Fish fatty acids and mental health in older people

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Thesis

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"Science is not the straight and narrow path to truth, but rather,
a trail fraught with many nooks, crannies and traverses.
After all, what is the purpose of science but to learn from
the universe the principles of love and meekness?
Could there be no higher purpose of scientists?"
(High Eagle)

"No is a word on your path to Yes. Don't give up too soon.
Not even if well-meaning parents, relatives, friends, and
colleagues tell you to get a real job.
Your dreams are your real job."
(Joyce Spizer)

"A successful career will no longer be about promotion.
It will be about mastery."
(Michael Hammer)

Abstract

Background

It has been suggested that the intake of fish and marine n-3 polyunsaturated fatty acids could protect against age-related cognitive decline and impaired mental well-being. However, results from observational studies are inconclusive and data from randomized controlled trials in older people without clinical dementia or depression are scarce. The objective of this thesis was to investigate the effect of daily supplementation with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on cognitive performance and mental well-being in an older non-clinical population. We also examined the effect of fish oil on gene expression profiles in white blood cells to identify early changes in pathways possibly related to mental health. Furthermore, we assessed the association of fish and EPA+DHA intake with mental health in different aging populations.

Methods

The effect of low and high doses of EPA+DHA (400 and 1,800 mg per day, respectively) on cognitive performance, several measures of mental well-being, and gene expression was examined in a 26-week randomized, double-blind, placebo-controlled trial. This study was conducted in 302 individuals aged 65 years or older with no clinical diagnosis of dementia or depression. Furthermore, the cross-sectional association between fatty fish and EPA+DHA intake with cognitive performance and the association with cognitive change during 6 years of follow-up was assessed in 1,025 aging US men who participated in the Veterans Affairs Normative Aging Study (NAS). In addition, the associations of EPA+DHA and fish intake with depressive symptoms and dispositional optimism were assessed in 644 free-living Dutch subjects with a history of myocardial infarction.

Results

Daily intake of low or high doses of EPA+DHA did not affect cognitive performance, mental well-being, anxiety, or quality of life, after 13 or 26 weeks of intervention. However, treatment with EPA+DHA for 26 weeks altered gene expression in white blood cells to a more anti-inflammatory and more anti-atherogenic profile. In elderly US men we found no association of fatty fish or EPA+DHA intake with cognitive performance or 6-year cognitive change. Intake of EPA+DHA was positively associated with dispositional optimism in subjects with a history of myocardial infarction. There was also a tendency for less depressive symptoms with a higher EPA+DHA or fish intake, but this association was no longer statistically significant after controlling for confounders.

Conclusion

Supplemental intake of EPA+DHA is unlikely to have a short-term impact on cognitive performance or mental well-being of older people without a clinical diagnosis of dementia or depression. Whether long-term intake of EPA+DHA and fish could be beneficial to the maintenance of cognitive performance or mental well-being of older people in Western populations still needs to be established.

Contents

1

Introduction

The importance of good nutrition for the maintenance of health has long been advocated. Decades ago specific attention was drawn to dietary influences of fish consumption on health by observation of benefits of fish oil on the risk for cardiovascular disease in the Japanese[1,2] and in the Inuit of Greenland[3,4], populations with a high consumption of fish. Fish oil has also been inversely associated with atopic eczema[5], hypercholesterolemia[6], and arthritis[7]. Moreover, a low risk of Alzheimer's disease has been reported in the Cree in northeastern Canada, another population with high fish intake[8]. These observations led to an increase in research on the beneficial effects of n-3 polyunsaturated fatty acids (n-3 PUFA) from fish on various health conditions. Beneficial effects include anti-atherogenic, anti-arrhythmic, anti-thrombotic and anti-inflammatory actions[9]. Also, evidence is accumulating for a potential benefit of n-3 PUFA on mental health in older individuals; they may slow down cognitive decline, delay the onset of dementia and improve mental well-being. In this thesis we have further examined the intake of fish and the n-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in relation to cognitive decline and mental well-being of older people by means of observational and intervention studies.

About n-3 PUFA

About 90% of fats in our diet come in the form of triglycerides, which consist of fatty acids and glycerol. N-3 PUFA (also called omega-3 PUFA) are polyunsaturated fatty acids where the first double bond is located at the third carbon atom, counting from the methyl end (**Figure 1.1**).

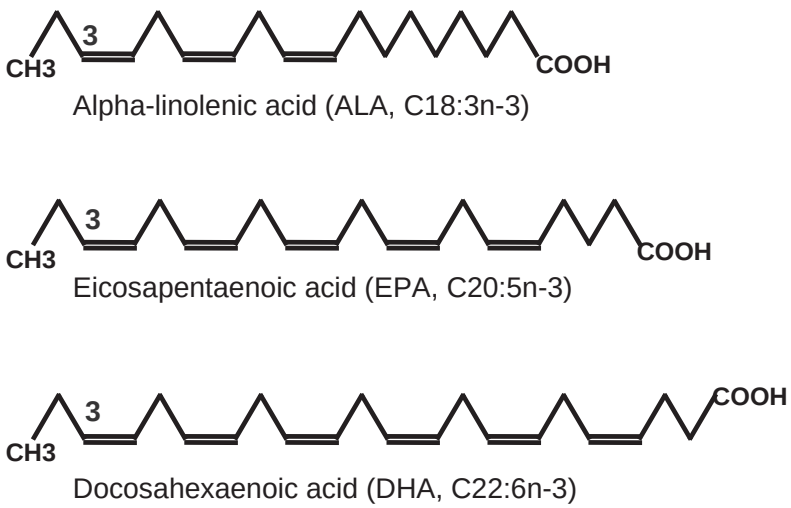

Figure 1.1 Structure of the n-3 PUFA alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

N-3 PUFA are present in the diet as alpha-linolenic acid (ALA; C18:3n-3) of which vegetable oils and nuts are main sources and as very long-chain polyunsaturated fatty acids, primarily eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA; C22:6n-3), which are mainly supplied by (fatty) fish. ALA is an essential fatty acid, which can be converted into EPA and DHA by a series of desaturations and elongations. However, in humans the conversion of ALA to EPA and DHA is very limited; only 5 to 10% of ALA is converted into EPA, and 1 to 5% of ALA or EPA is converted into DHA[10-14]. This conversion may also be influenced by the amount of ALA and linoleic acid in the diet[15,16]. Because EPA and DHA are not efficiently synthesized in humans it is more effective when they are obtained directly from the diet or otherwise e.g. from fish oil capsules.

The Netherlands Health Council recommends an intake of at least 450 mg EPA+DHA from fish per day, which is roughly equivalent to eating two portions of fish per week, one of which is oily fish[17]. The recommended EPA+DHA values that have been proposed by different organizations are globally in the range of 200 to 600 mg/d[18]. There is a large variation in intake across studies and countries, but in most western populations intakes are far below recommendations. In the US and Europe, the mean intake of EPA+DHA varies between 100 and 500 mg per day[19-22] while in Japan intakes are around 1 g per day[22]. Average fish intake of the general Dutch population as estimated in 1998 was 10 g per day[23]. In a population of Dutch older men, assessed in 2000, 28% did not consume fish at all and 41% consumed >20 g per day, only 11% of which comprised fatty fish[24].

EPA and DHA need to be obtained from the diet. There is a large variation in intakes between countries. Average intake in the Netherlands and other western countries is far below the recommendation of 450 mg per day.

Dementia, cognitive decline and depression

Dementia and cognitive decline

Because of the aging population there is an increase in age-related diseases, of which dementia is one of the most common. Dementia is a progressive neurodegenerative disease characterized by loss of function and death of nerve cells in several areas of the brain, complicated by inflammatory reactions[25]. This results in a loss of mental functions such as memory and learning, which together with a decline in activities of daily living and behavioral changes impair every day functioning. Different types of dementia exist, including Alzheimer's disease (AD), vascular dementia, dementia with Lewy bodies, and frontotemporal dementia[26].

At present, AD accounts for 70% of prevalent dementias. In 2006 the estimated number of Alzheimer's disease patients worldwide was nearly 27 million. The

incidence is increasing markedly due to aging populations, and the forecast for the future is that the numbers might have increased fourfold by 2050 to 106.8 million, thus affecting 1 in every 85 older persons[27]. AD is characterized by the presence of neurofibrillary tangles and amyloid plaques that are clearly visible by microscopy in brains of those afflicted with AD. Neurofibrillary tangles are aggregates of the protein *tau* which has become hyperphosphorylated and accumulates inside the cells. Plaques are dense, mostly insoluble deposits of the protein amyloid-beta and cellular material outside and around neurons. AD is distinguished from vascular dementia, the second most common form of dementia, which is characterized by cerebrovascular lesions secondary to vascular disease. However, this distinction is not complete since associations between vascular risk factors and Alzheimer's disease have been demonstrated and therefore pathologies may overlap[28].

Dementia is preceded by impaired cognitive functioning: the process of receiving, processing, storing and using information. Main cognitive functions are memory and learning, attention and concentration, thinking, language, and visual and spatial skills. There are different cognitive stages ranging from normal cognitive functioning to cognitive impairment and dementia or AD (**Figure 1.2**). These stages are often age-related, but the level of cognitive functioning, the onset of cognitive decline and the rate of cognitive decline differ between persons of the same age, which indicates that also other factors are of influence. The pathogenesis of neurological damage and relevance to cognitive impairment is still uncertain. A better understanding of the risk factors is needed to maintain good cognitive performance as long as possible. The social, medical, and

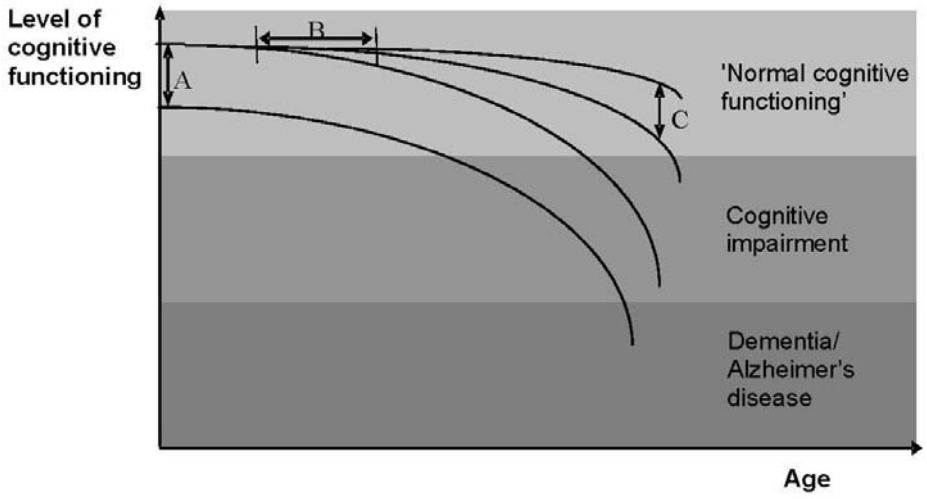

Figure 1.2 Differences in cognitive functioning over time between persons. A = difference in level of cognitive functioning; B = difference in onset of cognitive decline; C = difference in rate of cognitive decline.

economical impact of dementia and cognitive impairment is huge[29]. Since no effective treatment for dementia is available there is an urgent need to develop strategies to prevent or postpone the onset of cognitive decline. A delay in onset by 1 year would reduce the prevalence of dementia by 25%, and a 5-year delay would decrease the prevalence by 50%[30].

Depression

The WHO estimates that 121 million people worldwide are affected by depressive disorders[31], which was the fourth leading contributor to the global burden of disease in high-income countries in 2000[32]. The main symptoms of depression are depressed mood, loss of interest or pleasure, feelings of guilt or low self-worth, disturbed sleep or appetite, fatigue or low energy, and poor concentration[31].

There are different forms of depression, which mainly differ in severity and duration of symptoms. Bipolar disease is characterized by alternating periods of depression and mania (feeling of extreme elation and euphoria) and unipolar depression, also called major depressive disorder, occurs without periods of mania. Major depressive disorder is defined by having one core symptom (depressed mood or loss of interest or pleasure) and at least four other symptoms for at least two weeks according to the criteria of the 4th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)[33]. This distinguishes major depression from the somewhat less severe type, the so-called minor depressive disorder, which is defined by having at least one core symptom, but only one to three other symptoms for two weeks or longer. When the symptoms of minor depression last 2 years or longer it is called dysthymia.

The prevalence of clinical depression ranges from 1-15% in the general elderly population[34,35] and because of the aging population this prevalence is likely to increase. In the Netherlands the number of depressed people amounted to 856,000 in 2003 which is 6.3% of the inhabitants aged 13 years or older. It is expected that the incidence of depressive disorders will increase by 4% between 2005 and 2025[36]. Despite the high prevalence the majority of depressed older patients remains undetected and does not receive treatment[37]. Comorbidity with physical illness and cognitive impairment are often mentioned as factors hindering the detection of depressive symptoms[37,38].

It appears that anti-depressant therapy is not always effective; it is estimated that 20-30% of those with major depressive disorders treated with antidepressant medication continue to experience depressive symptoms. In addition, persons with a history of depression have a more than threefold higher risk of recurrence[39]. Furthermore, long term use of antidepressant medication gives rise to a number of common and unpleasant side effects, such as weight gain, gastrointestinal disturbances, blurred vision, drowsiness and dizziness[40]. Also, depression has a major influence on a person's quality of life, their relatives, and society. This calls for effective preventive measures.

Other measures of mental well-being

Anxiety

Anxiety is a psychological and physiological state characterized by cognitive, somatic, emotional, and behavioral components[41]. These components together create an unpleasant feeling that is typically associated with uneasiness, fear, or worry. Anxiety occurs without an identifiable triggering stimulus, which distinguishes it from fear, which occurs in the presence of an observed threat. Furthermore, it is distinct from depression, because depression is dominated by the emotion of sadness and is associated with feelings of sorrow, hopelessness, and gloom. However, separating these two states strictly on clinical grounds is not always straightforward, because anxiety is also a common symptom of depressive illness, and depression is a common complication in anxiety states.

Dispositional optimism

Dispositional optimism is defined in terms of generalized positive expectancies for one's future[42] and has been associated with healthy lifestyle and dietary habits[43,44]. It is not simply the reverse of depression, but both affect mental well-being. Optimism has been shown to protect against the development of depressive symptoms[45]. Because a low intake of fish or n-3 PUFA may be beneficial to depression, it may also be related to a lack of optimism.

Quality of life

Depression and dementia often coincide and it is difficult to disentangle which disease came first[46,47]. The impact of both diseases on quality of life (QOL) is large and comparable to other serious diseases. QOL is a broader concept than mental well-being; it encompasses physical health, psychological state, level of independence, social relationships, and relationship to salient features of the environment[48]. It assesses the individual's perceptions in the context of their culture and value systems, and their personal goals, standards and concerns. Effective interventions that would prevent cognitive impairment and depression, consequently improving QOL, are needed.

The worldwide prevalence of dementia and depression is high and increasing rapidly due to the aging population. Both diseases affect quality of life. This calls for effective preventive measures.

Risk factors for cognitive decline, dementia and depression

Epidemiological studies could make a valuable contribution to understanding the etiology and pathogenesis of dementia and depression and the identification of risk factors. During the past decades several risk factors for these diseases have been identified, which are summarized below.

Non-modifiable risk factors. Major risk factors for dementia and depression are

increasing age[49,50] and female gender[50,51]. Another major risk factor for dementia is the apolipoprotein E-ε4 (APOE4)[52-54]. APOE is a plasma protein involved in the metabolism of cholesterol and triglycerides, which exists in three allelic forms (ε2, ε3, and ε4). Individuals with two copies of the APOE-ε4 allele (2% of the total population) have a 50% to 90% chance of developing AD by the age of 85, and those with one copy of APOE-ε4 (15% of the total population) have 45% chance of developing the disease[52], compared with 20% in the general population[55].

Sociodemographic and lifestyle factors. There is convincing observational evidence for a relation between low educational level and dementia[50,51,56]. Furthermore, marital status[57,58], living situation[57-59], smoking[60,61], high alcohol consumption[62,63], low physical activity level[64,65], frailty[66], low walking speed[67,68], underweight or weight loss[69-72], having few social relationships or social activities[73,74] or the absence of intellectual or other activities[75-77] have all been suggested as risk factors, also for the occurrence of depression[78]. For depression additional risk factors are specific personality traits and stressful life events[79].

Nutritional factors. Current epidemiological data are in favor of a protective role of certain micronutrients, such as B-vitamins (related to the homocysteine metabolism), the antioxidant vitamins C and E and flavonoids[80]. Cochrane reviews that have evaluated the trial evidence available for these micronutrients do not support these associations, but they are also based on a very limited amount of RCTs. Furthermore, coffee consumption[81,82] and more recently vitamin D[83,84] have been inversely associated with cognitive decline. Fish and n-3 PUFA from fish (EPA+DHA) have also been identified as potentially important candidate nutrients for the prevention of cognitive decline[80], dementia[85] and mood disorders[86]. However, also here evidence has mainly come from observational studies and data from randomized controlled trials (RCTs) in healthy adults are limited[87] and inconsistent[88].

Underlying mechanisms

The nervous system has the highest lipid content of all organs in the human body. Approximately 50 to 60% of the dry weight of the brain consists of lipids and PUFA constitute approximately 35% of that lipid content. DHA is the major constituent as an important building block of neuronal membranes[89]. N-3 PUFA are very important for brain development during both the foetal and postnatal period[90,91]. With aging and late onset AD, DHA levels in the brain tend to decrease[90,92-94], suggesting that a drop in DHA levels may contribute to a deterioration in memory and other cognitive functions.

Several biological mechanisms for the possible association between fish or n-3 PUFA and mental health have been proposed. Most likely a combination of various mechanisms could play a role in mental health. It is at present unknown to what extent these different mechanisms are shared by mental processes like cognitive decline, specific domains of cognitive functioning, and depression. Therefore, a number of the proposed mechanisms are described below, but

current knowledge is too limited to separate these for the specific impact on cognition, separate cognitive domains or depression. Proposed mechanisms are roughly classified as short-term (i.e. weeks to months) or long-term (months to years). Acute mechanisms influencing cognitive functioning or mood are beyond the scope of this thesis. It should be noted that fish is also rich in other nutrients than n-3 PUFA that could be beneficial to health, such as proteins, selenium and vitamin D, but we will only discuss the mechanisms of n-3 PUFA, which are the main focus of this thesis.

'Short-term' mechanisms

Membrane fluidity. The high concentration of DHA in membrane phospholipids has a role in maintaining membrane integrity and fluidity. EPA and DHA are already incorporated in the phospholipid fraction of cell membranes within 2-4 weeks, thereby influencing physical properties and fluidity of the cells. As such they could affect the structure and functioning of proteins embedded in the membrane, including enzymes, receptors, and ion channels, thereby influencing cellular signaling and neurotransmission of for example serotonin, dopamine, and acetylcholine[95,96].

Cerebral blood flow. Neuroimaging studies have shown that impaired cerebral perfusion is related to worse cognitive functioning. N-3 PUFA have been shown to improve cerebral blood flow in animals within several weeks[97,98] or months[99] and it has been hypothesized that mental health benefits of n-3 PUFA may be mediated by improvements in cerebral vascular function[100].

Gene regulation. N-3 PUFA regulate the expression of genes and the binding of fatty acids to specific nuclear receptors in various tissues, including the liver, heart, adipose tissue, and brain. Modulation of gene transcription by EPA and/or DHA may eventually reduce the risk of various chronic diseases, such as AD, cardiac disease, and depression[101]. PUFA exert their effects on gene expression rapidly; in animals fed with diets rich in PUFA changes in expression of lipogenic genes have been observed within hours[102,103].

'Long-term' mechanisms

Vascular factors. Vascular disease appears to be closely related to the development of cognitive impairment and dementia[104]. The beneficial effects of n-3 PUFA in reducing vascular risk include anti-arrhythmic, anti-thrombotic, anti-inflammatory, and anti-atherogenic effects[105] resulting in a reduction of atherosclerosis[106] and risk of stroke[107]. N-3 PUFA can be incorporated into atherosclerotic plaques, enhancing their stability, which may reduce the risk of brain infarction[108]. Consumption of n-3 PUFA may also lower serum cholesterol and triglycerides levels and improve endothelial function by enhanced nitric oxide production. It has been shown that all these vascular risk factors also have measurable beneficial effects on the brain and are associated with cognitive functioning[109].

Inflammation. Inflammation plays a major role in the pathophysiology of

dementia[110] and n-3 PUFA may be protective through their anti-inflammatory properties. During aging, the increase of peripheral cytokines leads to an increase of brain cytokines that could be responsible for the development of cognitive disorders. EPA and DHA decrease the synthesis of inflammatory eicosanoids from arachidonic acid[111] and inhibit the release of pro-inflammatory cytokines[112].

Oxidative stress. Oxidative stress is a condition of increased free radicals production and/or decrease of availability of antioxidants. Oxidative stress is a key mechanism underlying the aging process and the aging brain is more vulnerable to oxidative stress. N-3 PUFA can protect against the damaging effects of oxidative stress by reducing lipid peroxidation[113]. However, in high doses n-3 PUFA can deplete antioxidant levels because they are easily oxidized.

Plaques and tangles. The protein beta-amyloid is toxic to nerve cells and when levels become excessive, large areas of brain cells are destroyed, resulting in amyloid plaques. N-3 PUFA may directly limit AD pathology by reducing the production of β-amyloid, minimizing its aggregation into plaques, and increasing its clearance. N-3 PUFA may also reduce the accumulation of the protein *tau*, thereby inhibiting the formation of neurofibrillary tangles[114].

There are several plausible mechanisms that support a role for EPA and/or DHA in cognitive performance and mental well-being. Some mechanisms may exert an effect in weeks or months, whereas other processes take years.

Scientific evidence

Numerous studies have addressed the potential benefits of n-3 PUFA in relation to cognitive impairment, dementia and depression. We will give an overview of observational and intervention studies (RCTs) that have been performed with dementia, cognitive decline or depression as outcome measures. Both kind of studies are complementary, not only because epidemiological studies cannot prove causation, while RCTs studies can, but also because longitudinal observational studies could provide insight into long-term underlying mechanisms, while the time window of RCTs matches more with short-term mechanisms.

Dementia and cognitive decline: observational evidence

Most of the evidence originates from a number of different epidemiological investigations (**Table 1.1**). The association of fish and EPA+DHA with cognitive decline and dementia has been examined in different populations, both cross-sectionally[93,94,115-120] and prospectively[116-119,121-129].

In five out of eight cross-sectional studies of cognitive performance or dementia in relation to intake of fish, dietary n-3 PUFA, or blood n-3 PUFA concentrations an inverse association was observed[93,94,115,116]. The other three studies showed no association[117-119]. In 12 out of 16 longitudinal population-based studies with 4 to

Table 1.1 Observational studies on the association of fish and n-3 PUFA with cognitive performance in older adults

Author, year (study)	Study population (*n*)	n-3 PUFA assessment	Findings*
Cross-sectional studies			
Dullemeijer, 2007 (FACIT)[119]	Healthy elderly, 50-70y (*n*=807)	Plasma n-3 PUFA proportions	No association between plasma n-3 PUFA proportions and cognitive performance
van Gelder, 2007 (Zutphen study)[117]	Elderly men, 70-89y (*n*=210)	Fish and EPA+DHA intake, dietary history method	No difference in MMSE scores between categories of fish (>20g/d: 26.5 vs 0 g/d: 26.4, *P*=0.81) or EPA+DHA intake (Q1: 26.3 vs Q3: 26.6, *P*=0.36)
Nurk, 2007 (HUSK study)[120]	General population, 70-74y (*n*=2,031)	Fish intake, FFQ	Diet high in fish and fish products associated with better cognitive performance on several cognitive tests, association dose-dependent
Kalmijn, 2004 (Doetinchem study)[115]	Cognitive impairment (*n*=163), no cognitive impairment (*n*=1,450), 45-70y	Fish and n-3 PUFA intake, FFQ	Higher fish and n-3 PUFA intakes associated with lower risk of overall cognitive impairment (OR=0.77; 0.60-0.97) and 0.81; 0.66-1.00 respectively) and speed (OR=0.71; 0.55-0.92 and 0.72; 0.57-0.90 respectively)
Laurin, 2003 (CSHA)[118]	Selection of subjects with clinical evaluation, ≥65y; follow-up ≅ 5y (*n*=174): cognitive impairment (*n*=43), no cognitive impairment (*n*=79), dementia (*n*=52)	Plasma phospholipids	No significant difference in n-3 PUFA concentrations
Tully, 2003[94]	Irish AD patients, community-based (*n*=148)	Serum cholesteryl EPA+DHA	AD patients have lower EPA (*P*<0.05) and DHA (*P*<0.001) status
Conquer, 2000[93]	77-83y, cognitive impairment (*n*=36), no cognitive impairment (*n*=19)	Plasma n-3 PUFA phospholipids	Lower n-3 PUFA concentrations (*P*<0.05) in cognitively impaired groups
Kalmijn, 1997 (Zutphen study)[129]	Dutch population, 64-89y, cognitive impairment (*n*=153), no cognitive impairment (*n*=323)	Fish intake, dietary history method	High fish consumption, inversely correlated with prevalent cognitive impairment (OR=0.63; 0.33-1.21), n-3 PUFA intake not

Longitudinal studies

Devore, 2009 (Rotterdam Study)[132]	Dutch population, ≥55y, follow-up ≈9.6y (*n*=5,395)	Fish and n-3 PUFA intake, FFQ	No association between fish or n-3 PUFA intake and long-term dementia risk
Kröger, 2009 (CSHA)[131]	General population, ≥65y, follow-up ≈4.9y (*n*=663)	Erythrocyte membrane n-3 PUFA	No associations between n-3 PUFA and dementia or AD
Beydoun, 2007 (ARIC)[127]	Adults aged 50-65y, follow-up 6y; cognitive decline (*n*=140), no cognitive decline (*n*=2,111)	Plasma n-3 PUFA phospholipids and cholesteryl esters	Higher n-3 PUFA plasma cholesteryl ester (OR=0.74; 0.57-0.97) and plasma phospholipid concentrations (OR=0.73; 0.58-0.93) associated with lower risk of decline in verbal fluency
Dullemeijer, 2007 (FACIT)[119]	Healthy elderly, 50-70y, follow-up 3y (*n*=404)	Plasma n-3 PUFA proportions	Higher plasma n-3 PUFA proportions associated with less cognitive decline in speed related cognitive domains (*P*= 0.02 for sensorimotor speed and *P*<0.01 for complex speed)
Van Gelder, 2007 (Zutphen study)[117]	Elderly men, 70-89y, follow-up 5y (*n*=210)	Fish and EPA+DHA intake, dietary history method	Higher fish intake (>20g/d: -0.3 vs 0 g/d: -1.2, *P*=0.01) and n-3 PUFA intake (Q1: -0.9 vs Q3: 0.2, *P*-trend=0.01) associated with lower MMSE scores
Schaefer, 2006 (Framingham Heart Study)[126]	US population, follow-up 9.1y (*n*=899), 99 cases of dementia	Plasma phophatidylcholine (PC)	The top quartile of plasma PC DHA was associated with a significant risk reduction of all-cause dementia (RR=0.53; 0.29-0.97, *P*=0.04) and AD (RR=0.61; 0.31-1.18)
Huang, 2005 (CHCS)[123]	US population, ≥65y, follow-up 5.4y (*n*=2,233)	Fish intake, FFQ	Consumption of fatty fish was associated with a reduced risk of dementia (HR=0.72; 0.51-1.02) and AD (HR=0.59; 0.36-0.95), but only in those without the APOE-ε4 allele
Morris, 2005 (CHAP)[130]	US population, ≥65y, follow-up 6y (*n*=3,718)	Fish and n-3 PUFA intake, FFQ	Fish intake (1 fish meal/week 10%, *P*=0.03 and 13%, *P*=0.042 or more fish meals/week), but not n-3 PUFA intake, was associated with a slower rate of cognitive decline
Laurin, 2003 (CSHA)[118]	Selection of subjects with clinical evaluation, ≥65y; follow-up ≅ 5y (*n*=174): cognitive impairment (*n*=43), no cognitive impairment (*n*=79), dementia (*n*=52)	Plasma EPA+DHA phospholipids	Unexpectedly, higher EPA concentrations (31%, *P*<0.01) in cognitively impaired group and higher DHA concentrations (30%, *P*=0.07) in dementia cases.

Table 1.1 *Continued*

Author, year (study)	Study population (*n*)	n-3 PUFA assessment	Findings*
Heude, 2003 (EVA study)[125]	Cognitive decline (*n*=27), no cognitive decline (*n*=219), 63-74y, follow-up 4y	N-3 PUFA in erythrocyte membranes	Higher n-3 PUFA concentrations associated with lower risk of cognitive decline (OR=0.59; 0.38-0.93)
Morris, 2003 (CHAP)[122]	Elderly, ≥65y (*n*=3,718), follow-up 3.9y	Fish and EPA+DHA intake, FFQ	Fish intake ≥1/week (RR=0.4; 0.2-0.9, *P*-trend=0.07), and n-3 PUFA intake (RR Q5=0.4; 0.1-0.9, *P*-trend=0.01) associated with slower cognitive decline
Barberger-Gateau, 2002 (PAQUID)[121]	French, community dwelling population, ≥68y, follow-up 7y (*n*=1,416)	Fish intake, FFQ	Fish consumption ≥1/week had a borderline significant effect on risk of developing dementia (HR=0.73; 0.52-1.03) or AD (HR=0.69; 0.47-1.01)
Engelhart, 2002 (Rotterdam study)[128]	Dutch population, follow-up 6y (*n*=5,395)	N-3 PUFA intake, FFQ	No association (RR=1.07; 0.94-1.22) between n-3 PUFA intake and risk of dementia
Kyle, 1999[124]	US population, mean age 75y, follow-up 10y (*n*=1,188)	Serum phophatidylcholine (PC)	Low levels of PC-DHA associated with higher risk of AD (67%, *P*<0.05)
Kalmijn, 1997 (Zutphen study)[129]	Cognitive decline (*n*=51), no cognitive decline (*n*=291), 64-89y, follow-up 3y	Fish and n-3 PUFA intake, dietary history method	Intake of n-3 PUFA not associated, but high fish intake associated with cognitive decline (OR=0.45; 0.17-1.16)
Kalmijn, 1997 (Rotterdam study)[116]	Non-demented participants, ≥55y, follow-up 2.1y (*n*=5,386)	Fish intake, FFQ	Fish intake was inversely related to incident dementia (RR=0.4; 0.2-0.9) and AD (RR=0.3; 0.1-0.9)

Abbreviations: AD: Alzheimer's disease; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; MMSE: Mini-Mental State Examination; Q: quartile; FFQ: food frequency questionnaire

Study acronyms: FACIT: Folic Acid and Carotid Intima-media Thickness; HUSK: Hordaland Health Study; CSHA: Canadian Study of Health and Aging; ARIC: Atherosclerosis Risk in Communities Study; CHCS: Cardiovascular Health Cognition Study; CHAP: Chicago Health and Aging Project; EVA: Etude du Vieillissement Arteriel; PAQUID: Personnes Agées QUID.

* Shown are relative risks (RR), hazard ratios (HR), odds ratios (OR) or mean values based on the multivariate adjusted model (when adjusted), along with 95% confidence intervals or standard deviations between parentheses.

10 years of follow-up, a higher fish or n-3 PUFA intake or status was associated with less cognitive decline or a lower risk of dementia[116,117,119,121-127,129,130]. One study in 174 subjects could not demonstrate a relation between baseline plasma n-3 PUFA and cognitive impairment or dementia after ≈5 years of follow-up[118]. A second analysis in a larger sample (*n*=663) of the same study population also showed no association[131]. Another study that was considerably larger (*n*=5,395), did show an association of fish intake with incident dementia[116], but the relation was no longer present after 6 and ≈10 years of follow-up[128,132]. The authors ascribed this discrepancy to the small number of incident dementia cases in the first phase, making it more prone to bias.

All together, the results of cross-sectional studies lack consistency while longitudinal studies mostly show an inverse association. More longitudinal studies focusing on specific cognitive domains such as memory, speed, and attention, are needed.

Dementia and cognitive decline: trial evidence

RCTs of fish or EPA+DHA and cognition are limited (**Table 1.2**). Two trials were performed in elderly patients with dementia[133,134]. Though both trials observed a beneficial effect of n-3 PUFA supplementation on general cognitive outcomes they add little weight to the evidence, because the first trial was small in sample size (*n*=20) and the second trial was of short duration (4 weeks). In a 6-month trial in 174 subjects with mild to moderate AD no overall effect of n-3 PUFA on global cognitive performance was found, except in a small subgroup (*n*=32) of patients with only mild cognitive impairment[135]. Trials in subjects without cognitive impairment are lacking.

Depression: observational evidence

We identified 22 observational studies in non-clinical adult populations on the association between fish or EPA+DHA and depression, of which only one had a prospective design (**Table 1.3**). Most studies (*n*=17) showed an inverse association of fish or n-3 PUFA intake[136-152] with depression, whereas four studies did not show an association[153-156]. In some studies associations were only observed in women[138,139,141]. Gender differences have been attributed to the fact that the endogenous n-3 fatty acid status is higher in women[141] and the uptake of DHA is enhanced due to estrogens[157]. However, sex-specific findings are not completely consistent and do not fully explain a lack of associations in (predominantly) male populations. In 11 studies the intake of fish fatty acids was assessed by food frequency questionnaire or other dietary methods, in nine studies blood n-3 PUFA concentrations were measured and in two studies n-3 PUFA were assessed in adipose tissue. Fish intake was inversely related to depression in six studies[136-139,143,147]. Of five other studies focusing on n-3 PUFA intake[140,141,153-155], two studies did not find an association[153,154]. All studies using n-3 PUFA concentrations in the blood[145,148-151] or in adipose tissue[142,146] found higher concentrations to be associated with less depression, except one[156]. It should be noted, however, that most studies that used biomarkers were small (*n*=20 to *n*=192), except the study by Tiemeier et al. (*n*=3,884)[145].

Table 1.2 Intervention studies of n-3 PUFA supplementation and cognitive performance

Author, year	Design	Subjects (*n*)	Intervention	Findings
Freund-Levi, 2006 (OmegAD)[135]	Double-blind, 6 months, + open label next 6 months	Patients with mild to moderate AD (*n*=174)	1.7 g DHA + 0.6 g EPA	No effect, except in a subgroup (*n*=32) with the mildest cognitive impairment (difference –0.5 vs -2.6, *P*=0.01)
Terano, 1999[133]	Double-blind, 6 months	AD patients (*n*=20)	0.72 g DHA	Effect on MMSE (22.2 vs 19.6) and Hasegawa Dementia rating scale (difference 19.9 vs 16.7, *P*<0.05)
Yehuda, 1996[134]	Double-blind, 4 weeks	AD patients (*n*=100)	n-3/n-6 fatty acid compound	Effect on several cognitive outcomes, difference and *P* not reported
Ongoing studies				
Dangour, 2006 (OPAL)[177]	Double-blind, 24 weeks	Healthy older persons, 70-79y (*n*=867)	0.5 g DHA + 0.2 g EPA	–
MIDAS	Double-blind, 24 weeks	Healthy elderly with memory complaints, ≥55y (*n*=465)	900 mg DHA	–
DHA in slowing the progression of AD	Double-blind, 18 months	Mild to moderate AD, ≥50y (*n*=400)	2,040 mg DHA	–
Gillette-Guyonnet, 2009 (MAPT)[178]	Double-blind, 36 months	Frail elderly, ≥70y (*n*=1,200)	800 mg DHA	–
The Efficacy of Phosphatidylserine-Omega3 in Elderly With Age Associated Memory Impairment	Double-blind, 15 weeks	Patients with mild cognitive impairment, 50-90y (*n*=157)	300 mg phosphatidylserine-omega3	–

Abbreviations: EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AD: Alzheimer's Disease
Study acronyms: OmegAD: Omega-3 and Alzheimer's Disease; OPAL: Older People And n-3 Long-chain polyunsaturated fatty acids study; MIDAS: Memory Improvement with Docosahexaenoic Acid Study; MAPT: The Multidomain Alzheimer Preventive Trial.

To the best of our knowledge, there is only one prospective study of fish and n-3 PUFA intake and incident depression. This study in a population of 7,903 university graduates with a mean age 41 years suggested a potential benefit of moderate n-3 PUFA intake after 2 years of follow-up[158]. A major limitation in this study was that data on depression were obtained by self-report.

Five studies of n-3 PUFA concentrations and depression have been performed in coronary heart disease patients and all found depression to be related to lower plasma or erythrocyte n-3 PUFA levels, in particular DHA[159-163] (studies not included in **Table 1.3**). Depressed subjects had 13 to 25% lower DHA levels and 12 to 20% lower total n-3 PUFA levels compared with non-depressed subjects.

Depression: trial evidence

An overview of trials (*n*=12) in healthy adults and adults with depressive disorders is shown in **Table 1.4**. Trials in patients with other diseases than depression and in children or postpartum mothers were beyond the scope of our research and therefore not included. We identified one randomized trial on n-3 PUFA and depressed mood in a non-depressed population. Thirty-three subjects with a mean age of 33 years experienced an improvement in mood during daily supplementation with 1.6 g EPA and 0.8 g DHA for 35 days[164]. Recently, Rogers et al. performed a trial in a general population with mild to moderate depression and did not observe an effect after three months of daily supplementation with 1.5 g EPA+DHA[165].

Of 10 studies in clinical populations, five found a beneficial effect[166-170], and five did not find an effect of EPA+DHA supplementation[171-175]. Most participants had a diagnosis of unipolar or major depression. Trials were heterogeneous with respect to sample size (*n*=20 to 280) and duration (28 to 120 days) and EPA+DHA treatment was often provided as an adjunctive therapy to pharmacological treatment. The daily dose of supplementation ranged from 1.0 to 9.6 g n-3 PUFA and comprised EPA only, DHA only or the combination.

In 2006 Appleton et al. performed a meta-analysis of 12 studies, some of which were not included in our overview because of different inclusion criteria. They concluded that the available evidence provided little support for the use of n-3 PUFA to improve depressed mood, although the interpretation was hampered by the large heterogeneity of trials[176]. Effects were mainly observed in populations with depressive disorders, but it should be noted that there were only two trials in non-depressed populations of older people.

Table 1.3 Observational studies on the association of fish and n-3 PUFA intake with depression

Author, year (study)	Study population (*n*)	N-3 PUFA	Findings *
Cross-sectional studies			
Bountziouka, 2009[136]	Greek population (*n*=1,190)	N-3 PUFA intake, FFQ	1 Portion increase in fish consumption/week was associated with 0.58 times (95% CI 0.45-0.73) lower chance of having a GDS score >5
Colangelo, 2009[141]	US population (*n*=3,317)	N-3 PUFA intake, dietary history	Inverse association between fish intake and CES-D scores (OR=0.80; 0.61-1.06, *P*-trend 0.03 for EPA+DHA) , more pronounced in women (OR=0.75; 0.53-1.08, *P*-trend 0.008)
Appleton, 2008[156]	UK population with mild to moderate depressed mood (*n*=192)	N-3 PUFA concentrations in blood	No association between n-3 PUFA status and DASS (β=0.08 (–0.746-0.905), *P*=0.85) and BDI scores (β=–0.48 (–1.447-0.487), *P*=0.33)
Appleton, 2007[155]	UK population (*n*=2,982)	N-3 PUFA intake, FFQ	Inverse association for n-3 PUFA intake from fish and DASS scores, but not after adjustment for confounders (β=-0.47 (–1.31-0.37), *P*=0.28). No association for intake of fish + n-3 PUFA supplements (β=0.08 (-0.07-0.23), *P*=0.31).
Appleton, 2007[137]	Northern Ireland male (*n*=2,747) and French male population (*n*=7,855) (total *n*=10,602)	Fish intake, FFQ	Inverse association between fish intake and WPD scores in Northern Ireland (β=–0.09 (–2.25-–0.01), *P*=0.05) and French population (β=-0.14 (–2.73-–1.17), *P*<0.01)
Kamphuis, 2006 (Zutphen Study)[140]	Dutch cohort, male, 70-79y (*n*=332)	EPA+DHA intake, dietary history method	Inverse association between fish intake and Zung scores (OR=0.46; 0.22-0.95, *P*-trend=0.04)
Mamalakis, 2006[142]	Healthy adults from Crete, 22-58y (*n*=130)	Adipose tissue	Inverse association between adipose tissue and Zung scores (β=-0.22, *P*=0.008)
Barberger-Gateau, 2005 (Three City Study)[143]	French cohort (*n*=9,294)	Fish intake, FFQ	Inverse association between fish intake >1/week and CES-D scores (OR=0.63; 0.52-0.75, *P*<0.001)
Hakkarainen, 2004[153]	Finnish cohort, male, 50-69y, (*n*=29,133)	Fish and n-3 PUFA intake, dietary history	No association between fish fatty acid intake and depression (RR=1.02; 0.97-1.08)

Assies, 2004[144]	Subjects with recurrent major depression (*n*=44)	N-3 PUFA concentrations in blood	Lower levels of DPA and DHA in erythrocytes of depressed patients, quantitative results not reported
Jacka, 2004[154]	Australian cohort, women (*n*=755)	N-3 PUFA intake, FFQ	No differences in intake between depressed and non-depressed subjects (*P*=0.3)
Timonen, 2004[139]	Finnish cohort (*n*=5,689)	Fish intake, FFQ	Increased risk of depression (HSCL-25) with low fish intake, but only in women (OR=2.4; 1.4-4.2)
Tiemeier, 2003 (Rotterdam Study)[145]	Community-dwelling elderly, ≥60 y, case-control (*n*=3,884)	N-3 PUFA concentrations in blood	Lower levels of DHA in depressive subjects (5.2% vs 5.9%, *P*=0.02)
Mamalakis, 2002[146]	Healthy adults from Crete, mean age 39y (*n*=139)	Adipose tissue	Lower DHA in adipose tissue of depressed subjects (*P*<0.05)
Silvers & Scott, 2002[147]	New Zealand adults aged ≥15y (*n*=4,644)	Fish intake, FFQ	Inverse association between fish intake and MH scale (*P*=0.005)
Tanskanen, 2001[138]	Random sample of Finnish population, 25-64y (*n*=3,204)	Fish intake, FFQ	Frequent fish consumers had less depressive symptoms measured with the BDI (OR=1.31; 1.10-1.56, *P*=<0.01). Association only in women when analyzed separately (OR=1.40; 1.11-1.78, *P*<0.01)
Maes, 1999[148]	34 subjects with major depression, 14 healthy subjects, case-control (*n*=48)	N-3 PUFA concentrations in blood	Lower EPA and DHA in serum phospolipids and serum cholesteryl esters of depressed patients
Edwards, 1998[149]	10 Major depressed, 14 healthy subjects (*n*=24)	N-3 PUFA concentrations in blood	Lower levels of n-3 PUFA 5.39 (0.93) mg/100 g total phospholipid vs 7.60 (0.38), *P*=0.02 in RBCs of depressed subjects
Peet, 1998[150]	15 Major depressed, 15 healthy subjects, 18-65y , case-control (*n*=30)	N-3 PUFA concentrations in blood	Lower levels of n-3 PUFA 5.39 (4.16) mg/100 g total phospholipid vs 9.04 (3.40), *P*=0.02 in RBCs of depressive patients
Adams, 1996[151]	Moderately to severely depressed patients (*n*=20)	N-3 PUFA concentrations in blood	Higher AA/EPA ratio associated with higher scores on HRS (r=0.472, *P*<0.05)

Table 1.3 *Continued*

Author, year (study)	Study population (*n*)	N-3 PUFA	Findings *
Maes, 1996[152]	36 major depressed, 14 minor depressed, 24 healthy subjects, case-control (*n*=74)	N-3 PUFA concentrations in blood	Depressive patients had lower EPA 0.37 (0.22) vs 0.53 (0.36), *P*=0.02 in cholesteryl esters and in phospholipids 0.59 (0.40) vs 0.81 (0.74), *P*=0.004.
Longitudinal studies			
Sanchez, 2007 (SUN cohort)[158]	General population, mean age 41y, follow-up 2y (*n*=7,903)	Fish and n-3 PUFA intake, FFQ	Possible beneficial effect of a moderate consumption of n-3 PUFA intake in Q2 and Q4; OR Q2: 0.72; 0.52-0.99, OR Q4: 0.65; 0.47-0.90, *P*-trend=0.38.

Abbreviations: PUFA: polyunsaturated fatty acids; FFQ: food frequency questionnaire; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; DPA: docosapentaenoic acid; AA: arachidonic acid; RBCs: red blood cells; Q: quartile; CES-D: Center for Epidemiologic Studies Depression Scale; DASS: Depression Anxiety and Stress Scales; BDI: Beck Depression Inventory; WPD: Welsh Pure Depression sub-scale of the Minnesota Multiphasic Personality Inventory; Zung: Zung Self-rating Depression Scale; HSCL-25: Hopkins Symptom Checklist-25; MH: Mental Health Scale of SF-36; HRS: Hamilton depression rating scale.
* Shown are relative risks (RR), hazard ratios (HR), odds ratios (OR) or mean values based on multivariable models (when adjusted), along with 95% confidence intervals or standard deviations between parentheses.

Table 1.4 Intervention studies of n-3 PUFA supplementation and depression

Author, year	Design	Subjects (*n*)	Intervention	Findings
Rogers, 2008[165]	84 days	Non-clinical (*n*=218)	0.63 g EPA + 0.85 g DHA	No significant differences between groups on DASS (8.4 vs 9.6, *P*=0.27)
Grenyer, 2007[172]	112 days	Major depression (*n*=83)	0.6 g EPA + 2.2 g DHA	No significant differences on HDRS and BDI, Δ and *P* NR
Frangou, 2006[166]	84 days	Bipolar disorder (*n*=75)	1 or 2 g EPA, partly adjunctive	Significant decreases, (Δ NR, *P*=0.03) on HDRS in 1 g and 2 g EPA groups
Silvers, 2005[173]	84 days	Major depression, 18-65y (*n*=77)	0.6 g EPA + 2.4 g DHA, adjunctive	Fish oil did not improve HDRS-SF (Δ 0.3 vs 0.6) and BDI (Δ 0.3 vs 1.5), *P* NR
Fontani, 2005[164]	35 days	Non-clinical, healthy volunteers (*n*=33)	1.6 g EPA + 0.8 g DHA + 0.4 g other n-3 fatty acids	Better POMS scores after n-3 PUFA supplementation, Δ and *P* NR
Hirashima, 2004[174]	28 days	Bipolar disorder (*n*=21)	5.0-5.2 g EPA + 3.0-3.4 g DHA or 1.3 g EPA + 0.7 g DHA, partly adjunctive	No significant differences between groups on HDRS, Δ and *P* NR
Marangell, 2003[175]	42 days	Major depression, 18-65y (*n*=36)	2 g DHA	No significant effect of DHA on MADRS (Δ 9.1 vs 5.4, *P*=0.23) and HDRS (Δ 8.1 vs 5.8, *P*=0.43)
Su, 2003[167]	56 days	Major depression, 18-60y (*n*=28)	4.4 g EPA + 2.2 g DHA, adjunctive	Patients in the fish oil group had greater reductions on HDRS (Δ 13.6 vs 6.4, *P*=0.001)
Keck, 2002[171]	120 days	Bipolar disorder, (*n*=116)	6.0 g EPA, adjunctive	No significant differences on IDS-C (Δ NR, *P*=0.82)
Nemets, 2002[168]	28 days	Unipolar depression, 18-75y (*n*=20)	2 g EPA, adjunctive	Significant improvement on HDRS (Δ 12.4 vs 2.3, *P*<0.01) at weeks 2, 3, and 4 in EPA group
Peet, 2002[169]	84 days	Major depression (*n*=70)	1,2 or 4 g EPA, adjunctive	Only significant effects of 1 g EPA (Δ 9.9, 11.2, 12.5, *P*=0.06) not of 2 g (Δ 5.8, 3.0, 5.7, *P*=0.003) and 4 g (Δ 6.4, 8.5, 9.3, *P*=0.02) on HDRS, MADRS and BDI respectively
Stoll, 1999[170]	112 days	Bipolar disorder, 18-65y (*n*=30)	6.2 g EPA + 3.4 g DHA, adjunctive	Significant improvement in fish oil group on HDRS (Δ 4.6 vs 3.1, *P*=0.002)

Abbreviations: EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; Δ: change; NR: not reported; DASS: Depression Anxiety and Stress Scales; HDRS: Hamilton Depression Rating Scale; HDRS-SF: HDRS short form; BDI: Beck Depression Inventory; POMS: Profile of Mood States; MADRS: Montgomery-Åsberg Depression Rating Scale; IDS-C: Inventory of Depressive Symptomatology.

Rationale and outline of this thesis

This thesis addresses two primary research questions:

1) Is there a role for fish or EPA+DHA intake in the prevention of cognitive decline?

2) Is there a role for fish or EPA+DHA intake in the maintenance of mental well-being?

To answer these research questions we performed several studies, which are briefly described below.

Evidence from trials focusing on cognitive performance is limited and intervention studies on the effect of EPA+DHA on cognitive performance in non-demented older people are lacking. Trials with sufficient power are needed to ascertain whether treatment with EPA+DHA offers significant benefits to cognitive performance and mental well-being in older individuals. We performed a double-blind, placebo-controlled trial in 302 independently living individuals aged 65 years or older to study the effects on cognitive performance of a low daily dose of EPA+DHA (400 mg) or a high daily dose of EPA+DHA (1,800 mg) for 26 weeks (**chapter 2**). The high 'pharmacological' dose was chosen to maximize contrast between the groups and the low dose to comply with the recommended daily intake in the Netherlands of 450 mg EPA+DHA. With a study duration of 26 weeks we were only able to examine relatively short-term effects of EPA+DHA on cognitive performance.

Evidence from cross-sectional and prospective studies suggests that an increased intake of fish and n-3 PUFA could protect against age-related cognitive decline and dementia. However, the results are inconsistent and studies that address changes in specific cognitive domains are limited. We investigated the association of fish and n-3 PUFA intake with cognitive performance and cognitive decline by a prospective epidemiological study in 1,025 older US men. We were able to assess cognitive decline during 6 years of follow-up, thereby focusing on relatively long-term effects of fish and n-3 PUFA intake on cognitive performance. Also, we related fish consumption to different domains of cognitive functioning, to examine differential effects of n-3 PUFA or other components in fish in specific parts of the brain (**chapter 3**).

Trials of EPA+DHA supplementation and depression have mainly been performed in populations of depressed patients and results are ambiguous. There is an obvious need for trials in non-depressed older populations. In a double-blind, placebo-controlled trial we examined the effect of supplementation with 400 mg or 1,800 mg of EPA+DHA per day for 26 weeks on mental well-being in older individuals. In **chapter 4** we describe the effect of EPA+DHA on mental well-being, as assessed by several depression rating scales and in **chapter 5** we evaluate the reliability of the depression rating scales that we used, with a focus on the utility of these questionnaires in the general population.

Observational studies on fish and n-3 PUFA intake and mental well-being have

mainly been performed in the general population with a relatively low prevalence of mental disturbances. We performed a cross-sectional analysis of fish and EPA+DHA intake and mental well-being in a population of older subjects with a history of myocardial infarction that had a higher prevalence of depressive symptoms than the general population (**chapter 6**).

In **chapter 7** we report the effect of daily supplementation with 400 mg or 1,800 mg of EPA+DHA for 26 weeks on QOL. Improving QOL is becoming an increasingly important outcome in elderly research. For studies in health promotion, such generic outcomes may be considered more relevant than disorder-specific outcomes.

Furthermore, we investigated early process markers of mental deterioration by examining the effect of low- and high-dose supplementation with EPA+DHA on gene expression profiles in human blood mononuclear cells (**chapter 8**).

In the general discussion (**chapter 9**) the main findings of our studies are summarized, and a reflection on methodological issues is given. Also, directions for future research and implications for public health are addressed.

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2

Effect of fish oil on cognitive performance in older subjects: a randomized controlled trial

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Abstract

Background High intake of n-3 polyunsaturated fatty acids may protect against age-related cognitive decline. However, results from epidemiological studies are inconclusive and results from randomized trials in non-demented elderly are lacking.

Objective To investigate the effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) supplementation on cognitive performance.

Methods Double-blind, placebo-controlled trial involving 302 cognitively healthy (Mini-Mental State Examination score >21) individuals aged 65 years or older. Participants were randomly assigned to 1,800 mg/d EPA+DHA, 400 mg/d EPA+DHA, or placebo capsules for 26 weeks. Cognitive performance was assessed using an extensive neuropsychological test battery that included the cognitive domains of attention, sensorimotor speed, memory, and executive function.

Results The mean age of the participants was 70 years, and 55% were male. Plasma concentrations of EPA+DHA increased by 238% in the high-dose and 51% in the low-dose fish oil group compared with placebo, reflecting excellent compliance. Baseline scores on the cognitive tests were comparable in the three groups. Overall, there were no significant differential changes in any of the cognitive domains for either low-dose or high-dose fish oil supplementation compared with placebo.

Conclusions In this randomized, double-blind, placebo-controlled trial, we observed no overall effect of 26 weeks of eicosapentaenoic acid and docosaheaenoic acid supplementation on cognitive performance.

Introduction

Age-related cognitive impairment is considered a strong risk factor for the development of dementia[1]. It is estimated that 24 million people worldwide have dementia, and as the population of elderly people is growing rapidly, this number is expected to double every 20 years[2]. Because no effective curative approaches are available yet, it is of major importance to develop and study preventive measures.

Increased intake of fish and n-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), might play a protective role against age-related cognitive decline. DHA is the predominant n-3 fatty acid in the brain and is an integral component of neural membrane phospholipids[3]. Both DHA and EPA may reduce oxidative stress, have an anti-inflammatory action and have been linked with aspects of neuron function, including neurotransmission, membrane fluidity, ion channel and enzyme regulation, and gene expression[4].

In a number of cross-sectional studies, cognitive performance was examined in relation to dietary intake data of n-3 PUFAs and fish[5-7] or n-3 PUFA concentrations in the blood[8,9]. Results from these studies were inconsistent, showing either that a high intake of n-3 PUFAs was associated with less cognitive decline[6,8] or no associations[5,7,9]. Longitudinal studies of fish or n-3 PUFA intake and cognitive performance also yielded contradictory results. In some studies high fish or n-3 PUFAs consumption was protective against cognitive decline[7,10-13], whereas no association was found in other studies[6,14]. Most studies of n-3 PUFA concentrations in the blood showed high concentrations to be associated with a lower risk of cognitive decline[15-17].

A very small number of randomized trials with cognitive endpoints have been performed in elderly patients with established dementia[18,19]. These studies were limited because they were small in sample size (n=20 and n=100 respectively), were of short duration (1 year and 4 weeks respectively), or assessed cognitive performance using generalized tests. A trial conducted in subjects with mild to moderate Alzheimer's Disease (AD) included more subjects (n=174) and the duration was 6 months. In this trial, no effect of n-3 PUFAs on cognitive performance was found. However, a reduction in cognitive decline rate was observed in a small subgroup of dementia patients with the mildest cognitive impairment[20]. Therefore, n-3 PUFAs might be beneficial in primary prevention of cognitive decline. We conducted a double-blind, randomized, placebo-controlled trial to investigate the effect of daily supplementation with high or low doses of EPA+DHA for 26 weeks on cognitive performance in older individuals.

Methods

Participants

Subjects aged >65 years were mainly recruited through an existing database of volunteers with interest in participating in studies at Wageningen University. Main exclusion criteria were: [1] a score of >16 on the Centre for Epidemiologic Studies Depression Scale (CES-D)[21]; [2] score of <21 on Mini-Mental State Examination (MMSE)[22]; [3] current or recent (<4 weeks) use of fish oil supplements; [4] intake of more than 800 mg of EPA+DHA from fish per day, as estimated from a fish consumption questionnaire; [5] current use of pharmacologic antidepressants or medication for dementia; or [6] use of more than four glasses of alcohol per day. Additionally, self-reported compliance during the 2-week placebo run-in period had to be >80%. The Wageningen University Medical Ethical Committee approved the study, and subjects gave written informed consent.

Study design

An independent person randomized subjects by means of computer-generated random numbers in stratified permuted blocks of six. Stratification factors included age (< and ≥69 years), gender, MMSE score (< and ≥28) and CES-D screening test score (< and ≥5). Individuals were randomly allocated to receive a daily dose of fish oil containing either approximately 400 mg or approximately 1,800 mg EPA+DHA, or a placebo oil (high oleic sunflower oil) for 26 weeks (Lipid Nutrition, Wormerveer, the Netherlands). The high daily dose of fish oil provided 1,093 ± 17 mg EPA and 847 ± 23 mg DHA, and the low daily dose provided 226 ± 3 mg EPA and 176 ± 4 mg DHA: the mean EPA+DHA ± SD determined of 20 samples taken at regular times during the study. The placebo capsules contained mainly oleic acid (C18:1n-9). The oils were administered in soft gelatin capsules each containing 900 mg oil and 2.7 mg tocopherol as antioxidant (Banner Pharmacaps, Tilburg, the Netherlands). Capsules were packaged in foil strips containing the daily dose of six capsules per strip to facilitate compliance and recording of capsule use (Medipack, Gorredijk, the Netherlands). Capsules with fish oil or placebo oil were indistinguishable in appearance. Staff members and participants were blinded toward treatment allocation until completion of blind data analysis. At baseline and at 13 weeks, participants received a 14-week supply of capsules (1 week extra as reserve). Compliance was judged by capsule-return counts and a diary in which participants registered missed capsules.

Sample size calculation was based on the Word Learning Test, where a difference of four points was considered clinically relevant. With a mean ± SD of 45 ± 8[23], a minimum sample size of 63 subjects per group would be required to detect a difference (power 80%, two-sided α=0.05). We were able to include 100 subjects per treatment group which allowed subgroup analysis and anticipated drop-out.

Cognitive performance

Cognitive testing was performed at baseline and after 13 and 26 weeks of intervention by well-trained research assistants, under supervision of a neuropsychologist. An extensive cognitive test battery was used consisting of five tests that have no ceiling effects and are reported to be sensitive and robust in detecting small cognitive differences[24]. The Word Learning test measures the storage and retrieval of newly acquired verbal material[25]. The forward test of the Wechsler Digit Span task measures attention and the backward test measures working memory[26]. The Trail Making test version A measures sensomotoric speed and version B measures concept shifting interference (executive function)[27]. The Stroop Color-Word test measures selective attention and susceptibility to behavioral interference[28]. The Verbal Fluency test measures the ability to draw on one's encyclopedic memory in a strategy-based manner (executive, verbal reasoning)[29]. Participants were tested by the same research assistant in 69% of the cases at 13 weeks and 56% of the cases at 26 weeks and underwent measurements on the same day of the week and at the same time the morning, after a standardized small snack at the research centre. Coffee and tea were not allowed before and during testing. A standard protocol was used, and all tests were performed in the same quiet room, without any obvious distracters.

Blood

Fasting venous blood samples were collected at baseline and after 13 and 26 weeks of intervention. A blood sample for determination of n-3 PUFAs was collected into 10-ml ethylenediaminetetraacetic acid (EDTA) Vacutainers, immediately placed in ice water, centrifuged at 2,000 g at a temperature of 4°C, and stored at –80°C until laboratory analyses. n-3 PUFAs in plasma cholesteryl esters were determined as described previously[30]. A second blood sample was collected into a 4.5-ml EDTA Vacutainer and stored at –80°C for APOE genotype determination by the PCR-based restriction fragment length polymorphism method and restriction enzyme digestion with HhaI[31].

Other measurements

Information on medical history, drug use, alcohol consumption, smoking habits, educational level and marital status was obtained by questionnaire. Education was categorized according to Statistics Netherlands (CBS). Physical activity level was estimated by means of a previously described questionnaire[32].

Fish intake in the previous 3 months was estimated with a food frequency questionnaire. Participants had to indicate on a 60-item list which kinds of fish they had consumed and how often. These 60 items were categorized into three groups based on the amount of fat in the different types of fish, i.e. lean, medium-fat, and fatty fish. Furthermore, information was obtained on how the fish was consumed: as main meal component during dinner, as a snack, or on toast or with a bread meal. EPA+DHA intake was calculated by multiplying the frequencies of portions of fish per month per group by an EPA+DHA conversion factor[33].

Body height was measured at baseline with a wall-mounted stadiometer to the nearest 0.1 cm. Body weight and waist circumference were measured at each center visit in a standing position, with participants dressed in light clothing and without shoes. Body weight was measured to the nearest 0.1 kg with a calibrated digital scale. Waist circumference was measured in duplicate to the nearest 0.1 cm at the midpoint between the lowest rib and the top of the iliac crest with a nonelastic tape.

Statistical analysis

Data analysis was performed by intention to treat and according to a predefined data analysis plan using SPSS 12.0.1. A two-sided *P*-value <0.05 was considered significant. Baseline characteristics of treatment groups were compared by analysis of variance (ANOVA) or Kruskal-Wallis for continuous variables and chi-square for categorical variables.

Crude cognitive test scores at baseline and after 26 weeks (or 13 weeks) were pooled to calculate the grand mean and SD per test. To compare the results of the individual cognitive tests and to limit the number of dependent variables, individual neuropsychological tests were clustered into compound Z scores for four primary composite endpoints on neuropsychological domains:

Sensorimotor speed =

$$\frac{\left(-Z_{\text{Trail Making-part A}}\right)+\left(-Z_{\text{Stroop-part 1}}\right)+\left(-Z_{\text{Stroop-part 2}}\right)}{3}$$

Memory =

$$\frac{Z_{\text{15WordLearning-immediate}}+Z_{\text{15WordLearning-delayed}}+Z_{\text{15WordLearning-recognition}}+Z_{\text{DigitSpan-backward}}}{4}$$

Executive function =

$$\frac{\left(-Z_{\text{Trail Making}\,\frac{\text{part B - part A}}{\text{part A}}}\right)+\left(-Z_{\text{Stroop-part 3 - }\frac{\text{part 1 + part 2}}{2}}\right)+\left(Z_{\text{WordFluency-animals}}+Z_{\text{WordFluency-letter}}\right)}{4}$$

Attention =

$$Z_{\text{DigitSpan-forward}}$$

To test differential changes among the three intervention groups after 13 and after 26 weeks, ANOVA was used with the treatment group as factor and scores on the different cognitive domains as dependent variables. A Dunnett post hoc test was conducted to compare mean changes in the two treatment groups with changes in the control group.

After performing the primary analysis, an additional per-protocol analysis was performed, as were preplanned subgroup analyses for gender and for (non) carriers of the APOE-ε4 allele.

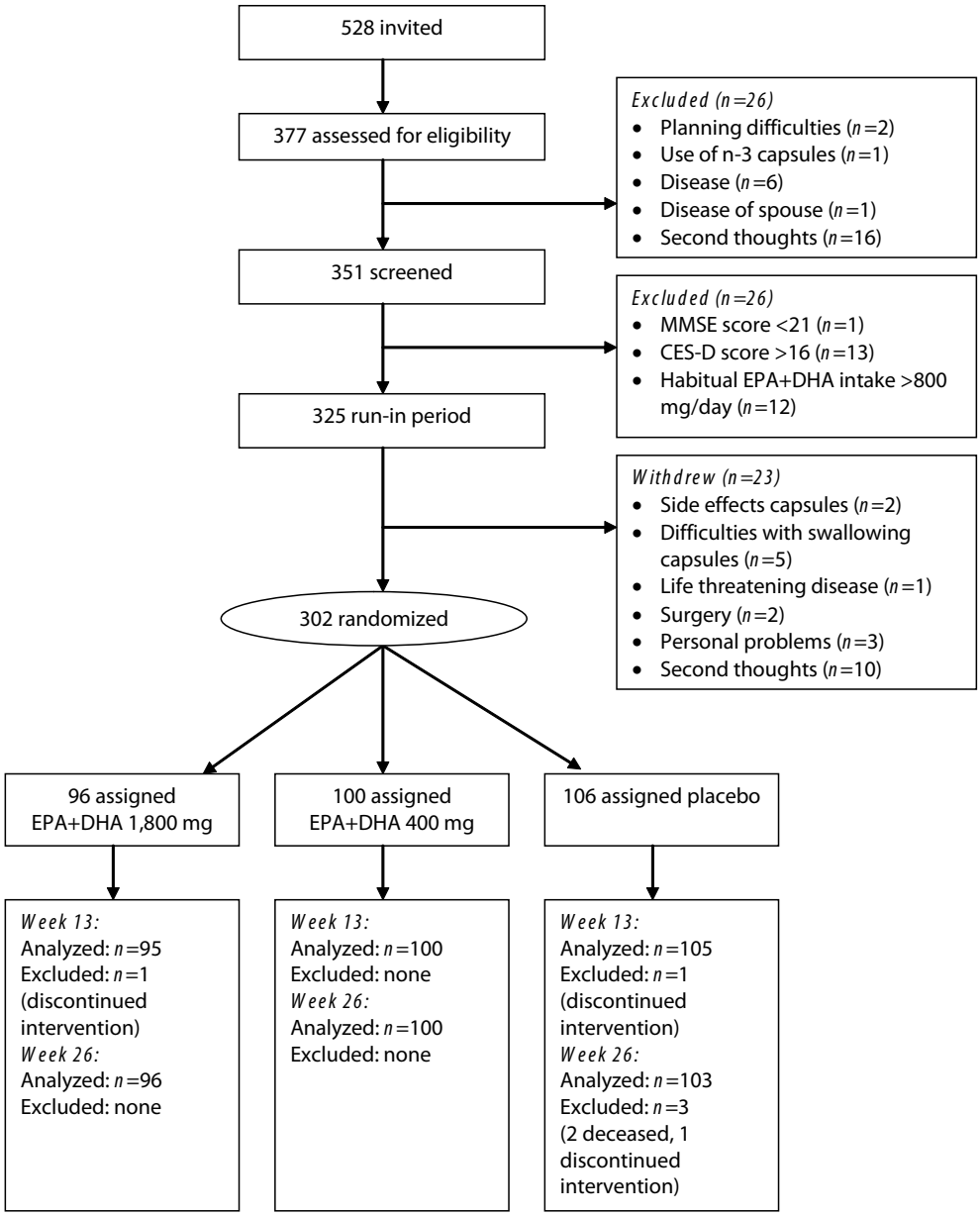

Figure 2.1 Flow of participants through study

Results

Subjects were screened between November 2005 and February 2006, and intervention took place between February and November 2006. **Figure 2.1** shows the participant flow through the study. Nine subjects discontinued the use of capsules: five discontinued use because of gastrointestinal complaints, two discontinued use because participation became too burdensome, and two died before the end of the intervention.

Apart from the individuals who stopped treatment prematurely, the average adherence to treatments based on counts of returned capsules was high (99%, with only three subjects <80%) and did not differ among the treatment groups.

Table 2.1 Characteristics of 302 subjects participating in a randomized, placebo-controlled trial, by treatment group

	1,800 mg EPA+DHA (*n*=96)	400 mg EPA+DHA (*n*=100)	Placebo (*n*=106)
Age (years)	69.9 ± 3.4[a]	69.5 ± 3.2	70.1 ± 3.7
Sex, Male (%)	55	55	56
Married/ living together (%)	80	81	77
Education Low/ Middle/ High (%)	10/ 54/ 35	11/ 49/ 40	5/ 59/ 37
BMI (kg/m^2)	26.1 ± 3.0	26.2 ± 3.4	26.5 ± 3.9
Waist circumference (cm)	94.5 ± 11.4	94.2 ± 10.6	95.9 ± 12.1
Physical activity score	11.5 ± 6.4	11.1 ± 6.2	11.4 ± 6.2
Smoking behavior (%)			
Smoker	8	8	10
Ex-smoker	64	54	56
Never smokers	28	38	34
Alcohol consumers (%)	80	87	88
Median alcohol consumption (glasses/ week)[b]	10 (6-14)[c]	8 (4-14)	8 (4-14)
Coffee consumers (%)	96	96	97
Median coffee consumption (cups/day)[b,d]	4 (3-5)	3 (3-4)	4 (3-4)
Fish consumption (times/ month)	7 (4-9)	5 (3-9)	6 (4-8)
EPA+DHA intake (mg/day)	306 (131-592)	278 (103-487)	316 (166-584)
Plasma EPA+DHA (mass%)	1.9 ± 0.9	1.9 ± 1.1	1.9 ± 1.1
APOE-ε4 allele 0/ 1/ 2 (%)	68/ 29/ 3	69/ 30/ 1	71/ 26/ 2[e]
MMSE (Mini-Mental State Examination) score	28 (27-29)	28 (27-29)	28 (27-29)
Range	23-30	24-30	23-30
Self-perceived memory impairment (%)[d]	53	50	68

[a] Mean ± SD (all such values)
[b] Mean consumption in consumers only
[c] Median (Q1-Q3) (all such values)
[d] Significant difference among the three treatment groups (one-way ANOVA/ Kruskal Wallis for continuous variables and chi-square analysis for categorical variables)
[e] Missing value for one participant

Compliance was confirmed by a change in the proportion of EPA+DHA in plasma cholesteryl esters of +51% in the low-dose fish oil group (from 1.88 ± 1.12 to 2.83 ± 1.03 g/100g fatty acids), +236% in the high-dose fish oil group (from 1.90 ± 0.86 to 6.40 ± 1.53 g/100g fatty acids) and -2% in the placebo group (from 1.91 ± 1.15 to 1.87 ± 1.11 g/100g fatty acids). The supplements were well tolerated; main complaints concerned mild gastrointestinal discomfort. Adverse events were reported by 14% of the subjects in the high-dose fish oil group (gastrointestinal problems [*n*=10], poly-urination, restless and weight gain); 13% of the subjects in the low-dose fish oil group (gastrointestinal problems [*n*=9], feeling lifeless, blurred vision, sore throat and muscle pain) and 15% of the subjects in the placebo group (gastrointestinal problems [*n*=12], skin irritations, blurred vision, TIA and muscle pain). At the end of the study, blinding of subjects toward treatment allocation (fish oil, placebo or no idea) was evaluated. The proportion of participants who thought they had received fish oil or placebo did not differ among the groups.

At baseline, treatment groups were equally distributed with regard to demographic, anthropometric and lifestyle factors (**Table 2.1**). Baseline scores on the cognitive tests were comparable among the three groups. In general, cognitive test scores in all three groups improved, but changes were not significantly different among the groups and were probably mostly due to learning effects because scores in the placebo group increased to the same extent (**Table 2.2**).

Mean baseline scores of all cognitive domains did not differ among the treatment groups. After 13 weeks, there was a decline in the domain of memory in the group supplemented with 400 mg EPA+DHA compared with the placebo group (*P*=0.03; difference in Z scores –0.18 [95% CI –0.34 to 0.01]). However, after 26 weeks, this difference was no longer observed. After 13 and 26 weeks of supplementation, there were no significant differential changes for fish oil vs placebo in any of the other cognitive function domains (**Table 2.3**).

However, an interaction effect between treatment and APOE genotype on the cognitive domain of attention was observed, i.e., APOE-ε4 carriers in the low-dose fish oil group (*n*=31; *P*=0.03; 0.47 [95%CI 0.03-0.91]) and in the high-dose fish oil group (*n*=31; *P*=0.04; 0.49 [0.01-0.96]) showed an improvement after 26 weeks of intervention compared with placebo. An interaction effect was also demonstrated between treatment and sex on the cognitive domain of attention; i.e., an improvement in men (*n*=167; *P*=0.05; 0.36 [0.01-0.72]) after 26 weeks of intervention for the low-dose fish oil group compared with placebo.

Discussion

In this randomized, controlled trial in elderly people, daily supplementation with EPA+DHA had no overall effect on any of the domains of cognitive function. However, we cannot exclude the possibility of a favorable effect of EPA+DHA on the cognitive domain of attention in subjects carrying the APOE-ε4 allele and in male subjects.

We examined cognitive performance in the present study by means of sensitive

Table 2.2 Crude scores from neuropsychological tests of cognitive function in older Dutch adults at baseline and after 13 and 26 weeks of EPA+DHA supplementation, by treatment group[a]

Test, maximum score		1,800 mg EPA+DHA[b]	400 mg EPA+DHA[c]	Placebo[d]
Sensorimotor Speed[e]				
Trail Making, part A, seconds to complete the task	Baseline	47.4 ± 16.3	48.3 ± 14.1	46.9 ± 16.8
	13 weeks	44.9 ± 16.0	44.7 ± 14.2	43.2 ± 14.2
	26 weeks	43.4 ± 15.1	42.3 ± 14.0	40.9 ± 16.2
Stroop part 1, seconds to complete the task	Baseline	51.5 ± 9.2	50.0 ± 7.7	50.9 ± 8.0
	13 weeks	50.8 ± 8.3	49.9 ± 7.7	49.5 ± 7.6
	26 weeks	50.3 ± 8.5	49.6 ± 7.3	49.7 ± 7.9
Stroop part 2, seconds to complete the task	Baseline	66.0 ± 12.0	63.6 ± 9.6	64.5 ± 11.5
	13 weeks	64.0 ± 11.0	62.7 ± 9.5	62.8 ± 11.2
	26 weeks	63.7 ± 10.9	61.6 ± 9.5	62.4 ± 10.8
Executive Function				
Trail Making Test (Part B-Part A/Part A)[f]	Baseline	0.73 ± 0.62	0.60 ± 0.53	0.65 ± 0.56
	13 weeks	0.76 ± 0.60	0.58 ± 0.48	0.59 ± 0.64
	26 weeks	0.73 ± 0.62	0.62 ± 0.46	0.66 ± 0.51
Stroop (Part 3 – [Part 1 + Part 2/2])[f]	Baseline	50.8 ± 21.1	53.9 ± 24.4	51.9 ± 21.9
	13 weeks	48.0 ± 19.8	47.4 ± 16.9	49.8 ± 21.4
	26 weeks	47.1 ± 25.0	45.8 ± 15.7	45.8 ± 17.7
Word Fluency – Animals, number of words	Baseline	23.9 ± 6.0	23.3 ± 6.0	23.2 ± 6.0
	26 weeks	24.2 ± 5.7	23.6 ± 5.8	24.0 ± 5.8
Word Fluency – Letter P, number of words	Baseline	15.7 ± 5.6	16.5 ± 5.4	16.0 ± 5.5
	26 weeks	16.4 ± 5.8	15.9 ± 4.7	16.4 ± 5.5
Memory				
15 Word Learning – Immediate recall, 75 words	Baseline	39.3 ± 8.8	40.8 ± 8.6	39.6 ± 9.7
	13 weeks	43.2 ± 8.8	43.2 ± 9.3	43.7 ± 9.4
	26 weeks	44.9 ± 9.9	46.1 ± 10.1	44.8 ± 9.4
15 Word Learning – Delayed Recall, 15 words	Baseline	7.6 ± 3.1	8.1 ± 2.9	7.9 ± 3.1
	13 weeks	8.4 ± 3.0	8.5 ± 2.8	8.9 ± 3.1
	26 weeks	9.1 ± 3.2	9.2 ± 3.0	9.0 ± 3.2
15 Word Learning – Recognition, 30 words	Baseline	28.3 ± 2.4	28.7 ± 1.4	28.7 ± 1.8
	13 weeks	28.4 ± 1.8	28.6 ± 1.4	28.8 ± 1.6
	26 weeks	28.4 ± 2.1	28.7 ± 1.5	28.6 ± 1.7
Digit Span Backward, 14 points	Baseline	5.6 ± 1.8	5.9 ± 2.0	5.9 ± 1.8
	13 weeks	6.0 ± 2.0	6.0 ± 1.8	6.3 ± 2.1
	26 weeks	5.8 ± 1.8	6.2 ± 1.9	6.4 ± 1.9
Attention				
Digit Span Forward, 16 points	Baseline	8.2 ± 2.0	8.4 ± 1.9	8.5 ± 1.9
	13 weeks	8.4 ± 2.1	8.4 ± 1.8	8.5 ± 2.0
	26 weeks	8.6 ± 2.0	8.6 ± 1.8	8.5 ± 2.0

[a] Values are mean ± SD. No significant differences among the three treatment groups were observed for any of the neuropsychological tests (ANOVA)
[b] For treatment group 1; *n*=96 at baseline (*n*=94 for TMT part B and TMT interference), *n*=95 after 13 weeks and *n*=96 after 26 weeks.
[c] For treatment group 2; *n*=100 at baseline, after 13 and after 26 weeks.
[d] For treatment group 3; *n*=106 at baseline (*n*=105 for 15 WLT-Recognition and Stroop part 1 and *n*=104 for Stroop part 2, Stroop part 3 and Stroop interference), *n*=105 after 13 weeks and *n*=103 after 26 weeks.
[e] Higher scores indicate more time needed to complete a task, i.e. poorer performance.
[f] Higher scores indicate poorer interference abilities.

Table 2.3 Changes in Z scores of cognitive performance domains in older Dutch adults after 13 and 26 weeks of EPA+DHA supplementation by treatment group[a]

Cognitive domain		1,800 mg EPA+DHA[b]	400 mg EPA+DHA[c]	Placebo[d]
Memory	13 weeks - baseline	0.25 ± 0.52	0.10 ± 0.44[e]	0.27 ± 0.52
	26 weeks - baseline	0.31 ± 0.48	0.26 ± 0.49	0.28 ± 0.51
Executive function	13 weeks - baseline	0.05 ± 0.52	0.16 ± 0.65	0.09 ± 0.63
	26 weeks - baseline	0.09 ± 0.39	0.07 ± 0.46	0.13 ± 0.39
Attention	13 weeks - baseline	0.08 ± 0.85	0.01 ± 0.75	–0.03 ± 0.66
	26 weeks - baseline	0.16 ± 0.83	0.06 ± 0.71	–0.07 ± 0.83
Sensorimotor speed	13 weeks - baseline	0.15 ± 0.45	0.11 ± 0.40	0.23 ± 0.41
	26 weeks - baseline	0.21 ± 0.47	0.21 ± 0.34	0.28 ± 0.40

[a] Values are mean ± SD
[b] For treatment group 1; *n*=96 at baseline, *n*=95 after 13 weeks and *n*=96 after 26 weeks.
[c] For treatment group 2; *n*=100 at baseline, after 13 and after 26 weeks.
[d] For treatment group 3; *n*=106 participants at baseline (*n*=105 for Sensorimotor speed), *n*=105 after 13 weeks and *n*=103 after 26 weeks.
[e] Significant difference among the three treatment groups were observed (ANOVA with Dunnett's post hoc test)

tests that were reported to decline with age[24]. Parallel versions of these tests were used to minimize learning effects. Despite these parallel versions, improvements in test scores were seen in all treatment groups, indicating that learning effects did occur. This emphasizes the necessity of the placebo group with which to compare all measurements with during intervention.

We improved the robustness of the underlying cognitive constructs by clustering crude test scores for several tests in compound performance measures, namely cognitive domains that are sensitive to aging[24]. This procedure decreased variation associated with the individual tests and aggregated our cognitive performance measures to four a priori defined outcomes. Furthermore, cognitive function tests were performed under standardized conditions that reduced variation due to factors such as caffeine, various types of breakfasts[34], and the influence of the research assistant and test room.

Because fish intake seems to be low in Dutch elderly[35], it was likely that many of our subjects had a suboptimal EPA+DHA status, which was also reflected in low plasma levels of EPA+DHA that were comparable to other general elderly populations[17,36]. Plasma cholesteryl esters are generally considered to adequately reflect intake of dietary fatty acids during the prior weeks[37]. The Spearman correlation between baseline EPA+DHA intake from the FFQ and baseline EPA+DHA plasma concentrations was 0.57 ($P<0.01$), which is adequate.

We selected subjects who were cognitively still intact, as indicated by MMSE scores above 23. It is suggested that in subjects who already have cognitive impairment or dementia it might be too late for dietary supplementation to counteract the process of cognitive decline. Conversely, cognitive functioning of our population may have been too good for nutrient supplementation to be effective. However, in a study of n-3 PUFAs among patients with mild to

moderate AD, cognitive decline was only reduced in subgroup (*n*=32) with the best-preserved cognitive function (MMSE score >27)[20]. Our study population was comparable with these subjects because 87% had a MMSE score of 27 or higher. However, the subjects in their study had mild to moderate dementia, and 72% were APOE-ε4 carriers. We also found an effect of EPA+DHA supplementation in subjects who carried the APOE-ε4 allele, but only on the cognitive domain of attention. Fish oil may be beneficial in these subjects who are most sensitive to developing dementia. Conversely, in an epidemiologic study, it was observed that consumption of fish was associated with a reduced risk of AD only in subjects without the APOE-ε4 allele[13]. Furthermore, because multiple comparisons were made in our study, it should be noted that our finding could also be a chance finding.

Compliance in our study was high. The dropout rate in this study was only 3%, and seven of the nine subjects who discontinued capsule use returned for their follow-up measurements. Therefore, bias that could result from withdrawal of participants with poor cognitive function[38] is likely to be negligible. Per-protocol analysis excluding the dropouts and noncompliant subjects did not change the results.

We examined the effect of both a high (1,800 mg) and a low dose (400 mg) of EPA+DHA on cognitive functioning. The high "pharmacologic" dose was chosen to ensure maximal contrast between the groups to pick up an effect, if present. The low dose corresponds to the recommended intake in the Netherlands of 450 mg/d EPA+DHA[39]. Such a dose is roughly equivalent to eating two portions of fish per week (one of which is oily fish) and can be more easily translated into dietary advice. Yet it is not yet clear which dose would be sufficiently high and most effective to influence cognitive performance. Moreover, because the causal mechanisms are not yet elucidated, it is not even clear whether an effect of n-3 PUFAs on the brain would be caused by either EPA or DHA or the combination of the two.

A limitation of this study is that the duration of intervention may have been too short. Mechanisms on how EPA+DHA might influence cognitive performance are not completely understood, and both short-term as well as long-term mechanisms have been proposed that could explain such an association[4]. With the present study duration of 26 weeks, it would only be possible to detect short-term effects. We compared our study with other randomized trials of EPA+DHA supplementation in elderly patients. Two studies, performed in patients with established dementia, found a beneficial effect. One study on several cognitive outcomes after using an n-3/n-6 fatty acids compound for 4 weeks[19] and another study observed that 0.72 g DHA per day during 6 months significantly improved scores of the MMSE and Hasegawa Dementia rating scale[18]. A more recent and larger study found no effect of daily supplementation of 1.7 g DHA and 0.6 g EPA after 6 months, except in a subgroup of 32 subjects with very mild cognitive dysfunction (MMSE score >27)[20].

In this randomized, controlled trial, we observed no effect of EPA+DHA

supplementation for 26 weeks on cognitive performance in healthy older individuals. However, our data suggested that in subjects carrying the APOE-ε4 allele and in male subjects, EPA+DHA may improve attention. Based on these findings, longer-term EPA+DHA supplementation studies to investigate effects on cognitive performance are warranted, especially in groups at higher risk for cognitive decline.

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3

Intakes of n-3 fatty acid and fatty fish are not associated with cognitive performance and 6-year cognitive change in men participating in the Veterans Affairs Normative Aging Study

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Abstract

High intake of fish and n-3 polyunsaturated fatty acids (PUFAs) may protect against age-related cognitive decline. However, results are inconsistent and limited data exist regarding changes in multiple cognitive functions over a longer period of time. In this study, we assessed the association between fatty fish intake as well as n-3 PUFA intake with cognitive performance and cognitive change over 6 years in 1,025 elderly men. Participants were from the Veterans Affairs Normative Aging Study (NAS). Cognitive function was assessed with a battery of cognitive tests focusing on factors representing memory/language, speed and visuospatial/attention. Dietary intakes were assessed with a validated food frequency questionnaire. General linear models were used to assess cross-sectional associations and mixed models were used to assess the associations over time. Models were adjusted for age, education, BMI, smoking, diabetes, and intake of alcohol, saturated fat, vitamin C and vitamin E. The mean age of participating men was 68 years at baseline. Median fish consumption ranged from 0.2 to 4.2 servings per week across quartiles. Cross-sectional analyses showed no association between fatty fish or n-3 PUFA intake and cognitive performance. Longitudinal analyses, over 6 years of follow-up, also did not show any significant associations between fatty fish or n-3 PUFA intake and cognitive change. In this population of elderly men, intake of neither fatty fish nor n-3 PUFAs was associated with cognitive performance.

Introduction

Age-related cognitive impairment is considered to be a strong risk factor for the development of dementia[1]. There has been increasing scientific interest in the hypothesis that fish consumption, particularly fatty fish, and the intake of the marine n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) might play a protective role against age-related cognitive decline and dementia. Cognitive performance has been examined in relation to dietary intake of n-3 PUFAs and fish[2-5] or n-3 PUFA concentrations in the blood in several cross-sectional studies[6,7]. Results have been inconsistent, showing either that high intake or high concentrations of n-3 PUFAs in the blood were associated with better cognitive performance[2,3,5,6] or no association[4,7].

Longitudinal studies examining the association between fish or n-3 PUFA intake and cognitive performance have also yielded contradictory results. In some studies, high fish and/or n-3 PUFA intake was protective against 5 or 6-y cognitive decline[4,8], whereas no association was found in 3-y cognitive decline in the Zutphen Elderly Study[3]. Studies using n-3 PUFA concentrations in the blood have generally shown higher concentrations are associated with lower risk of cognitive decline[9,10]. Cognitive function in older adults declines differentially in specific cognitive domains[11]. If n-3 PUFAs are associated with cognitive functioning, they may affect specific cognitive domains differently, as different mechanisms could underlie each domain. However, data on these effects are limited because most studies have used only a global measure of cognitive performance. We know of only three studies that have addressed the association between n-3 PUFAs and cognitive performance in different domains. Two showed a reduced risk of impaired speed with higher intake of fatty fish or n-3 PUFAs[2], or with higher plasma n-3 PUFA proportions over 3 years[12] and one showed that higher proportions of n-3 PUFAs reduced the risk of decline in verbal fluency[10]. This prospective cohort study of elderly men was based on longitudinal data with three measurements over a 6-year follow-up period. We examined the association between fatty fish intake as well as n-3 PUFA intake with changes in multiple factors of cognitive functioning.

Subjects and methods

Subjects

The Normative Aging Study (NAS), a longitudinal study of aging established by the Veterans Administration (now Department of Veterans Affairs), started in 1963 by recruiting men in the Boston area who were originally free of heart disease or other major health problems. The study cohort initially consisted of 2,280 community-dwelling men who were between 21 and 81 years of age (mean 42 y) on entry during 1963-1970. Participating men return every 3-5 years for a health examination, at which time they complete several questionnaires. Dietary intake data have been collected since 1987. Since 1993, a battery of cognitive tests was added to these visits and cognitive performance was

assessed in three cycles, approximately 3 years apart. Relations of B vitamin and homocysteine concentrations with cognitive performance[13] and cognitive decline[14] in a subgroup of this NAS population have been previously reported. The Institutional Review Boards of both the Boston Veterans Affairs Medical Center and Tufts New England Medical Center approved the study and all subjects gave written informed consent.

Cognitive measures

The battery of cognitive tests was designed to be appropriate for an aging population, including tests specifically chosen to assess cognitive status and changes in adults with various pathologic conditions such as Alzheimer's Disease [Consortium to Establish a Registry for Alzheimer's Disease [CERAD[15]]. Some tests from the Neurobehavioral Evaluation System were also included [NES2[16]] Selected tests focus on language, speed, attention, memory, and spatial copying. The Vocabulary, Boston Naming, Continuous Performance and Pattern Memory tests were administered only once, the others tests repeatedly. The Mini-Mental State Examination (MMSE) was used as a global measure of cognitive function[17].

Memory tests

Word List Memory Test (adapted from CERAD): Ten words are presented on a computer screen consecutively, for 2 s each, and participants are then asked to recall these words. Three consecutive trials are administered, and the score is the sum of words remembered (maximum score 30). After an intervening spatial copying task, subjects are asked again to recall the memorized words (delayed recall, maximum score 10).

Backward Digit Span test (WAIS-R): Participants are read a list of digits and asked to recall these in backward sequence. The score is the total span of digits recalled correctly in backward order, with a maximum of eight.

Pattern Memory (NES2): One pattern is presented on the computer screen, which is followed after a brief interval by three similar patterns, from which subjects are asked to identify the original pattern. The scores are the number of correct responses (maximum 25) and mean response latency for correct decisions.

Language tests

Verbal fluency (CERAD): Subjects are asked to name as many animals as possible within 1 min.

Boston Naming Test-short form (CERAD): Subjects are asked to identify 15 line-drawn objects by name (maximum score 15).

Vocabulary [Wechsler Adult Intelligence Scale-Revised (WAIS-R[18])]: Subjects are asked to define words of increasing difficulty, which are scored according to quality of definition (maximum score 70).

Tests of perceptual speed and attention

Pattern Comparison (NES2): Subjects are asked to choose the odd pattern from three similar patterns displayed on a computer monitor. The scores are the number of correct responses (maximum 25) and the mean response latency for correct decisions.

Continuous Performance Test (NES2): Subjects are asked to press a button when they see a large letter "S", but no other letter, on a computer monitor. The score is the mean response latency for items in the best two of six trials (10 target items in each trial). Best trials are defined as trials on which no or minimal errors are made and for which the mean response latencies are the fastest[19].

Spatial copying task-Constructional Praxis (CERAD)

Subjects are asked to copy a circle, crossed rectangles, a vertical diamond, and a cube. These figures are augmented by the tilted triangles, 8-dot circle, horizontal diamond, and tapered box (from the Developmental Test of Visual-Motor Integration; VMI[20]) and the overlapping pentagons from the MMSE[17]. The accuracy of the copied figures is scored by trained staff using criteria from the CERAD and VMI. The resulting score is the total number of figures drawn correctly (maximum score 9). A second score is weighted by the degree of difficulty of the figure, resulting in a maximum score of 26.

Dietary assessment

The subject's average frequency of consumption during the previous year is estimated with the 126-item semi-quantitative Willett food-frequency questionnaire (FFQ)[21,22]. This questionnaire requests participants to record the number of times they consume each of the food items using seven response categories ranging from rarely/never to 2 or more per day. The form was mailed to the participants before their examination visit and checked for completeness at the examination. Forms were processed through a nutrient database at the Channing Laboratory at Harvard University to obtain estimates of usual daily nutrient intake. Four fish items were included: dark-meat fish (e.g., bluefish, mackerel, salmon, sardines, or swordfish; 1.37 g of n-3 fatty acids per portion); canned tuna (0.69 g); other fish (0.17 g); and shrimp, lobster, or scallops (0.46 g). We calculated average daily fish intake by summing these four fish items and we calculated fatty fish intake by summing the frequencies of tuna and dark-meat fish.

Other measurements

Information on education level, smoking history and medical history was obtained by questionnaire. Height and weight were measured to calculate body mass index (BMI, kg/m^2). Fasting plasma samples were drawn at the VA field site and stored at –80°C. These samples were then taken to the Nutrition Evaluation Laboratory at Tufts University Human Nutrition Research Center on Aging for analysis. Total homocysteine (Hcy) in plasma was measured using an

adaptation of a previously described method[23]. Plasma folate and vitamin B12 concentrations were measured by radio assay with the use of a commercially available kit from Bio-Rad (Hercules, CA).

Statistical analysis

Differences in baseline characteristics among different categories of fatty fish consumers and n-3 PUFA intake were compared by analysis of variance (ANOVA) or Kruskal-Wallis for continuous variables and chi-square for categorical variables. For the cognitive measures Tukey's post hoc test was used to examine differences between quartiles. For privacy reasons, subjects aged >89 years (n=1 for cross-sectional analysis and n=4 for the longitudinal analysis) were recoded to 89 years. For the cross-sectional analysis, principal components analysis (SAS PROC FACTOR procedure) with varimax rotation was used to create composite scores for separate dimensions of cognitive function while retaining as much of the individual test's variance as possible. This analysis included 451 men in total (who had baseline scores on all included tests) and resulted in three factors: 1) a memory/ language factor; 2) a visuospatial/attention factor; and 3) a speed factor.

We used general linear models (GLM) to investigate the association between fatty fish or n-3 PUFA intake (independent) and cognitive performance (dependent) at baseline. To test for linear trend, the quartile median value for dietary n-3 PUFA or fatty fish was assigned to each subject in that quartile. To investigate the association of fatty fish and n-3 PUFA intake on 6-year cognitive change, a repeated mixed coefficients model (SAS PROC MIXED procedure) was used. This procedure takes into account the intra-individual correlation of repeated measurements and does not exclude subjects with incomplete data at follow-up. Logarithmic transformation was applied to all dietary measures before including them in the regression models to improve linearity. We calculated energy adjusted intake for all dietary measures with the residual method[24] and created quartiles for fatty fish intake as well as n-3 PUFA intake. Fatty fish consumption and intake of n-3 PUFA were entered as class variables into the model, and the outcome variables, i.e. baseline cognitive functioning and 6-y cognitive change, were treated as continuous variables. Users of cod liver oil (n=29) and fish oil capsules (n=11) were excluded from the analysis.

Models were adjusted for age, educational level (<12, 12, 13-15, ≥16 y), BMI (kg/m^2), smoking (current, past, or never), diabetes (yes/no), alcohol intake (g/d), saturated fat intake (g/d), vitamin C intake (mg/d) and vitamin E intake (mg/d). These confounders were included based on previously published associations, as well as associations with the exposure and outcome (change in the β coefficient ≥10%) in the current data set. We checked for interaction between fish intake, as well as n-3 PUFA intake, with age, education, BMI and diabetes as categorical variables and no significant interactions were observed (P>0.05).

We also analyzed models additionally adjusted for physical activity, plasma folate, Hcy and vitamin B12. However, because including these variables did

not affect results they were removed from the final models. All statistical analyses were carried out using SAS (version 9.1; SAS Institute Inc, Cary, NC). Two-sided *P*-values ≤0.05 were considered statistically significant.

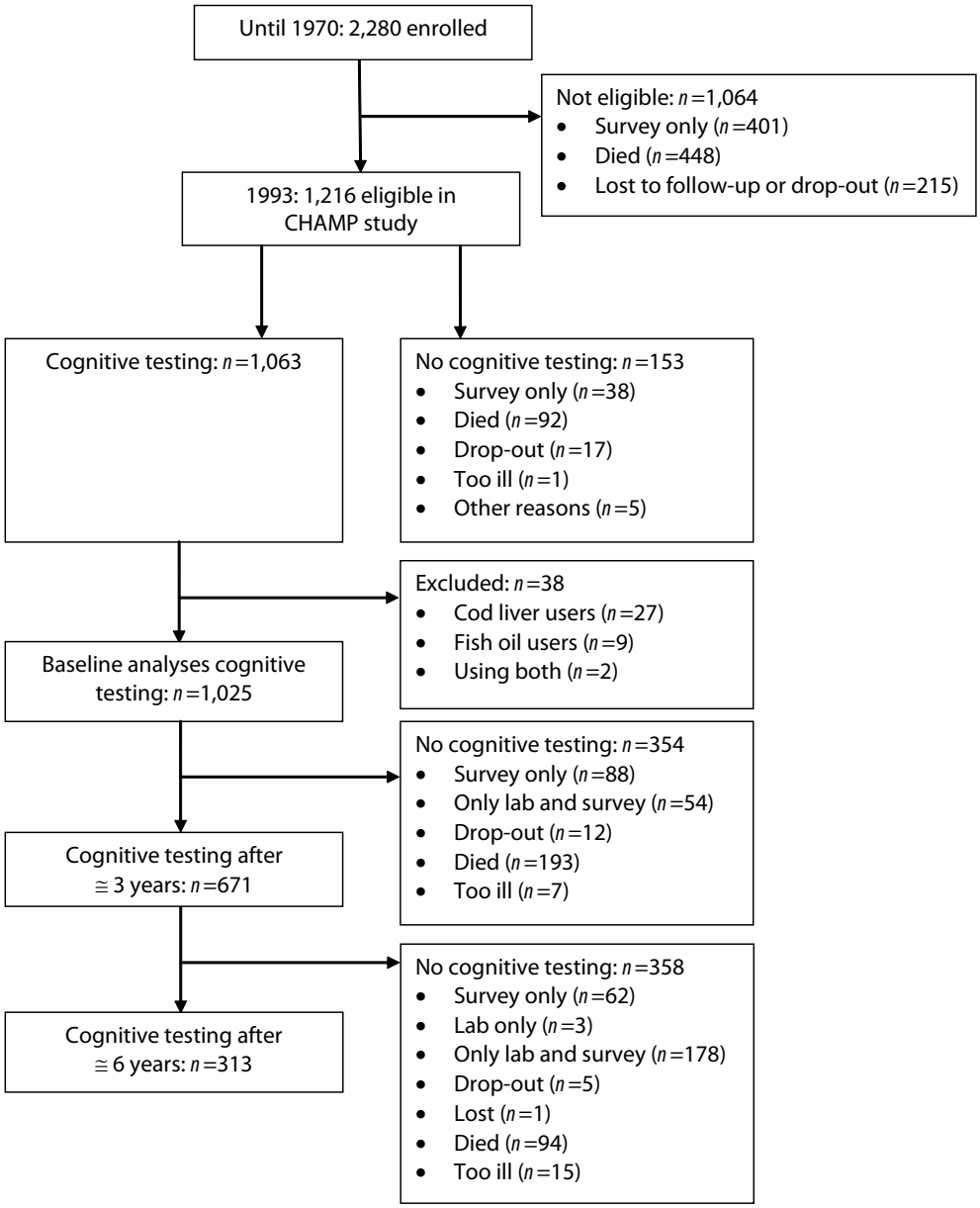

Figure 3.1 Participant flow in the study from initiation to cognitive measures.

Table 3.1 Baseline characteristics of 1,025 men in the Normative Aging Study, by n-3 PUFA intake[a]

	Energy-adjusted quartiles of n-3 PUFA intake (g/d), median (range)				
	1 0.10 (0.06-0.13) *n*=256	2 0.19 (0.17-0.21) *n*=256	3 0.29 (0.26-0.31) *n*=257	4 0.45 (0.40-0.55) *n*=256	*P*[b]
Age (y)	68.9 ± 7.3[c]	67.5 ± 7.1	67.8 ± 7.3	68.5 ± 7.3	0.12
Education (y) *n* (%)					0.05
<12	25 (10)	22 (9)	19 (7)	16 (6)	
12	87 (34)	80 (31)	72 (28)	51 (20)	
13-15	62 (24)	61 (24)	59 (23)	81 (32)	
≥16	82 (32)	93 (36)	107 (42)	108 (42)	
BMI (kg/m^2)	27.8 ± 4.1	27.9 ± 3.7	27.9 ± 3.6	27.7 ± 3.9	0.92
Smoking status *n* (%)					0.01
Current	26 (10)	17 (7)	16 (6)	5 (2)	
Former	153 (60)	148 (58)	160 (62)	159 (62)	
Never	76 (30)	91 (36)	81 (32)	92 (36)	
Alcohol (g/d)	4.9 (0.5-15.8)[d]	7.8 (2.5-18.2)	8.0 (3.0-17.8)	7.5 (2.7-15.1)	0.33
Physical activity score[e]	3 (2-6)	3 (2-5)	2 (1-4)	2 (1-3)	0.0007
Diabetes *n* (%)	38 (15)	40 (16)	37 (15)	51 (20)	0.32
MMSE score	28 (27-29)	28 (27-29)	28 (27-29)	28 (27-29)	0.66
Plasma folate (nmol/L)	21 (14-30)	21 (13-32)	20 (12-29)	21 (14-32)	0.66
Plasma vitamin B12 (pmol/L)	309 (241-394)	289 (225-378)	330 (243-409)	347 (286-451)	0.0018
Plasma homocysteine (µmol/ml)	10 (9-12)	10 (9-12)	10 (8-12)	10 (9-12)	0.33
Total energy intake (kcal/d)	2,070 (1,644-2,548)	1,776 (1,333-2,135)	1,881 (1,496-2,236)	1,941 (1,544-2,315)	<0.0001
Saturated fat intake (g/d)[f]	23.7 (19.6-27.8)	22.2 (19.2-26.0)	21.2 (17.6-24.3)	19.7 (16.1-22.6)	<0.0001
Vitamin C intake (mg/d)[f]	168 (121-261)	183 (126-277)	190 (136-311)	219 (155-305)	0.0042
Vitamin E intake (mg/d)[f]	12.4 (1.2-37.5)	17.6 (8.5-39.1)	18.6 (5.8-48.3)	19.7 (7.6-153)	0.13
Total fish intake (servings/wk)[fg]	0.8 (0.4-1.1)	1.8 (1.5-2.1)	2.3 (1.9-2.8)	4.1 (3.2-5.1)	<0.0001
Fatty fish intake (servings/wk)[fg]	0.4 (0.1-0.7)	0.8 (0.5-1.1)	1.3 (1.0-1.5)	1.9 (1.5-3.6)	<0.0001

[a] Total *n*=1,025; but *n*=1,016 for education, 1,007 for BMI, 1,009 for alcohol, 735 for physical activity, 966 for MMSE, 663 for plasma folate, 668 for plasma vitamin B12 and 688 for plasma homocysteine; [b] Based on ANOVA, Kruskal-Wallis test, or chi-square test; [c] Mean ± SD; [d] Median (Q1-Q3); [e] Physical activity score: 1=daily; 2=3-4 x/wk; 3=1-2 x/wk; 4=2-3 x/mo; 5=once/mo; 6=<once/mo; 7=never; [f] Adjusted for total energy intake using the residual method[32]; [g] One serving of fish is 100-150 g

Results

Baseline characteristics

Of the 2,280 men originally enrolled, 1,216 were still participating in the on-site examination portion of the study in 1993 when the cognitive measures were initiated. Of these, 87% (1,063) completed baseline cognitive testing. After exclusion of fish and cod liver oil users 1,025 remained for our baseline analyses. See **Figure 3.1** for the flow of participants. Of these, 671 completed a second battery at 3 years and 313 at 6 years. Reasons for loss to follow-up were mainly due to death or movement from the area. Men who moved continued participation by mail survey, but did not continue the in-person exam. Due to the high participant burden, not all men completed all tests. A shorter battery was offered to those refusing the full battery. Individual test completion ranged from 487 to 982 and when combined to create factors, this resulted in a sample size of 451 with complete measures for all included tests.

The mean age of completing participants at baseline cognitive measurement was 68 y. Total mean fish consumption was 2.4 servings/week of total fish and 1.3 servings/week of fatty fish. Mean total n-3 PUFA intake was 0.28 g/day. Median fatty fish consumption ranged from 0.2 to 2.8 portions/week across quartiles. Men in the higher quartiles of n-3 PUFA consumption had more years of education and were less likely to be smokers than those in the lower quartiles. Men with low n-3 PUFA intake had higher intakes of total energy and saturated fat, and lower intakes of alcohol, and vitamins C and E (**Table 3.1**). Unadjusted baseline cognitive scores for Word List Memory tests (P=0.01 and 0.03), the Boston Naming Test (P=0.01) and Continuous Performance Test differed (P=0.01) among quartiles of fatty fish consumers (**Table 3.2**). Unadjusted baseline scores across the quartiles of n-3 PUFA intake did not differ significantly for any of the cognitive tests (data not shown).

Cognitive performance (cross-sectional analysis)

After adjusting for age and education, as well as other potential confounding variables, there were no significant protective associations between quartiles of fatty fish or n-3 PUFA intake and cognitive dimensions, as measured by the factor scores. Rather, and in contrast to our hypothesis, performance tended to be impaired on the cognitive factor memory/language with greater intake of fatty fish (P=0.09) or n-3 PUFAs (P=0.17) (**Table 3.3**). However, examination of the main contributing cognitive test for each of the cognitive factors as the dependent variable did not show this (**Table 3.4**).

Change in cognitive performance over 6 y (prospective analysis)

Of the 1,025 men included in the cross-sectional analysis, 671 returned for follow-up measurements after 3 years and 313 returned after 6 years. The 313 men with complete follow-up were, on average, 2 years younger than those who did not return for the follow-up measurements, but did not differ significantly on any other variable examined here. Cognitive tests did not differ across quartiles

Table 3.2 Baseline cognitive measures of aging men across energy-adjusted quartiles of fatty fish intake[a,b]

	Energy-adjusted quartiles of fatty fish intake (servings/wk)[c], median (range)				
	1 0.21 (–0.02-0.38)	2 0.80 (0.66-0.90)	3 1.25 (1.11-1.38)	4 2.79 (1.76-3.61)	*P*
Memory					
Word list memory, total 3 trials (*n*=871)	18.5 ± 4.0[1] (16-21)	19.6 ± 3.5[2] (17-22)	19.1 ± 3.6 (17-21)	18.5 ± 3.9[1] (16-21)	0.01
Word list delayed recall (*n*=869)	6.1 ± 2.0[1] (5-7)	6.6 ± 1.8[2] (5-8)	6.5 ± 1.8 (5-8)	6.3 ± 2.0 (5-8)	0.03
Backward digit span, total span (*n*=766)	5.1 ± 2.2 (3-7)	5.2 ± 2.3 (4-7)	5.0 ± 2.4 (3-7)	4.8 ± 2.3 (3-6)	0.31
Pattern memory, total correct (*n*=488)	18.6 ± 3.0 (17-21)	19.2 ± 3.2 (18-21)	19.5 ± 3.3 (18-22)	19.4 ± 2.6 (18-21)	0.12
Language					
Verbal fluency, total correct (*n*=873)	18.2 ± 5.2 (15-22)	19.0 ± 4.7 (15-22)	19.1 ± 4.9 (16-22)	18.7 ± 5.0 (15-22)	0.26
Boston naming test, total correct (*n*=488)	19.1 ± 1.1 (19-20)	19.3 ± 0.9[2] (19-20)	19.3 ± 0.9[2] (19-20)	18.9 ± 1.5[1] (18-20)	0.01
Vocabulary, total correct (*n*=489)	50.5 ± 8.8 (45-57)	51.5 ± 9.5 (46-59)	51.6 ± 10.5 (47-59)	49.5 ± 10.1 (43-57)	0.28
Speed					
Pattern memory, mean response time correct trials, s (*n*=488)	6.1 ± 1.7 (5.0-6.8)	5.8 ± 1.4 (4.7-6.7)	5.6 ± 1.4 (4.8-6.5)	5.7 ± 1.4 (4.5-6.6)	0.06
Pattern comparison test, mean response time correct trials, s (*n*=880)	5.8 ±1.7 (4.6-6.7)	5.7 ± 1.6 (4.6-6.4)	5.5 ± 1.5 (4.5-6.3)	5.7 ± 1.5 (4.7-6.5)	0.33
Continuous performance test, mean response time two best trials (*n*=487)	354 ± 58[2] (315-373)	333 ± 43[1] (309-354)	338 ± 52 (304-361)	349 ± 59 (306-380)	0.01
Visuospatial					
Pattern comparison test, total correct (*n*=880)	23.8 ± 1.6 (23-25)	24.0 ± 1.7 (24-25)	24.0 ± 1.3 (24-25)	23.9 ± 1.7 (24-25)	0.42
Sum of drawings 1-9 (*n*=982)	5.5 ± 1.7 (4-7)	5.6 ± 1.8 (4-7)	5.7 ± 1.8 (5-7)	5.7 ± 1.7 (5-7)	0.63
Sum of weighted drawings 1-9 (*n*=982)	14.6 ± 5.6 (10-19)	14.8 ± 5.6 (11-19)	15.3 ± 5.9 (12-19)	15.1 ± 5.5 (12-19)	0.53

[a] All values are mean ± SD. Within a row, quartiles with differing superscripts differ at $P<0.05$, tested with ANOVA and Tukey's post hoc test; [b] Analyses were repeated by quartile of n-3 PUFA intake, with no significant results (all $P>0.2$); [c] One serving of fish is 100-150 g

Table 3.3 Baseline cognitive factors for 451 elderly men in the Normative Aging Study, by n-3 PUFA and fatty fish intake[a]

	Energy-adjusted quartiles, median (range)				
	1	2	3	4	*P*-trend
n-3 PUFA intake (g/d)	0.10 (0.06; 0.13)	0.19 (0.17; 0.21)	0.29 (0.26; 0.31)	0.45 (0.40; 0.55)	
Memory/ language[b]					
Model 1[c]	0.05 (–0.13; 0.23)	0.02 (–0.16; 0.19)	0.01 (–0.16; 0.18)	–0.07 (–0.25; 0.10)	0.35
Model 2[d]	0.08 (–0.10; 0.27)	0.02 (–0.16; 0.19)	0.02 (–0.16; 0.19)	–0.12 (–0.30; 0.06)	0.17
Visuospatial/ attention[e]					
Model 1[c]	–0.04 (–0.22; 0.14)	0.10 (–0.08; 0.27)	–0.05 (–0.22; 0.12)	–0.01 (–0.18; 0.17)	0.98
Model 2[d]	–0.03 (–0.21; 0.16)	0.11 (–0.06; 0.28)	–0.06 (–0.23; 0.11)	–0.02 (–0.20; 0.16)	0.76
Speed[f]					
Model 1[c]	0.00 (–0.18; 0.18)	–0.03 (–0.21; 0.14)	0.09 (–0.08; 0.26)	–0.07 (–0.24; 0.11)	0.83
Model 2[d]	–0.01 (–0.19; 0.17)	–0.04 (–0.21; 0.14)	0.08 (–0.09; 0.25)	–0.04 (–0.22; 0.14)	0.90
Fatty fish intake (servings/wk)[e]	0.21 (–0.02; 0.38)	0.80 (0.66; 0.90)	1.25 (1.11; 1.38)	2.79 (1.76; 3.61)	
Memory/ language[b]					
Model 1[c]	0.02 (–0.16; 0.20)	0.18 (0.01; 0.35)	0.05 (–0.13; 0.22)	–0.23 (–0.40; –0.07)	0.17
Model 2[d]	0.05 (–0.13; 0.24)	0.21 (0.04; 0.38)	0.02 (–0.15; 0.20)	–0.27 (–0.44; –0.10)	0.09
Visuospatial/ attention[f]					
Model 1[c]	0.02 (–0.16; 0.21)	–0.02 (–0.19; 0.16)	0.02 (–0.16; 0.20)	–0.02 (–0.20; 0.15)	0.75
Model 2[d]	0.07 (–0.12; 0.26)	0.00 (–0.17; 0.18)	0.01 (–0.17; 0.18)	–0.07 (–0.24; 0.10)	0.33
Speed[g]					
Model 1[c]	0.14 (–0.04; 0.32)	–0.01 (–0.18; 0.16)	–0.10 (–0.27; 0.08)	–0.03 (–0.19; 0.14)	0.10
Model 2[d]	0.13 (–0.05; 0.32)	–0.02 (–0.19; 0.16)	–0.09 (–0.27; 0.08)	–0.01 (–0.18; 0.16)	0.16

[a] Adjusted means (95% CI)
[b] High factor loadings for Boston Naming test, Verbal fluency, Vocabulary, Word list total 3 trials and Word list delayed recall
[c] Adjusted for age and education
[d] Also adjusted for BMI, smoking, diabetes, and intake of alcohol, saturated fat, vitamin C and vitamin E
[e] One serving of fish is 100-150 g
[f] High factor loadings for Backward Digit Span, Pattern memory (# correct), Pattern comparison (# correct) and Spatial copying
[g] High factor loadings for mean response time on Continuous performance, Pattern memory and Pattern comparison test

Table 3.4 Representative baseline tests/cognitive factor for men in the Normative Aging Study, by n-3 PUFA and fatty fish intake[a]

	Energy-adjusted quartiles, median (range)				
	1	2	3	4	*P*-trend
n-3 PUFA intake (g/d)	0.10 (0.06; 0.13)	0.19 (0.17; 0.21)	0.29 (0.26; 0.31)	0.45 (0.40; 0.55)	
Word list total 3 trials					
Model1[b] (*n*=871)	18.9 (18.4; 19.3)	19.3 (18.8; 19.7)	18.9 (18.5; 19.4)	18.7 (18.3; 19.2)	0.61
Model 2[c] (*n*=848)	19.0 (18.5; 19.5)	19.3 (18.8; 19.7)	18.9 (18.4; 19.4)	18.8 (18.3; 19.2)	0.45
Spatial copying, sum of drawings					
Model1[b] (*n*=982)	5.7 (5.4; 5.9)	5.6 (5.4; 5.9)	5.6 (5.4; 5.8)	5.6 (5.4; 5.8)	0.58
Model 2[c] (*n*=960)	5.7 (5.5; 5.9)	5.6 (5.4; 5.9)	5.5 (5.3; 5.8)	5.5 (5.3; 5.8)	0.21
Pattern comparison test, mean response time					
Model1[b] (*n*=880)	5.7 (5.5; 5.9)	5.6 (5.4; 5.8)	5.8 (5.6; 6.0)	5.5 (5.4; 5.7)	0.75
Model 2[c] (*n*=853)	5.6 (5.4; 5.8)	5.6 (5.4; 5.8)	5.8 (5.6; 6.0)	5.5 (5.4; 5.7)	0.97
Fatty fish intake (servings/wk)[d]	0.21 (–0.02; 0.38)	0.80 (0.66; 0.90)	1.25 (1.11; 1.38)	2.79 (1.76; 3.61)	
Word list total 3 trials					
Model1[b] (*n*=871)	18.8 (18.3; 19.3)	19.6 (19.1; 20.1)	18.8 (18.4; 19.3)	18.5 (18.1; 19.0)	0.73
Model 2[c] (*n*=848)	18.9 (18.4; 19.4)	19.7 (19.2; 20.2)	18.8 (18.3; 19.3)	18.6 (18.1; 19.0)	0.66
Spatial copying, sum of drawings					
Model1[b] (*n*=982)	5.6 (5.4; 5.8)	5.6 (5.4; 5.8)	5.7 (5.5; 5.9)	5.6 (5.4; 5.8)	0.99
Model 2[c] (*n*=960)	5.6 (5.4; 5.9)	5.6 (5.4; 5.9)	5.6 (5.4; 5.8)	5.5 (5.3; 5.8)	0.60
Pattern comparison test, mean response time					
Model1[b] (*n*=880)	5.7 (5.5; 5.9)	5.7 (5.5; 5.9)	5.7 (5.5; 5.8)	5.6 (5.4; 5.8)	0.53
Model 2[c] (*n*=853)	5.7 (5.5; 5.9)	5.6 (5.4; 5.8)	5.7 (5.5; 5.9)	5.6 (5.4; 5.8)	0.67

[a] Adjusted means (95% CI)
[b] Adjusted for age and education
[c] Also adjusted for BMI, smoking, diabetes, and intake of alcohol, saturated fat, vitamin C and vitamin E
[d] One serving of fish is 100-150 g

Table 3.5 Six-year cognitive change for 1,025 men in the Normative Aging Study, by n-3 PUFA and fatty fish intake[a]

	Energy-adjusted quartiles, median (range)				
	1	2	3	4	*P*
n-3 PUFA intake (g/d)	0.10 (0.06; 0.13)	0.19 (0.17; 0.21)	0.29 (0.26; 0.31)	0.45 (0.40; 0.55)	
Word list total 3 trials					
Model 1[b]	19.0 (18.6; 19.3)	18.9 (18.5; 19.2)	19.3 (19.0; 19.7)	18.9 (18.5; 19.2)	0.09
Model 2[c]	19.0 (18.6; 19.4)	19.0 (18.6; 19.3)	19.3 (19.0; 19.7)	18.9 (18.5; 19.3)	0.17
Spatial copying, Sum of drawings					
Model 1[b]	5.8 (5.6; 6.0)	5.9 (5.7; 6.1)	5.9 (5.8; 6.1)	5.9 (5.7; 6.1)	0.70
Model 2[c]	5.8 (5.6; 6.0)	5.9 (5.7; 6.1)	5.9 (5.8; 6.1)	5.8 (5.7; 6.0)	0.70
Pattern comparison test, mean response time					
Model 1[b]	5.8 (5.6; 5.9)	5.6 (5.5; 5.8)	5.6 (5.5; 5.8)	5.6 (5.4; 5.7)	0.21
Model 2[c]	5.7 (5.6; 5.9)	5.6 (5.5; 5.7)	5.6 (5.5; 5.8)	5.6 (5.4; 5.7)	0.32
Fatty fish intake (servings/wk)[d]	0.21 (–0.02; 0.38)	0.80 (0.66; 0.90)	1.25 (1.11; 1.38)	2.79 (1.76; 3.61)	
Word list total 3 trials					
Model 1[b]	19.0 (18.6; 19.3)	19.2 (18.9; 19.6)	19.1 (18.8; 19.5)	18.7 (18.4; 19.1)	0.09
Model 2[c]	19.0 (18.7; 19.4)	19.3 (18.9; 19.6)	19.2 (18.8; 19.5)	18.7 (18.4; 19.1)	0.08
Spatial copying, Sum of drawings					
Model 1[b]	5.8 (5.6; 6.0)	5.8 (5.7; 6.0)	6.0 (5.8; 6.1)	5.9 (5.7; 6.1)	0.54
Model 2[c]	5.8 (5.7; 6.0)	5.8 (5.7; 6.0)	5.9 (5.8; 6.1)	5.8 (5.7; 6.0)	0.70
Pattern comparison test, mean response time					
Model 1[b]	5.7 (5.6; 5.9)	5.8 (5.6; 5.9)	5.5 (5.4; 5.7)	5.6 (5.5; 5.8)	0.08
Model 2[c]	5.7 (5.5; 5.8)	5.7 (5.6; 5.8)	5.5 (5.4; 5.7)	5.6 (5.5; 5.8)	0.18

[a] Adjusted means (95% CI). Differences in cognitive measures among different quartiles of n-3 PUFA intake were compared with the mixed longitudinal repeated coefficient model (SAS PROC MIXED)
[b] Adjusted for age and education
[c] Also adjusted for BMI, smoking, diabetes, and intake of alcohol, saturated fat, vitamin C and vitamin E
[d] One serving of fish is 100-150 g

of fish intake or n-3 PUFA intake (**Table 3.5**). As in the cross-sectional analysis, performance on the cognitive factor memory/language tended to be impaired with greater intake of fatty fish (P=0.09) or n-3 PUFAs (P=0.17). Repeating these analyses with only the subjects who had complete follow-up over the 6 years did not show different results.

Discussion

The present study is one of the few prospective cohort studies to examine prospective associations between fish and n-3 PUFA intake with cognitive change in multiple cognitive factors. Our findings in this sample of aging men do not support the hypothesis that higher fish/ n-3 PUFA intake is associated with better cognitive function or with less cognitive decline on any of the cognitive tests.

Strengths of the current study include the ability to assess cognitive performance not only cross-sectionally, but also prospectively with multiple repeated measurements. Furthermore, we used an extensive battery of cognitive tests, appropriate for an aging population. This is an important advantage over the use of only general cognitive tests such as the MMSE, which was originally designed as a screening tool and not as a measure of change[17]. To limit the number of outcome variables, we defined three cognitive factors which account for most of the variance observed. However, because not all cognitive tests were performed in all subjects, this also decreased the number of subjects for each cognitive factor, compared with the number of subjects that performed some of the individual cognitive tests. Therefore, we repeated our analyses with the cognitive test that contributed most to each cognitive factor. However, despite the fact that this increased the number of subjects, these analyses did not show different results.

The Willett FFQ includes questions on different fish items and has been validated in several studies[21,25,26]. In a validation study with a random sample of 127 men aged 45 to 70 years living in the Boston area, these four items on seafood intake were shown to be reproducible and useful measures of seafood intake. Correlations between two administrations of the questionnaire 1 year apart ranged from 0.48 (fish) to 0.67 (shellfish)[26]. The correlation between fish intake as reported on the questionnaire with fish intake assessed with two 1-week dietary records was 0.61[25]. Although plasma, erythrocyte or phospholipid proportions of n-3 PUFAs are often considered more objective measures of n-3 PUFA intake[27], these values were not available in the present study. However, it has been shown that these biochemical measures may provide only limited information about absolute values of n-3 PUFA intake, because they may vary for participants with similar dietary intake due to other participant characteristics[28]. Welch et al. found that only ≈25% of the variation in plasma n-3 PUFA was explained by fish and fish-oil consumption[29]. Furthermore, because they only reflect intake over the past week or two, they may be less indicative of long term exposure than responses to a food frequency questionnaire for the past year[27].

Because the number of subjects participating in the consecutive cognitive test

series of our study decreased substantially (from 1,025 to 671 after 3 years to 313 after 6 years of follow-up) bias due to incomplete follow-up may have influenced our results. However, except for the fact that subjects with complete follow-up were on average 2 years younger, they were not different from the subjects with incomplete follow-up. An under- or overestimation of the effect is unlikely, because fish intake and cognitive scores were also comparable between completers and non-completers.

The trend for the impaired, though not significantly, memory/ language factor with greater fish or n-3 PUFA intake was unexpected and has, to our knowledge, not been shown before. We have no clear explanation for this result. Also, when we performed the same analysis with only the cognitive test that contributed most to each factor, we did not observe this trend, despite larger sample size. Because we made multiple comparisons it may also have been a chance finding. Our cross-sectional results are in agreement with those of two other studies, which also observed no significant associations[3,4]. They are also in agreement with two studies that evaluated n-3 concentrations in the blood and saw no association with cognitive function[7,12]. However, other studies have found positive associations with n-3 PUFA concentrations and cognitive function[6] and with fish or n-3 PUFA intake and global cognitive function and speed[2].

The null results of our longitudinal analyses are in line with the first publication from the Zutphen study data, which also showed no clear association between fish consumption and 3-y cognitive decline[3]. However, 5-y cognitive decline was later inversely associated with EPA+DHA intake in that study population[4]. The Chicago Health and Aging Project showed that fish consumption, but not intake of n-3 PUFAs, was associated with less cognitive decline[8]. A few studies have shown higher n-3 PUFA[9] or EPA concentrations in blood[7] to be associated with less cognitive decline. Dullemeijer et al. observed an association between n-3 PUFA plasma concentrations and sensorimotor and complex speed, but did not observe associations for memory, information-processing speed, or verbal fluency[12]. Beydoun et al. observed that lower concentrations of plasma n-3 fatty acids were associated with greater decline in verbal fluency, but not in memory or psychomotor speed[10]. The findings of these few studies assessing different cognitive domains are not consistent. More prospective studies, as well as intervention studies investigating the association between n-3 PUFAs and domain-specific measures are needed.

Our study population consumed an average of 2.4 servings of total fish per week, which is relatively high compared to other study populations in the Netherlands and the United States, where average fish intake was once per week or less[3,8,30], but lower than in a Norwegian population, where intake was on average 5 servings/week[5]. Our study population consisted of men only. The Zutphen study also included only men, but they observed a protective effect of a higher fish and n-3 PUFA intake on 5-y cognitive decline[4]. Relative to that study, the mean age of our men was 7 years younger, 68 compared to 75 years, and the mean MMSE was 1.3 points higher. The subjects of the Chicago Health

and Aging project, where fish consumption was also associated with slower cognitive decline, had a mean age of 74 years, also several years older than in our study[8]. It is, therefore, possible that a protective effect of fish intake may be more apparent with advancing age and cognitive decline. On the other hand, there is increasing concern with contaminants, such as mercury and dioxins in certain types of fish, although a review performed in 2006 showed that the benefits of moderate (1-2 servings/week) fish consumption exceed the potential risks, at least for cardiovascular and neurological outcomes[31]. Although we cannot assess this exposure directly, it remains possible that the benefits of the n-3 PUFA intake among the relatively high fish consumers in this study may have been attenuated by negative effects of contaminants. Further investigation in this area is needed.

In conclusion, higher intake of fish or n-3 PUFAs was not associated with better cognitive performance at baseline or with lower 6-year cognitive decline in any of the cognitive tests in this population of aging men. Studies with longer observation of changes in specific cognitive domains are needed to clarify the current conflicting results observed in the literature.

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4

Effect of fish oil supplementation on mental well-being in older subjects: a randomized, double-blind, placebo-controlled trial

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Abstract

Background It is suggested that a low intake of fish and/or n-3 PUFA is associated with depressed mood. However, results from epidemiologic studies are mixed, and randomized trials have mainly been performed in depressed patients, yielding conflicting results.

Objective We investigated the effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on mental well-being in a double-blind, placebo-controlled trial.

Design Independently living individuals (*n*=302) aged ≥65 y were randomly assigned to consume 1,800 mg/d EPA+DHA, 400 mg/d EPA+DHA, or placebo capsules for 26 weeks. Changes in mental well-being were assessed as the primary outcome with the Center for Epidemiologic Studies Depression Scale (CES-D), Montgomery-Åsberg Rating Scale (MADRS), Geriatric Depression Scale (GDS-15), and Hospital Anxiety and Depression Scale (HADS-A).

Results Plasma concentrations of EPA+DHA increased by 238% in the high-dose and 51% in the low-dose fish oil group compared to placebo, reflecting excellent compliance. Baseline CES-D scores ranged from 5.9 to 6.8 in the three groups and were not significantly different between groups. Mean changes in CES-D scores after 26 weeks were –0.2, 0.2 and –0.4 (*P*=0.87) in the high-dose fish oil, low-dose fish oil, and placebo groups, respectively. Treatment with neither 1,800 mg nor with 400 mg EPA+DHA differentially affected any of the measures of mental well-being after 13 or 26 weeks of intervention compared to placebo.

Conclusions In this randomized, double-blind, placebo-controlled trial we observed no effect of EPA+DHA supplementation for 26 weeks on mental well-being in the general older population studied.

Introduction

Depression is a common mental disorder with a complex etiology. Depressive disorders affect 121 million people worldwide[1] and are the third leading cause of burden of disease in high-income countries[2]. Depression can be diagnosed reliably in primary care, but not all treatments are effective. According to a WHO report, 36-50% of serious cases in developed countries and 76-85% of serious cases in less-developed countries do not receive treatment[3]. Therefore, it is important to focus on factors that may help to prevent the development of depressive disorders.

Several studies showed depletions in n-3 polyunsaturated fatty acids (PUFAs) in blood or adipose tissue to be associated with depression[4-10]. PUFA are very-long-chain fatty acids found in plants and marine sources. The marine-based n-3 PUFAs primarily consist of eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Because n-3 PUFAs are a major component of neural membranes and act as precursors of compounds involved in immune and inflammatory responses, it is biologically plausible that they could play a role in mood and behavioral disorders[11].

Most observational studies showed an inverse association of fish intake with prevalence of depression[12-15]. Three epidemiologic studies with 3,204, 5,689, and 29,133 subjects, respectively, have been performed in the general Finnish population[12,13,16], and one has been performed in 332 Dutch elderly men[15]. Tanskanen et al. and Timonen et al. found effects only in women[12,13], which could explain why Hakkarainen et al.[16], who studied a male population, did not find an association. However, this is contradictory to the findings of Kamphuis et al. who found an association in a male population. The age of this population (70-79 y) could explain the difference in results found. Hibbeln made a cross-national comparison of 13 countries including 35,000 subjects and showed a negative association between fish intake and prevalence of major depression[14]. Studies using n-3 PUFA concentrations in the blood also found higher concentrations to be associated with less depression[4-8], as was the case for n-3 PUFAs in adipose tissue[9,10].

Until now, 19 randomized trials investigating the effects of n-3 PUFAs on depressed mood have been performed. Twelve studies, most of them performed in depressed patients, were included in a recent meta-analysis by Appleton et al. who stated that the evidence available provided little support[17]. Fontani et al. performed the only trial in healthy subjects (n=33) with a mean age of 33 y, who experienced an improvement in mood during daily supplementation of 1.6 g EPA and 0.8 g DHA for 35 days[18]. Recently, Rogers et al. performed a trial in mild to moderately depressed individuals from the general population and did not observe an effect after 3 months of supplementation with 1.5 g EPA+DHA/d[19].

The aim of the present study was to investigate whether supplementation of n-3 PUFAs would be beneficial in improving the mental well-being of nondepressed, older individuals. We conducted a double-blind, randomized, placebo-controlled trial in 302 Dutch individuals who were administered a high

dose (1,800 mg/d) or a low dose (400 mg/d) of EPA+DHA or placebo capsules for 26 weeks.

Subjects and methods

Subjects

The subjects were screened between November 2005 and February 2006, and the intervention took place between February 2006 and November 2006. The subjects were aged ≥65 y, and were mainly recruited through an existing database of volunteers with interest in participating in studies of Wageningen University, the Netherlands. The exclusion criteria were as follows: [1] score of >16 on the Center for Epidemiologic Studies Depression Scale (CES-D)[20]; [2] score of <21 points on the Mini-Mental State Examination (MMSE)[21]; [3] current or recent (<4 weeks) use of fish oil supplements; [4] current use of antidepressant medication; [5] current use of medication for dementia; [6] serious liver disease; [7] consumption of >4 glasses of alcohol/d; [8] inability to participate as judged by the responsible medical doctor; [9] allergy to fish; [10] swallowing problems, [11] current or recent (i.e. < 2mo) participation in another clinical trial, or [12] intake of fish >4 times/week or >800 mg EPA+DHA/d from fish, as estimated from a fish consumption questionnaire. Additionally, compliance with capsule use during the 2-week placebo run-in period had to be ≥80% on the basis of self-report. The study was approved by the Medical Ethical Committee of Wageningen University and all subjects gave written informed consent.

Study design

An independent person randomized subjects by means of computer-generated random numbers in stratified permuted blocks of size 6. Stratification factors included age (< and ≥69 y), sex, MMSE (< and ≥28), and CES-D screening test score (<5 and ≥5). Individuals were randomly allocated to receive a daily dose of fish oil containing either a low dose of ~400 mg EPA+DHA, a high dose of ~1,800 mg EPA+DHA, or a placebo oil (sunflower oil high in oleic acid) for 26 weeks (Lipid Nutrition/Loders Croklaan, Wormerveer, the Netherlands). The oils were administered in six soft gelatin capsules daily, each containing 900 mg oil and 2.7 mg tocopherol as antioxidant (Banner Pharmacaps Europe BV, Tilburg, the Netherlands). Fish oil capsules provided a mean (± SD) daily intake of 1,093 ± 17 mg EPA and 847 ± 23 mg DHA in the high-dose group and 226 ± 3 mg EPA and 176 ± 4 mg DHA in the low-dose group, as estimated from 20 random samples taken at regular times during the study. The placebo capsules contained mainly oleic acid (C18:1n-9). The capsules were individually packaged in foil pill strips containing the daily dose of six pills per strip to facilitate compliance and recording of capsule use (Medipack, Gorredijk, the Netherlands). The capsules with fish oil or placebo oil were indistinguishable in appearance. Staff members and participants were blinded toward treatment allocation until completion of the trial and after completion of data analysis. Participants visited the research centre at baseline and after 13 and 26 weeks of intervention. At baseline and at

13 weeks, participants received a 14-week supply of capsules (one week extra as reserve). Compliance was judged by calculating the unused capsules in the returned foil strips and by reviewing a diary in which participants registered the number of capsules that were not consumed.

Sample size was calculated on the basis of the CES-D; a difference of three points was considered clinically relevant. With a mean (± SD) of 9 ± 7 for Dutch elderly[22], a minimum sample size of 85 subjects per group would be required to detect a difference (power=80%, 2-sided α=0.05). Taking into account a dropout rate of 15%, a sample size of 100 subjects per treatment group was considered adequate.

Assessment of mental well-being

Trained research assistants performed mental well-being tests at baseline and after 13 and 26 weeks of intervention under supervision of a neuropsychologist. The participants were tested by the same research assistant, whenever possible, using a standard protocol. To assess mental well-being, four questionnaires were used: [1] CES-D: a 20-item scale developed to measure depressive symptoms experienced in the past week[20]. The scale generates a total score ranging from 0 to 60; higher scores reflect more depressive symptoms. Adequate reliability and validity with elderly people was previously established[23-25], [2] The Montgomery-Åsberg Depression Rating Scale (MADRS): a 10-item, observer-rated scale that is explicitly designed to measure changes in depressive symptoms[26]. This scale generates a total score, which can range from 0 to 60; higher scores reflect more depressive symptoms. Adequate validity was established previously[27], [3] Short version of Geriatric Depression Rating Scale (GDS-15): a 15-item test with yes and no answers designed specifically for rating depression in the elderly. The total score ranges from 0 to 15, and the list has been tested and used extensively in the older population[28]. This scale is easy to administer and has been well validated in both home and clinical settings. The GDS has been used only at baseline and after 26 weeks of intervention, and [4] Subscale of the Hospital Anxiety and Depression Scale (HADS-A): used to identify anxiety symptoms; possible scores range from 0 to 21[29].

Additionally, in a subgroup of 104 subjects, the Dutch version of the Profile of Mood states short form (s-POMS)[30] was administered and the CES-D was repeated. These additional measurements were performed at weeks 17 and 21 of the study by telephone interview. The s-POMS is a 32-item questionnaire with good psychometric properties that assesses five components of mood: depression, fatigue, anger, tension and loss of vigor. The total scores range from 0 to 128.

Other measurements

Fasting venous blood samples were collected at baseline and after 13 and 26 weeks of intervention. A blood sample for measurement of n-3 PUFAs was collected into 10-mL EDTA-containing evacuated tubes and then immediately placed in ice water, centrifuged at 2,000 x g at a temperature of 4°C, and

then stored at –80°C until analyzed. Plasma cholesteryl ester n-3 PUFAs were measured as described previously[31].

Information on medical history, drug use, alcohol consumption, smoking habits, educational level and marital status was obtained by questionnaire and reviewed for completeness by a research assistant. Education was categorized into three categories according to criteria of Statistics Netherlands (CBS). The level of physical activity was estimated by means of a previously described questionnaire based on the duration and intensity of sport, household, and leisure-time activities[32].

A food-frequency questionnaire was administered at screening, at baseline, and after 13 and 26 weeks of intervention to estimate fish intake in the previous three mo. Research assistants, who were all trained by the same dietician, interviewed the subjects. After answering a single general question about their average frequency of fish consumption per month, participants had to indicate on a 60-item list which kinds of fish they had consumed and how often. These 60 items were categorized into three groups on the basis of the amount of fat in the different types of fish, i.e. lean, medium-fat and fatty fish. Furthermore, information was obtained on how the fish was consumed: as main meal component during dinner, as a snack, or on toast or with a bread meal. EPA+DHA intake was calculated by multiplying the frequencies of portions of fish consumed per month from each group by an EPA+DHA conversion factor[33].

Body height was measured at baseline with a wall-mounted stadiometer to the nearest 0.1 cm. Body weight and waist circumference were measured at each center visit while the subjects were in a standing position and wearing light clothing and no shoes. Body weight was measured to the nearest 0.1 kg with a calibrated digital scale. Waist circumference was measured in duplicate to the nearest 0.1 cm at the midpoint between the lowest rib and the top of the iliac crest with a nonelastic tape.

Statistical analyses

Data analysis was performed on an intention-to-treat basis and according to a predefined data analysis plan using SPSS 12.0.1 (SPSS Inc, Chicago, IL). A 2-sided *P*-value <0.05 was considered statistically significant.

Baseline characteristics of the treatment groups were compared by one-factor analysis of variance (ANOVA) or with a Kruskal-Wallis test for continuous variables and a chi-square for categorical variables. Because the mental well-being outcome measures were not normally distributed, differential changes between the three intervention groups were tested by using a Kruskal-Wallis test. These analyses were performed for effect after 13 and after 26 weeks. The six repeated CES-D measurements performed in a subgroup were also compared using Kruskal-Wallis as well as the two s-POMS measurements performed in the same subgroup in weeks 17 and 21. Secondary analysis involved a per-protocol analysis and exploratory post hoc analyses in a subgroup of individuals in the tertile with the highest CES-D scores at baseline.

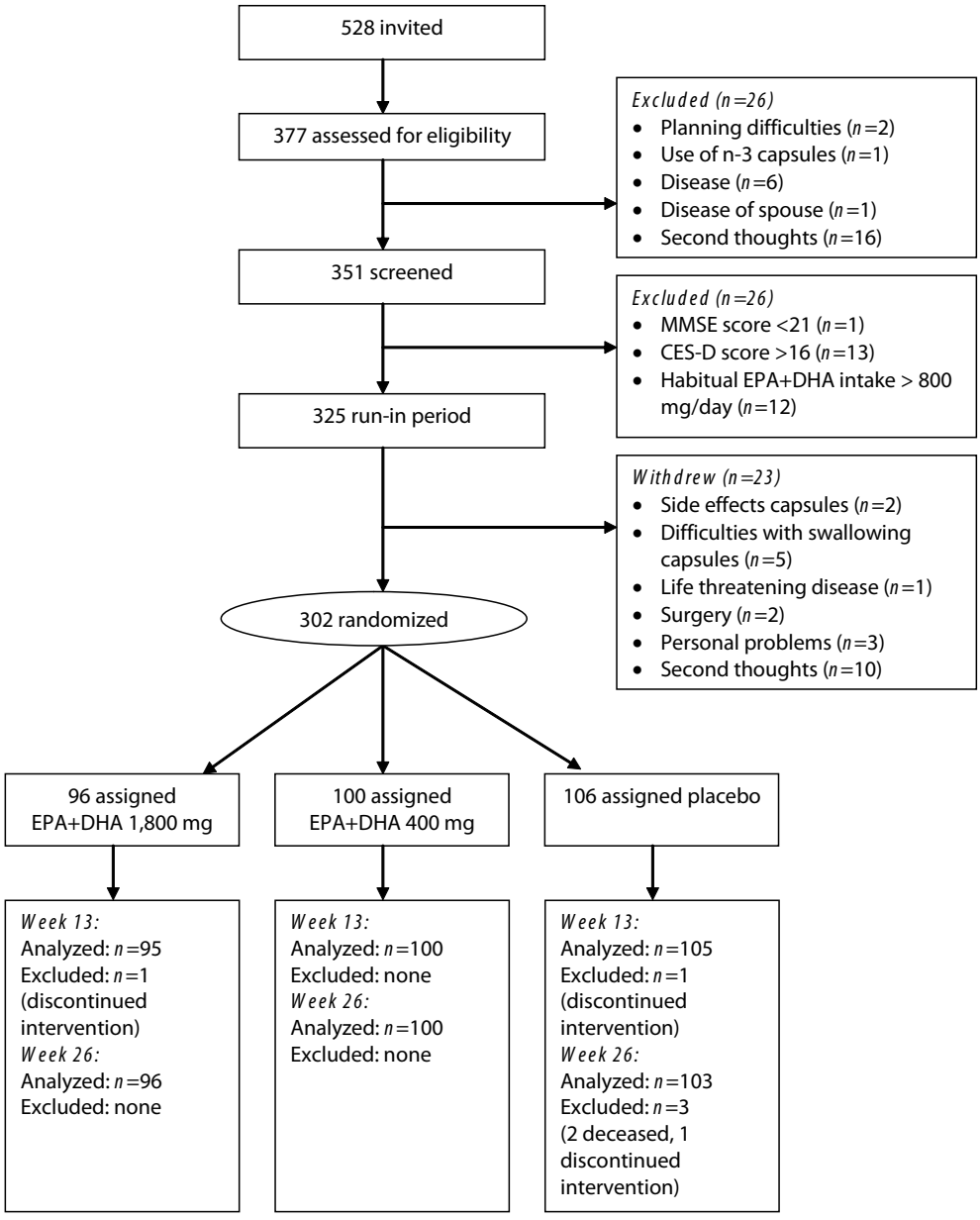

Figure 4.1 Flow diagram of participants throughout the study.
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CES-D, Center for Epidemiologic Studies Depression Scale; MMSE, Mini-Mental State Examination.

Results

Randomization and attrition

Of 528 individuals who expressed an interest in participating, 302 subjects who fulfilled the inclusion criteria were randomized to receive [1] 1,800 mg/ day EPA+DHA (*n*=96), [2] 400 mg EPA+DHA (*n*=100), or [3] placebo (*n*=106) (**Figure 4.1**). During the study nine subjects discontinued the use of capsules: five because of gastrointestinal complaints, two because participation became too burdensome and, two because they died before the end of the intervention. Of those subjects lost to drop-out, seven subjects were still tested after 13 weeks and six subjects after 26 weeks to be able to include them in the intention-to-treat analyses (*n*=300 at week 13 and *n*=299 at week 26).

Table 4.1 Characteristics of 302 subjects participating in a randomized, placebo-controlled trial, by treatment group[a]

	1,800 mg EPA+DHA (*n*=96)	400 mg EPA+DHA (*n*=100)	Placebo (*n*=106)
Age (years)	69.9 ± 3.4	69.5 ± 3.2	70.1 ± 3.7
Sex, Male (%)	55	55	56
Married, living together (%)	80	81	77
Education Low/ Middle/ High (%)	10/ 54/ 35	11/ 49/ 40	5/ 59/ 37
BMI (kg/m^2)	26.1 ± 3.0	26.2 ± 3.4	26.5 ± 3.9
Waist circumference (cm)	94.5 ± 11.4	94.2 ± 10.6	95.9 ± 12.1
Physical activity score	11.5 ± 6.4	11.1 ± 6.2	11.4 ± 6.2
Smoker/ Ex-smoker/ Never smoker (%)	8/64/28	8/54/38	10/56/34
Alcohol consumers (%)	80	87	88
Median alcohol consumption (glasses/ week)[b]	10 (6-14)	8 (4-14)	8 (4-14)
Fish consumption (times/ month)	7 (4-9)	5 (3-9)	6 (4-8)
EPA+DHA intake (mg/day)	306 (131-592)	278 (103-487)	316 (166-584)
Plasma EPA+DHA (mass%)	1.9 ± 0.9	1.9 ± 1.1	1.9 ± 1.1
MMSE (Mini-Mental State Examination)	28 (27-29)	28 (27-29)	28 (27-29)
CES-D (0-60)[c]	5.9 ± 5.5; *5.0 (2.0-8.8)*	6.1 ± 4.9; *5.0 (2.0-8.8)*	6.8 ± 5.2; *6.0 (2.0-10.0)*
MADRS (0-60)[c]	3.9 ± 4.4; *3.0 (0.0-6.0)*	3.8 ± 4.2; *3.0 (0.0-5.0)*	3.8 ± 3.5; *3.0 (1.0-7.0)*
GDS-15 (0-15)[c]	0.8 ± 1.2; *0.0 (0.0-1.0)*	0.9 ± 1.1; *0.5 (0.0-1.0)*	0.9 ± 1.2; *1.0 (0.0-1.0)*
HADS-A (0-21)[c]	2.1 ± 2.3; *1.0 (0.0-3.0)*	2.1 ± 2.1; *2.0 (0.0-3.0)*	2.7 ± 2.7; *2.0 (1.0-4.0)*

Values are mean ± SD and/or median (Q1-Q3), depending on the distribution

[a] No significant difference among the three treatment groups, $P<0.05$ (one-way ANOVA/ Kruskal-Wallis for continuous variables and chi-square analysis for categorical variables)

[b] Mean consumption in consumers only

[c] Higher scores indicate a poorer mental well-being

Participants' characteristics and compliance

The mean age of the participants was 70 y, and 55% of the population was male. At baseline, treatment groups were equally distributed with regard to demographic, anthropometric and lifestyle factors (**Table 4.1**).

Apart from the individuals who stopped treatment prematurely, the average adherence to treatments based on counts of returned capsules was high (96%; <80% for only three subjects) and did not differ between the treatment groups. Compliance was confirmed by a change in the proportion of EPA+DHA in plasma cholesteryl esters of 51% in the low-dose fish oil group (from 1.88 ± 1.12 to 2.83 ± 1.03 g/100g fatty acids), 236% in the high-dose fish oil group (from 1.90 ± 0.86 to 6.40 ± 1.53 g/100g fatty acids) and by a small change of –2% in the placebo group (from 1.91 ± 1.15 to 1.87 ± 1.11 g/100g fatty acids). The supplements were well tolerated; main complaints concerned mild gastrointestinal discomfort. In the high-dose fish oil group, 14% of the subjects reported adverse events, including gastrointestinal problem, 13% of the subjects reported adverse events, including gastrointestinal problems (*n*=9), feeling lifeless, blurred vision, sore throat, and muscle pain. In the placebo group, 15% of the subjects reported adverse events, including gastrointestinal problems (*n*=12), skin irritations, blurred vision, transient ischemic attack, and muscle pain. At the end of the study, blinding of subjects toward treatment allocation (fish oil, placebo or "no idea") was evaluated. The proportion of participants who thought they had received fish oil or placebo did not differ among the groups (*P*=0.15). In the high-dose fish oil group, 25% correctly thought that they had received fish oil and 54% had no idea. In the low-dose group 19% correctly thought that they

Table 4.2 Changes in scores on depression and anxiety questionnaires in Dutch elderly people after 13 and 26 weeks of supplementation, by treatment group: mean ± SD[a]

		1,800 mg EPA+DHA[b]	400 mg EPA+DHA[c]	Placebo[d]
Depression				
CES-D (0-60)[e]	13 weeks - baseline	–0.67 ± 3.57	0.74 ± 4.08	–0.54 ± 5.07
	26 weeks - baseline	–0.18 ± 4.81	0.20 ± 4.28	–0.43 ± 4.68
MADRS (0-60)[e]	13 weeks - baseline	–0.96 ± 3.52	–0.23 ± 3.65	–0.84 ± 3.50
	26 weeks - baseline	–1.03 ± 3.44	–0.82 ± 3.70	–0.83 ± 3.39
GDS-15 (0-15)[e,f]	26 weeks - baseline	–0.05 ± 1.08	0.01 ± 1.12	–0.12 ± 0.96
Anxiety				
HADS-A (0-21)[g]	13 weeks - baseline	–0.16 ± 1.59	0.30 ± 1.57	–0.30 ± 1.98
	26 weeks - baseline	0.10 ± 1.75	0.50 ± 2.48	–0.14 ± 1.89

[a] No significant differences between the three treatment groups were observed, *P*>0.05 (Kruskal-Wallis)
[b] For treatment group 1; *n*=96 at baseline, *n*=95 after 13 weeks and *n*=96 after 26 weeks.
[c] For treatment group 2; *n*=100 at baseline, after 13 and after 26 weeks.
[d] For treatment group 3; *n*=106 participants at baseline, *n*=105 13 weeks and *n*=103 after 26 weeks.
[e] Higher scores indicate a poorer mental well-being
[f] The GDS-15 has only been performed at baseline and after 26 weeks of intervention
[g] Higher scores indicate a higher anxiety level

had received fish oil and 64% had no idea. In the placebo group 25% correctly thought that they had received placebo and 60% had no idea.

Primary and secondary outcomes

Baseline scores on the depression questionnaires were comparable between the three groups. Mean CES-D scores ranged from 5.9 to 6.8, mean MADRS scores ranged from 3.8 to 3.9, mean GDS scores ranged from 0.8 to 1.1, and mean HADS-A scores ranged from 2.1 to 2.7 (**Table 4.1**). After 13 and 26 weeks of supplementation, there were no significant differential changes in the fish oil groups compared with the placebo group for any of the measures of mental well-being. Mean changes after 26 weeks were –0.2, 0.2, and –0.4 for the CES-D (*P*=0.87); –1.0, –0.8, and –0.8 for the MADRS (*P*=0.73); –0.1, 0.0, and –0.1 for the GDS (*P*=0.90), and 0.1, 0.5 and –0.1 for the HADS-A (*P*=0.26) in the high-dose fish oil, low-dose fish oil, and placebo group, respectively (**Table 4.2**).

When we performed per-protocol analyses excluding the drop-outs and noncompliant subjects, the results were not different from those of the intention-to-treat analyses. Exploratory analyses in a subgroup of individuals in the tertile with the highest CES-D scores (cut-off CES-D at baseline: ≥8) showed that mean changes were generally larger than changes in the total group, but changes were not significant between the intervention groups (**Table 4.3**). Mean changes after 26 weeks were –2.3 ± 6.7, –0.1 ± 5.4, and –2.4 ± 5.7 (*P*=0.23) in the high-dose fish oil, low-dose fish oil, and placebo groups, respectively.

The course of the CES-D scores in the subgroup of 104 subjects in whom the CES-D was administered five times, including the telephone interviews at

Table 4.3 Changes in scores on depression and anxiety questionnaires in the highest CES-D score tertile (CES-D ≥8) at baseline after 13 and 26 weeks of supplementation, by treatment group: mean ± SD[a]

		1,800 mg EPA+DHA[b]	400 mg EPA+DHA[c]	Placebo[d]
Depression				
CES-D (0-60)[e]	13 weeks - baseline	–2.52 ± 4.81	–0.28 ± 3.89	–1.70 ± 7.11
	26 weeks - baseline	–2.26 ± 6.72	–0.11 ± 5.36	–2.40 ± 5.69
MADRS (0-60)[e]	13 weeks - baseline	–2.66 ± 4.69	–0.92 ± 4.68	–0.98 ± 4.77
	26 weeks - baseline	–1.99 ± 5.05	–1.91 ± 5.19	–1.88 ± 3.76
GDS-15 (0-15)[e]	26 weeks - baseline	–0.04 ± 1.29	0.21 ± 1.67	–0.29 ± 1.17
Anxiety				
HADS-A (0-21)[f]	13 weeks - baseline	–0.44 ± 1.89	0.48 ± 1.73	–0.37 ± 2.54
	26 weeks - baseline	0.44 ± 2.61	0.91 ± 3.21	–0.37 ± 2.07

[a] No significant differences between the three treatment groups were observed, *P*>0.05 (Kruskal-Wallis)
[b] For treatment group 1; *n*=27.
[c] For treatment group 2; *n*=33.
[d] For treatment group 3; *n*=43 after 13 weeks and *n*=41 after 26 weeks of intervention.
[e] Higher scores indicate a poorer mental well-being
[f] Higher scores indicate a higher anxiety level

weeks 17 and 21, is shown in **Figure 4.2**. The course of CES-D scores was not significantly different between the intervention groups.

Total scores and scores for the s-POMS components depression, fatigue and loss of vigor at weeks 17 and 21 were not significantly different between the intervention groups. At week 21, scores for the mood component anger were significantly lower (*P*=0.01) in the low-dose fish oil group than in the placebo group (**Table 4.4**).

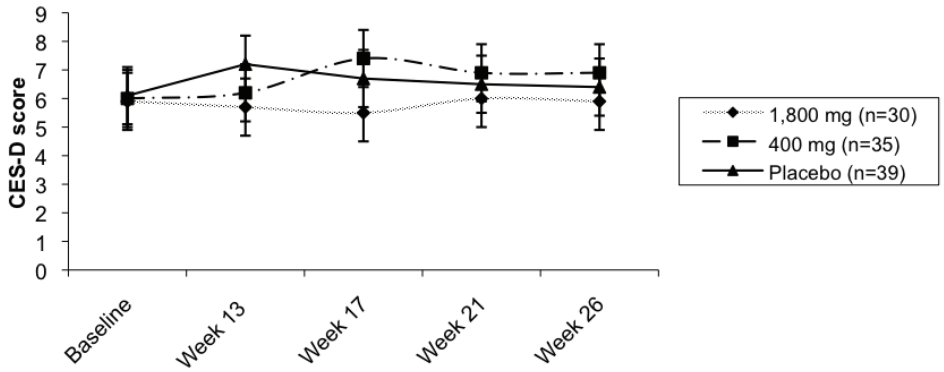

Figure 4.2 Course of mean Center for Epidemiologic Studies Depression Scale (CES-D) scores in Dutch elderly (*n*=104) during the 26-weeks intervention, by treatment group. No significant differences between interventions were observed (*P*>0.05, Kruskal-Wallis test).

Table 4.4 POMS scores in week 17 and 21 of intervention in Dutch community dwelling elderly, by treatment group, values are mean ± SD

POMS		1,800 mg EPA+DHA (*n*=30)	400 mg EPA+DHA (*n*=35)	Placebo (*n*=39)
Total score	Week 17	14.5 ± 11.2	19.6 ± 20.6	19.7 ± 14.2
	Week 21	17.5 ± 16.5	15.9 ± 12.8	18.7 ± 13.3
Depression	Week 17	1.7 ± 3.0	4.1 ± 6.0	2.6 ± 3.5
	Week 21	2.4 ± 4.1	3.0 ± 4.0	2.3 ± 3.1
Fatigue	Week 17	2.4 ± 3.4	3.0 ± 4.1	3.7 ± 3.8
	Week 21	2.5 ± 3.9	2.9 ± 3.8	3.8 ± 4.3
Anger	Week 17	3.4 ± 3.6	3.8 ± 6.1	3.6 ± 3.5
	Week 21	4.2 ± 5.2	1.8 ± 3.1[a]	3.9 ± 3.8
Tension	Week 17	1.5 ± 2.0	2.7 ± 4.4	3.3 ± 3.4
	Week 21	2.1 ± 3.3	2.1 ± 2.9	2.9 ± 3.1
Loss of vigor	Week 17	5.5 ± 3.1	6.0 ± 3.7	6.5 ± 3.4
	Week 21	6.3 ± 3.8	6.1 ± 3.8	5.8 ± 3.0

[a] Significant difference between the three treatment groups was observed, *P*<0.05 (Kruskal-Wallis)

Discussion

The present intervention study in older Dutch subjects showed no effect of daily supplementation with low or high doses of EPA+DHA on mental well-being as assessed by depression and anxiety questionnaires.

To the best of our knowledge, this is the first randomized, double-blind, placebo-controlled trial of fish oil supplementation and mental well-being in a nondepressed population of older adults. Compliance in our study was excellent and did not differ between treatment groups, which indicated that most subjects tolerated taking six daily capsules for 26 weeks. Drop-out rate in this study was 3%, and seven of nine subjects who discontinued capsule use were willing to undergo follow-up measurements.

We examined the effect of both a high (1,800 mg) and a low (400 mg) dose of EPA+DHA on mental well-being. The high "pharmacologic" dose, which corresponds to eating about eight portions of fish per week, was chosen to ensure maximum contrast between the groups to detect an effect, if present. The low dose corresponds to the recommended intake in the Netherlands of 450 mg EPA+DHA/d[34]. Such a dose is roughly equivalent to eating two portions of fish per week (one of which is oily fish) and can be more easily translated into dietary advice. Furthermore, Kamphuis et al. found that an intake of 400 mg n-3 PUFAs was associated with fewer depressive symptoms in an observational study of 332 older Dutch men[15]. Yet, it is not yet clear which dose would be sufficiently high and most effective to influence mental well-being, especially in nonpatient populations. Moreover, because the causal mechanisms have not yet been elucidated, it is not clear whether either EPA or DHA or the combination of the two may influence mood.

The state of mental well-being of the subjects was assessed by a series of questionnaires that have been validated and used frequently in this field and in nondepressed community-dwelling populations[25,35-38]. However, because the questionnaires were designed to primarily assess depressive symptoms, rather than mental well-being, we may not have been able to demonstrate an effect of our intervention in this nondepressive population.

We selected a population of individuals of ≥65 y, because depressive symptoms are highly prevalent in the elderly population and increase with age[39]. Our target group was a nondepressed population (CES-D score <16), because we aimed to investigate whether n-3 PUFA treatment could improve the state of mental well-being of older individuals in the general population. It was shown in a population-based study in the Netherlands that the distribution of CES-D scores in the general elderly population varied greatly, allowing room for improvement even among the nondepressed[40]. However, to be able to study a group of subjects more sensitive to change, it would have been sensible to use not only an exclusion criterion of CES-D >16, but possibly a CES-D <5 to exclude subjects with already optimal CES-D scores.

Our results contrast with those of another study that examined the effect of

n-3 PUFA supplementation in younger, healthy, nondepressed subjects, in which 33 subjects with a mean age of 33 y received a daily dose of 1.6 g EPA and 0.8 g DHA for 35 days. This intervention resulted in an increased mental well-being measured with a POMS questionnaire[18]. On the basis of the fact that we had a larger number of subjects in our study, who were older and were supplemented for a longer period, we expected an effect in our study. Power calculations performed by Appleton et al.[17] suggest that a sample size of ≈100 participants per group should be enough to show a clinically meaningful change in depressed mood, as indicated by a difference of 3-4 points on depression questionnaires.

In an exploratory post hoc analysis in subjects within the tertile with the highest CES-D scores (CES-D: ≥8), i.e., impaired mood, we observed that scores on the depression questionnaires changed more during intervention, although not significantly so. From this finding it may be concluded that subjects with an impaired mental well-being may indeed benefit more from n-3 PUFA supplementation and emphasizes that the relatively optimal CES-D scores measured in our study population may be a limitation of this study. However, Rogers et al., who performed a trial in mildly to moderately depressed individuals from the general population, neither observed an effect on mood assessed with the HADS-A after a shorter period of supplementation with 1.5 g EPA+DHA/d[19].

Other double-blind randomized controlled trials of fish oil and depressive mood were mainly performed in patients with depressive disorders[17]. The supplemental doses and contents varied, ranging from 0.2 g to 9.6 g PUFAs and consisted of EPA alone, DHA alone, or EPA plus DHA. The overall study duration ranged from 28 to 180 days, and beneficial effects appeared between 2 and 8 weeks, which is considerably sooner than our study.

In conclusion, in this randomized, double-blind, placebo-controlled trial, we observed no effect of EPA+DHA supplementation for 26 weeks on different measures of mental well-being in older individuals from the general population.

Acknowledgements

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5

The reliability of three depression rating scales in a general population of Dutch older persons

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Abstract

Objective To compare the reliability of three rating scales for assessing depressive symptoms in a community-based, non-clinically depressed older population.

Methods The study sample comprised of 302 independently living subjects aged 65 years or older. Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D), the Geriatric Depression Scale (GDS-15) and the Montgomery and Åsberg Depression Rating Scale (MADRS) at three time points: at baseline, after 13 weeks (except the GDS-15) and after 26 weeks. Three dimensions of reliability were compared: (i) internal consistency (Cronbach's alpha), (ii) reproducibility (Spearman correlations), and (iii) the intra- and inter-rater reliability (Spearman correlations to compare the differences between correlations of subjects tested by the same vs. different raters at three time points).

Results Cronbach's alpha was high for the CES-D (0.84), good for the MADRS (0.72) and relatively low for the GDS-15 (0.55). Reproducibility was also higher for the CES-D (0.71) than for the MADRS (0.61) and the GDS-15 (0.52). The rater had little influence on CES-D scores (intra/inter-rater ratio=0.99). The GDS-15 and the MADRS, however, performed better when administered by the same rater.

Conclusions The CES-D was the most reliable scale for measuring depressive symptoms in a non-clinically depressed older population.

Introduction

Depression is a common mental disorder affecting persons of all ages and backgrounds. Worldwide it is one of the most important causes of disability[1]. The prevalence of clinically relevant levels of depression ranges from 10-15% in the general elderly population[2,3]. Despite the high prevalence the majority of depressed older patients goes undetected and does not receive treatment[4]. Comorbidity with physical illness and cognitive impairment are often mentioned as factors hindering detection of depressive symptoms[4,5]. One way to improve detection of depressive symptoms is by using standard depression rating scales. Although many such instruments are available, only rarely have their reliabilities been directly compared.

In the present study we examined the reliability of three depression rating scales, which were used in a 6-month intervention study investigating the effect of fish oil on cognitive performance and mental well-being in a Dutch, non-clinically depressed, community-based, older population[6]. Three commonly used scales were administered to assess depressive symptoms, namely two self-report scales; the Center for Epidemiologic Studies Depression Scale (CES-D), the Geriatric Depression Rating Scale-15 (GDS-15), and one observer-rated scale; the Montgomery-Åsberg Depression Rating Scale (MADRS). Reliability was assessed by comparing their internal consistency, reproducibility, and intra- and inter-rater correlations.

Methods

Subjects

A total of 302 independently living subjects older than 65 years was enrolled in this study, of which the original aim was to investigate the effect of high and low doses of fish oil on depressive symptoms in a randomized controlled trial. Participants were recruited from an existing database of volunteers with interest in participating in studies of Wageningen University. Main exclusion criteria were: [1] score of >16 on the CES-D at time of screening[7]; [2] score of <21 on the Mini Mental State Examination (MMSE)[8]; [3] current use of anti-depressant medication; and [4] current use of medication for dementia. Subjects received either capsules with 400 mg EPA+DHA/day, 1,800 mg EPA+DHA/day, or placebo, and mental-well being was assessed at baseline and after 13 and 26 weeks. Treatment with fish oil did not affect depressive symptoms. A detailed description of this study has been reported elsewhere[6,9]. The study was approved by the Medical Ethical Committee of Wageningen University and all subjects gave written informed consent.

Assessment of depressive symptoms

Depressive symptoms were assessed by means of three different depression questionnaires that were administered three times with intervals of 13 weeks by trained research assistants using a standard protocol. For all scales higher

scores indicated more depressive symptoms.

The CES-D was developed as a screening tool to measure depressive symptoms in the community[7]. This short self-report scale consists of 20 items addressing depressive symptoms during the past week. There are four response categories ranging from 'rarely or never' (coded as 0) to 'most or all of the time' (coded as 3) with the total score ranging from 0 to 60. The CES-D is frequently used in elderly community-based populations[10,11] and adequate reliability and validity were established[12-14].

The Geriatric Depression Rating Scale (GDS) was specifically designed for elderly[15]. The original version is a self-report instrument containing 30 questions, with a total score ranging from 0 to 30. In order to limit burden to those interviewed, a short version consisting of 15 items was developed by Sheikh & Yesavage (GDS-15)[16]. This scale assesses depressive symptoms during the past week with simple yes/no questions and the total score varies between 0 and 15. The reliability and validity were tested as satisfactory in different elderly population samples[17-19]. The GDS-15 was only administered at baseline and after 26 weeks.

The Montgomery-Åsberg Depression Rating Scale (MADRS) is an observer rated scale that is designed to measure changes in severity of depressive symptoms[20]. It consists of ten questions concerning the last week and has a response scale of 0-6 ('no presence of symptoms' - 'high presence of symptoms'), resulting in a score ranging from 0 to 60.

Statistical analyses

We excluded subjects with a CES-D score ≥16 (n=19), because these scores are generally interpreted as suggestive for clinically significant depression. This resulted in a total of 283 subjects for the present analysis. Overall scores of the questionnaires in our population showed a strongly skewed distribution and log-transformations did not result in normality. Therefore, the actual scores were used and compared with non-parametric tests. Median scores with interquartile ranges (Q1-Q3) were calculated for each questionnaire on the three time points.

For each questionnaire we examined internal consistency, reproducibility and intra- and inter-rater reliability. Internal consistency was measured by calculating Cronbach's alpha[21], which reflects the average inter-item correlation score and as such it will increase when the correlations between the items increase. For a short scale Cronbach's alpha should be at least >0.70[22].

Reproducibility was assessed using Spearman correlation coefficients. This reflects the level of stability of the measure over time within the same subject[10]. We also examined reproducibility separately for the groups receiving fish oil treatment (high or low dose) or placebo, although no between-group differences were expected since treatment was ineffective.

The influence of the raters on the scores of the depression scales (the intra- and inter-rater reliability) was assessed by comparing Spearman correlation

coefficients. The correlations of scores of subjects who were rated by the same rater at the three time points (baseline, 13 weeks and 26 weeks) were compared with the correlations of scores of subjects who were rated by different raters at the three time points. To exclude effects of instability of the measure over time, an intra/inter rater ratio of the correlations was calculated. A ratio of 1 indicates no influence of the rater on the test scores. Statistical analyses were performed using SPSS 15.0 for Windows.

Results

Subjects' characteristics

Data from a total of 283 subjects were used in this analysis. Subjects had a mean age of 70 years and 57% was male. Seventy-eight percent was married or was living with a spouse. The median (Q1-Q3) scores of the CES-D, the MADRS and the GDS-15 at the three time points and the history of affective and psychiatric disorders are presented in **Table 5.1**. In the past, 3% of the subjects experienced a depression, 1% a psychiatric disorder, and 9% received therapy for psychiatric complaints. Further details on subject characteristics have been described elsewhere[6]. Spearman correlations of the CES-D, the MADRS and the GDS-15 between the different measurements are shown in **Table 5.2**.

Table 5.1 Characteristics of 283 Dutch non-clinically depressed, community-based, older subjects

Characteristics	
Age (years)[a]	71.8 ± 3.4
Sex, Male (%)	57
Married, living together (%)	78
Education Low/ Middle/ High (%)	9/ 53/ 38
CES-D (0-60)[b]	5.0 (2.0-8.0)
MADRS (0-60)[b]	2.0 (0.0-5.0)
GDS-15 (0-15)[b]	0.0 (0.0-1.0)
Depression in the past (%)	3
Psychiatric disorder in the past (%)	1
Therapy psychiatric complaints in the past (%)	9

[a] Mean ± SD
[b] Median (Q1-Q3), higher scores indicate more depressive symptoms

Table 5.2 Spearman correlation coefficients of the CES-D, MADRS and GDS-15 at the different time points

	T = 0 and T= 13 wk (*n*=300)	T = 0 and T = 26 wk (*n*=299)	T = 13 wk and 26 wk (*n*=298)
CES-D	0.73	0.67	0.72
MADRS	0.62	0.59	0.61
GDS-15	–	0.52	–

Table 5.3 Three dimensions of reliability of the CES-D, MADRS and GDS-15 in a Dutch, community-based, older population

		CES-D	MADRS	GDS-15
Cronbach's alpha	T = 0	0.74	0.64	0.40
	T = 13 wk	0.82	0.72	–
	T = 26 wk	0.82	0.67	0.41
	Average	0.79	0.68	0.41
Spearman correlation coefficients	T = 0 vs T = 13 wk	0.70	0.57	–
	T = 0 vs T = 26 wk	0.64	0.54	0.50
	T = 13 vs T = 26 wk	0.70	0.57	–
	Average	0.68	0.56	0.50
Spearman correlation coefficients	Same rater	0.66	0.59	0.55
	Different rater	0.67	0.50	0.41
	Intra/inter ratio	0.99	1.18	1.34

Reliability

The results of the three dimensions of reliability are presented in **Table 5.3**. Cronbach's alphas were on average 0.79, 0.68 and 0.41 for the CES-D, MADRS and the GDS-15, respectively. The GDS-15 scored lowest, both at baseline and after 26 weeks. The score of the MADRS was in between for all time points.

The average test-retest Spearman correlation coefficients for reproducibility in the group as a whole were 0.68, 0.56 and 0.50 for the CES-D, MADRS and the GDS-15, respectively. When examining only the placebo group, corresponding values were 0.70 and 0.57 and 0.49. In both fish oil groups combined, values were similar, i.e. 0.67, and 0.56 and 0.50, respectively.

For the CES-D the intra/inter rater ratio was 0.99, indicating that the rater was of no influence on the performance of this questionnaire. For the GDS-15 we observed a high intra/inter rater ratio (1.34), i.e. having different raters resulted in poorer correlations. The intra/inter rater ratio for the MADRS was 1.18.

Discussion

In this study the reliability of three commonly used rating scales of depressive symptoms was compared in a non-clinically depressed, community-based older population. On all three dimensions of reliability that we examined, CES-D performed best, followed by MADRS and GDS-15, respectively.

We assessed the reliability of the three depression scales in a large number of subjects (n=283) with a relatively long duration of follow-up and measurements at three time points. By assessing the reliability in an older, non-clinically depressed population, important knowledge is gained on which scales perform best in early detection of depressive symptoms in this kind of population. The present analysis was embedded in an intervention study that focused on the effect of fish oil on mental health. Since no changes in depressive symptoms

Table 5.4 Overview of studies in which the reliability of the CES-D, MADRS and GDS was examined

Study	Population	Alpha	Reproducibility	Inter-rater reliability	Conclusion	Remarks
CES-D						
Batistoni et al., 2007[26]	Community dwelling, Brazilian, elderly (aged 60-103, mean age 72.4y) (*n*=903)	0.86	NA[a]	NA	Psychometrically suitable for use in older people, because of satisfactory reliability and validity	
Beekman et al., 1994[11]	Dutch elderly >55y (*n*=224)		NA	NA	Dutch translation of the CES-D provides a useful tool for measuring depressive symptoms in the elderly. Normal answer format is recommended.	Not a replacement for psychiatric diagnosis.
	- simple answer format (yes/no)	0.82				
	- normal answer format (4 categories)	0.80				
	- normal answer format (different translation)	0.90				
Lewinsohn et al., 1997[10]	Community-based elderly ≥50y, mean age 64y (*n*=1,005)	0.82	0.52 (2.4y)	NA	Results support the use of CES-D as a screener for depression amongst community-residing elderly, but cannot be generalized to elderly people with illness	No significant gender differences were found for the prevalence rate
Radloff, 1977[7]	Q1 >18y (from Kansas and Washington) (*n*=2,514)	0.85	Q3: 0.48, 0.54, 0.49 (3,6,12 mo) Patients: 0.57 (2,4,6,8 wk)	NA	Not a clinical diagnostic tool, but a useful tool for epidemiologic studies of depression.	
	Q2 (only Washington, shortened version) (*n*=1,060)	0.85				
	Q3 (re-interview Q1 and Q2) (*n*=1,422)	0.84				
	Psychiatric patients (*n*=70)	0.90				
Schein & Koenig, 1997[32]	Elderly medically ill inpatients ≥60y, mean age 69.7y (*n*=76)	0.85	NA	NA	Limitations to the CES-D when standard scoring is used with medially ill older population because of high false-positive rate.	Items 2,4,5,8,9 fail to distinguish between depressed and non-depressed

Table 5.4 *Continued*

Study	Population	Alpha	Reproducibility	Inter-rater reliability	Conclusion	Remarks
Spijker et al., 2004[34]	Community residents, ≥55y, native Dutch (*n*=304), Turkish (*n*=330), Moroccan (*n*=299)	0.92	NA	NA	Satisfactory utility of the CES-D in elderly migrants or Turkish and Moroccan descent	
Thombs et al., 2008[34]	Patients with systemic sclerosis, mean age 55y (*n*=470)	0.88	NA	NA	CES-D is a reliable tool for measuring depressive symptoms in patients with systemic sclerosis.	
MADRS						
Hammond, 1998[27]	Acute medical inpatients ≥65y, mean age 80.5y (*n*=100)	0.61	NA	NA	Low internal consistency, with a modification it may be an appropriate measurement of depression severity	Alpha increased when 5 items were deleted.
Montgomery & Åsberg, 1979[20]	Depressed patients in England and Sweden (*n*=106 (33 outpatients, 73 inpatients))	NA	NA	0.89-0.97	Short scale developed to detect changes in depressive symptoms after treatment.	
Smalbrugge et al., 2008[29]	Nursing home patients ≥55y, mean age 79.3y (*n*=313)	0.85	NA	NA	MADRS seems the most appropriate tool for measuring (changes) in severity of depression	More time consuming and more difficult to administer.
Suzuki et al., 2005[28]	Japanese patients with unipolar depression, mean age 48y (*n*=132)	0.76	NA	NA	Gender difference in vegetative factor and age difference	Factor analysis showed three factors
GDS						
Batistoni et al., 2007[26]	Brazilian elderly aged 60-103, mean age 72.4y (n=446)	0.70	NA	NA	GDS was used as the reference scale for CES-D, because it had been validated and used in Brazil.	CES-D overestimates the percentage of depressed elderly.

Brown & Schinka, 2005[4]	Outpatients ≥65y, mean age 76.4y (*n*=147)	0.86	0.81	NA	The GDSI-15 may be a useful alternative to standard screening methods in assessing patients in outpatient settings.	Lower efficacy than the GDS-30
D'Ath et al., 1994[30]	Elderly subjects attending primary care (*n*=194)	0.80	NA	NA	GDS-15 useful for detecting depression.	Short scales (GDS10, GDS4 and GDS1) also useful.
Jongenelis et al., 2005[19]	Nursing home patients ≥55y, mean age 79.3y (*n*=333)	0.79	NA	NA	GDS-30 can reliable detect the presence of clinically relevant depression among nursing home patients with no, or with mild to moderate cognitive impairment	GDS-10 is an acceptable alternative method.
Malakouti et al., 2006[31]	Iranian subjects ≥59y (*n*=204)	0.90	0.58 (2 wks)	NA	GDS-15 has a good reliability and validity, usable for screening and clinical studies in elderly in urban Iranian areas	Higher cut-off scores with better sensitivity.
Sheikh & Yesavage, 1986[16]	Normal elderly from community, ≥55y (*n*=18) and elderly patients in treatment for depressive complaints (*n*=17)	0.79	NA	NA	GDS-15 is useful where time is limited.	Correlation GDS-15 and GDS-30 r=0.84
Smalbrugge et al., 2008[29]	Nursing home patients ≥55y, mean age 79.3y (*n*=313)	0.79	NA	NA	GDS-15 is an acceptable screening instrument, and is easy to administer.	MADRS is more appropriate for measuring severity of depression.
Pomeroy et al., 2001[35]	Subjects from a rehabilitation centre ≥60y, mean age 78.4y (*n*=87)	0.74	NA	0.68 (GDS-4)	Short scales performed just as well as the longer scales.	
Yesavage et al., 1982[15]	Normal elderly persons (*n*=40) Subjects under treatment for depression (*n*=60)	0.94	0.85 (1 wk)	NA	GDS is a reliable and valid self-rating depression screening scale for elderly populations.	Not a diagnostic instrument

[a] NA = not available

were observed in any of the treatment groups[6], the data of both the fish oil and placebo groups were suitable for examining the reliability of the three depression questionnaires, including the reproducibility.

Cronbach's alpha is a commonly used measure for internal consistency and reliability. However, its value has recently been debated[23-25]. Assumptions, like equal item variances, are often not met and the reliability of a test may be underestimated in case of single test administration. We calculated Cronbach's alpha at three time points, which gives a better estimate of the reliability.

The internal consistency of the CES-D in our study is adequate according to standard criterions ($\alpha>0.70$)[22] and in line with previous studies in non-patient older individuals (**Table 5.4**)[10,11,26]. The internal consistency of the MADRS ($\alpha=0.68$) was acceptable, indicating that the items of the MADRS reflect depressive symptoms in a healthy study population reasonably well. Three other studies on the internal consistency of the MADRS showed inconsistent results in different study populations (**Table 5.4**)[27-29]. The Cronbach's alpha of 0.41 for the GDS-15 in our study is low, which might be attributed to the strongly skewed distribution and the small variance, probably due to the yes/no answer format. Other studies reported better results in various study populations with a higher number of depressed subjects (**Table 5.4**)[15,19,26,29,30].

The reproducibility correlation coefficients of the CES-D in the present study were higher than in previous studies (**Table 5.4**)[7,10]. Although Radloff used almost the same intervals as we did (2, 3 and 6 months), our reproducibility may be better because of our non-clinically depressed and probably more stable population. The lower reproducibility of Lewinsohn and colleagues may be due to the long time interval of 2.4 years, during which more actual changes in mood are likely to occur. The MADRS had a moderate reproducibility (correlation of 0.56), which probably reflects the higher sensitivity of the MADRS for detecting small changes of depressive symptoms, where the MADRS was designed for. No comparable studies investigating the reproducibility of the MADRS were found. The reproducibility correlation of the GDS-15 of 0.50 is lower than reproducibility correlations of the two other questionnaires and also lower compared to GDS-15 in other studies, although these data are not very consistent (**Table 5.4**)[4,15,31]. The reproducibility is likely to be largely influenced by the yes/no answer format.

The intra/inter rater reliability of the CES-D in the present study was close to one, so the effect of having different raters is practically of no influence. This was expected because it is a self-report scale, and different raters used a standardized protocol for administering the test. Conversely, on the GDS-15, which is also a self-report scale, the rater was of influence, which was an unexpected result. For the MADRS the intra/inter rater ratio was 1.18, which is most likely caused by the fact that the rater's opinion forms part of the test score. Comparing our inter-rater reliability with findings in other studies is difficult, because we did not test the inter-rater reliability by doing a joint interview with two raters at the same time, but by single raters at three different time points and calculation

of the intra/inter rater ratio. Montgomery and Åsberg observed an inter-rater reliability between the 0.89 and 0.97, but their correlations were also based on a joint interview with two raters[20].

Conclusion

Based on our results, the CES-D appeared to be a better tool than the GDS-15 and MADRS for measuring depressive symptoms in a non-clinically depressed, healthy older population.

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6

Association of n-3 long-chain polyunsaturated fatty acid and fish intake with depressive symptoms and low dispositional optimism in older subjects with a history of myocardial infarction

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Abstract

Background Individuals with coronary heart disease are at increased risk of poor mental well-being. Dietary intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the main n-3 fatty acids from fish, may be beneficial to mental well-being.

Method In 644 participants, aged 60-80 years, with a history of myocardial infarction, the association of EPA+DHA and fish intake with mental well-being was examined. Habitual food intake was assessed with a 203-item food frequency questionnaire. Depressive symptoms were assessed with the self-report Geriatric Depression Scale (GDS-15) and dispositional optimism with the Revised Life Orientation Test (LOT-R) and a 4-item questionnaire (4Q). In Cox-regression models modified for cross-sectional analyses we adjusted for sex, age, energy intake, body mass index, family history of depression, education, marital status, smoking, physical activity, and intake of saturated fat, alcohol and fiber.

Results Compared with the lower tertile, subjects in the higher tertile of EPA+DHA intake had a lower prevalence of depressive symptoms, but this association was not statistically significant (prevalence ratio [PR] 0.78; 95% Confidence Interval [CI] = 0.50; 1.22, *P*-trend 0.27). The higher tertile of EPA+DHA intake was positively associated with dispositional optimism measured with the 4Q (PR 0.69; 95% CI 0.46-1.03, *P*-trend 0.05), but not according to the LOT-R. Fish intake was not related to either depressive symptoms or dispositional optimism.

Conclusion Intake of EPA+DHA was positively associated with dispositional optimism assessed with the 4Q, but not with optimism assessed with the LOT-R or with depressive symptoms.

Introduction

Poor mental well-being and coronary heart disease (CHD) often co-occur. Depression is a strong risk factor for the development of CHD and is associated with a worse prognosis of CHD[1]. In post-myocardial infarction (MI) patients the risk of depression is three times as high as compared to the general healthy population[2]. Therefore, the American Heart Association recommends to routinely screen for depressive disorders in coronary patients[3].

A low intake of fish and marine n-3 polyunsaturated fatty acids (n-3 PUFA), especially of eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), could increase the risk of CHD[4] and may also predispose to depression. Because n-3 PUFAs are a major component of neural membranes and act as precursors of compounds involved in immune and inflammatory responses, it is biologically plausible that these PUFA could play a role in mood and behavioral disorders[5]. Appleton et al. reviewed the clinical and epidemiological evidence of n-3 PUFA and fish with depression until the beginning of 2008 and concluded that in both clinical as well as in observational studies depression may be associated with n-3 PUFA and fish intake and n-3 PUFA status, but results are inconclusive[6]. A meta-analysis of randomized controlled trials on n-3 PUFA supplementation and depression, which were mainly performed in depressed patients, pointed towards a beneficial effect, although the pooling of results was hampered by the heterogeneity of populations and type of interventions[7]. More recently, the results of a trial performed in 302 Dutch older subjects provided with 400 mg/d or 1,800 mg/d of EPA+DHA for 26 weeks have been published and did not show an effect on mental well-being[8]. Studies that focused on the association between n-3 PUFA and depression in coronary patients showed that depression was associated with lower plasma or membrane n-3 PUFA levels, in particular of DHA[9-13].

Depression is not simply the reverse of optimism, but both affect mental well-being and because the intake of fish or n-3 PUFA may alleviate depressive symptoms, it may also enhance optimism. Dispositional optimism is defined in terms of generalized positive expectancies for one's future[14] and has been associated with more healthy dietary and other lifestyle habits[15,16], and also with less cardiovascular mortality[17]. In a prospective study in 773 community-dwelling elderly men it was shown that dispositional optimism was associated with healthy lifestyle and dietary habits, including eating ≥400 mg EPA+DHA/day[16]. Another study in 8,690 men and women aged 31 years showed that lack of optimism was associated with unhealthy dietary and other habits in general, including a low fish intake, but only in women[15].

In the present study, we examined the association of mental well-being, based on the lack of depressive symptoms and on the presence of dispositional optimism, with EPA+DHA and fish intake in an older population with a history of coronary heart disease.

Subjects and methods

Subjects

We used baseline data of the Alpha Omega Trial, a randomized placebo-controlled double-blind intervention study designed to investigate the effect of the n-3 PUFA alpha linolenic acid (ALA), EPA and DHA on cardiovascular mortality. Participants were free-living men and women aged 60-80 years with a documented history of myocardial infarction within the past 10 years. Main exclusion criteria were: [1] habitual fish intake >150 g/d; [2] habitual alcohol intake >6 drinks/d; [3] recent (within 2 weeks before study entry) or current use of fish oil or other n-3 capsules; or [4] dementia or severe cognitive impairment (Mini-Mental State Examination (MMSE) score <22[18]). The Medical Ethics Committee South West Holland approved the study and all participants gave written informed consent.

The sample examined in the present analysis comprised 791 participants who were enrolled in 2006, during the final part of the recruitment phase and who completed questionnaires on mental well-being. We excluded those with missing EPA+DHA intake data (*n*=97), and those who reported improbable energy intakes (*n*=10) (<500 or>3,500 kcal/d for women and <800 or >4,000 kcal/d for men)[19]. Furthermore, we excluded current users of anti-depressant medication (*n*=18) and subjects without complete data on all three measures of mental well-being (*n*=22). This resulted in a total of 644 subjects for the current analyses.

Assessment of mental well-being

Depressive symptoms were measured using the Geriatric Depression Screening Scale (GDS-15)[20]. The GDS-15 is a 15-item yes/no self-report questionnaire designed to screen for depressive symptoms during the past week in elderly subjects. The total score ranges from 0 to 15, with higher scores indicating more depressive symptoms. In elderly people a good sensitivity (0.88) and specificity (0.76) for depression was found for a cut-off point of ≥4[21].

Dispositional optimism was assessed by using two questionnaires: the Life Orientation Test – Revised (LOT-R) and a 4-item questionnaire (4Q). The LOT-R consists of 10 coded items of which four statements are filler items not used in scoring[22]. Of the six items that are scored, three are keyed in a positive direction and three in a negative direction. There are five answer categories ranging from "strongly disagree" (coded as 0) to "strongly agree" (coded as 4). Negatively worded items (i.e. items 3, 7 and 9) are reversely coded before scoring. The total score ranges from 0 to 24 and higher scores indicate greater optimism. Because there is, to the best of our knowledge, no cut-off point for the LOT-R, we defined low dispositional optimism as the ≈20% of the subjects with the lowest scores yielding <12 as a cut-off.

The 4Q was previously used in the Zutphen Elderly Study and was predictive of both subsequent depressive symptoms[23] and cardiovascular mortality[17]. It consists of the following four questions: "I still expect much from life"; "I do

not look forward to the years to come"; "My days seem to pass by slowly", and "I am still full of plans". The response format is a 3-point scale ranging from "fully in agreement" (coded as 0) to "not in agreement" (coded as 2). The additional answer category "do not know" is also coded as the midpoint (coded as 1). The two negatively worded items (i.e. items 2 and 3) are reversely coded before scoring, so higher scores again indicate greater optimism. A score <6, as previously defined in the Zutphen Elderly Study[24], was used as cut-off to indicate low dispositional optimism.

Assessment of diet, EPA+DHA, and fish consumption

Habitual food intake during the previous month was estimated with a 203-item food frequency questionnaire (FFQ), which was specifically developed for the Alpha Omega Trial. This list is based on a previously validated FFQ that was designed to estimate the intake of total energy, total fat, cholesterol, and saturated, monounsaturated, and polyunsaturated fatty acids in adults[25]. This questionnaire was updated, adapted for people aged 60-80 years, and extended with questions to estimate intakes of ALA, EPA, and DHA. Questions on frequency, amount, type, and cooking method of fish were also included.

Trained dieticians checked the returned questionnaires and obtained information by phone on important unclear or missing important items. The food consumption data were converted into nutrient intakes by using the Netherlands Food Composition Table 2001[26].

Assessment of lifestyle and health

Trained research nurses performed baseline examinations at the hospital or at the subjects' home. Body weight and height were measured and body mass index (BMI) was calculated as weight (in kg) divided by height squared (m^2). Blood pressure was measured twice and the average was calculated. Blood samples were obtained for measurement of glucose, total cholesterol, and HDL cholesterol. Self-administered questionnaires were used to collect information on medical history, medication use, smoking habits (current/ former/never), educational level (low/ intermediate/high), and marital status (living together with wife, partner, or others/ alone). Subjects were considered physically active when they reported 30 minutes of moderate or heavy exercise per day as assessed by the physical activity scale for the elderly (PASE) questionnaire[27]. Hypercholesterolemia was defined as total serum cholesterol of ≥6.5 mmol/l or use of lipid-lowering medication. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or use of antihypertensive medication. Diabetes was assessed by self-report or serum glucose ≥11.1 mmol/l, or present treatment with oral antidiabetic medication or insulin.

Statistical analyses

If mental well-being measures had one missing response, the missing item was assigned the mean value of the other items on that particular scale for that participant. When >1 item on a particular questionnaire was missing, no total score was computed and the participant was not included in the analyses.

Baseline characteristics of the subjects were calculated for all subjects and for men and women separately. To examine the agreement between the LOT-R and the 4Q we used Spearman rank correlation and cross tabulations to examine whether subjects were categorized into the same tertile of dispositional optimism by the two questionnaires. The associations of the prevalence of depressive symptoms (GDS-15 ≥4) and the absence of dispositional optimism (4Q <6 or LOT-R <12) with tertiles of EPA+DHA and fish were examined using a Cox-regression model with robust error variance, modified for cross-sectional analyses[28,29]. Associations are presented as prevalence ratios (PR) with 95% confidence intervals (95% CI) with the lower tertile of intake as reference group. Analyses were adjusted for sex, age, and family history of depression in the first model and in the second model more socio-demographic, lifestyle, and food intake factors were added, namely, BMI, education, marital status, smoking status, physical activity, energy intake, intake of saturated fat, alcohol, and fiber. Tests of linear trend across increasing tertiles of EPA+DHA and fish intake were performed by using the median values of intake for each tertile.

A two sided *P*-value of <0.05 was considered statistically significant and the statistical analyses were performed using SAS Version 9.1 (SAS Institute Inc., Cary, North Carolina, USA).

Results

Population characteristics

The study population comprised 644 participants, 500 men with a mean age of 69 years and 144 women with a mean age of 70 years (**Table 6.1**). Based on GDS-15 scores with a cut-off point ≥4, 17% of the subjects had depressive symptoms and this was twice as high in women (29%) compared to men (14%). Based on LOT-R scores with cut-off point <12 the percentage of low dispositional optimism was 15% and based on 4Q scores with cut-off point <6 the percentage of low dispositional optimism was 22%. Women were slightly less optimistic than men as measured with the LOT-R (16% vs. 15%) and as well as with the 4Q (32% vs. 19%). Median fish intake was 15 g/d and 40% of the participants consumed fish less than once per week, 40% consumed fish once per week and 20% consumed fish more than once per week. The median intake of EPA+DHA was 130 mg/d, women consuming slightly less than men (120 vs.140 mg/d).

Table 6.1 Characteristics of 644 Dutch subjects aged 60-80 years with a history of myocardial infarction[a]

	Total (*n*=644)	Men (*n*=500)	Women (*n*=144)
Age (y)	69 ± 6	69 ± 6	70 ± 6
Score GDS-15 (0-15)[b]	1 (0-3)	1 (0-3)	2 (1-4)
Score ≥4 (%)	17	14	29
Score LOT-R (0-24)[c]	15 (13-17)	15 (13-17)	14 (12-16)
Score ≥12 (%)	85	85	84
Score 4Q (0-8)[d]	7 (6-8)	7 (6-8)	6 (5-8)
Score ≥6 (%)	78	81	68
Family history depressive symptoms (%)	16	16	16
Previous treatment for depressive symptoms (%)	13	12	17
Living alone (%)	17	10	44
MMSE score (0-30)[e]	28.5 (27-29)	29 (27-30)	28 (27-29)
BMI (kg/m^2)[f]	28 ± 4	28 ± 4	28 ± 5
Physical activity (%)[g]	66	68	60
Education (%) low/ intermediate/ high[h]	55/ 33/ 12	52/ 35/ 14	66/ 27/ 7
Smoking status (%) current/ former/ never	15/ 67/ 18	13/ 74/ 13	20/ 44/ 36
Hypercholesterolemia (%)[i]	87	86	90
Blood pressure (mmHg)			
Systolic	139 ± 22	138 ± 21	140 ± 23
Diastolic	78 ± 11	79 ± 11	75 ± 12
Hypertension (%)[j]	93	93	90
Diabetes mellitus (%)[k]	20	18	27
Dietary intake			
Energy intake (kJ/d)	8,002 ± 2,090	8,330 ± 2,086	6,864 ± 1,669
Saturated fat (g/d)	26 ± 10	28 ± 10	21 ± 6
EPA (mg/d)[l]	50 (20-80)	50 (20-80)	50 (20-90)
DHA (mg/d)[m]	80 (40-130)	90 (50-130)	70 (40-145)
Fiber (g/d)	22 ± 7	22 ± 7	21 ± 7
Daily alcohol intake:			
0 glasses (%)	28	20	54
0.1-2.9 glasses (%)	65	70	44
≥3 glasses (%)	8	9	1
Total fish consumption (g/d)	15 (8-17)	15 (8-18)	15 (5-17)
Fish intake:			
<1/ week (%)	40	40	41
1/ week (%)	40	41	36
>1/ week (%)	20	19	23

[a] Values are mean ± SD, median (interquartile range: Q1-Q3) for skewed data, or percentages.
[b] GDS-15: Geriatric Depression Scale-15, higher scores indicate poorer mental well-being[20].
[c] LOT-R: Life Orientation Test - Revised, higher scores indicate greater optimism[22].
[d] 4Q: 4-item questionnaire on dispositional optimism, higher scores indicate greater optimism[23].
[e] MMSE: Mini-Mental State Examination, higher scores indicate better cognitive function[18].
[f] BMI: body mass index.
[g] Defined as ≥30 minutes/d of physical activity.
[h] Low: primary education or less; intermediate: secondary general or vocational education; high: higher vocational education, university.
[i] Defined as total serum cholesterol concentrations ≥6.5 mmol/l or use of lipid-lowering medication.
[j] Defined as systolic ≥140 mmHg or diastolic ≥90 mmHg or use of antihypertensive medication.
[k] Defined by self-report, as serum glucose ≥11.1 mmol/l or as treatment with oral antidiabetic medication or insulin.
[l] EPA: eicosapentaenoic acid.
[m] DHA: docosahexaenoic acid.

Depressive symptoms

Compared with the lowest tertile, subjects in the highest tertile of EPA+DHA intake had a significantly lower prevalence of depressive symptoms (prevalence ratio [PR] 0.56; 95% CI = 0.36; 0.88, *P*-trend 0.009), which was no longer significant after adjustment for confounders (PR 0.78; 95% CI = 0.50; 1.22, *P*-trend 0.27) (**Table 6.2**). For fish intake the unadjusted PR of depressive symptoms for the highest tertile was 0.73 (95% CI = 0.48; 1.12, *P*-trend 0.14) and the multivariable adjusted PR was 0.94 (95% CI = 0.62; 1.42, *P*-trend 0.57) (**Table 6.3**). Adding the 18 users of anti-depressant medication to the depressive symptoms group did not change these results (data not shown).

Dispositional optimism

Of the subjects 46% was categorized in the same tertile with the LOT-R and 4Q. Furthermore, 47% was categorized in the adjacent tertile for the LOT-R and 4Q and 7% was categorized into the opposite tertile. Spearman rank correlation

Table 6.2 Prevalence ratios[a] for the association of EPA+DHA intake with depressive symptoms[b] and dispositional optimism[c] in older subjects with a history of myocardial infarction

	EPA+DHA (mg/d)[d]			*P*-trend[e]
	≤90 40 (10;70) (*n*=221)	90-180 140 (120;160) (*n*=214)	≥180 360 (220;460) (*n*=209)	
	Depressive symptoms on the GDS-15			
Crude association	1	0.86 (0.59;1.25)	0.56 (0.36;0.88)	0.009
Model 1[f]	1	0.92 (0.64;1.34)	0.60 (0.39;0.94)	0.02
Model 2[g]	1	1.07 (0.74;1.56)	0.78 (0.50;1.22)	0.27
	Low optimism on the 4Q			
Crude association	1	0.93 (0.67;1.28)	0.52 (0.35;0.78)	0.003
Model 1[f]	1	0.98 (0.72;1.33)	0.57 (0.39;0.85)	0.003
Model 2[g]	1	1.05 (0.76;1.44)	0.69 (0.46;1.03)	0.05
	Low optimism on the LOT-R			
Crude association	1	0.84 (0.54;1.30)	0.86 (0.55;1.34)	0.57
Model 1[f]	1	0.84 (0.54;1.31)	0.88 (0.57;1.37)	0.65
Model 2[g]	1	0.92 (0.59;1.42)	1.13 (0.72;1.79)	0.53

[a] Prevalence ratios (95% CI) obtained using Cox regression with robust error variance.
[b] Depressive symptoms are defined as a score of ≥4 on the Geriatric Depression Screening Scale (GDS-15).
[c] Low optimism is defined as a score <6 on the 4-item questionnaire (4Q) or a score <12 on the Life Orientation Test - Revised (LOT-R).
[d] Cut-off and median (Q1-Q3) per tertile.
[e] *P*-trend refers to a linear trend in regression coefficients across tertiles of intake using the median values of each tertile.
[f] Adjusted for sex, age, and family history of depression.
[g] Adjusted for BMI, education, marital status, smoking status, physical activity, energy intake, intake of saturated fat, alcohol, and fiber (*n*=636).

between the two questionnaires was 0.44 (P<0.0001).

For dispositional optimism measured with the 4Q the unadjusted PR for the highest tertile of EPA+DHA intake as compared to the reference group was 0.52 (95% CI = 0.35; 0.78, P-trend 0.003) and the statistical significant trend remained after adjustment for confounders (PR 0.69; 95% CI = 0.46; 1.03, P-trend 0.05) (**Table 6.2**). However, dispositional optimism measured with the LOT-R was not associated with EPA+DHA intake. Fish intake was not related to dispositional optimism neither measured with the LOT-R nor with 4Q (**Table 6.3**).

Discussion

In this study in Dutch, older subjects with established coronary heart disease there was a significant positive association between EPA+DHA intake and dispositional optimism assessed with the 4Q, but not with the LOT-R. The inverse association of depressive symptoms with EPA+DHA intake was no longer statistically significant after adjusting for confounders.

Table 6.3 Prevalence ratios[a] for the association of fish intake with depressive symptoms[b] and dispositional optimism[c] in older subjects with a history of myocardial infarction

	Fish (g/d)[d]			
	≤9.8 4.3 (0.0;7.7) (*n*=214)	9.8-<16.2 15.0 (11.8;15.5) (*n*=214)	≥16.2 36.8 (17.4;40.5) (*n*=216)	*P*-trend[e]
Depressive symptoms on the GDS-15				
Crude association	1	0.91 (0.61;1.34)	0.73 (0.48;1.12)	0.14
Model 1[f]	1	0.94 (0.64;1.39)	0.75 (0.50;1.14)	0.13
Model 2[g]	1	1.19 (0.81;1.75)	0.94 (0.62;1.42)	0.57
Low optimism on the 4Q				
Crude association	1	0.80 (0.57;1.13)	0.71 (0.49;1.01)	0.07
Model 1[f]	1	0.84 (0.60;1.16)	0.74 (0.52;1.04)	0.09
Model 2[g]	1	0.93 (0.66;1.30)	0.85 (0.60;1.21)	0.37
Low optimism on the LOT-R				
Crude association	1	1.00 (0.65;1.55)	0.85 (0.54;1.34)	0.44
Model 1[f]	1	1.02 (0.66;1.57)	0.86 (0.54;1.35)	0.47
Model 2[g]	1	1.13 (0.73;1.76)	1.03 (0.65;1.63)	0.96

[a] Prevalence ratios (95% CI) obtained using Cox regression with robust error variance.
[b] Depressive symptoms are defined as a score of ≥4 on the Geriatric Depression Screening Scale (GDS-15).
[c] Low optimism is defined as a score <6 on the 4-item questionnaire (4Q) or a score <12 on the Life Orientation Test - Revised (LOT-R).
[d] Cut-off and median (Q1-Q3) per tertile.
[e] *P*-trend refers to a linear trend in regression coefficients across tertiles of intake using the median values of each tertile.
[f] Adjusted for sex, age, and family history of depression.
[g] Adjusted for BMI, education, marital status, smoking status, physical activity, energy intake, intake of saturated fat, alcohol, and fiber (*n*=636).

To assess depressive symptoms we used the self-administered GDS-15 which has a good sensitivity (0.88) and specificity (0.76) to screen for depression in an older population[21]. To assess dispositional optimism two questionnaires were used. The LOT-R assesses respondents' expectations for the future and is considered the standard psychological optimism test with good psychometric properties[22]. The other optimism test, the 4Q, was shown to represent a rather stable personality measure over time, and was predictive of subsequent depressive symptoms and cardiovascular mortality in the Zutphen Elderly Study[17,23], but has not been validated against other measures of mental well-being.

Dietary intake of the previous month was assessed by a FFQ that was based on a validated FFQ for estimating total energy and fatty acid intake[25]. A general disadvantage of FFQs is that they are memory based which could result in over- or underestimation of intake. Fish consumption may also be subject to reporting bias due to the known possible health benefits of fish. However, for ranking individuals according to their intake FFQs are suitable. Fish consumption was assessed including the frequency, amount, and type of fish, but our sample size and the range of intake were too small to separately analyze different types of fish in relation to mental well-being. We observed that associations between fish intake and mental well-being were weaker than associations between EPA+DHA and mental well-being, which supports the idea that EPA+DHA, as the major nutrient in fish, attributed mostly to the beneficial association with mental well-being.

Other observational studies showed that both depression[30] and lack of dispositional optimism are associated with unhealthy lifestyle and dietary habits[15,16]. Fish and EPA+DHA intake may be considered part of a healthy diet and lifestyle, which may explain why our associations between mental well-being and EPA+DHA intake and fish were attenuated after adjusting for many important lifestyle and dietary variables in the multivariable adjusted models. Appleton et al. concluded that depression was associated with fish intake both directly and indirectly, as a result of the absence of fish in a diet associated with depressed mood and in a lifestyle associated with depressed mood[30]. Thus, it is possible that mental well-being is more influenced by a healthy dietary pattern and lifestyle, than exclusively by fish or EPA+DHA intake. Conversely, MI patients with depression were found to be less likely to adhere to dietary and lifestyle recommendations than MI patients without depression[31]. Thus, more optimal mental well-being might lead one to use a healthy diet.

Our results, though not significant, pointed to the same direction as other studies on depression in coronary heart patients, which all showed that depression was associated with lower plasma or membrane levels of n-3 PUFA, in particular DHA[9-13]. However, one study also observed that the effect was no longer significant after adjustment for confounders, whereas only two of these studies adjusted for lifestyle confounders and none for other nutritional confounders. EPA+DHA intake is associated with blood levels of moderate strength[32]. The correlation between EPA+DHA intake and EPA+DHA concentrations in plasma

cholesteryl esters in our study was 0.37 (unpublished results). Our results are consistent with some cross-sectional studies in general populations that also found no association between intake and depression[33-35]. Yet, several other cross-sectional studies did observe an inverse association[30,36-39]. Our study population predominantly comprised men and some studies observed an association between fish and EPA+DHA intake and depressive symptoms only in women[36,37,40]. Contrarily, Astorg et al. observed stronger associations of fatty fish or n-3 PUFA intake with (recurrent) depressive episodes in men compared to women[41]. Sex may thus interact with the association between depression and EPA+DHA and fish. In a stratified analysis in male participants only (data not shown) we observed weaker associations between EPA+DHA and fish intake compared to our whole study population so a gender difference may indeed be an explanation for the fact that we did not observe a significant association in our total population.

To the best of our knowledge, there is only one other study that found an association between dispositional optimism and EPA+DHA intake[16]. In this study dispositional optimism was also measured with the 4Q and higher mean dispositional optimism scores were only observed with EPA+DHA intakes ≥400 mg/day compared with EPA+DHA intakes <400 mg/day. However, we did not observe an association of EPA+DHA intake and dispositional optimism measured with the LOT-R. These questionnaires may differ, because the 4Q also captures life engagement, vitality, motivation, and feeling a purpose in life[17], rather than generalized positive outcome expectancies that is the focus of the LOT-R[22]. Nevertheless, 46% of the participants were classified into the same tertile with both the LOT-R and 4Q and Spearman rank correlation was 0.44 indicating reasonable agreement between the two questionnaires.

The present study also has some limitations that need to be considered. First, we performed a cross-sectional analysis from which causality cannot be inferred. As pointed out by Kamphuis et al. reverse causality is an alternative explanation, because depression may induce loss of appetite, decreased food consumption, and weight loss[39]. Second, although we adjusted our analyses for the most important potential confounders residual confounding cannot be completely ruled out. Third, the differences in intakes of fish and EPA+DHA between the tertiles may have been too small to find an association. Fourth, we did not assess clinical depressive disorders through structured clinical interviews based on the Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria. Finally, selection bias could be an issue in our study since depressive subjects could be less willing to participate in a 3-year intervention study, questioning generalizability.

In conclusion, in this population of Dutch older subjects with a history of coronary heart disease there was a significant positive association between EPA+DHA intake and dispositional optimism assessed with the 4Q, but not with the LOT-R. There was also a tendency for less depressive symptoms with a higher EPA+DHA or fish intake, but after adjustment for confounders these

associations were no longer significant. We recommend performing longitudinal and additional large intervention studies in high risk populations with low EPA+DHA biochemical status and with different states of mental well-being.

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7

Effect of fish oil supplementation on quality of life in a general population of older Dutch subjects: a randomized, double-blind, placebo-controlled trial

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Abstract

Objectives To investigate the effect of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) supplementation on quality of life (QOL).

Design Randomized, double-blind, placebo-controlled trial.

Setting Independently living individuals from the general older Dutch population.

Participants Three hundred two individuals aged 65 years and older without depression or dementia.

Intervention 1,800 mg/d EPA+DHA (*n*=96), 400 mg/d EPA+DHA (*n*=100), or placebo capsules (*n*=106) for 26 weeks.

Measurements QOL was assessed using the short version of the World Health Organization QOL questionnaire (WHOQOL-BREF). The WHOQOL-BREF covers four domains: physical health, psychological health, social relationships and satisfaction with environment. The total score range is 26 to 130 with higher scores indicating a more favorable condition.

Results Mean age of the participants was 70, and 55% were male. Plasma concentrations of EPA+DHA increased 238% in the high-dose and 51% in the low-dose EPA+DHA group, reflecting excellent adherence. Median baseline total WHOQOL scores ranged from 107 to 110 in the three groups and were not significantly different from each other. After 26 weeks, the mean difference from placebo was –1.42 (95% confidence interval (CI) = –3.40; 0.57) for the high-dose and 0.02 (95% CI = –1.95; 1.99) for the low-dose fish oil group. Treatment with 1,800 mg or 400 mg EPA+DHA did not affect total QOL or any of the separate domains after 13 or 26 weeks of intervention.

Conclusion Supplementation with high or low doses of fish oil for 26 weeks did not influence the QOL of healthy older individuals.

Introduction

The World Health Organization (WHO) has defined quality of life (QOL) as an individual's perception of their position in life in the context of culture and value systems in which they live and in relation to their goals, expectations, standards and concerns[1]. It is a broad-ranging concept affected in a complex way by physical health, psychological state, level of independence, social relationships, and relationship to salient features of their environment[2]. Improving QOL is becoming an increasingly important outcome in elderly research, and for studies of health promotion, generic outcomes are even more relevant than disorder-specific outcomes.

Current evidence indicates that low intake and low levels of n-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), are associated with mental health problems, which are among the leading causes of impaired QOL in old age[3]. Because n-3 PUFAs are a major component of neural membranes and act as precursors of compounds involved in immune and inflammatory responses, it is biologically plausible that they could play a role in mental processes and thus affect QOL[4].

Two observational studies performed in the same population of New Zealand adults have assessed the association between n-3 PUFAs and QOL. The Medical Outcomes Short Form (36) Health Survey (SF-36) was used and indicated a significant positive association between fish intake and self-reported scores on the mental health part of the SF-36 in 4,644 subjects[5] and found a significant positive trend across quintiles of the proportion of EPA in serum phospholipids for self-reported scores on the physical health part, but not for the mental health part of the SF-36 in 2,793 subjects[6]. Furthermore, several observational studies relating n-3 PUFAs to mood, which is a strong determinant of impaired QOL, provide support for an association between depressed mood and fish intake or between depressed mood and n-3 PUFAs concentrations in the blood[7]. Clinical trials have mainly been conducted in patients with depressive disorders and yielded inconsistent findings for n-3 PUFA supplementation[8]. Recently, a trial in mild to moderately depressed individuals from the general population showed no effect after 3 months of supplementation with 1.5 g EPA+DHA per day[9]. Only one trial has been performed in healthy subjects (*n*=33; mean age 33), who experienced an improvement in mood during daily supplementation of 1.6 g EPA and 0.8 g DHA for 35 days[10].

Despite the availability of valid and reliable QOL instruments, they have seldom been applied in efficacy trials that address mental well-being[11], but such data are sorely needed because caregivers and professionals may wish to improve the patients' QOL, especially for patients with dementia or cognitive impairment, for which pharmacological treatment is often ineffective. A double-blind randomized placebo-controlled trial of the effect of fish oil supplementation on cognitive performance and depressive symptoms was conducted[12,13]. In this trial of 302 elderly Dutch subjects, QOL was additionally assessed using the

WHOQOL-BREF questionnaire. Effects of high and low doses of n-3 PUFA on QOL, including subdomains, are presented here.

Methods

Participants

Between November 2005 and February 2006, subjects were screened; intervention took place between February 2006 and November 2006. All subjects were aged 65 and older. The main exclusion criteria were a score greater than 16 on the Centre for Epidemiological Studies Depression Scale (CES-D)[14]; a score of less than 21 points on the Mini-Mental State Examination (MMSE)[15]; current or recent (<4 weeks) use of fish oil supplements; intake of fish more than four times per week or more than 800 mg of EPA+DHA from fish per day, as estimated from a fish consumption questionnaire; current use of pharmacological antidepressants; current use of medication for dementia; and consumption of more than four glasses of alcohol per day. Additionally, adherence to capsule use during the 2-week placebo run-in period had to be at least 80% on the basis of self-report. The Medical Ethical Committee of Wageningen University approved this study and all subjects gave written informed consent.

Study design

Primary outcomes of this trial were cognitive performance and depressive symptoms; the results, together with a detailed description of this study, have been reported elsewhere[12,13]. An independent person randomized subjects using computer-generated random numbers in stratified permuted blocks of size six. Stratification factors included age (<69 and ≥69y), sex, MMSE (<28 and ≥28), and CES-D screening test score (<5 and ≥5). Individuals received a daily dose of fish oil containing a high dose of EPA+DHA (~1,800 mg), a low dose of EPA+DHA (~400 mg), or a placebo oil (high oleic sunflower oil) for 26 weeks (Lipid Nutrition, Wormerveer, the Netherlands). The high daily dose of fish oil provided 1,093 ± 17 mg EPA and 847 ± 23 mg of DHA, which corresponds to eating approximately eight portions of fish per week. The low daily dose of fish oil provided 226 ± 3 mg EPA and 176 ± 4 mg of DHA, which is roughly equivalent to eating one to two servings of oily fish per week. The capsules with fish oil or placebo oil were indistinguishable in appearance. Adherence was judged according to counts of capsules returned and a diary in which participants registered the number of capsules that they did not consume.

Sample size calculation was based on the two primary outcomes of this study: cognitive performance (Word Learning Test: difference of four points; mean ± SD of 45 ± 8)[16] and depressive symptoms (CES-D: difference of three points; mean ± SD of 9 ± 7)[17]; a minimum sample size of 63 and 85 subjects per group, respectively, was required to detect a difference (power 80%, two-sided α=0.05). Anticipating a possible dropout rate of 15%, 100 subjects per treatment group were included. With this sample size we would have been able to detect a difference of 2.4 points on the WHOQOL questionnaire.

Assessment of QOL

In this study, the short version of the WHOQOL, the WHOQOL-BREF, was used[18]. The WHOQOL is a 100-item QOL instrument developed by the WHO to facilitate cross-cultural comparisons in QOL research[19]. The WHOQOL instruments focus on an individual's own views of their well-being. The WHOQOL-BREF was developed to enable a brief but accurate assessment of QOL in routine clinical work, epidemiological studies, and clinical trials. The questionnaire comprised 26 items, including two general items and 24 items covering four domains: physical health, psychological health, social relationships, and environment. The scores of the two general items range from 1 to 5 and the domain scores range from 7 to 35, 6 to 30, 3 to 15 and 8 to 40, respectively. The total score range of the WHOQOL-BREF is 26 to 130 with higher scores indicating a favorable condition. The reference period is the previous 2 weeks and questions are scored on a 5-point Likert scale. Studies have shown good content validity, discriminant validity and test-retest reliability[20,21]. The QOL questionnaire was self-administered and checked for completeness by a research assistant at the study center at baseline and after 13 and 26 weeks of intervention.

Other measurements

A fasting venous blood sample for determination of n-3 PUFAs was collected at baseline and after 13 and 26 weeks of intervention. N-3 PUFAs in plasma cholesteryl esters were determined as described previously[22].

Information on medical history, drug use, alcohol consumption, smoking habits, educational level, marital status and physical activity level was obtained using a questionnaire and reviewed by a research assistant for completeness. A food-frequency questionnaire was administered at screening, at baseline, and after 13 and 26 weeks of intervention to estimate fish intake in the previous 3 months. EPA+DHA intake was calculated by multiplying the frequencies of portions of fish per month from each group by a conversion factor[23].

Height was measured at baseline and weight and waist circumference were measured at each center visit in a standing position and with participants dressed in light clothing and without shoes. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, and weight was measured to the nearest 0.1 kg using a calibrated digital scale. Waist circumference was measured in duplicate to the nearest 0.1 cm at the midpoint between the lowest rib and the top of the iliac crest using a nonelastic tape.

Statistical analysis

Data analyses were performed on an intention-to-treat basis and according to a predefined data analysis plan using SPSS 12.0.1 (SPSS, Inc., Chicago, IL). A two-sided *P*-value <0.05 was considered statistically significant. Staff members and participants were blinded to treatment allocation until completion of the trial and after completion of blind data analysis.

Baseline characteristics of treatment groups were compared by one-way analysis of variance (ANOVA) or Kruskal-Wallis for continuous variables and chi-square for categorical variables. Differential changes in QOL between the three intervention groups were tested using ANOVA, and the Dunnett test was used to compare mean changes within the treatment groups with that of the placebo group. These analyses were performed for effects of fish oil treatment after 13 and after 26 weeks.

After performing the primary intention-to-treat analysis an additional per-protocol analysis, including adherent subjects only, was performed. Furthermore, some exploratory post hoc analyses were performed for subgroups of men and women separately, because based on literature, more effect could be expected in women[24-27], and in a subgroup of individuals in the tertile with the highest total WHOQOL-BREF scores at baseline.

Results

Randomization and attrition

Three hundred two subjects who fulfilled the inclusion criteria were randomized to receive 1,800 mg per day of EPA+DHA (*n*=96), 400 mg per day of EPA+DHA (*n*=100), or placebo (*n*=106). For more details on participant flow see Figure 2.1 and 4.1 in one of the previous publications on this trial[12,13]. Nine subjects did not complete the study: five stopped because of gastrointestinal complaints and two because participation became too burdensome, and two died before the end of the intervention. Nevertheless, seven dropouts visited the study center after 13 weeks, and six dropouts visited the study center after 26 weeks. Therefore, for intention-to-treat analyses, data of 300 participants were analyzed after 13 weeks and of 299 participants after 26 weeks of intervention.

Participant characteristics and compliance

The mean age of the participants was 69.8 and 55% were male. Baseline characteristics were well balanced between the three treatment groups (**Table 7.1**).

Apart from the individuals who stopped treatment prematurely, the average adherence to treatments based on counts of returned capsules was high (99%, with only 3 subjects <80%) and did not differ between the treatment groups. Compliance was confirmed by an increase in the proportions of EPA and DHA in plasma cholesteryl esters of 62% and 28% in the low-dose fish oil group (EPA from 1.3 ± 0.9 to 2.0 ± 0.9 and DHA from 0.6 ± 0.2 to 0.8 ± 0.2 g/100g fatty acids), and 305% and 94% in the high-dose fish oil group (EPA from 1.3 ± 0.7 to 5.2 ± 1.3 and DHA from 0.6 ± 0.2 to 1.2 ± 0.2 g/100g fatty acids) and by a small decrease of 2% and 3% in the placebo group (EPA from 1.3 ± 1.0 to 1.2 ± 0.9 and DHA from 0.7 ± 0.2 to 0.6 ± 0.2 g/100g fatty acids). The supplements were well tolerated; the main complaints were mild gastrointestinal discomfort, and the number of complaints was not different between the three groups. At the

Table 7.1 Characteristics of 302 subjects participating in a randomized, placebo-controlled trial according to treatment group[a]

	1,800 mg EPA+DHA (*n*=96)	400 mg EPA+DHA (*n*=100)	Placebo (*n*=106)
Age (years)	69.9 ± 3.4[b]	69.5 ± 3.2	70.1 ± 3.7
Male (%)	55	55	56
Married/ living together (%)	80	81	77
Education Low/ Middle/ High (%)	10/ 54/ 35	11/ 49/ 40	5/ 59/ 37
BMI (kg/m^2)	26.1 ± 3.0	26.2 ± 3.4	26.5 ± 3.9
Waist circumference (cm)	94.5 ± 11.4	94.2 ± 10.6	95.9 ± 12.1
Physical activity score	11.5 ± 6.4	11.1 ± 6.2	11.4 ± 6.2
Smoking behavior (%)			
Smoker/ ex-smoker/ never smoker	8/ 64/ 28	8/ 54/ 38	10/ 56/ 34
Alcohol consumers (%)	80	87	88
Median alcohol consumption (glasses/week)[c]	10 (6-14)[d]	8 (4-14)	8 (4-14)
Fish consumption (times/ month)	7 (4-9)	5 (3-9)	6 (4-8)
EPA+DHA intake (mg/day)	306 (131-592)	278 (103-487)	316 (166-584)
Plasma EPA (mass%)	1.3 ± 0.7	1.3 ± 0.9	1.3 ± 1.0
Plasma DHA (mass%)	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.2
Apolipoprotein E ε4 allele 0/ 1/ 2 (%)	68/ 29/ 3	69/ 30/ 1	71/ 26/ 2
MMSE (Mini-Mental State Examination)	28 (27-29)	28 (27-29)	28 (27-29)
CES-D (range 0-60)	5.0 (2.0-8.8)	5.0 (2.0-8.8)	6.0 (2.0-10.0)

[a] There were no significant differences between the three treatment groups, $P<0.05$ (one-way ANOVA or Kruskal Wallis for continuous variables and chi-square analysis for categorical variables).
[b] Mean ± SD (all such values).
[c] Mean consumption in consumers only.
[d] Median (Q1-Q3) (all such values).

end of the study the proportion of participants who thought they had received fish oil or placebo did not differ among the groups (P=0.15); the recognition scores do not differ from chance scores.

Quality of life

Total WHOQOL-BREF scores at baseline and after 13 weeks and after 26 weeks of intervention were comparable between the three groups, median total scores at baseline were 110.0 (Q1-Q3: 101.0-116.8), 107.0 (Q1-Q3: 99.3-114.0) and 107.0 (Q1-Q3: 100.0-113.3) in the high-dose fish oil, low-dose fish oil and placebo groups, respectively (**Table 7.2**). The scores on the four different domains of physical health, psychological health, social relationships and environment were also not significantly different from each other.

After 13 weeks and after 26 weeks of supplementation, neither of the fish oil groups showed better total WHOQOL-BREF scores or better on any of the four domains than the placebo group (**Table 7.3**). After 26 weeks, the mean difference from placebo for total WHOQOL-BREF was –1.42 (95% CI = –3.40; 0.57) in

Table 7.2 Scores for overall quality of life (QOL) and for the four separate domains of QOL studied in Dutch older adults at baseline and after 13 and 26 weeks of EPA+DHA supplementation according to treatment group: median (Q1-Q3)[a]

Quality of life (minimum-maximum)[b]		1,800 mg EPA+DHA[c]	400 mg EPA+DHA[d]	Placebo[e]
WHOQOL-BREF total (26-130)	Baseline	110.0 (101.0-116.8)	107.0 (99.3-114.0)	107.0 (100.0-113.3)
	13 weeks	111.0 (101.0-117.0)	107.0 (100.0-114.0)	108.0 (100.0-115.9)
	26 weeks	108.0 (100.0-118.0)	107.0 (101.3-114.0)	107.0 (100.0-115.0)
Domain I: Physical health (7-35)	Baseline	30.0 (27.0-33.0)	30.0 (27.0-32.0)	29.0 (26.8-31.3)
	13 weeks	31.0 (27.0-33.0)	30.0 (27.0-32.0)	30.0 (27.0-32.0)
	26 weeks	30.0 (27.0-33.0)	30.0 (28.0-32.0)	29.0 (27.0-31.0)
Domain II: Psychological health (6-30)	Baseline	24.0 (22.0-26.0)	23.0 (21.0-25.0)	24.0 (21.0-25.0)
	13 weeks	24.0 (21.0-26.0)	23.0 (21.0-25.0)	23.5 (21.3-26.0)
	26 weeks	24.0 (21.0-26.0)	23.0 (21.3-25.0)	23.0 (22.0-26.0)
Domain III: Social relationships (3-15)	Baseline	12.0 (11.0-13.0)	12.0 (11.0-13.0)	12.0 (11.0-13.0)
	13 weeks	12.0 (11.0-13.0)	12.0 (10.0-13.0)	12.0 (11.0-13.0)
	26 weeks	12.0 (11.0-13.0)	12.0 (11.0-13.0)	12.0 (11.0-13.0)
Domain IV: Environment (8-40)	Baseline	35.0 (32.0-38.0)	35.0 (32.3-36.0)	34.0 (31.0-36.0)
	13 weeks	35.0 (33.0-38.0)	34.0 (31.0-37.0)	34.0 (30.9-37.0)
	26 weeks	35.0 (31.0-37.8)	34.0 (31.3-37.0)	34.0 (31.0-37.0)

[a] No significant differences between the three treatment groups were observed, $P>0.05$ (Kruskal-Wallis).
[b] Higher scores indicate better quality of life.
[c] n=96 at baseline, n=95 after 13 weeks, and n=96 after 26 weeks.
[d] n=100 at baseline, and after 13 and 26 weeks.
[e] n=106 at baseline, n=104 after 13 weeks, and n=103 after 26 weeks.

the high-dose fish oil group and 0.02 (95% CI = –1.95; 1.99) in the low-dose fish oil group.

Per-protocol analysis, excluding the dropouts and nonadherent subjects, did not change the results. Exploratory analysis in a subgroup of 98 individuals in the tertile with the highest total WHOQOL-BREF scores at baseline (≥113) yielded similar findings. Mean differences from placebo for total WHOQOL-BREF after 26 weeks in this subgroup were –2.60 (95% CI = –5.79; 0.59) for the high-dose fish oil group (n=38) and –1.59 (95% CI = –4.95; 1.78) for the low-dose group (n=31). Exploratory analysis according to sex showed a significant change after 26 weeks in men in the high-dose fish oil group (n=53) with a difference from placebo of –3.28 (95% CI = –5.85; 0.71). After 26 weeks, a significant difference in men in the high-dose fish oil group was also observed for the domain physical health (–1.00 (95% CI = –1.95; –0.05)) and for the domain environment (–1.45 (95% CI = –2.55; –0.35)).

Table 7.3 Changes in scores for overall quality of life (QOL) and for the four separate domains of QOL studied in Dutch older adults at baseline and after 13 and 26 weeks of EPA+DHA supplementation according to treatment group: mean (95% CI)

Quality of life (minimum-maximum)[a]	Change attributed to treatment	1,800 mg EPA+DHA[b]	*P*[c]	400 mg EPA+DHA[d]	*P*
WHOQOL-BREF total (26-130)	After 13 weeks	–0.98 (2.89; 0.94)	0.42	–1.07 (–2.97; 0.82)	0.35
	After 26 weeks	–1.42 (–3.40; 0.57)	0.20	0.02 (–1.95; 1.99)	1.00
Domain I: Physical health (7-35)	After 13 weeks	–0.57 (–1.33; 0.19)	0.17	–0.57 (–1.31; 0.19)	0.17
	After 26 weeks	–0.39 (–1.26; 0.49)	0.51	0.25 (–0.61; 1.12)	0.75
Domain II: Psychological health (6-30)	After 13 weeks	–0.25 (–0.85; 0.35)	0.56	–0.09 (–0.68; 0.51)	0.93
	After 26 weeks	–0.17 (–0.78; 0.45)	0.77	0.21 (–0.40; 0.81)	0.66
Domain III: Social relationships (3-15)	After 13 weeks	–0.19 (–0.61; 0.23)	0.51	–0.33 (–0.74; 0.09)	0.15
	After 26 weeks	–0.09 (–0.51; 0.34)	0.87	–0.02 (–0.44; 0.40)	0.99
Domain IV: Environment (8-40)	After 13 weeks	0.17 (–0.70; 1.03)	0.88	0.08 (–0.77; 0.94)	0.97
	After 26 weeks	–0.67 (–1.51; 0.17)	0.14	–0.37 (–1.20; 0.46)	0.51

[a] Higher scores indicate better quality of life.
[b] *n*=96 at baseline, *n*=95 after 13 weeks, and *n*=96 after 26 weeks.
[c] 2-sided *P*-values for the difference from placebo with the Dunnett test.
[d] *n*=100 at baseline, and after 13 and 26 weeks.

Discussion

To the best of the authors' knowledge, this is the first randomized, double-blind, placebo-controlled trial to assess the effect of n-3 PUFAs supplementation on the QOL of older adults. It showed that daily supplementation with a low or a high dose of EPA+DHA for 26 weeks had no effect on QOL in healthy older subjects. Adherence was excellent and did not differ between the treatment groups, which indicated that most subjects tolerated taking six capsules daily for 26 weeks. The dropout rate was low, at 3%, and cannot therefore explain the findings.

QOL was assessed using the WHOQOL-BREF, a questionnaire that comprises physical, psychological, social, and environmental domains and studies of which have shown good internal consistency, excellent discriminant validity and good sensitivity[20,21]. Most of the questionnaires that assess QOL assess health-related QOL, whereas the WHOQOL includes a strong mental health component and emphasizes the perception of the individual. Because of the implications for an association between omega-3 PUFAs and mental well-being, the WHOQOL questionnaire was considered most appropriate for addressing the hypothesis.

Systematic qualitative and quantitative research in different cultures and populations, have generated the aspects included in the WHOQOL questionnaire

and the psychometric properties have been validated in several studies[20,21,28,29], although QOL is a subjective concept to measure because it depends on self-report. Mood state and an individual's perception of QOL are strongly correlated. Individuals with depressive symptoms (CES-D score >16) were excluded from this study, reducing the chance of measuring distorted QOL scores, because depression leads to lower QOL scores.

Although there is some evidence that fish oil may have a role as an adjuvant treatment for depression, there is little evidence to support a role in the prevention of depressive symptoms or promotion of mental health. To the best of the authors' knowledge, this study is the first to assess whether fish oil has a generic effect on the QOL of older people without depression or dementia. These are disorders that, together with coronary heart disease, constitute the main causes of impaired QOL in the Netherlands[30]. It could be that QOL of the study sample was close to optimal and that further improvement could not be achieved with fish oil supplementation. If true, it can be concluded that low or high doses of fish oil are of little added value to mental health in a generally healthy population, although it might also be that the duration of our study was too short to observe changes, although no trend was observed either.

The observation of a significant decrease of QOL measures in men in the high-dose fish oil group is in contrast with what would be expected. Several previous studies observed an association between high fish intake and depression in women but not in men[25-27], although QOL is a broader concept than depression, and these studies were all observational studies; sex effects have not been shown in trials before. Moreover, it could also be a chance finding because multiple comparisons were made in the current study.

The results are not consistent with two observational studies performed in a population of healthy New Zealand adults that showed a beneficial relationship between n-3 PUFAs and QOL[5,6], and also with most randomized controlled trials that investigated the association between n-3 PUFAs and depressed mood[8], but most of these trials were not performed in healthy subjects but in patients with depressive disorders with n-3 PUFA treatment on top of drug treatment. Of these only one examined the effect of n-3 PUFAs supplementation in 33 healthy subjects without depression (mean age 33) who received a daily dose of 1.6 g of EPA and 0.8 g of DHA for 35 days[10]. The current study, with a larger number of subjects, who were older and supplemented for a longer period, did not confirm these findings, although in the previous study mental well-being was measured using a questionnaire that focuses only on mood (Profile of Mood States (POMS)), whereas the questionnaire used in the current study covers more aspects affecting QOL.

Based on this randomized double-blind placebo-controlled trial, it can be concluded that supplementation with high or low doses of fish oil is unlikely to influence the QOL of healthy older individuals. More studies investigating the effects of n-3 PUFAs on QOL are warranted, especially in subjects with impaired QOL.

Acknowledgements

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8

Fish oil supplementation induces anti-inflammatory gene expression profiles in human blood mononuclear cells

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Abstract

Background Polyunsaturated fatty acids (PUFAs) can have beneficial effects on human immune cells, such as peripheral blood mononuclear cells (PBMCs). However, the mechanisms of action of PUFAs on immune cells are still largely unknown.

Objective The objective was to examine the effects of supplementation with the PUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on whole-genome PBMC gene expression profiles, in healthy Dutch elderly participating in a double-blind trial, by using whole-genome transcriptomics analysis.

Design The subjects were randomly allocated to one of three groups: 1) consumption of 1.8 g EPA+DHA/d (*n*=36), 2) consumption of 0.4 g EPA+DHA/d (*n*=37), or 3) consumption of 4.0 g high-oleic acid sunflower oil (HOSF)/d (*n*=38). All supplements were given in capsules. Before and after 26 weeks of intervention, blood samples were collected. Microarray analysis was performed on PBMC RNA from 23 subjects who received 1.8 g EPA+DHA/d and 25 subjects who received HOSF capsules. Quantitative real-time polymerase chain reaction was performed on all 111 subjects.

Results A high EPA+DHA intake changed the expression of 1040 genes, whereas HOSF intake changed the expression of only 298 genes. EPA+DHA intake resulted in decreased expression of genes involved in inflammatory- and atherogenic-related pathways, such as nuclear transcription factor κB signaling, eicosanoid synthesis, scavenger receptor activity, adipogenesis, and hypoxia signaling.

Conclusion These results are the first to show that intake of EPA+DHA for 26 weeks can alter the gene expression profiles of PBMCs to a more antiinflammatory and antiatherogenic status.

Introduction

The beneficial effects of long chain n-3 poly-unsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), on inflammation[1] and cardiovascular diseases[2] are generally accepted. Immune cells, such as peripheral blood mononuclear cells (PBMCs), play a vital role in inflammation and the development of cardiovascular diseases. However, the cellular mechanisms of action of PUFAs on immune cells are not completely understood[3]. It is recognized that PUFAs can modulate cellular function through changes in gene expression, eg, via binding and subsequent activation of peroxisome proliferator-activated receptors (PPARs)[4]. Activation of these nuclear receptors is known to up- and down-regulate the expression of genes involved in lipid metabolism and inflammation, respectively. Moreover, PUFAs, such as DHA and EPA are stronger natural ligands for PPARs than are monounsaturated or saturated fatty acids[5]. We recently showed that PPARα, one of the subtypes of PPARs, has a functional role in human PBMCs[6].

Various long-term intervention trials have been performed to elucidate the mode of action of PUFAs, such as EPA and DHA, in human immune cells[7]; however, most of these trials only studied the ex vivo immunological function of these cells. To obtain a more comprehensive overview of the processes that are modulated by EPA and DHA in immune cells such a PBMCs, in vivo, a whole-genome transcriptomic analysis would be more valuable. In a previous study we showed that the effects of endogenous fatty acids on human PBMCs can be elucidated by using a genomics approach[8]. However, the effects of the long-term intake of fatty acids on PBMC gene expression profiles have not yet been studied. Therefore, we examined the effects of EPA+DHA supplementation for 26 weeks on whole-genome PBMC gene expression profiles in a Dutch elderly population and compared the effects with those of control supplementation with high-oleic acid sunflower oil (HOSF).

Subjects and methods

Subjects

Three hundred fifty-one subjects ≥65 y were screened, of whom 189 were men and 162 were women. Three hundred two subjects (167 men and 135 women) were included in the study, and detailed baseline characteristics of these participants were described elsewhere[9]. From these participants, 111 subjects were randomly included in the present study (**Figure 8.1**). The participants were recruited according to the following exclusion criteria: current or recent (<4 weeks) use of fish-oil supplements or intake of fish >4 times/week or >800 mg EPA+DHA/d from fish as estimated by using a fish-consumption questionnaire, serious liver disease, consumption >4 glasses of alcohol-containing beverages per day, unable to participate as judged by the responsible medical physician, allergy to fish or fish oil, swallowing problems, or participation in another clinical trial <2 mo before the start of the trial or at the same time. Cognitive exclusion criteria were also used, as described elsewhere[9]. Additionally, compliance with

capsule use during a 2-week placebo run-in period had to be ≥80% on the basis of self-report. All subjects gave written informed consent to participate in the study, and the study protocol was approved by the Medical Ethical Committee of Wageningen University, the Netherlands.

Study design

The subjects were randomly allocated to receive a daily dose of fish oil containing either a low dose of EPA+DHA (0.4 g; *n*=36), a high dose of EPA+DHA (1.8 g; *n*=37), or HOSF (*n*=38) for a period of 26 weeks (Lipid Nutrition/ Loders Croklaan, Wormerveer, the Netherlands) (**Figure 8.1**). The oils were administered in six soft gelatin capsules daily, each of which contained 900 mg oil and 2.7 mg tocopherol as antioxidant (Banner Pharmacaps Europe BV, Tilburg, the Netherlands). The fatty acid composition of the capsules was measured in 20 capsules taken at regular times during the study.

Blood sampling and PBMC isolation

Fasting venous blood samples were collected at baseline and after 26 weeks of intervention. A blood sample for measurement of n-3 PUFAs was collected into 10-mL EDTA-Vacutainers (Becton Dickinson, Breda, the Netherlands), immediately placed on ice, centrifuged at 2,000 x g at a temperature of 4°C and then stored at –80°C until laboratory analyses. N-3 PUFAs in plasma

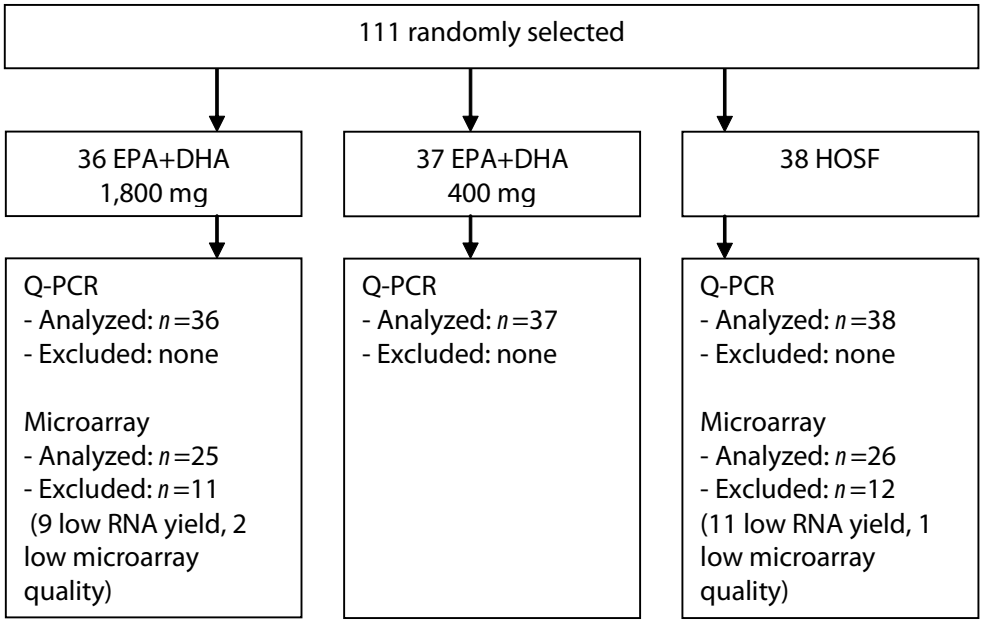

Figure 8.1 Overview of the subgroup used for the present study. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Q-PCR, quantitative real-time polymerase chain reaction.

cholesteryl esters were measured as described previously[10]. Plasma free fatty acids and triglycerides were measured by gas–liquid chromatography and C-reactive protein (CRP) concentrations were determined from measurements of high-sensitivity CRP (hsCRP). For PBMC isolation, 4 mL blood was collected into Becton Dickinson Vacutainer Cell Preparation Tubes with sodium citrate. PBMCs were isolated immediately after blood collection according to the manufacturer's instructions.

Total RNA isolation

Trained research nurses performed baseline examinations at the hospital or at tPBMC RNA was isolated from all PBMC samples by using a Qiagen RNeasy Micro kit (Qiagen, Venlo, the Netherlands). The RNA yield was quantified with a Nanodrop ND 1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and integrity was measured with an Agilent 2100 Bioanalyzer with RNA 6000 Nano chips (Agilent Technologies, South Queensferry, United Kingdom).

Microarray processing

PBMC samples from 77 subjects yielded enough RNA to perform microarray analysis. Deblinding showed that 25 subjects were in the high EPA+DHA group, 26 in the low EPA+DHA group, and 26 in the HOSF group. Microarray analysis was performed on baseline samples and on samples after 26 weeks of intervention in the high EPA+DHA group and the HOSF group. Total RNA from the PBMCs of these 51 subjects was labeled using a one-cycle cDNA labeling kit (MessageAmpTM II-Biotin Enhanced Kit; Ambion Inc, Nieuwekerk a/d IJssel, the Netherlands) and hybridized to human whole-genome NuGO GeneChip arrays encoding 17,699 genes, designed by the European Nutrigenomics Organisation and manufactured by Affymetrix (Affymetrix Inc, Santa Clara, CA). Sample labeling, hybridization to chips and image scanning were performed according to the manufacturer's GeneChip Expression Analysis Technical Manual (Affymetrix).

Microarray analysis

Microarrays from three subjects, two from the high EPA+DHA group, and one from the HOSF group were excluded because they did not fulfill our quality-control criteria. Microarrays were analyzed by using the reorganized oligonucleotide probes as described by Dai et al.[11]. Expression values were calculated by using the Robust Multichip Average method. The Robust Multichip Average signal value estimates are based on a robust average of background-corrected perfect match intensities, and normalization was performed using quantile normalization[12]. Only genes with normalized signals >20 on ≥10 arrays were defined as "expressed" and selected for further analysis. Genes were defined as "changed" when comparison of the normalized signal intensities showed a false discovery rate Q value[13] <0.05 in a 2-tailed paired *t* test with Bayesian correction (Limma)[14]. Pathway analysis was performed by using GenMAPP 2.1

(www.genmapp.org), Ingenuity Pathway Analysis 5.5 (www.ingenuity.com), and Gene Set Enrichment Analysis 2.0.1 (www.broad.mit.edu/gsea/)[15]. Pathways were defined as significantly changed when $P<0.05$ (Ingenuity Pathway Analysis and Gene Set Enrichment Analysis) or the Z score was >1 (GenMAPP).

cDNA synthesis and real-time polymerase chain reaction

RNA from all subjects was reverse transcribed with use of a cDNA synthesis kit (Promega, Leiden, the Netherlands). Standard quantitative real-time polymerase chain reaction (Q-PCR) was performed with use of Platinum Taq DNA polymerase and SYBR Green on an iCycler PCR machine (Bio-Rad Laboratories BV, Veenendaal, the Netherlands) and duplicated at least twice. Primer sequences used in the PCRs were chosen based on the sequences available in PRIMERBANK (http://pga.mgh.harvard.edu/ primerbank/index.html). Q-PCR data were normalized by measuring cycle threshold ratios between candidate genes and a housekeeping gene, human ribosomal protein LP0.

Statistical analyses

Differences between end and baseline values of plasma metabolites and Q-PCR results were tested by using a paired *t* test. Statistical significance was accepted at $P<0.05$. Differences between groups were tested by using a 2-factor ANOVA with a Tukey honestly significant difference post hoc test to correct for multiple testing of 3 treatment groups with SPSS software (version 12.0.1; SPSS Inc, Chicago, IL). Statistical significance was accepted at $P<0.05$.

Results

Subject characteristics

Of the 302 participants enrolled in the study, blood samples were randomly collected from 111 healthy men and woman varying in aged 66-80 y. A subset of these samples was used to perform microarray hybridizations (**Figure 8.1**). The baseline characteristics of the participants are shown in **Table 8.1**. No significant differences were observed between the intervention groups, both for the whole group and for the subgroup in which the microarrays were performed. To confirm the amount of the various fatty acids the participants consumed daily, the fatty acid composition of the capsules was measured (**Table 8.2**). The high-dose EPA+DHA supplement provided 1,093 ± 17 mg EPA (mean ± SD) and 847 ± 23 mg DHA daily, and the low-dose EPA+DHA supplement provided 226 ± 3 mg EPA and 176 ± 4 mg of DHA daily.

Plasma measurements

To check individual compliance with intake, the plasma fatty acid composition was determined in cholesteryl esters at baseline and after 26 weeks (**Figure 8.2, A and B**). Both EPA and DHA were significantly different ($P<0.01$) between the three intervention groups. Mean plasma EPA concentrations increased by 3.43%

Table 8.1 Baseline characteristics of subjects in the three groups whose peripheral blood mononuclear cells underwent microarray analysis or quantitative real-time polymerase chain reaction (Q-PCR)[a]

	HOSF group		Low EPA+DHA group	High EPA+DHA group	
	Q-PCR	Microarray	Q-PCR	Q-PCR	Microarray
Subjects (*n*)	38	25	37	36	23
Sex (M/F)	25/13	15/10	20/17	21/15	15/8
Age (y)	70.9 (66-80)	70.4 (67-77)	70.5 (66-79)	70.3 (67-76)	69.9 (67-76)
Smokers (*n*)	3	2	4	1	0
Weight (kg)	79.1 (59.1-108.6)	81.1 (59.1-109.5)	75.3 (47.6-101.8)	76.6 (60.1-106.7)	78.1 (60.1-106.7)
Height (m)	1.73 (1.57-1.87)	1.72 (1.57-1.87)	1.70 (1.53-1.87)	1.71 (1.58-1.86)	1.72 (1.58-1.86)
BMI (kg/m^2)	26.5 (20.2-37.2)	27.6 (20.2-42.3)	25.8 (19.9-34.9)	26.2 (21.12-33.6)	26.5 (21.7-33.6)
Triglycerides (mmol/L)	1.18 (0.4-2.7)	1.20 (0.5-2.7)	1.17 (0.2-3.8)	1.14 (0.4-3.3)	1.02 (0.4-2.0)
FFAs (mmol/L)	0.34 (0.06-0.98)	0.36 (0.06-0.98)	0.36 (0.08-0.99)	0.38 (0.12-0.71)	0.35 (0.12-0.59)
EPA (% by wt)	1.30 (0.4-8.5)	1.38 (0.4-8.5)	1.43 (0.4-5.0)	1.26 (0.4-4.4)	1.44 (0.6-4.4)
DHA (% by wt)	0.64 (0.4-1.3)	0.63 (0.4-1.3)	0.66 (0.2-1.6)	0.62 (0.3-1.1)	0.65 (0.3-1.1)
hsCRP (mg/L)	1.99 (0.1-14.7)	2.07 (0.1-14.7)	2.91 (0.1-21.0)	2.86 (0.1-10.5)	2.85 (0.1-10.5)

[a] Ranges in parentheses. EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FFA, free fatty acids; HOSF, high-oleic sunflower oil; hsCRP, high-sensitivity C-reactive protein. None of the baseline characteristics differed significantly between groups (ANOVA).

Table 8.2 Fatty acid composition of the capsules for the three groups[a]

	High EPA+DHA	Low EPA+DHA	HOSF
SFAs	22.08	10.40	8.46
MUFAs	14.73	67.97	80.53
18:1n-9 (oleic acid)	4.66	64.38	79.63
PUFAs	56.05	20.23	11.02
c18:2n-6 (linoleic acid)	0.74	9.13	10.81
c20:5n-3 (EPA)	21.40	4.49	0.00
c22:6n-3 (DHA)	16.44	3.46	0.00

[a] SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HOSF, high-oleic sunflower oil.

by wt (95% CI: 2.93; 3.94) from a mean baseline concentration of 1.26% by wt in the high-dose EPA+DHA group and by 0.56% by wt (95% CI: 0.33; 0.80), from a baseline concentration of 1.43% by wt in the low-dose EPA+DHA group. The mean plasma DHA concentration increased by 0.54% by wt (95% CI: 0.47; 0.62) and 0.13% by wt (95% CI: 0.08; 0.18) from mean baseline concentrations of 0.62% by wt and 0.66% by wt in the high- and low-dose EPA+DHA groups,

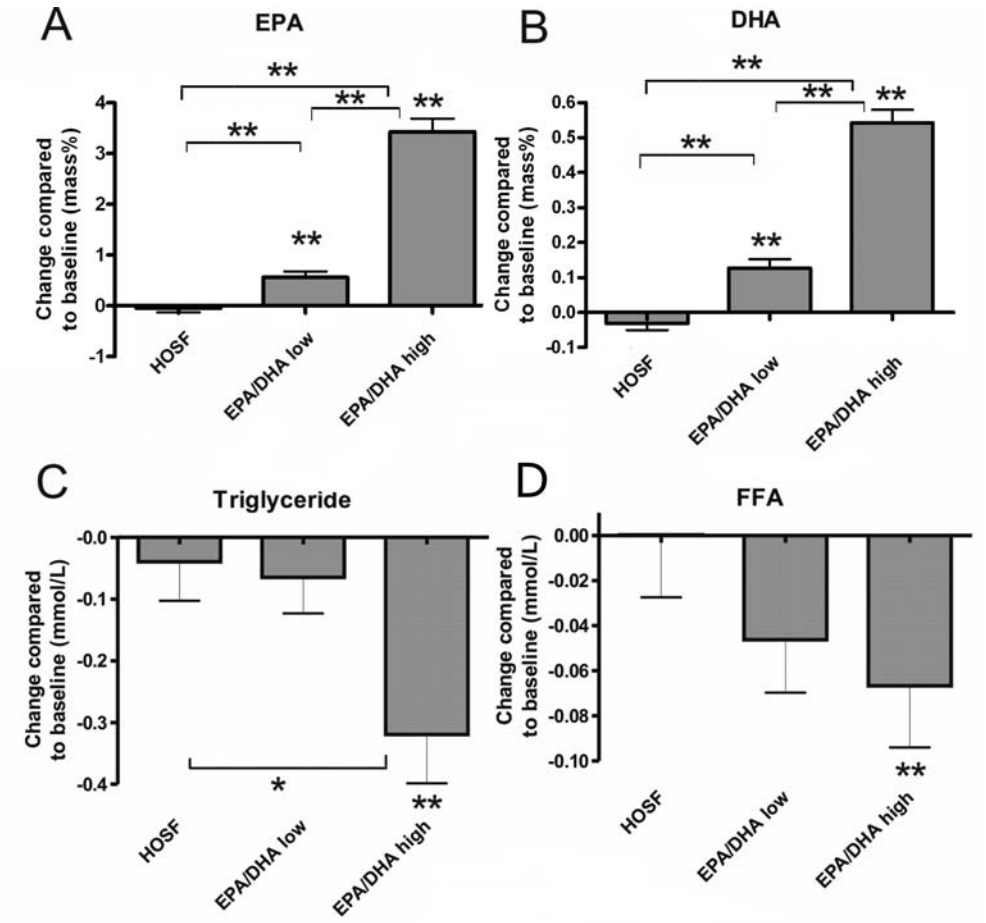

Figure 8.2 Mean (±SEM) changes from baseline in plasma lipid metabolites after intake of high-oleic sunflower oil (HOSF; *n*=38), low-dose eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) (*n*=37), or high-dose EPA+DHA (*n*=36) capsules for 26 weeks in all 111 participants. A: Plasma cholesterylesters of EPA B: plasma cholesterylesters of DHA. C: plasma triglyceride concentrations D: plasma total free fatty acid (FFA) concentrations. Brackets indicate differences between groups as determined with a 2-factor ANOVA with Tukey honestly significant difference correction (*P<0.05; ** P<0.01). Differences within groups were determined with a paired *t* test (** P<0.01). Note that the y axes of the graphs differ.

respectively. No significant changes were observed in the group that consumed the HOSF capsules: EPA decreased by 0.05% by wt (95% CI: –0.21; 0.12; baseline: 1.30% by wt), and DHA decreased by 0.03% by wt (95% CI: –0.07; 0.01; baseline: 0.64% by wt). Plasma triglyceride concentrations decreased significantly after 26 weeks in the high-dose EPA+DHA group (–0.32 mmol/L; 95% CI: –0.47; –0.16), but not in the low-dose EPA+DHA (–0.06 mmol/L; 95% CI: –0.18; 0.05) or HOSF (–0.04 mmol/L; 95% CI: –0.17; 0.09) group (**Figure 8.2C**). Plasma free fatty acids (FFAs) also decreased significantly after 26 weeks in the high-dose EPA+DHA group (–0.07 mmol/L; 95% CI: –0.12; –0.01), but not in HOSF group (–0.02 mmol/L; 95% CI: –0.06; 0.02). FFAs in the low-dose EPA+DHA group showed a tendency to decrease, although this was not statistically significant (*P*=0.06) (**Figure 8.2D**). Plasma CRP concentrations did not change significantly in any group. The mean decrease in the high-dose EPA+DHA group was –1.10 mg/L (95% CI: –2.68; 0.48), in the low-dose EPA+DHA group was –0.76 mg/L (95% CI: –1.72; 0.20), and in the HOSF group was -0.82 mg/L (95% CI: –4.29; 2.64).

Microarray analysis

Microarray hybridization was performed on PBMC RNA that was collected at baseline and after 26 weeks of supplementation from 25 subjects in the high EPA+DHA group and from 26 subjects from the HOSF group. Two arrays in the high-dose EPA+DHA group and one array in the HOSF group did not pass the quality-control criteria. Changes in gene expression were determined by comparing the microarray results of the samples after 26 weeks of intervention with the baseline samples, for both intervention groups. From the 17,699 genes present on the microarray, 12,256 genes were defined as expressed in PBMCs (**Figure 8.3**). Consumption of 1.8 g of EPA+DHA/d for 26 weeks resulted in gene expression changes of 1,040 genes, whereas consumption of the HOSF capsules resulted in expression changes of 298 genes (**Figure 8.3**). Of these genes, 140 were overlapping between the groups, which resulted in 900 uniquely changed genes in the EPA+DHA group. Except for one gene, the direction of change of the overlapping genes was the same in the EPA+DHA and the HOSF groups (data not shown).

Pathway analysis

To determine the role of the genes that changed with EPA+DHA supplementation, we performed pathway analysis. GenMAPP analysis showed that supplementation with a high dose of EPA+DHA for 26 weeks significantly decreased the expression of genes involved in inflammatory pathways, such as eicosanoid synthesis, interleukin signaling and MAP kinase signaling (**Figure 8.4**). In addition, a decreased expression of genes involved in pathways related to atherosclerotic processes, such as cell adhesion, scavenger receptor activity, and adipogenesis was observed. Ingenuity pathway analysis and Gene Set Enrichment Analysis showed similar decreases in inflammatory signaling pathways, such as eicosanoid metabolism and interleukin-6 and MAP kinase signaling, with additional

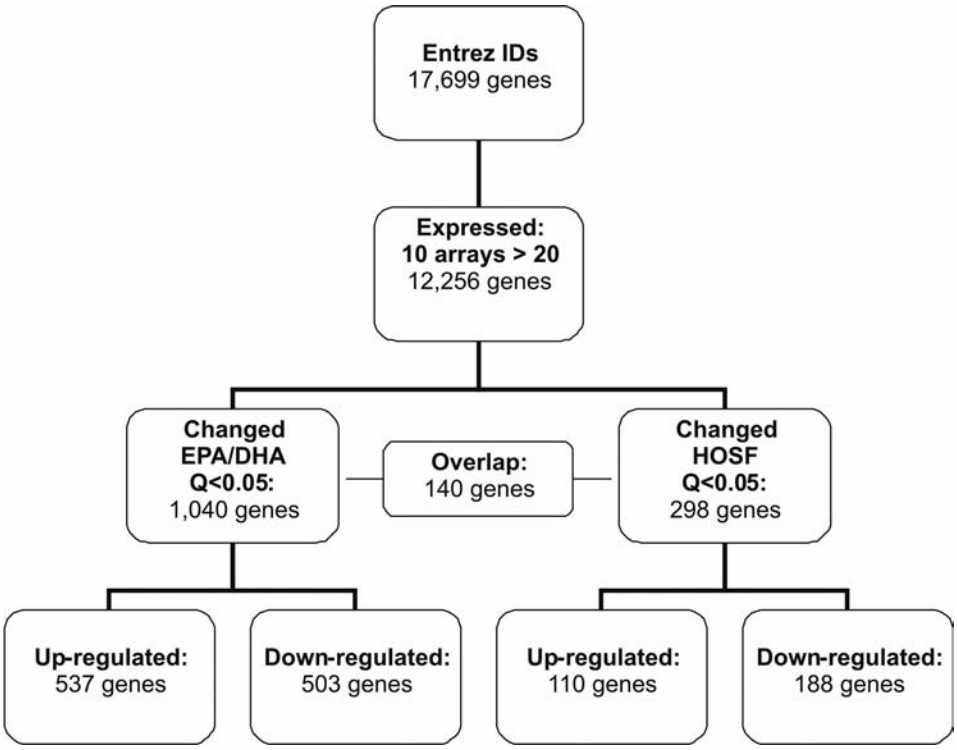

Figure 8.3 Flow chart of gene selection and number of genes changed in the microarray analysis. Q, false discovery rate value. HOSF, high-oleic sunflower oil; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

signaling routes such as nuclear transcription factor κB (NF- κB) and Toll-like receptor signaling. These programs also showed a decrease in oxidative stress, cell adhesion, PPAR signaling, LXR/RXR (liver X receptor/retinoid X receptor) activation, and hypoxia signaling in the cardiovascular system, which includes hypoxia initiation factor 1α signaling (data not shown).

In addition to the genes present in the above-described pathways, several other genes involved in similar processes, but not present in the pathways, were down-regulated (**Figure 8.5**). A list of inflammatory-related genes, such as NF-κB target genes, oncostatin M (*OSM*), Ig-like receptors, and interleukin receptors, that were down-regulated in the high-dose EPA+DHA group are shown in **Figure 8.5**. Several plaque stability-related metallopeptidases, which also decreased with EPA+DHA supplementation, are also listed in **Figure 8.5**.

Interestingly, pathway analysis of genes changed in the HOSF group also showed down-regulation of genes involved in inflammation and cell adhesion (**Figure 8.4**). However, the expression of fewer genes was changed with HOSF intake; consequently, fewer genes were observed in the changed pathways.

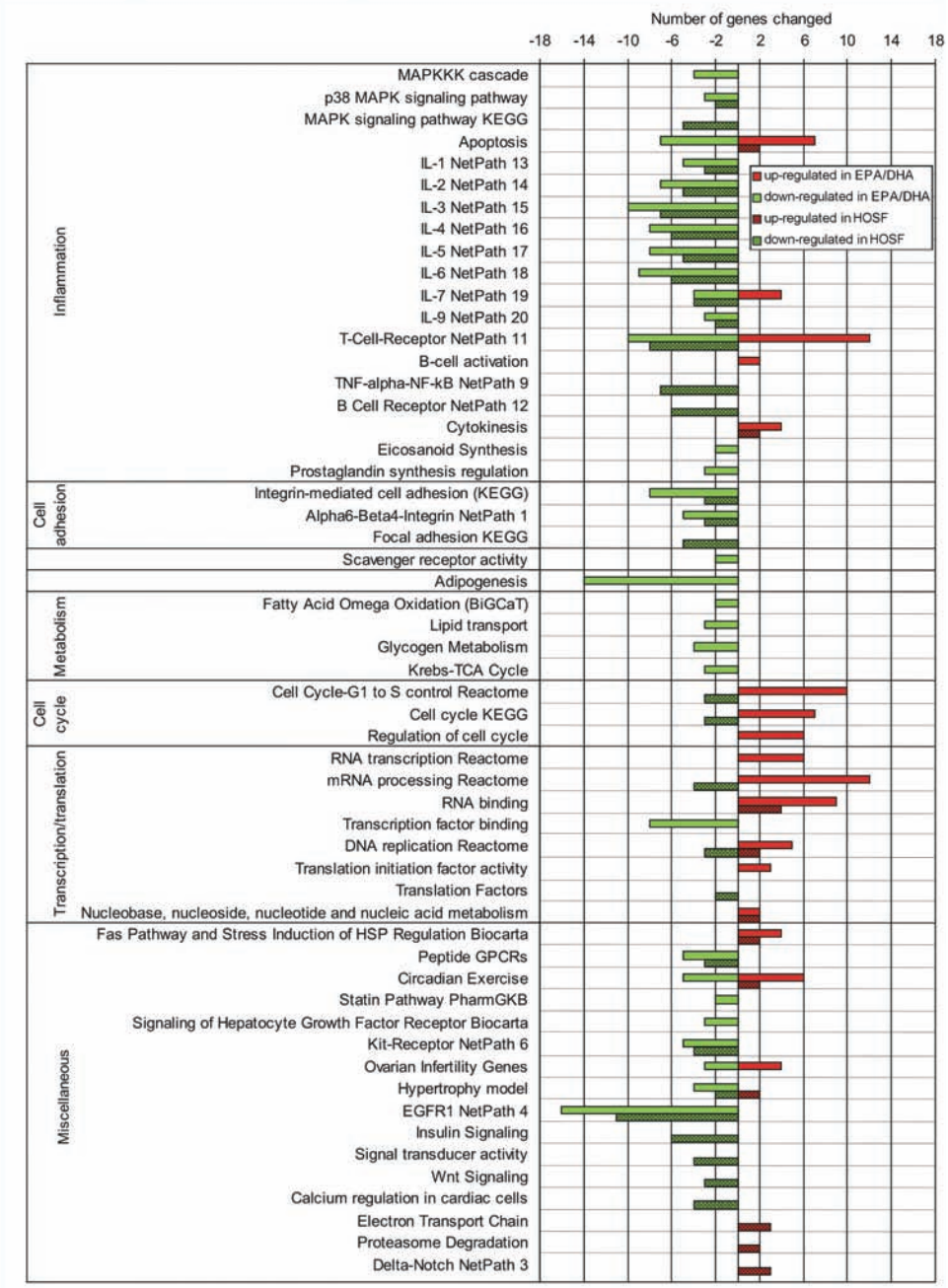

Figure 8.4 Ranking of differentially expressed pathways in peripheral blood mononuclear cells (PBMCs) after 26 weeks of supplementation with high-dose eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) or high-oleic sunflower oil (HOSF) capsules in elderly individuals. Pathway analysis was performed with the program GenMAPP (www.genmapp.org). The pathways included had a Z score of ≥1 and had ≥2 genes changed.

Additionally, we studied the expression changes of previously described PPARα target genes in PBMCs[6] and observed that 14 of these 106 PPAR response element-containing PPARα target genes were down-regulated, and two were up-regulated (**Table 8.3**).

To determine whether a low dose of 0.4 g EPA+DHA/d can induce gene expression changes similar to those seen with a high dose of 1.8 g EPA+DHA/d, 6 genes selected from several changed pathways were analyzed with Q-PCR in the whole study population of 111 individuals (**Figure 8.6**). Q-PCR of *CD36*, *PDK4*, *LTA4H*, *ADFP*, *CD14* and *HIF1α* showed that consumption of 0.4 g EPA+DHA/d for 26 weeks also resulted in a down-regulation of the expression of these genes and that the effects were intermediate between the effect of the high-dose EPA+DHA group and the HOSF group. These data also confirm the microarray results, as reflected by the same direction of gene expression changes.

Discussion

In this study we assessed changes in PBMC gene expression profiles after fish-oil or control-oil supplementation in a 26-week double-blind and randomized intervention trial in an elderly population. Supplementation of 1.8 g EPA+DHA/d, the equivalent of the consumption of 10 portions of fish weekly, resulted in antiinflammatory- and antiatherogenic-like changes in PBMC gene expression profiles. In addition, supplementation with 0.4 g EPA+DHA/d, the equivalent of the consumption of two portions of fish weekly, which approaches the average intake of EPA+DHA in various countries around the world[16], resulted in similar (but lower) gene expression changes.

The effects of fish oil on health have been extensively studied[17-19]. However, the effect of fish-oil supplementation on whole-genome gene expression profiles in human PBMCs has not been examined before. The antiinflammatory-like gene expression profile observed in the present study is mainly characterized by a decreased expression of inflammatory genes, including several NF-κB target genes, proinflammatory cytokines, and genes involved in eicosanoid synthesis. Several other intervention studies in healthy subjects with varying doses of n-3 fatty acids showed a reduction in cytokine formation after ex vivo stimulation of mononuclear cells[20,21]. The decrease in eicosanoid formation we observed was characterized by a down-regulation in the gene expression of enzymes involved in eicosanoid synthesis, such as LTA4H and ALOX-5. It is known that n-3 PUFA supplementation can result in a decrease in proinflammatory arachidonic acid-derived eicosanoids[1]. For example, various ex vivo stimulation studies with inflammatory cells in healthy volunteers showed that supplementation with fish oil or DHA for 4 to 17 weeks resulted in a decreased production of eicosanoids[1]. In addition, intake of n-3 fatty acids for 6 weeks reduced the urinary excretion of leukotriene E4[22]. Hence, our observations on PBMC gene expression are in line with previously observed n-3 PUFA-induced effects after ex vivo stimulation. The subjects who consumed the HOSF capsules showed a similar, albeit less

Table 8.3 Previously described PPARα target genes in PBMCs[6] found to be changed upon high EPA+DHA supplementation. FC, fold change; FDR Q, false discovery rate Q value[a]

Gene description	Gene name	Entrez ID	Mean FC	FDR Q
Heparin-binding EGF-like growth factor	HBEGF	1839	–1.40	0.021
Pyruvate dehydrogenase kinase isozyme 4	PDK4	5166	–1.25	0.027
Zinc finger E-box-binding homeobox 2	ZEB2	9839	–1.21	0.004
Vascular endothelial growth factor A	VEGFA	7422	–1.19	0.024
Small inducible cytokine B16 precursor	CXCL16	58191	–1.17	0.010
Transcription regulator protein BACH1	BACH1	571	–1.15	0.003
Microphthalmia-associated transcr. factor	MITF	4286	–1.15	0.021
Myeloid-assoc. differentiation marker	MYADM	91663	–1.14	0.003
Platelet glycoprotein 4	CD36	948	–1.13	0.049
MARVEL domain containing 1	MARVELD	83742	–1.12	0.027
TNF receptor superfamily member 8	TNFRSF8	943	–1.11	0.045
Dihydropyrimidinase-related protein 2	DPYSL2	1808	–1.10	0.026
Adipose differentiation-related protein	ADFP	123	–1.10	0.031
Suppressor of tumorigenicity protein 14	ST14	6768	–1.09	0.037
Uncharacterized protein C15orf17	C15orf17	57184	1.11	0.043
Protein Chibby	CBY1	25776	1.11	0.036

[a] SFC, fold change; FDR Q, false discovery rate Q value; EGF, endothelial growth factor; TNF, tumor necrosis factor.

pronounced, effect on some of the inflammatory pathways changed by EPA+DHA supplementation. The overlapping genes were almost all changed in the same direction, which suggested that some of the effects on inflammation, although clearly less apparent, were comparable with the EPA+DHA intervention. Beneficial effects of MUFAs on inflammation have been reported, but only limited data are available[23]. The antiatherogenic gene expression profile that we observed in this study after EPA+DHA intake is characterized by a reduced expression of genes that play a role in several processes known to be involved in atherosclerotic plaque formation. PBMC subpopulations are involved in these processes, which include adhesion, infiltration and subsequent foam cell formation[24]. We found an EPA+DHA-induced decrease in the expression of genes involved in integrin-mediated cell adhesion and foam cell formation. An n-3 PUFA-induced decrease in the expression of monocyte and macrophage adhesion molecules was previously reported, but only in animal and in vitro studies[25,26]. A reduction in foam cell formation is characterized by a lower gene expression of the scavenger receptors CD36 and the LDL receptors and a decrease in the expression of genes involved in lipid accumulation, such as adipose differentiation-related protein. It has been shown that incubation of monocytes with n-3 PUFA in vitro can reduce CD36 mRNA and protein expression[27]. Incubation of macrophages with PUFA-enriched chylomicron remnant-like particles in vitro resulted in reduced lipid accumulation in these cells[28]. The reduced gene expression of scavenger receptors and of adipose differentiation-related protein that we observed in our in vivo study are in line

Process	Gene name	Description	Entrez ID	Mean FC EPADHA	EPADHA: q-value	MUFA: q-value
Inflammation						
inflammatory signaling						
	CEBPB	CCAAT/enhancer binding protein (C/EBP), beta	1051	-1.11		0.24488
	DUSP1	dual specificity phosphatase 1	1843	-1.15		0.66208
	DUSP2	dual specificity phosphatase 2	1844	-1.50		
	MKNK1	MAP kinase interacting serine/threonine kinase 1	8569	-1.06		0.12427
	OSM	oncostatin M	5008	-1.24		
	IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	3572	-1.20		0.41624
	IL13RA1	interleukin 13 receptor, alpha 1	3597	-1.17		
	NFIL3	nuclear factor, interleukin 3 regulated	4783	-1.25		0.17716
	ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	2114	-1.16		0.14611
	EIF4EBP1	eukaryotic translation initiation factor 4E binding protein 1	1978	-1.12		0.17647
	H3F3B	H3 histone, family 3B (H3.3B)	3021	-1.09		0.10052
	LILRB3	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3	11025	-1.11		0.19059
	LILRA2	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	11027	-1.15		
	LILRA5	leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 5	353514	-1.32		0.17412
	C5AR1	complement component 5a receptor 1	728	-1.19		0.13402
	FADD	Fas (TNFRSF6)-associated via death domain	8772	1.15		
NF-kB signaling						
	IRAK3	interleukin-1 receptor-associated kinase 3	11213	-1.32		
	IRAK1	interleukin-1 receptor-associated kinase 1	3654	-1.09		
	MAP3K3	mitogen-activated protein kinase kinase kinase 3	4215	-1.08		
	GSK3B	glycogen synthase kinase 3 beta	2932	-1.14		
	TNFRSF1B	tumor necrosis factor receptor superfamily, member 1B	7133	-1.07		
	TNFAIP3	tumor necrosis factor, alpha-induced protein 3	7128	-1.34		
	PRKACB	protein kinase, cAMP-dependent, catalytic, beta	5567	1.08		0.12618
	PIK3C2B	phosphoinositide-3-kinase, class 2, beta polypeptide	5287	1.08		0.57405
	LTA	lymphotoxin alpha (TNF superfamily, member 1)	4049	1.19		0.20133
eicosanoid synthesis						
	LTA4H	leukotriene A4 hydrolase	4048	-1.06		0.87139
	ALOX5	arachidonate 5-lipoxygenase	240	-1.12		0.15431
	GPX3*	glutathione peroxidase 3 (plasma)	2878	-1.26		0.21764
	TBXAS1	thromboxane A synthase 1 (platelet, cytochrome P450, family 5, subfamily A)	6916	-1.12		0.42338
	CBR3	carbonyl reductase 3	874	1.15		0.62017
Toll like receptor signaling						
	TLR8	toll-like receptor 8	51311	-1.11		0.36478
	TLR5	toll-like receptor 5	7100	-1.18		0.11129
	CD14	CD14 molecule	929	-1.12		
Cell adhesion						
integrin mediated cell adhesion						
	ITGAM	integrin, alpha M (complement component 3 receptor 3 subunit)	3684	-1.08		0.14636
	ITGA7	integrin, alpha 7	3679	-1.06		0.44713
	GRB2	growth factor receptor-bound protein 2	2885	-1.07		
	GAB2	GRB2-associated binding protein 2	9846	-1.20		
	VAV2	vav 2 guanine nucleotide exchange factor	7410	-1.07		
	VAV3	vav 3 guanine nucleotide exchange factor	10451	-1.09		0.14406
	PAK1	p21/Cdc42/Rac1-activated kinase 1 (STE20 homolog, yeast)	5058	-1.13		0.15681
	CD2	CD2 molecule	914	1.11		0.59631
chemokine signaling						
	CXCL16*	chemokine (C-X-C motif) ligand 16	58191	-1.17		0.25727
	CXCR4	chemokine (C-X-C motif) receptor 4	7852	-1.28		
	CCR3	chemokine (C-C motif) receptor 3	1232	-1.15		0.36681
	PLCB1	phospholipase C, beta 1 (phosphoinositide-specific)	23236	-1.19		0.29959
	PLCL2	phospholipase C-like 2	23228	-1.07		
	CXCL5	chemokine (C-X-C motif) ligand 5	6374	1.18		0.43542
	CX3CR1	chemokine (C-X3-C motif) receptor 1	1524	1.14		
	CCR5	chemokine (C-C motif) receptor 5	1234	1.24		0.20481
	PF4	platelet factor 4 (chemokine (C-X-C motif) ligand 4)	5196	1.12		0.63284
	PF4V1	platelet factor 4 variant 1	5197	1.21		0.54134
	CXCR3	chemokine (C-X-C motif) receptor 3	2833	1.20		0.19436
Macrophage differentiation/foam cell formation						
scavenger receptor activation						
	CXCL16*	chemokine (C-X-C motif) ligand 16	58191	-1.17		0.25727
	CD36*	CD36 molecule (thrombospondin receptor)	948	-1.13		0.51754
	LDLR*	low density lipoprotein receptor (familial hypercholesterolemia)	3949	-1.18		0.63176
	CD163	CD163 molecule	9332	-1.12		0.1636
	SCARB2	scavenger receptor class B, member 2	950	-1.09		0.11173
adipogenesis						
	ADFP*	adipose differentiation-related protein	123	-1.10		0.41353
	SCD	stearoyl-CoA desaturase (delta-9-desaturase)	6319	-1.12		0.42498
	NCOA1	nuclear receptor coactivator 1	8648	-1.12		
	RXRA	retinoid X receptor, alpha	6256	-1.12		0.10428
	NRIP1	nuclear receptor interacting protein 1	8204	-1.28		
	IRS2	insulin receptor substrate 2	8660	-1.30		
	GADD45B	growth arrest and DNA-damage-inducible, beta	4616	-1.12		0.15614
	DDIT3	DNA-damage-inducible transcript 3	1649	-1.13		0.14013
	MIF	macrophage migration inhibitory factor (glycosylation-inhibiting factor)	4282	1.07		0.14753
plaque stability						
	MMP25	matrix metallopeptidase 25	64386	-1.07		0.36541
	MMP15	matrix metallopeptidase 15 (membrane-inserted)	4324	-1.07		0.66088
	TIMP2	TIMP metallopeptidase inhibitor 2	7077	-1.12		0.24743
	ADAM9	ADAM metallopeptidase domain 9 (meltrin gamma)	8754	-1.12		0.26681
	ADAMTSL4	ADAMTS-like 4	54507	-1.15		
	ADAM8	ADAM metallopeptidase domain 8	101	-1.05		0.42584

Oxidative stress						
ROS protection						
	SOD2	superoxide dismutase 2, mitochondrial	6648	-1.14		0.17534
	GPX3*	glutathione peroxidase 3 (plasma)	2878	-1.26		0.21704
	MAF	v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)	4094	-1.16		
	JUNB	jun B proto-oncogene	3726	-1.16		0.34339
	JUND	jun D proto-oncogene	3727	-1.06		0.15904
	FTH1	ferritin, heavy polypeptide 1	2495	-1.15		0.22615
	BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1	571	-1.15		0.10514
HIF signaling						
	HIF1A	hypoxia-inducible factor 1, alpha subunit (basic helix-loop-helix transcription factor)	3091	-1.10		
	VEGFA	vascular endothelial growth factor A	7422	-1.19		0.22122
	CREB5	cAMP responsive element binding protein 5	9586	-1.29		0.10884
	PTEN	phosphatase and tensin homolog (mutated in multiple advanced cancers 1)	5728	-1.14		0.14725
	UBE2E2	ubiquitin-conjugating enzyme E2E 2 (UBC4/5 homolog, yeast)	7325	-1.10		0.20161
	NFKBIA	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	4792	-1.17		0.64139
	UBE2D1	ubiquitin-conjugating enzyme E2D 1 (UBC4/5 homolog, yeast)	7321	-1.11		0.14451
nitrix oxide production						
	NOS3	nitric oxide synthase 3 (endothelial cell)	4846	1.10		0.41688
	NOSIP	nitric oxide synthase interacting protein	51070	1.07		0.51261
PPAR signaling						
	ACVR2A	activin A receptor, type IIA	92	-1.10		0.15009
	ADIPOR2	adiponectin receptor 2	79602	-1.06		0.21523
	CD36*	CD36 molecule (thrombospondin receptor)	948	-1.13		0.51754
	LDLR*	low density lipoprotein receptor (familial hypercholesterolemia)	3949	-1.18		0.68176
	ADFP*	adipose differentiation-related protein	123	-1.10		0.4152
Cell cycle						
	CDK2	cyclin-dependent kinase 2	1017	1.07		0.21646
	TP53	tumor protein p53 (Li-Fraumeni syndrome)	7157	1.07		0.64061
	CDK4	cyclin-dependent kinase 4	1019	1.08		
	PCNA	proliferating cell nuclear antigen	5111	1.09		
	HRAS	v-Ha-ras Harvey rat sarcoma viral oncogene homolog	3265	1.10		0.16298
	LCK	lymphocyte-specific protein tyrosine kinase	3932	1.11		0.43081
	MCM6	minichromosome maintenance complex component 6	4175	1.11		0.19694
	CCND2	cyclin D2	894	1.14		0.11551
	RPA2	replication protein A2, 32kDa	6118	1.06		0.20975
	RPA3	replication protein A3, 14kDa	6119	1.08		

down-regulated
not significant
p val <0.1 and >0.05
p val <0.05 and >0.01
p val < 0.01
up-regulated
not significant
p val <0.1 and >0.05
p val <0.05 and >0.01
p val < 0.01

Figure 8.5 Genes changed after eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) supplementation in various functional groups that were overrepresented by the pathway analyses software programs GenMAPP (www.genmapp.org), Ingenuity Pathway Analysis (www.ingenuity.com), and Gene Set Enrichment Analysis (www.broad.mit.edu.gsea/), supplemented with changed genes also related to these processes. Asterisks indicate that the genes appear in multiple functional groups. FC, fold change; NF-κB, nuclear transcription factor κB; ROS, reactive oxygen species; PPAR, peroxisome proliferator-activated receptors; HIF, hypoxia-induced factor.

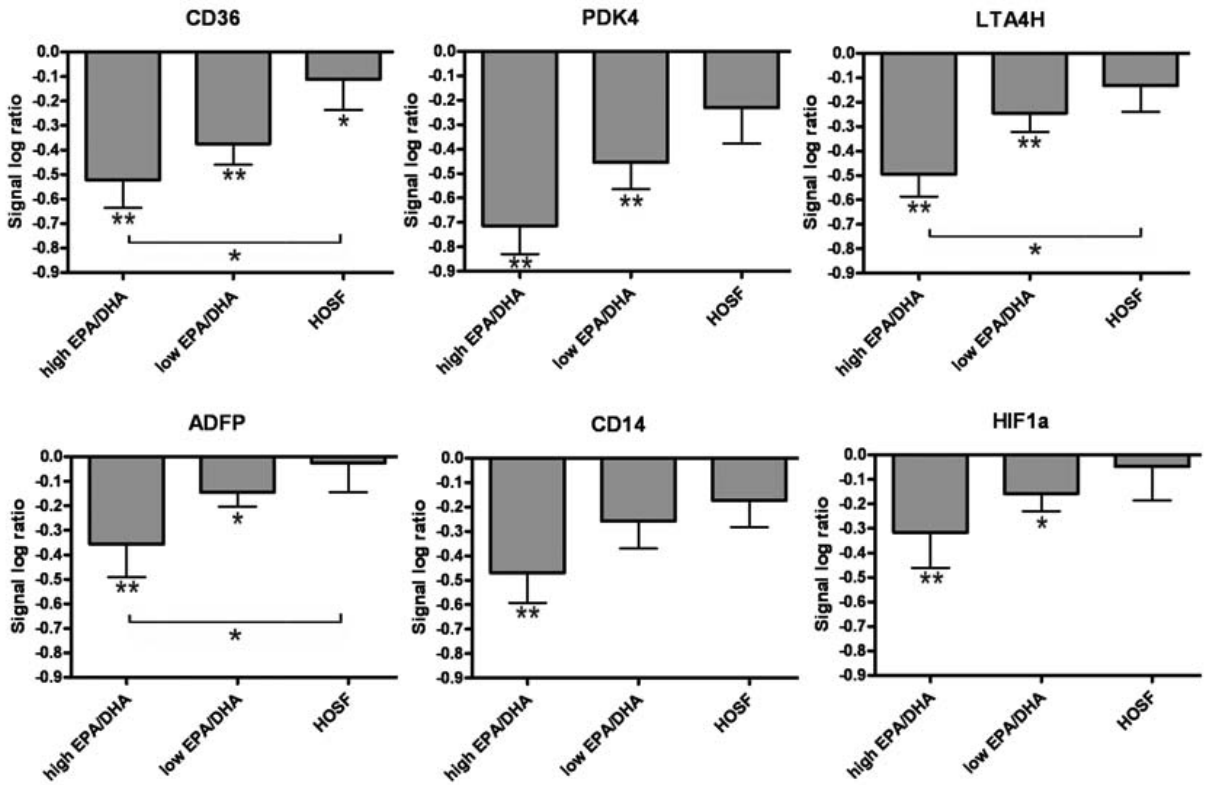

Figure 8.6 Mean (±SEM) changes in the expression of a selection of genes determined by quantitative real-time polymerase chain reaction changes after supplementation with a high dose of eicosapentaenoic acid (EPA) + docosahexaenoic acid (DHA) (*n*=36), a low dose of EPA+DHA (*n*=37), or high-oleic sunflower oil (HOSF; *n*=38). CD36, CD36 antigen; PDK4, pyruvate dehydragonase kinase 4; LTA4H, leukotriene A4 hydrolase; ADFP, adipose differentiation related protein; CD14, CD14 antigen; HIF1a, hypoxia-induced factor 1α. Brackets indicate differences between groups as determined with a 2-factor ANOVA with Tukey honestly significant difference correction (*P<0.05; ** P<0.01). Differences within groups were determined with a paired t test (** P<0.01; * P<0.05).

with these in vitro data and might imply that the monocytes present within the PBMCs are less prone to differentiate into foam cells after 26 weeks of EPA+DHA supplementation. Besides the abovementioned changes in foam cell formation, we also observed decreases in genes such as matrix metalloproteinases and disintegrin metalloproteinases, which are related to plaque stability, a process that normally occurs after infiltration and foam cell formation. These genes are known to destabilize atherosclerotic plaques, which can result in plaque rupture[29]. Also, the hypoxia inducible factor 1α gene (*HIF1α*) and its main target gene, the vascular endothelial growth factor gene (*VEGF*), were down-regulated[30]. Both genes are known to regulate atherosclerotic plaque angiogenesis, which destabilizes and progresses the lesion. Plaque stability has been found to increase after n-3 PUFA supplementation[31].

The high-dose EPA+DHA intake in our study also increased the expression of nitric oxide synthase 3, which plays a role in the protection of the vessel wall from atherosclerosis[32]. Increased expression of nitric oxide synthase 3 in murine macrophages and in vitro in human vascular wall cells after supplementation or incubation with EPA+DHA has been shown before[33-35]. Importantly, all studies described above illustrate short term in vitro experiments or animal studies, whereas we observed our effects in healthy humans after 26 weeks of n-3 PUFA intake. Because we observed changes in PBMC gene expression profiles with EPA+DHA supplementation that indicate antiinflammatory and antiatherogenic-like modifications, we speculate that EPA+DHA supplementation might improve the preatherosclerotic condition in elderly people.

An interesting point to discuss is whether the changes in gene transcription profiles were due to a direct effect of EPA+DHA on mononuclear cells or whether the changes reflected the response of PBMCs to EPA+DHA-induced systemic adaptations in the body. A nice illustration in this respect is the unexpected finding of down-regulation of PPARα target genes after EPA+DHA supplementation. On the basis of mice and in vitro studies, in which a more pronounced expression of PPAR target genes was observed on EPA and DHA intakes than on monounsaturated fatty acid intake[5], we expected an increase in PPARα target genes on EPA+DHA supplementation instead of the observed decreases. A possible explanation for these unexpected findings may lie in systemic long-term adaptations via effects of EPA+DHA on gut or liver, resulting in reduced plasma FFA and triglyceride (TG) concentrations. The observed down-regulation of PPAR target genes might have been caused by these reduced plasma FFA and TG concentrations. Hence, PBMC gene expression profiles could also be viewed as a reflection of the physiologic state of subjects, as we showed previously[8].

Microarrays were performed only in the subjects who consumed the high dose of EPA+DHA only, reflecting a relatively high consumption of 10 portions of fish weekly. However, Q-PCR results showed that a lower dose, representing the intake of two portions of fish weekly, resulted in lower, but similar, gene expression changes. This finding suggests that lower amounts of fish intake may

have similar effects on PBMC gene expression profiles, especially when taken for a longer period. In addition, our study population consisted of elderly individuals, which likely express a slightly more proinflammatory and proatherosclerotic gene expression profile, because aging is associated with chronic, low-grade increased inflammatory activity[36]. Therefore, the observed results cannot be generalized to the whole population. However, it does make the studied population suitable to detect nutrition-induced changes on transcriptional profiles, even when plasma CRP concentrations are not changed yet. It has been reported that the effects of PUFA supplementation are mainly detectable when CRP is already elevated at baseline[37-39], which was not the case in our population. Moreover, CRP is mainly produced by the liver, whereas a down-regulation of inflammatory markers was observed on PBMC transcriptomes. Because of the limited amount of PBMC material, confirmation on protein level could not be determined in PBMCs. A recent transcriptomics study in PBMCs showed that weight loss in obese men led to a similar reduction in the expression of inflammatory genes as observed in our study[40]. Interestingly, the effects in our study were obtained in lean subjects, merely by supplementation with 1.8 g EPA+DHA/d, and even with 0.4 g EPA+DHA/d. This emphasizes the potential of PBMC gene expression profiling to determine the effects of nutrition on human health status. In conclusion, this study showed that the intake of the n-3 PUFAs EPA and DHA for 26 weeks alters the gene expression profiles of PBMCs in the direction of a more anti-inflammatory- and antiatherogenic-like profile. Moreover, this nutrigenomics study showed that PBMC profiling cannot only reflect pathological conditions, but can also reflect metabolic changes due to long-term nutritional adaptations in healthy humans.

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9

General discussion

Driven by accumulating evidence of potential benefits of fish fatty acids on mental health in old age, we investigated the role of EPA+DHA and fish in relation to cognitive performance and mental well-being of older people. We examined this by means of a randomized, double-blind intervention study (RCT), a cross-sectional study and a prospective study in populations with no clinical diagnosis of dementia or depression. We also examined changes in gene expression profiles after supplementation with EPA+DHA, as an early marker of metabolic changes. In this final chapter the main findings of our studies are summarized and discussed in a broader context. Also, directions for future research and implications for public health are given.

Main findings

The main findings described in this thesis are presented in **Table 9.1**. The RCT performed in a population of 302 healthy older individuals showed no effect of 6 months supplementation with 400 mg or 1,800 mg EPA+DHA compared with placebo on cognitive performance (**chapter 2**), neither on depressive symptoms (**chapter 4**) nor on quality of life (**chapter 7**). We compared the reliability of the questionnaires used to measure depressive symptoms in the RCT, and the CES-D performed best in this population, followed by MADRS and GDS-15 (**chapter 5**). EPA+DHA supplementation for 6 months caused changes in gene expression profiles in peripheral blood mononuclear cells (PBMCs), indicating a less inflammatory and less atherogenic status (**chapter 8**). No association between fatty fish or n-3 PUFA intake and cognitive decline was found after a follow-up period of 6 years in a population of older US men (**chapter 3**). In a population of Dutch subjects with a history of coronary heart disease, intake of EPA+DHA was positively associated with dispositional optimism assessed with one of the two questionnaires used, but not with depressive symptoms (**chapter 6**).

The main conclusion of this thesis is that, based on the studies that we performed, low doses or high doses of EPA+DHA are unlikely to improve cognitive performance or mental well-being of older people without clinical diagnosis of dementia or depression on the short-term. Furthermore, our data did not confirm the hypothesis that intake of fish in the low range commonly consumed in Western populations is related to cognitive performance or mental well-being in populations of older people without clinical dementia or depression.

Intervention study: methodological considerations

A major part of this thesis is based on a 26-week RCT, which showed that low doses as well as high doses of supplemental EPA+DHA did not improve cognitive performance (**chapter 2**), depressive symptoms (**chapter 4**) or quality of life (**chapter 7**) of older people. The RCT was mainly designed based on available knowledge emerging from cross-sectional and prospective studies, and a limited number of trials with the same outcome measures. There are several considerations related to the methodology of the RCT and to the advanced scientific progress in the field that are relevant to discuss here.

Table 9.1 Main findings of the studies described in this thesis

Chapter	Study	Population	Exposure	Results
Cognitive performance				
2	RCT	302 Dutch elderly aged >65 years	400 mg EPA+DHA; 1,800 mg EPA+DHA or placebo for 26 weeks	No effect of EPA+DHA supplementation on cognitive performance
3	Prospective study with 6y follow-up	1,025 older men living in the Boston area, United States	Intake of EPA+DHA and (fatty) fish	Neither intake of EPA+DHA nor of fatty fish was associated with cognitive performance or with cognitive decline over 6 years of follow-up.
Mental well-being				
4	RCT	302 Dutch elderly aged >65 years	400 mg EPA+DHA; 1,800 mg EPA+DHA or placebo for 26 weeks	No effect of EPA+DHA supplementation on mental well-being
5	Study of reliability of depression scales	302 Dutch elderly aged >65 years	Not applicable	CES-D performed best to measure depression in this population, followed by MADRS and GDS-15
6	Cross-sectional study	644 Dutch elderly aged 60-80 years with a history of myocardial infarction	Intake of EPA+DHA and fish	No association between EPA+DHA or fish intake and depression, possible association between EPA+DHA, but not fish and optimism
Quality of life				
7	RCT	302 Dutch elderly aged >65 years	400 mg EPA+DHA; 1,800 mg EPA+DHA or placebo for 26 weeks	Quality of life was not influenced by EPA+DHA supplementation.
Gene expression				
8	RCT	111 Dutch elderly aged >65 years	400 mg EPA+DHA; 1,800 mg EPA+DHA or placebo for 26 weeks	Intake of EPA+DHA altered gene expression profiles of PBMCs to a more anti-inflammatory status

Abbreviations: RCT: Randomized controlled trial; CES-D: Center for Epidemiologic Studies Depression Scale; MADRS: Montgomery-Åsberg Depression Rating Scale; GDS: Geriatric Depression Scale; PCR: polymerase chain reaction; PBMCs: peripheral blood mononuclear cells

Study population

The RCT on EPA+DHA supplementation was set up to study cognitive performance and mental well-being in a general Dutch population of men and women aged 65 years and older, who had no clinical diagnosis of dementia or depression. Therefore, individuals with cognitive impairment (MMSE <21 points) and depression (CES-D >16) were excluded. In a population-based study in the Netherlands it has been shown that the CES-D scores in the elderly show large variation, allowing room for improvement also among the non-depressed[1]. Studying an apparently healthy population with a good mental health status may have the disadvantage that additional beneficial effects of EPA+DHA supplementation cannot be observed. On the other hand, there is the advantage of less interference with e.g. (co)morbidity or drug treatment. Moreover, it reduces the likelihood of intervening too late, when symptoms of cognitive impairment are no longer reversible.

We chose a study population with a suboptimal EPA+DHA status that is more likely to benefit from supplementation. EPA+DHA status was assessed during the screening phase by means of a FFQ that was specifically designed to measure fish intake. Subjects with an intake of more than 800 mg of EPA+DHA from fish per day were excluded from our study. Furthermore, we determined EPA+DHA in plasma cholesteryl esters, which reflect EPA+DHA intake over the past weeks[2]. These measurements confirmed the suboptimal EPA+DHA status of our subjects, as expected in Dutch older people because of their low habitual fish intake[3]. The plasma EPA+DHA levels in our subjects corresponded to those of other older populations in Western countries where fish intake is low[4-6].

Study duration

When we designed our 26-week RCT, two other trials of EPA+DHA supplementation and cognitive performance had been performed that reported beneficial effects after respectively 4 weeks[7] and 6 months[8]. A more recent trial showed no effect after 6 months of supplementation[9]. RCTs of cognitive performance and other nutrients such as B-vitamins, acetyl-L-carnitine or foods or drinks fortified with multiple micronutrients, showed effects after 4 weeks to 3 years[10-13]. In these studies measured effects were not clearly linked to study duration. In RCTs of EPA+DHA supplementation and depression, effects (if any) became already visible within 2-8 weeks. With a study duration of 26 weeks we were only able to examine relatively short-term changes in cognitive performance or mental well-being. Therefore, we cannot exclude a possible beneficial effect of long-term intake of EPA+DHA on cognitive performance or depressive symptoms.

Dose and type of intervention

We used a low (400 mg) as well as a high (1,800 mg) daily dose of EPA+DHA. The low dose corresponds to the recommended intake by the Netherlands Health Council of at least 450 mg EPA+DHA from fish per day, which is roughly

equivalent to eating two portions of fish per week, one of which is oily fish[14]. The high dose corresponds to eating about ten portions of fish per week, which is hardly achievable via dietary intake. With this high dose we aimed at maximum contrast between the groups to detect an effect, if present. In the few RCTs on cognitive performance daily doses ranged from 0.7 g DHA[8] to 1.7 g DHA + 0.6 g EPA[9]. Effective doses in RCTs on depression ranged from 1.0 g to 9.6 g EPA+DHA per day. It is not yet clear whether beneficial effects of fish or fish oil are due to EPA, DHA or a combination of both. Effects of the type of n-3 PUFA may also be different per outcome measure, favoring a more beneficial effect of DHA for cognitive performance, while for depression generally a more favorable effect of EPA has been shown[15]. Since we included cognitive performance as well as depression as primary outcome measures in our RCT, we chose the combination of EPA and DHA in the same form and ratio (3:2) as it appears in fish.

Placebo group

Randomization and inclusion of a control group is of utmost importance for the internal validity of a RCT to ensure that observed effects, if any, are fully attributable to the treatment and not to other factors. We used high oleic sunflower oil (HOSF) as the placebo, which is high in monounsaturated n-9 fatty acids of which regular consumption in the diet is ≈30 g/d. Therefore, the additional ≈2 g provided with our placebo added relatively little to this amount. Moreover, human body fat stores contain approximately 50% oleic acid[16], which is about 7 kg in an average 70 kg human and compared to this amount our daily dose would equal less than 0.03% of the total oleic acid stores in the body. Also, HOSF is considered a relatively neutral oil that has not been associated with mental health, contrary to for example olive oil which contains, next to oleic acid, also antioxidants and phenolics which have anti-inflammatory actions[17,18]. In our RCT all treatment groups showed improvements in test scores, which emphasizes the necessity of a placebo group for comparative purposes. Improvements may be due to learning effects, though we used parallel versions of cognitive tests to minimize these effects. Improvements may also be attributed to the subjects' belief in a beneficial effect of fish oil ("expectation bias").

Blinding

RCTs should preferably be double-blind, i.e. subjects and research staff must be unaware about the type of treatment until data-analysis is completed. In previous RCTs of fish oil the maintenance of blinding was shown to be difficult, because of adverse effects related to high doses of fish oil, mainly gastrointestinal, and because of a "fishy" aftertaste[19,20]. An evaluation after our RCT indicated that adverse effects were not different between the intervention and placebo groups and also that the proportion of participants who thought they had received fish oil or placebo did not differ among the groups. This could be due to the high quality of the fish oil that we used (Marinol®) or to the fact that the dose we provided was lower than doses of EPA+DHA that were provided in several other studies[19,21-23].

Randomization

To prevent selection bias in a RCT, subjects should be randomly divided over the intervention and placebo groups. To ensure an equal balance of baseline values that may be of influence on the response of mental health to EPA+DHA supplementation, we stratified subjects by age, gender, MMSE score and CES-D score prior to randomization. Randomization in our study was successful, because demographic, anthropometric and lifestyle characteristics of the subjects were well balanced over the three intervention groups. Randomization was maintained throughout the study period since there was no selective drop-out related to the interventions.

Compliance

Subjects in our RCT consumed six large capsules per day (i.e. 900 mg), and because of this amount they were advised to consume the capsules three times a day during a meal. Compliance was checked by means of diaries in which subjects recorded capsule use, by counting remaining capsules that were returned by the subjects, and by n-3 PUFA assessment in plasma cholesteryl esters. All measures reflected high compliance; apart from the subjects who stopped prematurely, only three subjects consumed less than 80% of the capsules. We performed our primary analysis of the results according to the intention-to-treat (ITT) principle. In ITT analysis data of all randomized subjects are analyzed regardless of whether subjects followed the protocol or not. ITT has become the preferred way of analyzing RCTs, although there is also controversy on this issue. The main objection is that ITT analysis does not answer the essential question of RCTs, i.e. whether the treatments differ in efficacy[24]. This is especially the case if many subjects are non-compliant. In our RCT the dropout rate was only 3% and we did obtained follow-up measurements of seven of the nine subjects who dropped-out. Only three subjects had used <80% of the capsules. Thus, the majority of the subjects included in our ITT analysis still complied with our study protocol and therefore we believe that ITT analysis in our RCT provided us with a proper answer to the question whether EPA+DHA treatment had an effect on mental health. Furthermore, per-protocol analysis, that included only compliant subjects yielded similar results.

Assessment of cognitive performance

We examined cognitive performance by means of tests that had no 'ceiling effects' are were considered sufficiently sensitive to detect age-related cognitive decline (**chapter 2**)[25]. Standard protocols were used by research staff to reduce variation in assessment due to external factors and parallel versions of tests were used to minimize learning effects. We applied five different cognitive tests: the Word Learning Test, Wechsler's Digit Span test, the Trail Making Test, the Stroop Color Word test, and the Verbal Fluency test. These tests covered four neuropsychological domains, namely sensorimotor speed, memory, executive function, and attention. For each domain we calculated a compound cognitive score by means of Z scores. All cognitive domains, except attention, were

assessed by at least three tests that measured different aspects and degrees of complexities, which has been proposed as the preferred method of cognitive testing[26]. By using compound scores, the number of cognitive test outcomes and, consequently, the risk of chance findings is reduced. Focusing on separate cognitive domains is important, because different mechanisms could affect decline of specific cognitive domains and could therefore be influenced by different treatments and within a different time-span.

Assessment of mental well-being

Among the numerous tests that are available to assess depression, we selected the Center for Epidemiologic Studies Depression Scale (CES-D)[27], Geriatric Depression Scale (GDS-15)[28], and Montgomery-Åsberg Rating Scale (MADRS)[29] (**chapter 4**). Additionally, we administered the Hospital Anxiety and Depression Scale (HADS-A) to assess anxiety[30]. All tests were administered by trained research assistants in a standardized way, which was especially important for the MADRS, because this is an observer rated scale. The CES-D is a commonly used screening tool in elderly populations. The GDS-15 is designed to detect depression in the elderly and it has been shown to detect changes in depressive symptoms[31]. The MADRS is considered more sensitive to measure treatment effects[29]. We have assessed the reliability of the three depression rating scales, CES-D, GDS-15 and MADRS, by comparing the internal consistency (Cronbach's alpha), test-retest reliability, and the intra- and inter-rater reliability (**chapter 5**). On all three items the CES-D was the most reliable scale for measuring depressive symptoms in our older population without clinical depression. There are several other depression scales that are commonly used, but that we did not select for different reasons. The Zung Self-Rating Depression Scale[32] and the Beck Depression Inventory (BDI)[33] are intended for measuring the severity of depression in psychiatric patients and they have been shown to be less sensitive to changes following treatment[34]. For the Zung scale this could be due to the fact that symptoms over an undefined time frame are assessed. The Hamilton Depression Scale (HDS, HAMD or HDRS)[35] has been considered the gold standard, but disadvantages are that the HDS contains many somatic items and has been shown to have a low internal consistency[36]. Moreover, it is an observer-rated scale intended to be used in a clinical setting by health care professionals to assess the severity of depressive symptoms.

In a later phase we administered the short form of the Profile of Mood States (s-POMS)[37] in a subgroup of 104 subjects after 17 and 21 weeks of intervention, by means of telephone interviews. The s-POMS is an instrument for monitoring changes in mood, which can easily be applied in different settings[38]. Because baseline data were lacking, we could not assess intra-individual changes after 26 weeks. However, scores on the POMS after 17 and 21 weeks were similar in all three groups, indicating no effect of EPA+DHA on mood, which was in line with the results of the other mental well-being questionnaires.

Also, as a secondary outcome, we assessed quality of life (QOL) (**chapter 7**) using the short version of the World Health Organization QOL questionnaire

(WHOQOL-BREF)[39]. Most questionnaires that assess QOL focus on health-related QOL, whereas the WHOQOL includes a strong mental health component and emphasizes the perception of the individual. Because our aim was to examine the association between EPA+DHA and mental health, the WHOQOL questionnaire was considered the most appropriate scale. EPA+DHA supplementation during 26 weeks had no effect on QOL, which was also in line with the other mental well-being questionnaires.

Comparison with other studies

We investigated the effects of EPA+DHA supplementation on mental health in a non-demented and non-depressed population. To the best of our knowledge, no other RCTs of EPA+DHA and cognitive performance have been performed in a non-demented population. Two RCTs in demented subjects showed a beneficial effect of EPA+DHA supplementation on cognitive performance, but they add little weight to the evidence because one was small in sample size (n=20)[8] and the other one was of short duration (4 weeks)[7]. Another RCT in 174 subjects with mild to moderate AD did not show an effect after 6 months of supplementation, except in a subgroup of 32 subjects with very mild cognitive dysfunction (MMSE >27 points)[9]. Data from other RCTs are awaited, including an ongoing 2-year RCT in the UK in 868 non-demented older people who are supplemented with EPA and DHA[40].

With regard to mental well-being in non-clinical populations, data are available from two RCTs[41,42]. The first study was conducted in a young non-depressed population (mean age 33 years) and found a beneficial effect after 35 days of supplementation with 2.8 g/d total n-3 PUFA. The second study in subjects with mild to moderate depression failed to show an effect after 84 days of daily supplementation with 1.5 g EPA+DHA. More RCTs have been performed in clinically depressed populations, where EPA and/or DHA were provided as an adjunctive to usual medical treatment. These RCTs were combined in a meta-analysis and here some evidence for a beneficial effect of EPA+DHA was found[43]. It has been speculated that n-3 PUFA status may be important in clinical or severe levels of depression, but not at less severe levels[44]. This is supported by a cross-sectional study where the inverse association of plasma EPA concentrations with depressive symptoms was stronger in subjects treated with antidepressant drugs[45].

Novel findings on gene expression profiles

We examined the effect of EPA+DHA supplementation on gene expression in a random subgroup of 111 subjects who participated in our RCT (**chapter 8**). EPA+DHA supplementation for 26 weeks altered gene expression profiles in peripheral blood mononuclear cells (PBMCs) to a more anti-inflammatory and anti-atherogenic status in a dose-dependent way. PBMCs could be a model for gene expression elsewhere in the body and the impact of fish oil supplementation on whole genome gene expression profiles in human PBMCs has not been

examined before. Our findings of the effect of EPA+DHA supplementation on gene expression related to inflammation and atherogenesis may provide preliminary clues to EPA+DHA activated mechanisms that could be involved in mental health. These findings merit further investigation.

Observational studies: methodological considerations

Observational epidemiological studies could provide relevant information for the design of RCTs and are also useful for getting insight into long-term effects. Part of this thesis is based on observational studies, which may have weaknesses related to the study design that could affect the validity of the findings. The strengths and limitations of the prospective study (**chapter 3**) and a cross-sectional study (**chapter 6**) that we have performed are discussed below.

Study design

A major limitation of observational studies as compared with RCTs is that no definite conclusions can be drawn about the causality of the relation. We examined the association of EPA+DHA and fish intake with depression and dispositional optimism in a population of older subjects with a history of myocardial infarction, using cross-sectional baseline data of a large intervention study (Alpha Omega Trial) (**chapter 6**). A main limitation of this design is that exposure and outcome are assessed at the same moment in time which makes it difficult to assess the temporality of the association. It is possible that low fish consumption is a predisposing factor for depression, but it could also be that the presence of depressive symptoms decreases dietary intake of fish fatty acids due to loss of appetite or other reasons (reverse causation). Therefore, we excluded subjects using anti-depressant medication from the analysis.

We examined the association between EPA+DHA and fish intake and cognitive performance and cognitive change in older men of the Normative Aging (NAS) study, using a prospective design (**chapter 3**). We examined fish intake at baseline and cognitive performance during 6 years of follow-up. Reverse causation is not an issue here, because intake was assessed prior to the assessment of cognitive performance. Furthermore, the advantage of a longitudinal design is that changes over time can be assessed and for changes in cognitive performance 6 years of follow-up is a reliable period.

Internal validity

Subjects in our cross-sectional study on depression and dispositional optimism (**chapter 6**) had a history of myocardial infarction within the past 10 years and are of older age (mean age 69 years), which may cause survival bias. Our prospective study on cognitive change (**chapter 3**) was performed in older US men who participated in the Normative Aging Study (NAS). A total of 2,280 men had been enrolled in 1970. Cognitive testing started in 1993, after nearly 25 years of follow-up, and at that time 1,216 men were still in the study. Missing data during these years were mostly due to mortality (42%), follow-up

measurements of mailed questionnaires only (38%), or loss to follow-up and drop-out (20%). During the 6-years of follow-up for our prospective analysis the number of subjects participating in the consecutive cognitive test series decreased substantially (from 1,025 to 671 after 3 years to 313 after 6 years of follow-up). Therefore, bias due to incomplete follow-up may have influenced our results. However, we compared completers with non-completers for differences in age, education, fish intake, and cognitive scores and except for the fact that subjects with complete follow-up were on average 2 years younger; they were not different from the subjects with incomplete follow-up. To prevent bias due to selective drop-out, we analyzed the data with a repeated mixed coefficients model (SAS PROC MIXED procedure) for our analyses, that took account of subjects with incomplete follow-up data.

A second potential source of bias in an observational study may occur from misclassification of the exposure. Our cross-sectional study on depressive symptoms and dispositional optimism (**chapter 6**), and the prospective study of US men (**chapter 3**) did not include cognitively impaired subjects. We assume that these subjects were able to adequately recall their dietary pattern, including fish consumption. In both observational studies fish intake was assessed by a FFQ. The FFQ in the Normative Aging Study (NAS) (**chapter 3**) focused on consumption of foods over the past year and has been validated in several studies[46-48]. The four questions on seafood intake were sufficient to characterize habitual intake of seafood and correlations between two measurements 1 year apart ranged from 0.48 (fish) to 0.67 (shellfish)[48]. The correlation of fish intake estimated with the FFQ validated against two 1-week dietary records was 0.61[47]. The FFQ in the Alpha Omega trial (**chapter 6**) focused on the past month and was based on a validated FFQ for estimating the intake of total energy and fatty acids[49]. This FFQ was modified for estimating intake of ALA, EPA and DHA of the older population in the Alpha Omega Trial. The correlation between EPA+DHA intake and plasma EPA+DHA was 0.37. Although a FFQ cannot accurately assess absolute dietary intakes, it is a suitable tool to rank individuals.

Our observational findings can be confounded by factors that are both related to fish intake and mental health. When examining cognitive decline (**chapter 3**) and mental well-being (**chapter 6**), we adjusted for established confounders like age, gender, educational level, BMI, smoking, alcohol consumption, physical activity, and total energy. Additionally, we added indicators for the type of dietary pattern, such as saturated fat, vitamin C, vitamin E, and fiber. As in all observational studies there may still be residual confounding due to unknown confounders or imprecise measurement of confounders. Improving the accuracy of measurement of confounders would be preferable, but this is often not feasible in large epidemiological studies.

Assessment of cognitive performance

In many observational studies cognitive functioning is assessed using the Mini-Mental State Examination (MMSE)[50] or other tests of global cognitive function. Originally, the MMSE was designed as a screening tool to assess the global

cognitive state of subjects by the clinician, but now it is also frequently used as a measure of cognitive performance or cognitive change in epidemiological studies[51]. However, it has been shown that it is not a sensitive tool for the assessment of short-term cognitive impairment[52]. Also, the MMSE as well as other global measures of cognitive function cannot detect changes in different cognitive domains. We used an extensive battery of nine cognitive tests and their subtests, which were appropriate for an aging population, and focused on specific cognitive domains. We calculated a compound cognitive score for each domain by means of factor analysis (**chapter 3**). This resulted in three factors: a memory/language factor; a visuospatial/attention facto, and a speed factor. This made the cognitive construct more robust and reduced the number of outcome variables, which reduced chance findings.

Assessment of mental well-being

We used the GDS-15 to measure depression, as an indicator of mental well-being[28]. The GDS-15 is easy to administer and designed for use in an older population. We assessed dispositional optimism by means of the Revised Lifestyle Orientation Test (LOT-R)[53] and a 4-item questionnaire (4Q) that was previously used in the Zutphen elderly study[54]. The LOT-R is a commonly used psychological optimism test with good psychometric properties[53]. Scores on the 4Q have been shown to be relatively stable over time[55], but have not yet been validated against other measures of mental well-being. Including measures of dispositional optimism is new in the field of fish fatty acids research and though depression is not simply the reverse of optimism, optimism has been shown to protect against the development of depressive symptoms[54]. Furthermore, it has also been associated with healthy lifestyle and dietary habits[56].

Comparison with other studies

The association between EPA+DHA and fish and cognitive performance in older adults has been examined in several epidemiological studies of which an overview is given in **chapter 1** (**Table 1.1**). All together, the results of cross-sectional studies observed either an inverse association or no association, whereas longitudinal studies mostly showed an inverse association. Most studies have only used a global measure of cognitive performance, which we consider a limitation because cognitive function in older adults could decline differentially in specific cognitive domains[25]. Also, if n-3 PUFA are associated with cognitive functioning, they may affect specific cognitive domains differently, as different mechanisms could underlie specific domains. It is, however, unclear yet which mechanisms may principally underlie this association in general and which mechanisms may underlie changes in specific cognitive domains. To the best of our knowledge, three studies addressed the association between n-3 PUFA and cognitive performance in specific cognitive domains. Two showed a reduced risk of impaired cognitive performance on speed related tests with higher intake of fatty fish or n-3 PUFA[57], or with higher plasma n-3 PUFA levels over 3 years[58] and one showed that higher proportions of plasma n-3 PUFA

reduced the risk of decline in verbal fluency over 6 years of follow-up[4]. These studies were all conducted in healthy, middle-aged populations with a mean age between 56 and 60 years. The NAS population was about 10 years older with a mean age of 68 years. Two of these studies[4,58] assessed n-3 PUFA status by plasma cholesterylesters or plasma phospholipids. Studies using biomarkers often observe stronger associations, because biomarkers are less prone to misclassification than dietary intake data. The other study, conducted by Kalmijn et al., assessed intake of fatty fish and marine n-3 PUFA with a FFQ, like we did, and they observed an inverse association with cognitive performance[57]. Mean intake values in their population were 11 g of total fish, 3 g of fatty fish, and 165 mg of EPA+DHA per day. Intake in the Normative Aging Study population was considerably higher with an average intake of 43 g of total fish, 23 g of fatty fish and 280 mg of EPA+DHA per day. The range of intake was larger and included also non-fish eaters. However, we have to note that comparing actual intakes based on FFQs, and in this case two different FFQs, should be done cautiously. Another limitation for a good comparison between these studies is that different cognitive tests were used to measure the same cognitive domains. Thus, the studies investigating effects from n-3 PUFA in older people on separate cognitive domains are too scarce and heterogeneous to draw definite conclusions.

With regard to depression, observational studies are summarized in **Table 1.3** in **chapter 1**. All studies, except one, were cross-sectional and most studies (*n*=17) showed an inverse association of EPA+DHA or fish intake with depression, whereas four studies did not. Ten studies focused on intake of fish and/or n-3 PUFA like we did and all, except two, showed an inverse association. In nine studies n-3 PUFA were measured in blood and all, except one, were performed in populations with (major) depression. These studies consistently showed an inverse association. In the other two studies in healthy adults, n-3 PUFA was measured in adipose tissue, which was also inversely related to depression. Fish and EPA+DHA intake in our study was lower compared to most other cross-sectional studies on EPA+DHA or fish intake and mental well-being where mean intakes ranged from 1 to ≥2 portions of fish per week[59-64]. However, Colangelo et al. and Kamphuis et al. observed an inverse association in populations who consumed less EPA+DHA (mean intake of 143 and 101 mg/d, respectively) than our study population[65,66]. Studies of fish fatty acids were all performed in the general population, while our cross-sectional study was performed in a population of subjects with a history of coronary heart disease who had a higher prevalence of depression, i.e. 17% compared with 1 to 15% in the general population[67,68]. There are five other cross-sectional studies on n-3 PUFA and depression in patients with acute coronary syndromes, i.e. myocardial infarction or unstable angina[69-73]. In these studies serum n-3 PUFA concentrations were examined and all studies showed significantly lower concentrations in depressed individuals.

Reflection and future directions

Our RCT did not show an effect of EPA+DHA supplementation on cognitive performance or mental well-being. This result was confirmed by the observational studies we conducted. Nevertheless, we cannot deny the total body of evidence indicating a beneficial effect, although overall results lack consistency. More research is therefore needed, and several aspects that could be relevant for future studies are discussed below.

What kind of studies? For cognitive performance, especially RCTs are lacking, but some are ongoing and results are awaited for. For depression more RCTs, particularly in non-depressed populations, are needed. Moreover, prospective studies will be of added value to study associations over several years, a time period needed to capture long-term effects that cannot be covered by RCTs. Furthermore, results from these observational studies could yield relevant information on EPA+DHA dose and type of study population for future RCTs. As long as no effective treatments are available, epidemiological research should be continued to identify modifiable risk factors.

Which populations? We assessed the association between fish fatty acids and mental health in populations with no clinical diagnosis of dementia or depression. We aimed to examine effects in the preclinical phase to obtain insight in possibilities for prevention. However, it may be that in individuals who are still too healthy no changes can be detected within a time-span of 26 weeks. Conversely, in already cognitively impaired or demented subjects it may be too late for dietary supplementation to counteract the process of cognitive decline. Future RCTs in non-clinical populations may focus on more sensitive groups, such as subjects with amnestic Mild Cognitive Impairment (MCI) or subjective memory complaints, subjects with a suboptimal n-3 PUFA status or carriers of the APOE-ε4 allele. Amnestic MCI patients or subjects with subjective memory complaints show deterioration in specific cognitive functions and this pathological process could possibly be influenced by EPA+DHA treatment. In depleted subjects there is more room for improvement of n-3 PUFA status. In our RCT we observed an effect of EPA+DHA supplementation specifically on the cognitive domain of 'attention' in a subgroup of carriers of the APOE-ε4 allele. Conversely, Huang et al. found in an epidemiological study, that consumption of fish was associated with a reduced risk of AD only in subjects without the APOE-ε4 allele[74]. A differential effect of n-3 PUFA for APOE genotypes warrants further investigation.

How long? The European task force under the auspices of the European Alzheimer Disease Consortium, agreed that the study duration of RCTs focusing on cognitive decline should be at least 18 months[75]. Such period is considered to be sufficient to detect changes, if any, for slowly developing disorders like cognitive decline and AD. For studying genetic determinants cross-sectional studies are suitable since 'reverse causation' cannot occur. To capture long-term effects of EPA+DHA or fish intake on slowly developing processes like age-related cognitive decline, however, prospective studies of sufficiently long duration

are needed[76]. Currently, it is not clear which short-term (weeks or months) or long-term mechanisms (years) could underlie an effect of EPA+DHA on mental health, if any. When designing future studies, one should carefully think about the appropriate time window to capture the effect of EPA+DHA, depending on whether short-term or long-term mechanisms are involved. These considerations also influence the choice of specific dimensions of brain function that are to be studied. With regard to short-term mechanisms, gene expression studies, as described in **chapter 8** are a promising field, because these may elucidate early changes that could lead to changes in mental health.

Which exposure? Daily doses of EPA+DHA supplementation ranged from 0.7 to 2.3 g in RCTs focusing on cognitive performance and from 1.0 to 9.6 g in RCTs focusing on depression. It is not clear which dose is most effective and this could also depend on the habitual fish intake of the target group. Future dose-finding trials will be helpful in establishing optimum doses for use in RCTs. One should also take into account that high doses of fish oil could have more adverse effects and are more prone to a fishy aftertaste, which could cause deblinding of treatment. However, these effects could be reduced when high quality, refined fish oils with a high concentration of EPA+DHA are used, also to reduce the amount of capsules to be ingested each day[20]. EPA and DHA can be used as separate or combined treatments. It has been speculated that DHA mainly exerts an effect on cognitive performance, whereas EPA seems to be more effective in mood disorders[15], but more comparisons of EPA and DHA are needed to clarify the combined and separate effects on different outcome measures. With regard to observational studies, the range of fish intake should be sufficiently large, and non-fish eaters should be included to be able to detect associations for small amounts of EPA+DHA intake.

Which endpoints? We assessed cognitive performance, depression, and QOL as measures of mental health. There is no gold standard for the validation of cognitive tests and mental well-being questionnaires. For depression often a diagnostic judgment by a clinician using the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)[77] is used, but this is usually not feasible in large studies. The wide variety of tests that are used hinders the comparisons between studies and meta-analyses in this field. We propose to use a standard battery of cognitive tests that include domain-specific outcome measures. These tests should preferably be applied in combination with imaging techniques, such as MRI, Trans Cranial Doppler ultrasound (TCD), or biological markers such as tau isoforms and amyloid β, to get more insight in underlying mechanisms of action. However, suitable biomarkers in the field of nutritional research and mental health still need to be established and validated. If this can be achieved, a whole array of opportunities will become available to obtain insight in processes in specific brain areas that can be modified by nutrient intake. Although imaging techniques are relatively expensive, they have the additional advantage that study sample size or duration of follow-up can be reduced. Several previous studies suggested that multiple factors might be involved in the occurrence of AD at late ages. Because of the probable multi-factorial nature of AD, it seems logical to

initiate multi-domain interventions, including for instance nutritional, physical, and cognitive training to examine their potential synergistic effects[78].

Public health implications

The prevalence of depression and dementia is high and due to the aging of the population, these numbers will likely increase in the coming years. For dementia there is a lack of effective treatment. For depression current treatment is often not satisfactory, despite the fact that more than 40 antidepressants are available on the market. The recurrence rate in people with a history of depression is high[80], and less than 50% of patients achieve full remission[79]. Moreover, pharmacological treatment often has unpleasant side effects[81]. Depression in older people can remain undetected and only part of the elderly in need receives treatment[82]. This calls for effective preventive measures. The importance of a healthy lifestyle is advocated for the maintenance of a good mental and physical health. The Netherlands Health Council recommends in the 2006 Dietary Guidelines an intake of at least 450 mg EPA+DHA from fish per day, which is roughly equivalent to eating two portions of fish per week, one of which is oily fish[14]. This advice is mainly based on the beneficial effects of EPA+DHA on cardiovascular health. Based on the results of this thesis and other scientific evidence, there is no reason to change the current recommendation to improve mental health of the general population.

The focus of research so far has mainly been on health effects of n-3 PUFA from fish, but fish is also a good source of proteins, vitamin D and selenium, which could also be beneficial to health[83]. Moreover, fish is a good alternative protein source for meat that is a major source of saturated fat. Therefore, we would recommend a food-based approach for increasing the intake of EPA+DHA, except for individuals who do not eat fish, for whom foods fortified with n-3 PUFA or fish oil capsules may be good alternatives. Incorporating fish in a dietary pattern may have additional advantages due to interactions with micro- and macronutrients from other foods, which also need to be considered in future studies. Three studies have examined cognitive impairment in relation to the Mediterranean diet, that includes fish. Adherence to this diet reduced the risk of developing Mild Cognitive Impairment (MCI) and AD, mortality from AD, and reduced conversion of MCI to AD[84-86]. However, in etiologic research where one aims at elucidating the causal relationships with individual foods or nutrients, a "reductionist approach" is required. Both complementary approaches are needed to come to effective public health measures.

Fish may have several health benefits, but there is also concern about contaminants, such as methyl mercury, dioxins, and polychlorinated biphenyls. These substances are mainly found in fish species that are high in the food chain, such as swordfish, shark, tuna and king mackerel[87]. Mozaffarian et al. reviewed the risks and benefits of fish consumption in relation to cardiovascular disease and neurodevelopment and neurologic function. They concluded that the benefits of modest fish consumption (1-2 servings/week) outweigh the risks and,

except for few selected fish species, even for sensitive groups such as women of childbearing age[88]. High quality fish oil is usually purified from contaminants, but it has the disadvantage that it lacks other healthy nutrients from fish (e.g. vitamin D, selenium).

Currently, the average intakes EPA+DHA in most Western countries do not meet the recommendations. Increasing fish consumption, however, raises the concern that edible fish stocks become depleted. Fish farming is not the solution since wild fish is needed to feed farmed stocks[89]. Algae would be a good alternative source of EPA+DHA, which also suits vegetarians. Furthermore, n-3 PUFA from plants (alpha-linolenic acid, ALA) could be obtained via genetic engineering. In the near future, production of EPA-rich rapeseed oil and fungal treatment of biodiesel waste may give us another source of EPA. Also, fortified foods are now available on the market, such as eggs from chickens fed n-3 fatty acids[90] and margarines, dairy, and meat products enriched with n-3 PUFA. However, most of these products contain ALA rather than EPA+DHA. It is questionable whether ALA is a suitable replacement for EPA+DHA, because in humans the conversion from ALA to EPA is only 5 to 10% and from ALA to DHA only 1 to 5%[91-93].

Concluding remarks

This thesis makes a valuable contribution to the field of nutrition and mental health by providing experimental data for the effect of low and high EPA+DHA intake on cognitive performance and mental well-being in a general older population with no dementia or depression. In a well-controlled RCT we found no evidence for a beneficial effect on clinically relevant outcomes related to mental health. Examination of gene expression profiles in white blood cells of supplemented subjects, however, revealed a more anti-inflammatory and anti-atherogenic status which is a novel finding in this field. Our observational studies in populations with a low fish intake did not provide evidence for an important role of EPA+DHA in cognitive performance or mental well-being. Based on this thesis and the total evidence in the field, we conclude that the beneficial effect of EPA+DHA on mental health in the general population still needs to be established.

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Summary

The growing aging population will lead to an increase in age-related diseases, including dementia and depression. Both diseases have a serious impact on mental health and quality of life. Evidence is accumulating that mental health is favorably affected by intake of very-long-chain n-3 polyunsaturated fatty acids from fish, i.e. eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Epidemiological and experimental studies suggest that EPA+DHA may slow down cognitive decline, delay the onset of dementia and improve mental well-being. In this thesis we further investigated the potential benefits of fish and EPA+DHA on mental health of older people without clinical dementia or depression.

In **chapter 2** of this thesis, we examined the effect of EPA+DHA supplementation on cognitive performance in a double-blind, placebo-controlled trial (RCT). A total of 302 individuals aged 65 years or older without cognitive impairment were randomly allocated to capsules with a low dose of EPA+DHA (400 mg per day) or a high dose of EPA+DHA (1,800 mg per day), or placebo for 26 weeks. Cognitive performance was assessed by means of an extensive neuropsychological test battery that included the cognitive domains of attention, sensorimotor speed, memory and executive function. Overall, there was no significant effect on any of these cognitive domains for either low-dose or high-dose fish oil supplementation compared to placebo.

Evidence from cross-sectional and prospective studies suggests that an increased intake of fish and EPA+DHA could protect against age-related cognitive decline and dementia. However, the results are inconsistent and studies that address changes in specific cognitive domains are limited. We investigated the association of fish and EPA+DHA intake with cognitive performance and 6-year cognitive decline in specific domains of cognitive functioning in 1,025 older US men (**chapter 3**). The fatty fish consumption ranged from 0.2 to 4.2 servings per week. The intake of fatty fish or EPA+DHA was not related to cognitive performance or change in cognitive function during 6 years of follow-up.

Trials of EPA+DHA supplementation and depression have mainly been performed in depressed patients, with conflicting results. In non-clinically depressed populations trials are scarce. In the previously mentioned RCT we additionally investigated the effect of EPA+DHA supplementation on mental well-being (**chapter 4**). Changes in different aspects of mental well-being (depressive symptoms, anxiety, and mood) were assessed by means of the Center for Epidemiologic Studies Depression Scale (CES-D), Montgomery-Åsberg Rating Scale (MADRS), Geriatric Depression Scale (GDS-15), Hospital Anxiety and Depression Scale (HADS-A), and the short form of the Profile of Mood States (s-POMS). CES-D scores were around 6-7 and did not differ significantly between the low dose EPA+DHA, high dose EPA+DHA and placebo group at the start of the study. Mean changes in CES-D scores after 26 weeks were –0.2, 0.2 and –0.4 (P=0.87), respectively. Treatment with 400 or 1,800 mg/d of EPA+DHA had no effect on any of the measures of mental well-being after 13 or 26 weeks of intervention.

In **chapter 5** we evaluated the reliability of three depression rating scales (CES-D, MADRS, and GDS-15) in the general older population of our RCT. Three dimensions of reliability were compared: 1) internal consistency by means of Cronbach's alpha, 2) reproducibility by means of Spearman correlations (r_s), and 3) the intra- and inter-rater reliability. The internal consistency was high for the CES-D (Cronbach's alpha of 0.84), good for the MADRS (0.72) and relatively low for the GDS-15 (0.55). Reproducibility was also higher for the CES-D (r_s=0.71) than for the MADRS (0.61) and the GDS-15 (0.52). The rater had little influence on CES-D scores (intra-/inter-rater ratio=0.99). The GDS-15 and the MADRS, however, performed better when administered by the same rater. We conclude that CES-D is the preferred scale for measuring depressive symptoms in a non-clinically depressed older population.

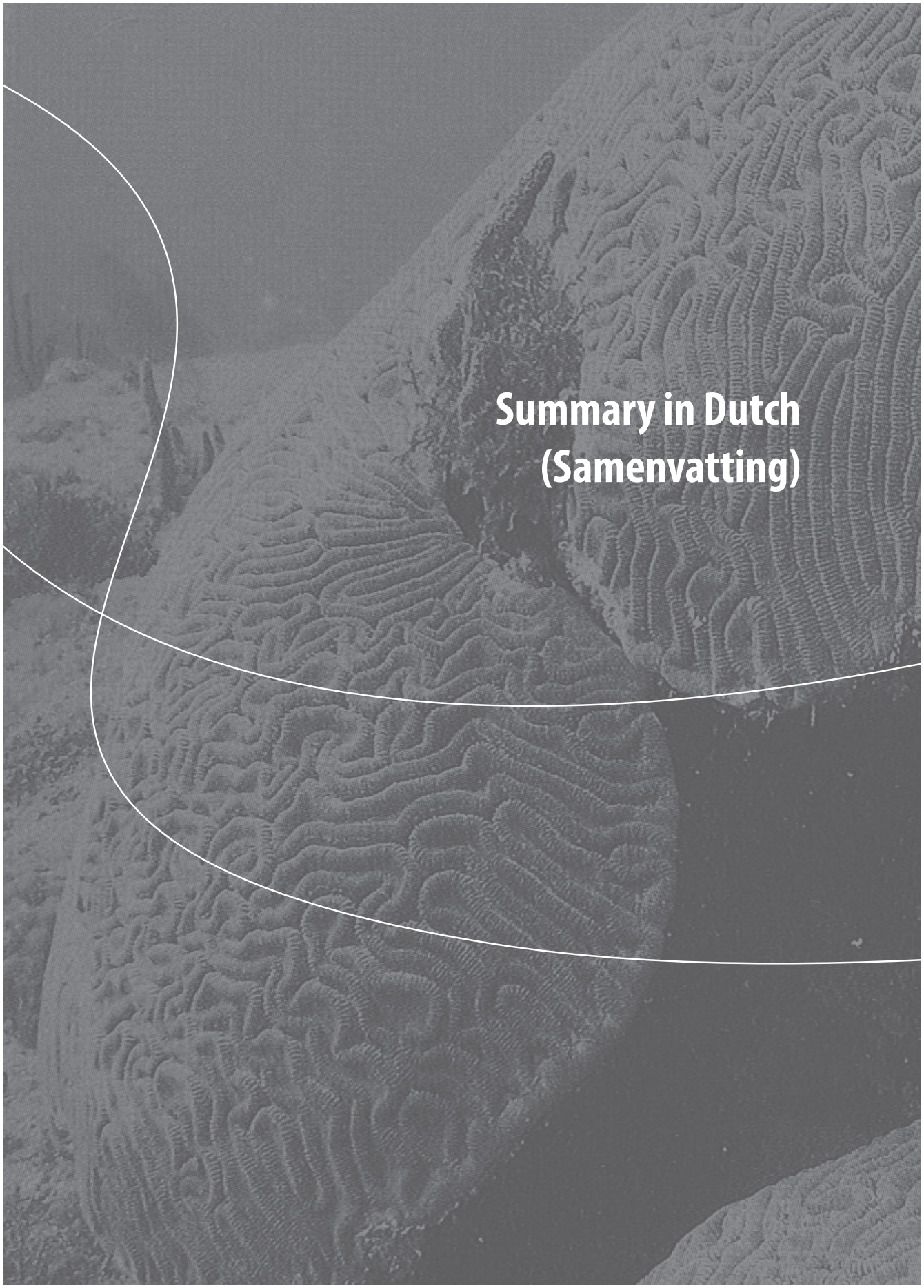
Epidemiological studies generally point toward a beneficial effect of fish and EPA+DHA intake against depression. Most observational studies have been performed in populations with a low prevalence of mental disturbances. We examined the association of EPA+DHA and fish intake with mental well-being in 644 free-living subjects aged 60-80 years, who had a history of myocardial infarction (**chapter 6**) and a higher prevalence of depressive symptoms (17%) compared with the general population (3 to 12%). Depressive symptoms were assessed with the self-report Geriatric Screening Scale (GDS-15) and dispositional optimism with the Revised Life Orientation Test (LOT-R), and a 4-item questionnaire (4Q). Compared with the lower tertile, subjects in the higher tertile of EPA+DHA intake had a lower prevalence of depressive symptoms, but this was not statistically significant (prevalence ratio [PR] 0.78; 95% Confidence Interval [CI]: 0.50; 1.22, *P*-trend 0.27). Findings on dispositional optimism were only statistically significant when assessed with the 4Q (PR 1.09; 95% CI: 1.01; 1.23, *P*-trend 0.05). For fish intake, similar though weaker associations were found.

Cognitive impairment and depression both affect quality of life. We assessed quality of life (QOL) in the RCT by using the short version of the World Health Organization QOL questionnaire (WHOQOL-BREF) (**chapter 7**). After 26 weeks, the mean difference in score compared with placebo was 0.02 (–1.95; 1.99) for the low-dose and an improvement of –1.42 (95% CI: –3.40; 0.57) for the high-dose fish oil group. Treatment with 400 mg or 1,800 mg EPA+DHA did not affect total QOL after 13 or 26 weeks of intervention.

Furthermore, we investigated early effects of EPA+DHA on processes that could be related to mental health by examining the effect of low- and high-dose supplementation with EPA+DHA on gene expression profiles in human white blood cells (**chapter 8**). We found that subjects who had been supplemented with EPA+DHA during 6 months had a less inflammatory and less atherogenic status.

The main findings, methodological considerations and interpretation of the findings of the studies described in this thesis are discussed in **chapter 9**. Also, directions for future research and implications for public health are considered.

The main conclusion of this thesis is that in the short-term, low or high doses of supplemental EPA+DHA are unlikely to improve cognitive performance or mental well-being of older people without a clinical diagnosis of dementia or depression. Our observational studies in populations with a low fish intake did not provide evidence for an important role of EPA+DHA in cognitive performance or mental well-being. Based on this thesis and the total evidence in the field, we conclude that the beneficial effect of EPA+DHA on mental health in the general population still needs to be established.



Summary in Dutch (Samenvatting)

Door de groeiende populatie ouderen zal ook het aantal leeftijdsgerelateerde ziektes stijgen, waaronder dementie en depressie. Beide hebben een grote invloed op de mentale gezondheid en de kwaliteit van leven. De laatste jaren staan de langketenige meervoudig onverzadigde omega-3 vetzuren, in het bijzonder de visvetzuren eicosapentaeenzuur (EPA, C20:5n-3) en docosahexaeenzuur (DHA, C22:6n-3), in de belangstelling vanwege hun mogelijk gunstige invloed op de mentale gezondheid. Epidemiologisch en experimenteel onderzoek suggereert dat EPA+DHA de cognitieve achteruitgang en het ontwikkelen van dementie vertragen en het mentaal welbevinden kunnen verbeteren. In dit proefschrift hebben we de invloed van vis en EPA+DHA op de mentale gezondheid van ouderen zonder klinische dementie of depressie verder onderzocht.

In **hoofdstuk 2** is het effect van dagelijkse suppletie met een lage en een hoge dosis EPA+DHA op cognitief functioneren bij ouderen onderzocht in een dubbelblind, gerandomiseerd, placebo-gecontroleerd interventieonderzoek. Op basis van loting zijn 302 deelnemers ingedeeld in drie groepen die gedurende 26 weken capsules kregen met daarin een lage dosis EPA+DHA (400 mg/dag), een hoge dosis EPA+DHA (1800 mg/dag) of zonnebloemolie (placebo). Cognitief functioneren is gemeten door middel van een uitgebreide set cognitieve testen die waren gericht op de cognitieve domeinen "aandacht", "snelheid", "geheugen" en "executief functioneren". De lage en hoge dosis EPA+DHA hadden geen effect op deze cognitieve domeinen.

Resultaten uit epidemiologisch onderzoek doen vermoeden dat een verhoogde consumptie van EPA+DHA en vis mogelijk beschermt tegen leeftijdsgerelateerde cognitieve achteruitgang en dementie. De bevindingen zijn echter niet consistent en met name onderzoeken naar veranderingen in specifieke cognitieve domeinen ontbreken. We hebben in 1025 oudere Amerikaanse mannen onderzocht of de inname van EPA+DHA en vis samenhangt met cognitief functioneren en cognitieve achteruitgang in specifieke cognitieve domeinen over een periode van 6 jaar (**hoofdstuk 3**). De consumptie van vette vis varieerde van 0,2 tot 4,2 porties per week. Het eten van vette vis of EPA+DHA bleek niet gerelateerd aan cognitief functioneren of cognitieve achteruitgang.

Experimenteel onderzoek naar het effect van EPA+DHA suppletie op depressie is voornamelijk gedaan in depressieve patiënten en de resultaten ervan zijn niet eenduidig. In personen zonder klinische depressie is nog nauwelijks interventieonderzoek gedaan. Wij hebben het effect van een lage en hoge dosis EPA+DHA op mentaal welbevinden onderzocht in een 6 maanden durend interventieonderzoek (**hoofdstuk 4**). Veranderingen in verschillende aspecten van mentaal welbevinden (depressieve symptomen, angst en stemming) zijn onderzocht met behulp van de "Center for Epidemiologic Studies Depression Scale" (CES-D), "Montgomery-Åsberg Rating Scale" (MADRS), "Geriatric Depression Scale" (GDS-15), "Hospital Anxiety and Depression Scale" (HADS-A) en de verkorte versie van de "Profile of Mood States" (s-POMS). De deelnemers hadden een CES-D score van 6-7 bij aanvang van het onderzoek. De gemiddelde veranderingen in CES-D scores na 26 weken waren –0,2 in de groep met de

lage dosis EPA+DHA, +0,2 in de groep met de hoge dosis EPA+DHA en –0,4 in de placebogroep, hetgeen niet significant verschilde (*P*=0,87). Behandeling met 400 of 1800 mg EPA+DHA per dag had na 13 en 26 weken geen effect op het mentaal welbevinden gemeten met bovenstaande vragenlijsten.

In **hoofdstuk 5** hebben we de betrouwbaarheid van drie depressievragenlijsten (CES-D, MADRS, and GDS-15) onderzocht in de niet-klinische populatie van ons interventieonderzoek. Drie aspecten van betrouwbaarheid zijn vergeleken, namelijk 1) interne consistentie met Cronbach's alpha, 2) reproduceerbaarheid met Spearman correlaties (r_s) en 3) de betrouwbaarheid binnen en tussen de testafnemers. De interne consistentie was hoog voor de CES-D (Cronbach's alpha van 0,84), goed voor de MADRS (0,72) en relatief laag voor de GDS-15 (0,55). De reproduceerbaarheid was hoger voor de CES-D (r_s=0,71) dan voor de MADRS (0,61) en de GDS-15 (0,52). De testafnemer had weinig invloed op de CES-D scores. De scores op de GDS-15 en de MADRS waren echter betrouwbaarder wanneer de vragenlijsten door dezelfde persoon werden afgenomen. We concluderen dat de CES-D de voorkeur verdient boven de andere genoemde vragenlijsten als het gaat om het meten van depressieve symptomen in een algemene oudere populatie.

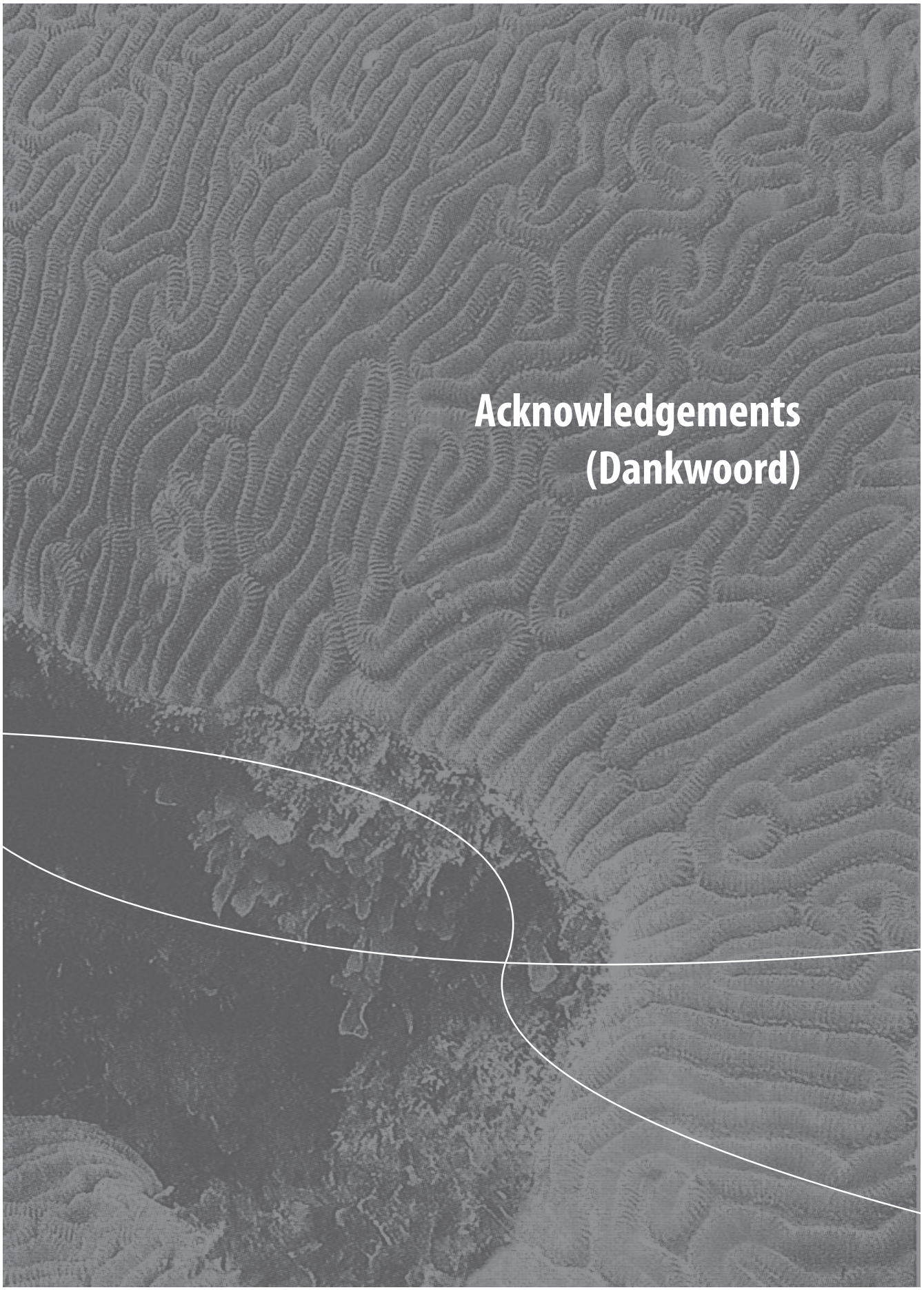
Epidemiologisch onderzoek laat over het algemeen een gunstig effect zien van EPA+DHA- en visinname op depressie. Veel van dit onderzoek is uitgevoerd in populaties waarin depressie weinig voorkomt. Wij hebben de inname van EPA+DHA en vis in relatie tot depressie onderzocht in 644 zelfstandig wonende ouderen (60-80 jaar) die in de voorafgaande 10 jaar een hartinfarct hadden doorgemaakt (**hoofdstuk 6**). Deze populatie heeft een hogere prevalentie van depressie symptomen (17%) vergeleken met de algemene populatie (3 tot 12%). Naast depressieve symptomen, gemeten met de "Geriatric Screening Scale" (GDS-15), is de aanwezigheid van dispositioneel optimisme vastgesteld met de "Revised Life Orientation Test" (LOT-R) en een 4-item vragenlijst (4Q). Personen met de laagste inname van EPA+DHA (eerste tertiel) hadden minder depressieve symptomen dan personen met de hoogste inname van EPA+DHA, maar dit was niet statistisch significant (prevalentieratio [PR]: 0,78; 95% betrouwbaarheidsinterval [CI]: 0,50; 1,22, *P*-trend 0,27). Voor dispositioneel optimisme gemeten met de 4Q werd een gunstig verband met EPA+DHA gevonden (PR 1,09; 95% CI 1,01; 1,23, *P*-trend 0,05), maar dit werd niet bevestigd door de LOT-R. De associaties voor visconsumptie waren zwakker maar wezen in dezelfde richting.

Cognitieve achteruitgang en depressie hebben beide een grote invloed op de kwaliteit van leven. Bij de deelnemers aan het bovengenoemde interventieonderzoek hebben we de kwaliteit van leven gemeten met behulp van de verkorte versie van de "World Health Organization Quality of Life questionnaire" (WHOQOL-BREF) (**hoofdstuk 7**). Na 26 weken zagen we een verandering van 0,02 (95% CI: –1,95; 1,99) in de groep met de lage dosis en –1,42 (–3,40; 0,57) in de groep met de hoge dosis EPA+DHA. Suppletie met 400 mg of 1800 mg EPA+DHA had dus geen meetbaar effect op de kwaliteit

van leven na 13 of 26 weken interventie.

Verder hebben we onderzocht of EPA+DHA van invloed kunnen zijn op mogelijke mechanismen die gerelateerd zijn aan de mentale gezondheid, door het bestuderen van de genexpressie in witte bloedcellen na suppletie met een lage of hoge dosis EPA+DHA (**hoofdstuk 8**). We vonden dat EPA+DHA suppletie gedurende zes maanden resulteerde in een genexpressieprofiel dat minder inflammatoir en minder atherogeen was.

De belangrijkste resultaten en methodologische aspecten van de onderzoeken in dit proefschrift worden besproken in **hoofdstuk 9**. Verder worden hier suggesties gedaan voor toekomstig onderzoek en wordt de betekenis van de bevindingen voor de volksgezondheid bediscussieerd. De belangrijkste conclusie van dit proefschrift is dat het verhogen van de inname van EPA+DHA op relatief korte termijn weinig invloed heeft op het cognitief functioneren en mentaal welbevinden van ouderen zonder klinische dementie of depressie. Onze epidemiologische bevindingen bevestigen daarnaast de hypothese dat visconsumptie van belang is voor de mentale gezondheid van ouderen in Westerse landen niet. Gebaseerd op dit proefschrift en het totaal aan wetenschappelijk bewijs op dit gebied, concluderen we dat het tot op heden niet duidelijk is of EPA+DHA een gunstig effect op de mentale gezondheid van de algemene populatie hebben.



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Niemand is een eiland. Om de goede strijd te strijden hebben we hulp nodig.
(De pelgrimstocht naar Santiago, Paulo Coelho)

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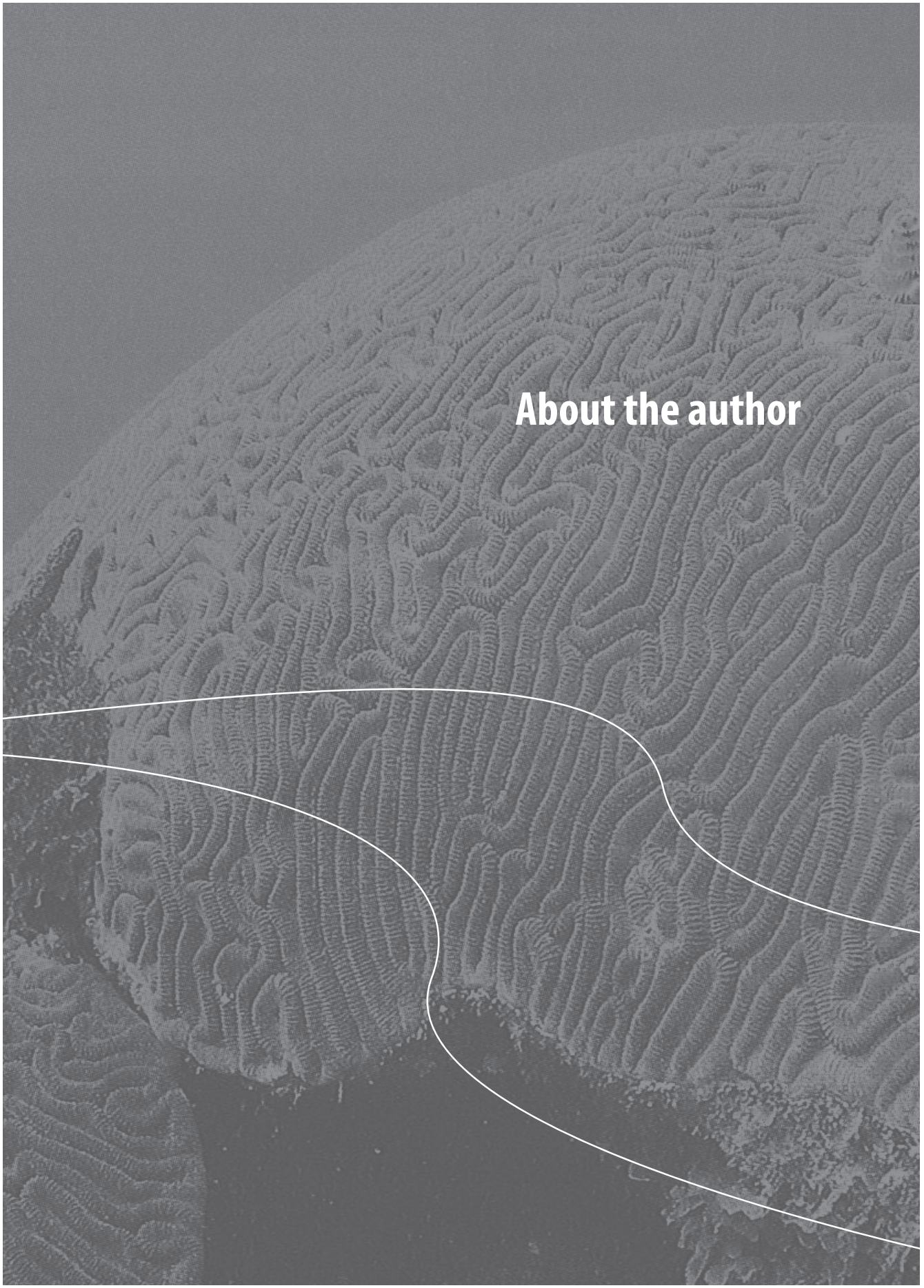

About the author

Curriculum vitae

Ondine van de Rest was born on the 28th of November 1975 in Breda, the Netherlands. After completing secondary school at the "Onze Lieve Vrouwe Lyceum" in Breda in 1994, she started her studies on Human Nutrition at Wageningen University. During her MSc training, she studied food consumption during an intervention study on the bioavailability of β-carotene and retinol in school children at Bogor, West Java, Indonesia. For her second MSc thesis she investigated the influence of sugar and orange aroma concentration of drink yoghurts on sensory specific satiety and ad libitum consumption. Ondine graduated in September 1999 in both the Majors "Nutrition and Health" as well as "Nutrition Behaviour". After her graduation she started as trial manager of the FACIT-study, a large intervention study on the influence of folic acid on atherosclerosis, at the Division of Human Nutrition of Wageningen University/ Wageningen Centre for Food Sciences (WCFS). In January 2005 she started her PhD research on the effect of omega-3 fatty acids on cognitive performance and mental well-being in older people, of which the results are described in this thesis. During this period she conducted an intervention study and she also performed observational data analyses. She conducted part of these analyses during a 4-month stay at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University in Boston, USA. Ondine joined several (international) conferences, followed courses on epidemiology, statistics and geriatrics and participated in several committees and discussion groups of the Division of Human Nutrition. She won the Student Travel Award in the Student Prize Paper competition at the IANA 2006 Symposium on Nutrition and Cognitive Decline/ Alzheimer Disease in Chicago and was nominated for the Encouragement Prize for Dementia Research by Alzheimer Centre Nijmegen. In 2009, she was selected for the European Nutrition Leadership Program. Furthermore, she was appointed by Top Institute Food and Nutrition (TIFN) as assistant project leader of an intervention trial on calcium, probiotics and acute diarrheal disease, performed in Jakarta, Indonesia. Currently, she is employed as a postdoctoral fellow at the Division of Human Nutrition to continue her research focusing on cognitive performance in older people. Also, she will continue the collaboration with UMC St Radboud to complete the challenge study on fish oil and cerebral blood flow in MCI patients, which she initiated during her PhD.

List of publications

Original research papers

van de Rest O, de Goede J, Sytsma F, Oude Griep L, Geleijnse JM, Kromhout D, Giltay EJ. Association of n-3 long-chain polyunsaturated fatty acid and fish intake with depressive symptoms and low dispositional optimism in older subjects with a history of myocardial infarction. Br J Nutr; in press.

van de Rest O, van der Zwaluw N, Beekman ATF, de Groot CPGM, Geleijnse JM. The reliability of three depression rating scales in a general population of Dutch older persons. Int J Geriatr Psychiatry; in press.

van de Rest O, Spiro III A, Krall-Kaye E, Geleijnse JM, de Groot CPGM, Tucker KL. Intakes of n-3 fatty acid and fatty fish are not associated with cognitive performance and 6-year cognitive change in men participating in the Veterans Affairs Normative Aging Study. J Nutr; in press.

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Bouwens M, **van de Rest O**, Dellschaft N, Grootte Bromhaar M, de Groot CPGM, Geleijnse JM, Müller M, Afman LA. Fish oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. Am J Clin Nutr 2009;90:415-24.

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Dullemeijer C, Durga J, Brouwer IA, **van de Rest O**, Kok FJ, Brummer RM, van Boxtel MPJ, Verhoef P. N-3 fatty acid levels in plasma and cognitive performance in older adults. Am J Clin Nutr 2007;86:1479-85.

van de Rest O, Durga J, Verhoef P, Melse-Boonstra A, Brants HAM. Validation of a food frequency questionnaire to assess folate intake of Dutch elderly people. Br J Nutr 2007;98:1014-20.

Ozturk H, Durga J, **van de Rest O**, Verhoef P. The MTHFR 677 C→T genotype modifies the relation of folate intake and status with plasma homocysteine in middle-aged and elderly people. Nederlands Tijdschrift voor Klinische Chemie en Laboratoriumgeneeskunde 2005;30(3):208-17.

Book chapter

Eussen SJPM, **van de Rest O**, Manders M, de Groot CPGM. Nutrition, Aging and Cognitive Function. The Whitehall-Robins Report 2006;15(2).

Abstracts in published conference proceedings

van de Rest O, Spiro III A, Krall-Kaye E, Geleijnse JM, de Groot CPGM, Tucker KL. Intake of fatty fish and n-3 fatty acids in relation to cognitive performance and 6 year cognitive change in aging men: the Veterans Affairs Normative Aging Study. Eur J Clin Nutr 2009;63(Supplement 3):S21.

van de Rest O, Geleijnse JM, Kok FJ, van Staveren WA, Beekman ATF, Hoefnagels WHL, de Groot CPGM. The efficacy of omega-3 fatty acids in maintaining optimal mental health in elderly people: a double-blind placebo-controlled trial. J Nutr Health Aging 2008;12(7):420.

van de Rest O, Geleijnse JM, Kok FJ, van Staveren WA, Beekman ATF, Hoefnagels WHL, de Groot CPGM. The efficacy of omega-3 fatty acids on cognitive performance and mental well-being in elderly people: a double-blind placebo-controlled trial. Ann Nutr Met 2007;51(Supplement 1):76.

van de Rest O, Geleijnse JM, Kok FJ, van Staveren WA, Beekman ATF, Hoefnagels WHL, de Groot CPGM. The efficacy of omega-3 fatty acids in maintaining optimal mental health in elderly people: a double-blind placebo-controlled trial. J Nutr Health Aging 2006;10(3):209.

Educational program

Discipline specific activities

Courses

- Nutritional and Lifestyle Epidemiology, VLAG; Wageningen (NL), 2003
- Masterclass Geriatric Nutrition: diet, functionality and disease, VLAG; Wageningen (NL), 2004
- Principles of epidemiologic data analysis by Dr. K. Rothman, NIHES; Lunteren (NL), 2007
- N-3 fatty acids and the brain, VLAG; Wageningen (NL), 2007
- Linear regression, Erasmus Summer Programme; Rotterdam (NL), 2007

Scientific meetings

- Annual meetings NWO Nutrition; Arnhem/ Deurne (NL), 2005, 2006, 2007
- Annual symposiums of the Netherlands Epidemiology Society (WEON); Wageningen (NL), 2005 and Maastricht (NL), 2007
- Nutrition and Aging, "International Acadamy Nutrition and Aging (IANA)"; St Louis, (USA, 2005), Chicago (USA, 2006), and Albuquerque (USA, 2008)
- "Food for thought, de rol van onze voeding op de hersenen", NVD/ Alpro Soya; Groenekan (NL), 2005
- Prospective registration of trials in the Netherlands, Dutch Cochrane Centre; Amsterdam (NL), 2005
- Sixth International Conference on Dietary Assessment Methods (ICCDAM); Kopenhagen (Denmark), 2006
- Symposium Nutrition and ageing, NZO/WUR; Wageningen (NL), 2006
- 1st Nutrition & Health congress; Amsterdam (NL), 2006
- Lipids & Brain: PUFA metabolism, function and protection against diseases, EFECG/ DGF; Paris (France), 2007
- N-3 fatty acids and the brain, VLAG/WUR; Wageningen (NL), 2007
- 10th European Nutrition Conference, FENS; Paris (France), 2007
- Wageningen Nutritional Sciences Forum, Wageningen University; Arnhem (NL), 2009

General courses

- Good Clinical Practice, ICH-Training and Advice; Wageningen (NL), 2004
- VLAG PhD week; Bilthoven (NL), 2005
- Talent class "Media training", NWO; The Hague (NL), 2005
- Organizing and supervision thesis projects, OWU; Wageningen (NL), 2005

- Masterclass Nutrition Communication: Challenges and opportunities, VLAG; Wageningen (NL), 2005
- PhD Competence Assessment, WGS; Wageningen (NL), 2005
- Scientific writing, CENTA; Wageningen (NL), 2006
- Philosophy and Ethics of Food Science and Technology; VLAG, Wageningen (NL), 2008
- European Nutritional Leadership Programme (ENLP); Luxembourg (L), 2009

Optional courses and activities

- Preparation PhD research proposal; Wageningen (NL), 2005
- PhD study tour, Wageningen University; to United Kingdom and Ireland, 2005, and the USA, 2007
- Journal Club, Wageningen University; Wageningen (NL), 2005-2008
- N-3 journal Club, Wageningen University; Wageningen (NL), 2005-2007
- Oldsmobiles Club, Wageningen University; Wageningen (NL), 2005-2008
- Research presentations, Wageningen University; Wageningen (NL), 2005-2008
- Epi research meetings, Wageningen University; Wageningen (NL), 2005-2008
- Concept and methods in epidemiology (based on Epidemiology by Dr. K. Rothman), Wageningen University; Wageningen (NL), 2006-2007
- 4-month visit at Tufts University, Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging; Boston (USA), 2008

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