarker levels are described below.

25-hydroxyvitamin D

Electrochemiluminescence binding assays may be utilised for the in vitro determination of total 25-hydroxyvitamin D (e.g. using the commercially available Cobas 8000, Roche). For example, Vitamin D-binding protein (VDBP) may be employed to capture both 25-hydroxyvitamin D3 and D2 with the intention to quantify total vitamin D. Briefly, the sample may be incubated with a pre-treatment reagent to denature the natural VDBP in the sample to release the bound vitamin D. The sample may then be further incubated with a recombinant ruthenium-labelled VDBP to form a complex of 25-hydroxyvitamin D (25-OH-D) and the ruthenylated-VDBP. Addition of a biotinylated 25-OH-D creates a complex consisting of the ruthenium-labelled VDBP and the biotinylated 25-OH-D. The entire complex may be bound to a solid phase by the interaction of biotin and streptavidin-coated microparticles, which may be captured on the surface of an electrode. After removal of unbound substances, adding voltage to the electrode induces chemiluminescent emission which may be measured by a photomultiplier. Results may be determined via an instrument-specific calibration curve.

Homocysteine

Total plasma homocysteine may be measured using an enzymatic cycling assay. Briefly, oxidised homocysteine may be first reduced and then reacted with S-adenosylmethionine to form methionine and S-adenosyl homocysteine (SAH) in the presence of homocysteine S-methyl transferase. SAH may then be assessed by coupled enzyme reactions where SAH is hydrolysed into adenosine and homocysteine by SAH hydrolase and homocysteine is cycled back into the homocysteine conversion reaction, which serves to amplify the detection signal.

The formed adenosine may be hydrolysed into inosine and ammonia, and glutamate dehydrogenase may then be used to catalyse the reaction of ammonia with 2-oxoglutarate and NADH to form NAD+. The concentration of homocysteine in the sample is directly proportional to the amount of NADH converted to NAD+, which may be measured spectroscopically at an absorbance of 340 nm.

Omega 3 fatty acids

Omega 3 fatty acids such as EPA and DHA, for example expressed as a weight percentage of total fatty acids, may be quantified using gas chromatography coupled with a flame ionisation detector. Briefly, erythrocytes may be separated from plasma by centrifugation and washed before lipid extraction by the Folch method including a mixture of hexane and isopropanol after acidification. Margaric acid may be added as an internal standard. Total lipid extracts may then be saponified and methylated, and fatty acid methyl esters (FAME) may be extracted with pentane and analysed by gas chromatography (GC). An example protocol may use a gas chromatograph with a split injector, a bonded silica capillary column (BPX 70, 60 m x 0.25 mm; 0.25 µm film thickness) and a flame ionisation detector; helium may be used as a carrier gas; and the column temperature program may be started at 150°C, increased by 1.3°C/min to 220°C and held at 220°C for 10 min. Identification of FAME may be based on retention times obtained for FAME prepared from fatty acid standards.

Samples

The invention comprises a step of determining the level of two or more biomarkers in one or more samples obtained from a subject.

In one embodiment, the one or more samples are independently selected from the group consisting of a blood sample, plasma sample and serum sample.

Techniques for collecting samples from a subject are well known in the art.

Comparison to reference values

The present method may comprise a step of comparing the levels of omega-3 fatty acids, vitamin D or a metabolite thereof, and/or homocysteine in the test sample with one or more reference or control values. The term “reference value” is synonymous with “control value” and broadly includes data that the skilled person would use to facilitate the accurate interpretation of technical data.

Typically, a reference value for each individual biomarker determined in the method is used. The reference value may be a normal level of that biomarker, e.g. a level of the biomarker in the same sample type (e.g. blood, serum or plasma) in a normal subject. The reference value may, for example, be based on a mean or median level of the biomarker in a control population of subjects, e.g. 5, 10, 100, 1000 or more normal subjects (who may either be age- and/or gender-matched or unmatched to the test subject. It is known in the art how to assign correct reference values as they will vary with gender, race, genetic heritage, health status or age, for example.

The reference value may be determined using corresponding methods to the determination of biomarker levels in the test sample, e.g. using one or more samples taken from normal subjects. For instance, in some embodiments biomarker levels in control samples may be determined in parallel assays to the test samples. Alternatively, in some embodiments reference values for the levels of individual biomarkers in a particular sample type (e.g. blood, serum or plasma) may already be available, for instance from published studies. Thus, in some embodiments, the reference value may have been previously determined, or may be calculated or extrapolated, without having to perform a corresponding determination on a control sample with respect to each test sample obtained.

The control or reference values for a biomarker as described herein in a particular sample may be stored in a database and used in order to interpret the results of the method as performed on the subject.

The level of a biomarker in a test sample, for example the level of the omega-3 fatty acids, vitamin D or a metabolite thereof, and/or homocysteine in a sample from the subject, may be compared to the respective level of the same target in one or more cohorts (populations/groups) of control subjects.

The comparison of the level of the omega-3 fatty acids, vitamin D or a metabolite thereof, and/or homocysteine in a sample from the subject may comprise comparing the level to reference values from a population of control subjects that have been divided into quartiles.

In one embodiment, a level of omega-3 fatty acids is determined and a level of omega-3 fatty acids in the sample from the subject that is in the lowest quartile of reference values from a control population is indicative of pre-disposition to cognitive decline.

In one embodiment, a level of vitamin D or a metabolite thereof is determined and a level of vitamin D or a metabolite thereof in the sample from the subject that is in the lowest quartile

of reference values from a control population is indicative of pre-disposition to cognitive decline.

In one embodiment, a level of homocysteine is determined and a level of homocysteine in the sample from the subject that is in the highest quartile of reference values from a control population is indicative of pre-disposition to cognitive decline.

In one embodiment, the method of the invention comprises calculating an index (referred to herein as a Nutritional Risk Index, NRI) comprising the steps:

(a) a level of omega-3 fatty acids is determined and a level of omega-3 fatty acids in the sample from the subject that is in the lowest quartile of reference values from a control population is assigned a score of n, and a level of omega-3 fatty acids in the sample from the subject that is outside the lowest quartile is assigned a score of zero;

(b) a level of vitamin D or a metabolite thereof is determined and a level of vitamin D or a metabolite thereof in the sample from the subject that is in the lowest quartile of reference values from a control population is assigned a score of n, and a level of vitamin D or a metabolite thereof in the sample from the subject that is outside the lowest quartile is assigned a score of zero; and/or

(c) a level of homocysteine is determined and a level of homocysteine in the sample from the subject that is in the highest quartile of reference values from a control population is assigned a score of n, and a level of homocysteine in the sample from the subject that is outside the highest quartile is assigned a score of zero,

wherein n is a positive integer (e.g. +1), wherein the index is calculated as the sum of scores obtained from steps (a), (b) and/or (c), and wherein a greater index score is indicative of greater pre-disposition to cognitive decline.

In one embodiment, a reference value for erythrocyte EPA and DHA is about 4.82 weight percent of total fatty acids. A level lower than this reference value may be assigned a score of n in the index disclosed herein. In one embodiment, a reference value for plasma 25-hydroxyvitamin D is about 15 ng/mL. A level lower than this reference value may be assigned a score of n in the index disclosed herein. In one embodiment, a reference value for plasma

homocysteine is about 18.1 µmol/L. A level higher than this reference value may be assigned a score of n in the index disclosed herein.

The reference value for the level of the biomarker as described herein is preferably measured using the same units used to characterise the level of biomarker in the test sample. Thus, if the level of the biomarker as described herein is an absolute value, such as the µmol/L (µM), the reference value may also be based upon the units µmol/L (µM) in individuals in the general population or a selected control population of subjects.

The extent of the difference between the subject's biomarker levels and the corresponding reference values is also useful for determining which subjects would benefit most from certain interventions. The level of the biomarker in the test sample may be increased or decreased by, for example, at least 1%, at least 5%, at least 10%, at least 20%, at least 30%, at least 50% or at least 100% compared to the reference value.

Method of treatment

The omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels may be administered simultaneously, sequentially or separately.

The term "combination" or phrases "in combination", "used in combination with" or "combined preparation" as used herein refer to the combined administration of two or more of omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels simultaneously, sequentially or separately.

The term "simultaneous" as used herein means that the agents are administered concurrently, i.e. at the same time. The term "sequential" as used herein means that the agents are administered one after the other. The term "separate" as used herein means that the agents are administered independently of each other but within a time interval that allows the agents to show a combined, preferably synergistic, effect. Thus, administration "separately" may permit one agent to be administered, for example, within 1 minute, 5 minutes or 10 minutes after the other.

It is to be appreciated that all references herein to treatment include curative, palliative and prophylactic treatment. The treatment of mammals, particularly humans, is preferred. Both human and veterinary treatments are within the scope of the invention.

Dosage

The skilled person can readily determine an appropriate dose of one of the agents of the invention to administer to a subject without undue experimentation. Typically, a physician will determine the actual dosage which will be most suitable for an individual patient and it will depend on a variety of factors including the activity of the specific agent employed, the metabolic stability and length of action of that agent, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy. There can of course be individual instances where higher or lower dosage ranges are merited, and such are within the scope of the invention.

Subject

A "subject" (or "individual") refers to either a human or non-human animal.

Examples of non-human animals include an avian, bovine, canine, equine, feline, hicrine, lupine, murine, ovine or porcine animal. A "companion animal" is any domesticated animal, and includes, without limitation, cats, dogs, rabbits, guinea pigs, ferrets, hamsters, mice, gerbils, horses, cows, goats, sheep, donkeys, pigs and the like.

Preferably, the subject is a human.

In one embodiment, the subject is an ageing human subject. The term "ageing human subject" may mean a human subject of 50 years of age or more. In one embodiment, the subject is a human subject of at least 50, 55, 60, 65, 70, 75, 80, 85, 90 or 95 years of age. In a preferred embodiment, the subject is a human subject of 50 years of age or more. In a particularly preferred embodiment, the subject is a human subject of 70 years of age or more.

The term "elderly" in the context of a human means an age from birth of at least 60 years, preferably above 63 years, more preferably above 65 years, and most preferably above 70 years. The term "older adult" in the context of a human means an age from birth of at least 45 years, preferably above 50 years, more preferably above 55 years, and includes elderly individuals.

For other animals, an "older adult" has exceeded 50% of the average lifespan for its particular species and/or breed within a species. An animal is considered "elderly" if it has surpassed 66% of the average expected lifespan, preferably if it has surpassed the 75% of the average expected lifespan, more preferably if it has surpassed 80% of the average expected lifespan. An elderly cat or dog has an age from birth of at least about 7 years.

Dietary intervention

The term “dietary intervention” as used herein refers to an external factor applied to a subject which causes a change in the subject’s diet.

In one embodiment, the dietary intervention is a diet supplemented with an omega 3 fatty acid. In one embodiment, the dietary intervention is a diet supplemented with vitamin D. In one embodiment, the dietary intervention is a diet supplemented with an agent capable of reducing plasma homocysteine levels.

In one embodiment, the dietary intervention comprises increasing omega-3 fatty acid intake by the subject, preferably by administering an omega 3 fatty acid supplement. In one embodiment, the dietary intervention comprises increasing vitamin D intake by the subject, preferably by administering a vitamin D supplement. In one embodiment, the dietary intervention comprises increasing intake of an agent capable of reducing plasma homocysteine levels by the subject, preferably by administering a supplement of an agent capable of reducing plasma homocysteine levels.

The diet may be one which is adjusted to the starting body weight of the subject.

The dietary intervention may comprise administration of at least one diet product. The diet product may be a meal replacement product or a supplement product. The diet product may include food products, drinks, pet food products, food supplements, nutraceuticals, food additives or nutritional formulae.

Compositions

The agents and compositions of the invention may increase cognitive function in an individual (e.g. a non-demented individual) susceptible to or suffering from a decline in cognitive function, such as that brought about by the ageing process. The agents and compositions of the invention may prevent, reduce or delay a decline in cognitive function in an individual (e.g. a non-demented individual) susceptible to or suffering from a decline in cognitive function, such as that brought about by the ageing process. In some embodiments, the methods of the invention comprise, prior to the administration, identifying the individual as having cognitive aging or being at risk of cognitive aging. For example, the methods can comprise, prior to the administration, identifying the individual as being in need of improved cognitive ability. The agents and compositions of the invention may decrease brain atrophy and neuroinflammation and increase amyloid-β phagocytosis and the number of synapses.

The omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels (i.e. agents of the invention) may be administered simultaneously, sequentially or separately.

The omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels may be comprised within one or more compositions.

In one embodiment, the omega 3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels is in the form of a food product, preferably further comprising an ingredient selected from the group consisting of protein, carbohydrate, fat and combinations thereof.

In one embodiment, the omega 3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels is in the form of a pharmaceutical composition further comprising a pharmaceutically acceptable carrier, diluent or excipient.

In various embodiments, the omega 3 fatty acid is 1 to 50 wt.% of the food product or composition, preferably 1 to 30 wt.% of the food product or composition, and most preferably 1 to 15 wt.% of the food product or composition. Preferably, the omega 3 fatty acid comprises at least one of eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA), and more preferably comprises both EPA and DHA. A daily dose of the food product or composition preferably provides 0.5 g to 1.0 g of DHA per day and/or 0.5 g to 1.0 g of EPA per day, more preferably 0.7 g to 1.0 g of DHA per day and/or 0.6 mg to 0.75 g of EPA per day, and most preferably about 770 mg of DHA per day and/or about 700 mg of EPA per day.

In one embodiment, the omega-3 fatty acids are administered at a dosage of 0.5 g to 2.0 g per day, such as 0.5 g to 1.5 g per day. In one embodiment, the subject is administered 0.5 g to 1.0 g of DHA per day and/or 0.5 g to 1.0 g of EPA per day, more preferably 0.7 g to 1.0 g of DHA per day and/or 0.6 mg to 0.75 g of EPA per day, and most preferably about 770 mg of DHA per day and/or about 700 mg of EPA per day.

The omega 3 fatty acid may comprise a blend of one or more sources of omega 3 fatty acids, and each of the one or more sources of omega 3 fatty acids can be natural (e.g. fish oil) or synthetic (i.e. formed through a process manipulated by a human, as opposed to those of natural origin). The term “fish oil” as used herein means a crude or purified fatty or oily extract rich in omega 3 fatty acids and obtained from a sea individual, preferably a cold-water fish such as, but not limited to, salmon, tuna, mackerel, herring, sea bass, striped bass, halibut, catfish and sardines, as well as shark, shrimp and clams, or any combination thereof.

In one embodiment, the food product or composition is administered in a daily dose that provides 60-2000 IU/day of the Vitamin D or metabolite thereof.

In one embodiment, the food product or composition is administered in a daily dose that provides 0.001 mg/day to 0.1 mg/day of the Vitamin D or metabolite thereof, for example 0.01 mg/day to 0.05 mg/day of the Vitamin D or metabolite thereof, preferably about 0.015 mg/day of the Vitamin D or metabolite thereof.

In one embodiment, the Vitamin D or metabolite thereof is administered at a dosage of 60-2000 IU/day.

In one embodiment, the Vitamin D or metabolite thereof is administered at a dosage of 0.001 mg/day to 0.1 mg/day, for example 0.01 mg/day to 0.05 mg/day, preferably about 0.015 mg/day.

In one embodiment, the agent capable of reducing plasma homocysteine levels is vitamin B6 and/or vitamin B9.

In one embodiment, the food product or composition is administered to the individual in a daily dose that provides at least 0.01 to 100 times the recommended daily requirement (RDA) of the Vitamin B6 per day, for example 10 to 80 times the RDA of the Vitamin B6, and/or 0.01 to 5.0 times the RDA of the Vitamin B9 per day, for example 1.0 to 2.5 times the RDA of the Vitamin B9. The RDA of Vitamin B6 is 1.3 mg/day, and thus the food product or composition can be administered in a daily dose that provides 0.13 mg/day to 130 mg/day of the Vitamin B6, for example 13 mg/day to 100 mg/day of the Vitamin B6. The RDA of Vitamin B9 is 0.4 mg/day, and thus the food product or composition can be administered in a daily dose that provides 0.004 mg/day to 2.0 mg/day of the Vitamin B9, for example 0.4 mg/day to 1.0 mg/day of the Vitamin B9. Nevertheless, the present disclosure is not limited to a specific daily dose of the Vitamin B6 or a specific daily dose of the Vitamin B9.

In one embodiment, the subject is administered at least 0.01 to 100 times the RDA of Vitamin B6 per day, for example 10 to 80 times the RDA of Vitamin B6, and/or 0.01 to 5.0 times the RDA of Vitamin B9 per day, for example 1.0 to 2.5 times the RDA of Vitamin B9. In one embodiment, the subject is administered 0.13 mg/day to 130 mg/day of Vitamin B6, for example 13 mg/day to 100 mg/day of Vitamin B6. In one embodiment, the subject is administered 0.004 mg/day to 2.0 mg/day of Vitamin B9, for example 0.4 mg/day to 1.0 mg/day of Vitamin B9.

In one embodiment, the food product or composition can optionally comprise a nitric oxide releasing compound. The nitric oxide releasing compound is any compound or compounds that cause or can result in the release of nitric oxide in an individual. The nitric oxide releasing compound preferably comprises one or more of arginine, citrulline, ornithine or a peptide or protein containing at least one of these amino acids, more preferably arginine and/or citrulline, and even more preferably comprises citrulline, which provides beneficial effects on the cardiovascular system, specifically in terms of improving blood flow, endothelial function and blood pressure. In various embodiments, the nitric oxide releasing compound is 1 to 20 wt.% of the food product or composition, preferably 1 to 15 wt.% of the food product or composition, and more preferably 1 to 10 wt.% of the food product or composition. In one embodiment, a daily dose of the food product or composition provides from 0.5 g to 10.0 g of the nitric oxide releasing compound (e.g., citrulline) per day, preferably 1.0 g to 5.0 g per day, more preferably 2.0 g to 4.0 g per day, and most preferably about 3.0 g per day.

The food product or composition can further comprise at least one B Vitamin additional to the Vitamin B6 and/or the Vitamin B9, for example one or more of Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin), Vitamin B5 (pantothenic acid), Vitamin B7 (biotin) and Vitamin B12 (Cobalamin) or salts, conjugates or derivatives thereof that have B vitamin activity. The food product or composition can comprise from 0.1 to 40 times the RDA of one or more of these additional B vitamins, preferably 1 to 20 times the RDA, and more preferably 1 to 10 times the RDA. In an embodiment, the food product or composition further comprises all of Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin), Vitamin B5 (pantothenic acid), Vitamin B7 (biotin) and Vitamin B12 (cobalamin).

In one embodiment, the food product or composition comprising a combination of an omega-3 fatty acid, at least 0.01 to 100 times the recommended daily requirement (RDA) of Vitamin B6 per day and/or 0.01 to 5.0 times the RDA of the Vitamin B9 per day, also preferably provides 0.1 to 40 times the recommended daily requirement (RDA) of Vitamin B12 per day, e.g. 1 to 10 times the recommended daily requirement (RDA) of Vitamin B12 per day.

The Vitamin B12 may thus be administered as a further B Vitamin in a daily dose of about 10, 20, 30 or 40 times the RDA of the Vitamin B12 per day. Preferably, the daily dose provides 10 to 40, more preferably 10 to 30 or even more preferably 10 to 25 times the RDA of the Vitamin B12 per day, most preferably about 12 to 21 times the RDA of the Vitamin B12 per day. The United States RDA of Vitamin B12 is 2.4 micrograms daily for humans of age 14 years and older, so such individuals may be administered a daily dose of the food product or composition that provides also about 0.002 mg to about 0.4 mg of Vitamin B12 per day, preferably 0.02 mg

to 0.07 mg of Vitamin B12 per day, more preferably 0.03 mg to 0.05 mg of Vitamin B12 per day.

In a preferred embodiment, the subject is further administered Vitamin B12. In one embodiment, the vitamin B12 is administered at a dosage of 0.1 to 40 times the RDA of vitamin B12 per day, preferably 10 to 40, 10 to 30 or 10 to 25 times the RDA of vitamin B12 per day, more preferably 12 to 21 times the RDA of vitamin B12 per day.

In one embodiment, the vitamin B12 is administered at a dosage of about 0.002 mg to about 0.4 mg per day, preferably 0.02 mg to 0.07 mg per day, more preferably 0.03 mg to 0.05 mg per day.

In one embodiment, the omega-3 fatty acid; vitamin D or a metabolite thereof; and/or agent capable of reducing plasma homocysteine levels are administered to the subject simultaneously, sequentially or separately with vitamin B12, wherein the vitamin B12 is administered at a dosage of 0.1 to 40 times the RDA of vitamin B12 per day, preferably 10 to 40, 10 to 30 or 10 to 25 times the RDA of vitamin B12 per day, more preferably 12 to 21 times the RDA of vitamin B12 per day.

In some embodiments, the food product or composition can further comprise one or more antioxidants to protect against oxidative damage and inflammation-induced damage. Non-limiting examples of suitable antioxidants include Vitamin C, Vitamin D, Vitamin E, selenium, and combinations thereof. For example, the food product or composition can comprise 0.0001 wt.% to 25 wt.% of the antioxidant, if present; preferably 0.0001 wt.% to about 15 wt.%; more preferably 0.001 wt.% to 5 wt.%; and most preferably 0.001 wt.% to 2 wt.%.

In one embodiment, the composition is a food composition (food product) for a human and/or a pet such as a companion individual. The food composition may comprise one or more additional substances such as a mineral, another vitamin, a salt, or a functional additive such as flavouring, a colourant, an emulsifier, or an antimicrobial compound or other preservative. Non-limiting examples of suitable minerals include calcium, phosphorous, potassium, sodium, iron, chloride, boron, copper, zinc, magnesium, manganese and iodine. Non-limiting examples of suitable additional vitamins include fat soluble vitamins as A, D, E and K.

In one embodiment, the composition is a pharmaceutical composition comprising one or more pharmaceutically acceptable carriers, diluents or excipients. Generally, pharmaceutical compositions are prepared by admixing the omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels with one or more of an

excipient, a buffer, a binder, a plasticiser, a colourant, a diluent, a compressing agent, a lubricant, a flavourant or a moistening agent.

The omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels may have an acute effect that can be seen in less than one month. Additionally or alternatively, the omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels can have a long-term effect, and thus various embodiments comprise administration to the individual (e.g. orally) for a time period of at least one month; preferably at least two months, more preferably at least three, four, five or six months; most preferably for at least one year. During the time period, the omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels can be administered to the individual at least one day per week; preferably at least two days per week, more preferably at least three, four, five or six days per week; most preferably seven days per week. The omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels can be administered in a single dose per day or in multiple separate doses per day.

Kit

In another aspect, the invention provides a kit comprising the omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels of the invention.

The omega-3 fatty acid, vitamin D or a metabolite thereof and/or agent capable of reducing plasma homocysteine levels may be provided in suitable containers.

The kit may also include instructions for use.

The skilled person will understand that they can combine all features of the invention disclosed herein without departing from the scope of the invention as disclosed.

Preferred features and embodiments of the invention will now be described by way of non-limiting examples.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of chemistry, biochemistry, molecular biology, microbiology and immunology, which are within the capabilities of a person of ordinary skill in the art.

EXAMPLES

Example 1

The Multi-domain Alzheimer Preventive Trial (MAPT) was designed to assess the efficacy of an omega 3 supplement (DHA 800 mg, EPA 200 per day), a multi-domain intervention (nutritional counselling, physical exercise, cognitive stimulation) or a combination of the supplement + multi domain versus a placebo in reducing rate of cognitive decline in adults of 70 years of age or older. Post hoc analysis of MAPT results suggest that omega 3 supplementation prevents cognitive decline in subjects with lower omega 3 status at baseline. The VITACOG trial demonstrated that homocysteine lowering B vitamins can attenuate total brain atrophy in subjects aged 70 and older with MCI and hyperhomocysteinemia. Two subsequent post hoc reports from VITACOG demonstrated that the B vitamin effects were most pronounced on total brain atrophy and cognitive decline in subjects in the highest tertile of baseline omega 3 status. Baseline nutritional status and nutrient interaction are legitimate design elements to consider for future nutritional interventions. These new insights have led to next generation clinical trials targeting subjects at “nutritional risk” (clinicaltrials.gov: NCT01953705) and nutrient combinations that leverage interactive metabolism may prove more capable of preventing cognitive decline. Implementing these clinical trial design elements (e.g., enrichment and or oversampling) first requires a strong scientific rationale for doing so since it adds cost and operational burden to any clinical trial. Using the MAPT trial data, we tested the hypothesis that baseline nutritional status, reflected by plasma homocysteine, serum vitamin D and erythrocyte omega 3 fatty acids are each independent risk factors for cognitive decline, but when combined using a “nutritional risk index” they compound the risk illustrated by acceleration in the rates of cognitive decline over 36 months.

Results

Complete nutritional biomarker and education information were available for 712/780 subjects. The mean age was 75 (4.5), 67% were women, 27.5% completed a University or higher education degree and 20.7% carried the APOE4 allele, which were all similar to the parent MAPT cohort (Table 1). The mean erythrocyte EPA+DHA, plasma homocysteine and serum 25-OH-vitamin D (Figures 2 and 3) was 5.8 (1.5) wt%, 15.8 (5.4) µmol/L, and 23.7 ng/ml (12.2), respectively (Table 1). The NRI increased by 1 point for each of the following: erythrocyte EPA+DHA ≤ 4.82, plasma homocysteine ≥ 18.1 umol/L, and plasma 25-hydroxyvitamin D ≤ 15 ng/mL. Each NRI point increase was incrementally associated with additional acceleration in cognitive decline in comparison with those without nutritional risk (NRI = 0) (e.g. NRI = 1 β = -0.04, p = 0.02; NRI = 2 β = -0.06, p = 0.009; NRI = 3 β = -0.18, p = 0.006) (Table 3). Further

controlling for APOE4, APOE4*time interaction, trial arm, and their interactions with time did not change these results.

Conclusions

A blood-based “nutritional risk index” composed of erythrocyte omega-3 fatty acids (EPA+DHA), plasma homocysteine and serum 25-hydroxyvitamin D identifies people with distinct trajectories of cognitive decline in older adults, independent of age, gender, education, APOE4 genotype and intervention arms. Each point increase in the NRI index was associated with more accelerated cognitive decline over 3-years (Figure 1). These data suggest that reducing nutritional risk attributable to low vitamin D3 and erythrocyte omega 3 fatty acids and high homocysteine may prevent age-related cognitive decline; a concept that could be facilitated by deploying a Nutritional Risk Index and a dietary supplement that addresses these areas.

Table 1. Baseline demographic, nutritional and genetic characteristics of MAPT[1]

	Total (n=712)	**RBC Omega 3 (n=712)**		**Serum vitamin D (n=712)**		**Plasma homocysteine (n=712)**	
		High[2] (n=524)	**Low** (n=188)	**High** (n=517)	**Low** (n=195)	**Low** (n=536)	**High** (n=176)
Age, years, mean (SD)[3]	75.6 (4.5)	75.5 (4.5)	76.1 (4.5)	75.2 (4.4)	76.8 (4.8)	75.3 (4.4)	76.7 (4.6)
Men, N (%)	232 (32.6)	168 (32.1)	64 (34.0)	162 (31.3)	70 (35.9)	155 (28.9)	77 (43.8)
Education							
No diploma/ primary school	168 (23.6)	104 (19.9)	64 (34.0)	111 (21.5)	57 (29.2)	130 (24.3)	38 (21.6)
Secondary education	244 (34.3)	184 (35.1)	60 (31.9)	183 (35.4)	61 (31.3)	172 (32.1)	72 (40.9)
High-school diploma	104 (14.6)	82 (15.7)	22 (11.7)	73 (14.1)	31 (15.9)	84 (15.7)	20 (11.4)
University level	196 (27.5)	154 (29.4)	42 (22.3)	150 (29.0)	46 (23.6)	150 (28.0)	46 (26.1)
Treatment arm							
DHA	180 (25.3)	126 (24.1)	54 (28.9)	136 (26.3)	44 (22.6)	130 (24.3)	50 (28.4)
Multi-domain	176 (24.7)	141 (26.9)	34 (18.2)	124 (24.0)	52 (26.7)	124 (23.1)	52 (29.6)
DHA+ Multi-domain	177 (24.9)	126 (24.1)	51 (27.3)	132 (25.5)	45 (23.1)	137 (25.6)	40 (22.7)
Placebo	179 (25.1)	131 (25.0)	48 (25.7)	125 (24.2)	54 (27.7)	145 (27.1)	34 (19.3)
RBC Omega 3, wt%	5.8 (1.5)	6.4 (1.2)	4.0 (0.6)	5.9 (1.5)	5.5 (1.3)	5.9 (1.5)	5.5 (1.3)
Serum vitamin D, ng/mL	23.7 (12.3)	24.1 (12.5)	22.6 (11.7)	28.6 (10.9)	10.8 (3.0)	23.8 (12.2)	23.4 (12.6)
Plasma homocysteine, µM/L	15.8 (5.3)	15.3 (5.3)	17.1 (5.3)	15.5 (5.0)	16.4 (6.1)	13.4 (2.7)	22.9 (5.2)

Table 2: Prevalence of subjects meeting each level of the nutritional risk index (n = 712)

RBC Omega 3 (EPA+DHA)		**Serum vitamin D**		**Plasma homocysteine**		**Sample size for each combination**
High > 4.82	Low	High ≥ 15	Low	High ≥ 18.1	Low	N (%)
Nutritional risk index = 0 (reference group)						
X		X			X	301 (42.3)
Nutritional risk index = 1						
	X[#]	X			X	91 (12.8)
X			***X***		X	101 (14.2)
X		X		***X***		85 (12.0)
Nutritional risk index = 2						
X			***X***	***X***		37 (5.2)
	X		***X***		X	43 (6.0)
	X	X		***X***		40 (5.6)
Nutritional risk index = 3						
	X		***X***	***X***		14 (2.0)

[#]Bold italic font indicates the respective nutrient contributing to the nutritional risk index

Table 3. Nutritional risk index of cognitive function and decline in MAPT[1]

	Estimated coefficients (95%CI)	**P-value**
Age, years	-0.037 (-0.048, -0.026)	<0.01*
Time, month	0.0012(-0.00076, 0.0032)	0.22
Men vs women	-0.19 (-0.29, -0.08)	<0.01*
Education		
Secondary education	0.094 (-0.041, 0.23)	0.17
High-school diploma	0.440 (0.27, 0.60)	<0.01*
College or more	0.440 (0.30, 0.58)	<0.01*
APOEe4 carrier vs non-carriers	-0.190 (-0.31, -0.064)	0.05
Baseline association		
Nutritional Risk Index		
0 (reference)	—	
1	-0.0059 (-0.11, 010)	0.91
2	-0.14 (-0.29, 0.0087)	0.07
3	-0.043 (-0.43, 0.34)	0.83
Longitudinal association		
Nutritional Risk Index * Time (month)		
0*Time (reference)	—	
1*Time	-0.0033 (-0.0062, -0.00038)	0.03*
2*Time	-0.0046 (-0.0087, -0.00042)	0.03*
3*Time	-0.023 (-0.036, -0.01)	<0.01*

[1]Nutritional risk index increases by 1 point for each of the following: Serum 25-hydroxyvitamin D ≤ 15 ng/mL; plasma homocysteine ≥ 18.1; RBC omega 3 ≤ 4.82; Primary outcome measure is the cognitive composite Z score.

Table 4. Independent and interactive nutritional risk for age related cognitive decline in MAPT (n=712)

	Educ	VitD Low	HCy High	Omega3 Low	NRI	Estimated coefficients	SE	*P* - value
Intercept						2.4044	0.4301	<.0001
Age at baseline, years						-0.0338	0.005622	<.0001
Time, Month						0.0009	0.000998	0.3466
Men vs. Women						-0.2087	0.05377	0.0001
Baseline association								
VitD*HCy*omega3		0	0	0	0	0	—	—
VitD*HCy*omega3		1	1	1	3	0.0177	0.1832	0.9229
VitD*HCy*omega3		1	1	0	2	-0.1413	0.1172	0.2281
VitD*HCy*omega3		1	0	1	2	-0.1968	0.1079	0.0682
VitD*HCy*omega3		1	0	0	1	-0.0698	0.07576	0.3570
VitD*HCy*omega3		0	1	1	2	-0.2887	0.1110	0.0094
VitD*HCy*omega3		0	1	0	2	0.0348	0.08176	0.6696
VitD*HCy*omega3		0	0	1	2	-0.0064	0.07924	0.9354
Longitudinal association								
Time*VitD*HCy*omega3		1	1	1	3	-0.0156	0.005679	0.0060
Time*VitD*HCy*omega3		1	1	0	2	-0.0060	0.003168	0.0585
Time*VitD*HCy*omega3		1	0	1	2	-0.0058	0.002949	0.0463
Time*VitD*HCy*omega3		1	0	0	1	-0.0033	0.001963	0.0870
Time*VitD*HCy*omega3		0	1	1	2	-0.0033	0.003222	0.3038
Time*VitD*HCy*omega3		0	1	0	1	-0.0037	0.002172	0.0817
Time*VitD*HCy*omega3		0	0	1	1	-0.0029	0.002122	0.1613
Time*VitD*HCy*omega3		0	0	0	0	0	—	—
Education								
No diploma/primary	0					0	—	—
Some secondary	1					0.1308	0.06718	0.0518
Completed secondary	2					0.4885	0.08341	<.0001
Completed University/Higher	3					0.5045	0.07115	<.0001

[1]Nutritional risk index increases by 1 point for each of the following: Serum 25-hydroxyvitamin D ≤ 15 ng/mL; plasma homocysteine ≥ 18.1; RBC omega 3 ≤ 4.82; Primary outcome measure is the cognitive composite Z score. Same conclusions with/without adjustment for trial arms.

Materials and methods

Biochemical assays and genetics in MAPT

25-hydroxyvitamin D. An electrochemiluminescence binding assay was utilised for the in-vitro determination of total 25-hydroxyvitamin D (Cobas 8000, Roche). This commercially available assay employs VDBP to capture both 25-hydroxyvitamin D3 and D2 with the intention to quantify total vitamin D (25-OH) in human serum and plasma. Briefly, the sample is incubated with a pre-treatment reagent for 9 minutes denaturing the natural VDBP in the sample to release the bound vitamin D (25-OH-D). The sample is then further incubated with a recombinant ruthenium-labelled VDBP to form a complex of 25-OH-D and the ruthenylated-VDBP. The addition of a biotinylated 25-OH-D creates a complex consisting of the ruthenium-labelled VDBP and the biotinylated 25-OH-D. The entire complex becomes bound to the solid phase by the interaction of biotin and streptavidin-coated microparticles, which are captured on the surface of the electrode. The unbound substances are removed. Adding voltage to the electrode induces chemiluminescent emission which is measured by a photomultiplier. Results are determined via an instrument-specific calibration curve which is generated by 2-point calibration and a calibration master curve provided via the reagent barcode. Units are expressed as ng/mL.

Homocysteine. Total plasma homocysteine was measured using a commercially available enzymatic cycling assay (Cobas 8000, Roche). The concentration of total plasma homocysteine was measured in µmol/L in plasma samples against a standard curve. Oxidised homocysteine was first reduced and then reacted with S-adenosylmethionine to form methionine and S-adenosyl homocysteine (SAH) in the presence of homocysteine S-methyl transferase. SAH is then assessed by coupled enzyme reactions where SAH is hydrolysed into adenosine and homocysteine by SAH hydrolase and homocysteine is cycled back into the homocysteine conversion reaction, which serves to amplify the detection signal. The formed adenosine is hydrolysed into inosine and ammonia. Glutamate dehydrogenase catalyses the reaction of ammonia with 2-oxoglutarate and NADH to form NAD+. The concentration of homocysteine in the sample is directly proportional to the amount of NADH converted to NAD+, which was read at an absorbance of 340 nm. Units are expressed as uM.

Erythrocyte membrane omega 3 fatty acids. Erythrocyte EPA+DHA expressed as a weight percentage of total fatty acids is quantified using gas chromatography coupled with a flame ionisation detector. Briefly, erythrocytes are separated from plasma by centrifugation and washed three times before lipid extraction by the Folch method including a mixture of hexane and isopropanol after acidification. Margaric acid (Sigma) is added as an internal standard. Total lipid extracts were saponified and methylated. Fatty acid methyl esters were extracted

with pentane and analysed by gas chromatography (GC) using an Agilent Technologies 6890N gas chromatograph with a split injector, a bonded silica capillary column (BPX 70, 60 m x 0.25 mm; 0.25 µm film thickness) and a flame ionisation detector. Helium was used as a carrier gas, the column temperature program started at 150°C, increased by 1.3°C/min to 220°C and held at 220°C for 10 min. Identification of FAME was based on retention times obtained for FAME prepared from fatty acid standards. The area under the curve was determined using ChemStation software (Agilent) and results are expressed as % of total fatty acids. DHA concentration was calculated using the internal standard and expressed as µg/g of red blood cells. FA methyl esters (FAME) are quantifiable after transmethylation using FAME analysis using a GC 2100 Gas Chromatograph (Shimadzu) equipped with a CP Wax 58CB 50 - m fused silica capillary column. Programmed temperature spray injector and a flame ionisation detector calculate the omega-3 index (EPA + DHA expressed as a percentage of total fatty acids). Fifteen membrane phospholipid fatty acids are also determined, including palmitic acid C16:0 and stearic acid C18:0; MUFAs (oleic acid C18:1 n - 9 and palmitoleic acid C16:1 n-7); n-6 PUFAs (LA C18:2, gamma linolenic acid C18:3, dihomo-gamma linolenic C20:3, AA C20:4, docosatetraenoic acid C22:4 and docosapentaenoic acid C22:5); and n-3 PUFAs (ALA C18:3, C20:5 EPA, docosapentaenoic acid C22:5, DHA C22:6); one n - 9 PUFA (eicosatrienoic acid C20:3).

Descriptive statistics

Prevalence of hyperhomocysteinemia and distribution of plasma HCy in the population. Distribution of demographics and clinical variables across the tertiles/quartiles of HCy and vitamin D at baseline are compared to identify potential effect modifiers. Distribution of demographics and clinical variables across tertiles of the cognitive composite z score and neuroimaging parameters are assessed to identify potential confounders of the relationship with cognitive Z.

Example 2

The following non-limiting example is illustrative of compositions for attenuating cognitive ageing in a non-demented individual, in embodiments provided by the present disclosure.

Ingredient	**Dose/Day**
DHA	770 mg
EPA	700 mg
Vitamin B1 (thiamin)	50 mg
Vitamin B2 (riboflavin)	15 mg
Vitamin B3 (niacin)	25 mg

Vitamin B5 (pantothenic acid)	23 mg
Vitamin B6 (pyridoxine)	18 mg
Vitamin B7 (biotin)	0.15 mg
Vitamin B9 (folic acid anhydrous)	0.4 mg
Vitamin B12 (cobalamin)	about 12 to 21 times RDA of VitB12
Vitamin C	500 mg
Vitamin D	0.015 mg
Vitamin E	82.6 mg
Selenium	0.08 mg
Citrulline	3000 mg
Choline bitartrate	85 mg

The present invention is further described by the following numbered paragraphs:

1. A method of attenuating, treating or preventing cognitive aging in a non-demented individual in need thereof or at risk thereof, the method comprising administering to the individual a therapeutically effective amount of a composition comprising an omega-3 fatty acid, Vitamin B6 and Vitamin B9.

2. The method of paragraph 1, wherein the individual is an older adult.

3. The method of any of paragraph 1 or 2, wherein the individual is an elderly human.

4. The method of any of paragraphs 1 to 3, wherein the composition is orally administered to the individual daily for at least one month.

5. The method of any of paragraphs 1 to 4, wherein the composition further comprises a nitric oxide releasing compound.

6. The method of paragraph 5 wherein the nitric oxide releasing compound comprises citrulline.

7. The method of any of paragraphs 1 to 6, wherein the omega-3 fatty acid comprises a fatty acid selected from the group consisting of docosahexaenoic acid, eicosapentaenoic acid and mixtures thereof.

8. The method of any of paragraphs 1 to 7, wherein the composition comprises one or more additional B vitamins selected from the group consisting of Vitamin B1, Vitamin B2, Vitamin B3, Vitamin B5, Vitamin B7 and Vitamin B12.

9. The method of any of paragraphs 1 to 8, wherein the composition comprises one or more antioxidants selected from the group consisting of Vitamin C, Vitamin D, Vitamin E, and selenium.

10. The method of any of paragraphs 1 to 9, wherein the individual has a low DHA status at baseline.

11. The method of any of paragraphs 1 to 10, wherein the individual has a Clinical Dementia Rating (CDR) of 0.5 at baseline.

12. The method of any of paragraphs 1 to 11, wherein the individual has a low a plasma homocysteine level at baseline of at least 12 µmol/L.

13. The method of any of paragraph 1 to 12, wherein the individual has a risk score in Cardiovascular Risk Factors, Aging and Dementia (CAIDE) of 10 to 15 at baseline.

14. The method of any of paragraph 1 to 13, wherein the individual is amyloid positive on amyloid PET scans at baseline.

15. The method of any of paragraph 1 to 14, wherein the individual has a genotype indicating risk of cognitive decline.

16. The method of any of paragraph 1 to 15, wherein the composition is administered to the individual in a daily dose that provides 0.13 mg/day to 130 mg/day of the Vitamin B6 and/or 0.004 mg/day to 2.0 mg/day of the Vitamin B9.

17. The method of any of paragraphs 1 to 16, wherein the composition is administered to the individual in a daily dose that provides 0.002 mg to 0.4 mg of Vitamin B12 per day, preferably 0.02 to 0.07 mg of Vitamin B12 per day, more preferably 0.03 to 0.05 mg of Vitamin B12 per day.

18. The method of any of paragraphs 1 to 17, wherein the administration leads to an improvement of neuronal fluidity, stimulation of neuronal plasticity and activity, improvement of the anti-inflammatory potential, support or maintenance of cognitive performance, support or maintenance of brain performance, slowing down aging of the brain, support of an active mind and brain fitness, support or maintenance of a healthy brain, enhancement of memory, enhancement of executive functions, enhancement of attention, maintenance of cognitive health, maintenance of brain cellular health.

19. A method of attenuating, treating or preventing cognitive aging in a non-demented individual in need thereof or at risk thereof, the method comprising administering to the individual a therapeutically effective amount of a composition comprising an omega-3 fatty acid, Vitamin B6 and Vitamin B9.

20. A method of achieving one or more of benefits selected from the group consisting of decreasing brain atrophy, increasing or maintaining number of synapses, increasing or maintaining amyloid-β phagocytosis, and decreasing neuroinflammation in a non-demented individual in need thereof, the method comprising administering to the individual a therapeutically effective amount of a composition comprising an omega-3 fatty acid, Vitamin B6 and Vitamin B12.

21. A composition comprising a combination of an omega-3 fatty acid, Vitamin B6 and Vitamin B12; and the composition comprises the combination in an amount effective to attenuate cognitive aging in a non-demented individual.

22. The composition of paragraph 21, wherein the composition comprises omega-3 fatty acid in an amount of 1 to 50 wt.% of the composition, 0.002 mg to 0.4 mg of Vitamin B12 per day, preferably 0.02 to 0.07 mg of Vitamin B12 per day

23. The composition of paragraph 21 or 22, wherein the composition is a food product comprising an ingredient selected from the group consisting of protein, carbohydrate, fat and combinations thereof.

24. The composition of paragraph 21 or 22, wherein the composition is a pharmaceutical composition comprising a component selected from the group consisting of pharmaceutically-acceptable carriers, diluents and excipients.

25. Composition of any of paragraphs 21 to 24 for use in attenuating, treating or preventing cognitive aging in a non-demented individual in need thereof or at risk thereof.

26. Composition of any of paragraphs 21 to 25 for use in an improvement of neuronal fluidity, stimulation of neuronal plasticity and activity, improvement of the anti-inflammatory potential, support or maintenance of cognitive performance, support or maintenance of brain performance, slowing down aging of the brain, support of an active mind and brain fitness, support or maintenance of a healthy brain, enhancement of memory, enhancement of executive functions, enhancement of attention, maintenance of cognitive health, maintenance of brain cellular health.

27. A method of making a food composition for attenuating cognitive aging in a non-demented individual, the method comprising adding an effective amount of a combination of an omega-3 fatty acid, Vitamin B6 and Vitamin B9 to at least one ingredient selected from the group consisting of protein, carbohydrate, and fat.

28. A method of making a pharmaceutical composition for attenuating cognitive aging in a non-demented individual, the method comprising adding an effective amount of a combination of an omega-3 fatty acid, Vitamin B6 and Vitamin B9 to at least one component selected from the group consisting of pharmaceutically-acceptable carriers, diluents and excipients.

29. A method of preventing dementia in an individual at risk thereof, the method comprising administering to the individual a therapeutically effective amount of a composition comprising an omega-3 fatty acid, Vitamin B6 and Vitamin B9.

30. The method of paragraph 29, wherein the dementia that is prevented is selected from the group consisting of Alzheimer's disease, vascular dementia, Lewy body dementia, frontotemporal dementia, and combinations thereof.

31. A method of improving cognitive ability in a non-demented individual, the method comprising administering to the individual a therapeutically effective amount of a composition comprising an omega-3 fatty acid, Vitamin B6 and Vitamin B9.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the disclosed agents, compositions, uses and methods of the invention will be apparent to the skilled person without departing from the scope and spirit of the invention. Although the invention has been disclosed in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the disclosed modes for carrying out the invention, which are obvious to the skilled person are intended to be within the scope of the following claims.

CLAIMS

1. A method for identifying pre-disposition to cognitive decline in a subject, the method comprising determining levels of:

   (a) omega-3 fatty acids, and vitamin D or a metabolite thereof;

   (b) omega-3 fatty acids, and homocysteine;

   (c) vitamin D or a metabolite thereof, and homocysteine; or

   (d) omega-3 fatty acids, vitamin D or a metabolite thereof, and homocysteine,

   independently in one or more samples obtained from the subject.

2. The method of claim 1, wherein the method comprises determining levels of omega-3 fatty acids, vitamin D or a metabolite thereof, and homocysteine.

3. The method of claim 1 or 2, wherein:

   (a) a level of omega-3 fatty acids is determined and a decrease in the level of omega-3 fatty acids in the sample from the subject compared to a reference value is indicative of pre-disposition to cognitive decline;

   (b) a level of vitamin D or a metabolite thereof is determined and a decrease in the level of vitamin D or metabolite thereof in the sample from the subject compared to a reference value is indicative of pre-disposition to cognitive decline; and/or

   (c) a level of homocysteine is determined and an increase in the level of homocysteine in the sample from the subject compared to a reference value is indicative of pre-disposition to cognitive decline.

4. The method of any preceding claim, wherein the one or more samples are independently selected from the group consisting of a blood sample, plasma sample and serum sample.

5. The method of any preceding claim, wherein the level of omega-3 fatty acids is determined in a blood sample, preferably an erythrocyte sample; the level of vitamin D or metabolite thereof is determined in a serum sample; and/or the level of homocysteine is determined in a plasma sample.

6. The method of any preceding claim, wherein the omega-3 fatty acid is eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA), preferably erythrocyte membrane EPA and/or erythrocyte membrane DHA.

7. The method of any preceding claim, wherein the vitamin D or metabolite thereof is vitamin D3, vitamin D2, 25-hydroxyvitamin D3 and/or 25-hydroxyvitamin D2, preferably 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2.

8. The method of any preceding claim, wherein the subject is an ageing human subject, preferably a human subject of 50 years of age or more.

9. An omega-3 fatty acid for use in reducing or preventing cognitive decline in a subject, wherein the omega-3 fatty acid is administered to the subject simultaneously, sequentially or separately with vitamin D or a metabolite thereof, and/or an agent capable of reducing plasma homocysteine levels.

10. Vitamin D or a metabolite thereof for use in reducing or preventing cognitive decline in a subject, wherein the vitamin D or metabolite thereof is administered to the subject simultaneously, sequentially or separately with an omega-3 fatty acid, and/or an agent capable of reducing plasma homocysteine levels.

11. An agent capable of reducing plasma homocysteine levels for use in reducing or preventing cognitive decline in a subject, wherein the agent capable of reducing plasma homocysteine levels is administered to the subject simultaneously, sequentially or separately with an omega-3 fatty acid, and/or vitamin D or a metabolite thereof.

12. A combination of (a) an omega-3 fatty acid; (b) vitamin D or a metabolite thereof; and (c) an agent capable of reducing plasma homocysteine levels for use in reducing or preventing cognitive decline in a subject, wherein (a), (b) and (c) are administered to the subject simultaneously, sequentially or separately.

13. The omega-3 fatty acid for use according to claim 9, vitamin D or metabolite thereof for use according to claim 10, agent capable of reducing plasma homocysteine levels for use according to claim 11, or combination for use according to claim 12, wherein the subject is a subject identified as pre-disposed to cognitive decline by a method of any one of claims 1-8.

14. The omega-3 fatty acid for use according to claim 9 or 13, vitamin D or metabolite thereof for use according to claim 10 or 13, agent capable of reducing plasma

homocysteine levels for use according to claim 11 or 13, or combination for use according to claim 12 or 13, wherein the omega-3 fatty acid is eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA).

15. The omega-3 fatty acid for use according to any one of claims 9, 13 or 14, vitamin D or metabolite thereof for use according to any one of claims 10, 13 or 14, agent capable of reducing plasma homocysteine levels for use according to any one of claims 11, 13 or 14, or combination for use according to any one of claims 12-14, wherein the vitamin D or metabolite thereof is vitamin D3, vitamin D2, 25-hydroxyvitamin D3 and/or 25-hydroxyvitamin D2.

16. The omega-3 fatty acid for use according to any one of claims 9 or 13-15, vitamin D or metabolite thereof for use according to any one of claims 10 or 13-15, agent capable of reducing plasma homocysteine levels for use according to any one of claims 11 or 13-15, or combination for use according to any one of claims 12-15, wherein the agent capable of reducing plasma homocysteine levels is vitamin B6 and/or vitamin B9.

17. The omega-3 fatty acid for use according to any one of claims 9 or 13-16, vitamin D or metabolite thereof for use according to any one of claims 10 or 13-16, agent capable of reducing plasma homocysteine levels for use according to any one of claims 11 or 13-16, or combination for use according to any one of claims 12-16, wherein the subject is an ageing human subject, preferably a human subject of 50 years of age or more.

18. The omega-3 fatty acid for use according to any one of claims 9 or 13-17, vitamin D or metabolite thereof for use according to any one of claims 10 or 13-17, agent capable of reducing plasma homocysteine levels for use according to any one of claims 11 or 13-17, or combination for use according to any one of claims 12-17, wherein the omega-3 fatty acid; vitamin D or a metabolite thereof; and/or agent capable of reducing plasma homocysteine levels are administered to the subject simultaneously, sequentially or separately with vitamin B12, wherein the vitamin B12 is administered at a dosage of 0.1 to 40 times the RDA of vitamin B12 per day.

DRAWINGS

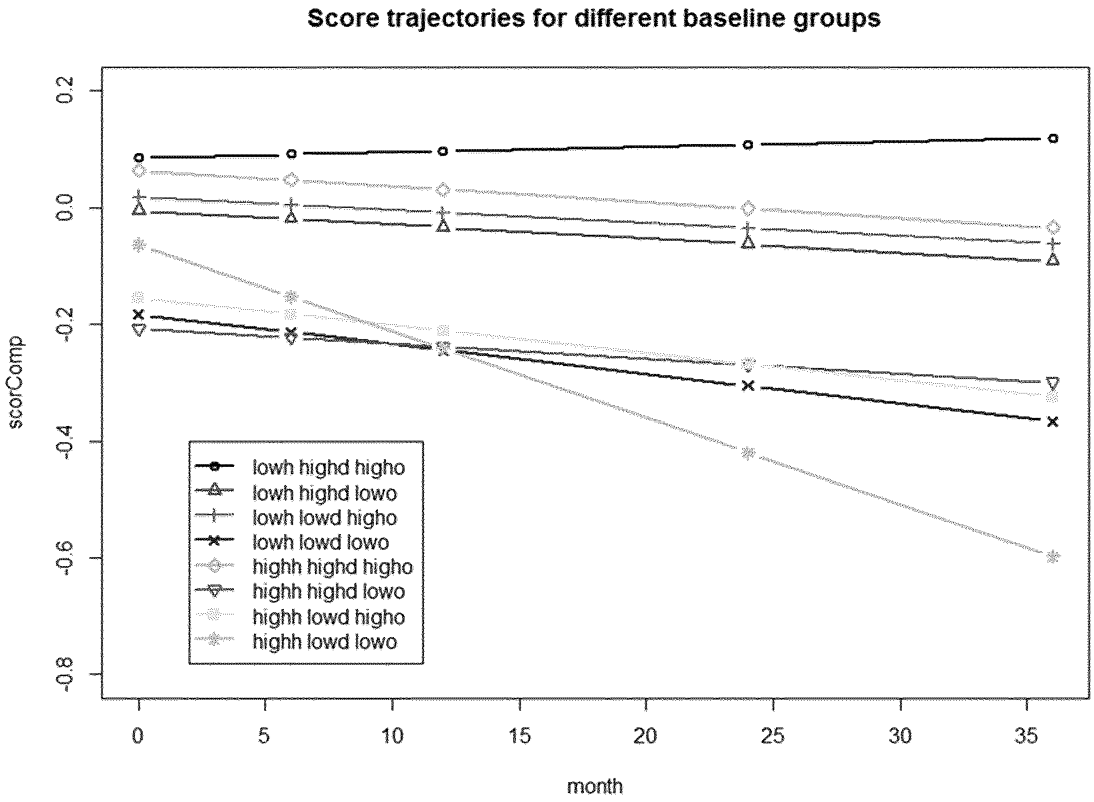


Figure 1

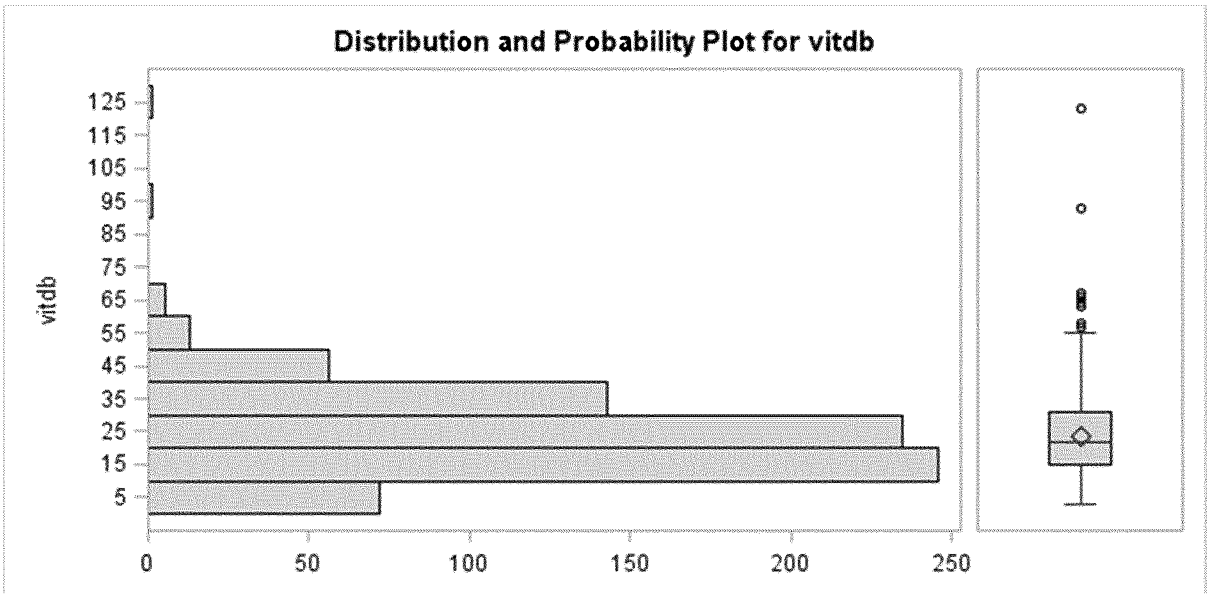
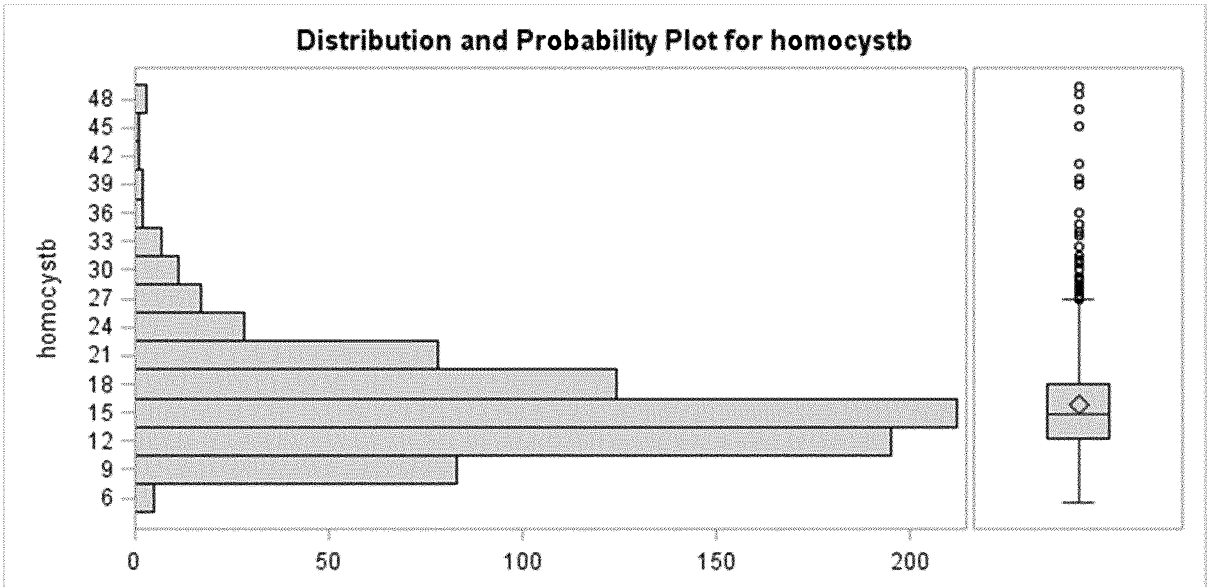

Distribution and Probability Plot for vitdb
vitdb
125
115
105
95
85
75
65
55
45
35
25
15
5
0
50
100
150
200
250

Figure 2


Distribution and Probability Plot for homocystb
homocystb
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45
42
39
36
33
30
27
24
21
18
15
12
9
6
0
50
100
150
200

Figure 3