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Fish, Docosahexaenoic Acid and Alzheimer’s Disease

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Abbreviations:

Aβ – amyloid beta
AD – Alzheimer’s disease
ApoE – apolipoprotein E
ARA – arachidonic acid (20:4ω6)
DHA – docosahexaenoic acid (22:6ω3)
EPA – eicosapentaenoic acid (20:5ω3)
GLUT1 – glucose transporter 1
MCI – mild cognitive impairment
MMSE – mini mental state examination
NPD1 – neuroprotectin D1
PE – phosphatidylethanolamine
PPAR – peroxisomal proliferator-activated receptor
PUFA – polyunsaturated fatty acid(s)
RAR – retinoic acid receptor
RCT – randomized clinical trial
RXR – retinoid X receptor
TAG – triacylglycerol(s) or triglyceride(s)

Key words: Alzheimer’s disease, docosahexaenoic acid, fish, aging, brain, ω3 fatty acids, eicosapentaenoic acid, cognition, dementia, cognitive decline, arachidonic acid.

Running Title: Fish, Docosahexaenoic Acid and Alzheimer’s Disease
Abstract

1. Introduction

2. Declining Cognitive Function in the Elderly
   2.1 Clinical Aspects
   2.2 Neuropathological Features
   2.3 Risk Factors

3. Biological Functions of DHA
   3.1 Neuronal Membranes
   3.2 Amyloid β
   3.3 Cardiovascular Properties
   3.4 Anti-inflammatory Properties
   3.5 Oxidative Stress
   3.6 Nuclear Receptors

4. Epidemiological Studies
   4.1 Prospective Studies
   4.2 Cross-sectional Studies
   4.3 Fish Intake Compared to DHA Intake

5. Biological Studies of DHA in AD and Other Forms of Cognitive Decline
   5.1 Blood DHA
      5.1.1 Prospective Studies
      5.2.2 Cross-sectional Studies
   5.2 Brain DHA
   5.3 Clinical Trials

6. Linking Epidemiological and Biological Studies
   6.1 Methodological Challenges
   6.2 Biological Issues
      6.2.1 DHA Metabolism During Healthy Aging
      6.2.2 Apolipoprotein E
      6.2.3 Other Nutrients in Fish
      6.2.4 Fish as a Component of a Healthy Diet
      6.2.5 Low DHA or High ω6 PUFA Intake?

7. Animal Models

8. Brain Glucose Uptake: An Emerging Mechanism

9. Discussion

10. References
ABSTRACT

Cognitive decline in the elderly, particularly Alzheimer’s disease (AD), is a major socio-economic and healthcare concern. We review here the literature on one specific aspect of diet affecting AD, that of the ω3 fatty acids, particularly the brain’s principle ω3 fatty acid - docosahexaenoic acid (DHA). DHA has deservedly received wide attention as a nutrient supporting both optimal brain development and for cardiovascular health. Our aim here is to critically assess the quality of the present literature as well as the potential of ω3 fatty acids to treat or delay the onset of AD. We start with a brief description of cognitive decline in the elderly, followed by an overview of well recognized biological functions of DHA. We then turn to epidemiological studies, which are largely supportive of protective effects of fish and DHA against risk of AD. However, biological studies, including blood and brain DHA analyses need careful interpretation and further investigation, without which the success of clinical trials with DHA may continue to struggle. We draw attention to some of the methodological issues that need resolution as well as an emerging mechanism that may explain how DHA could be linked to protecting brain function in the elderly.
1. INTRODUCTION

Cognitive decline, particularly in the form of Alzheimer's disease (AD), has emerged in the past 20 years as a major challenge to the quality of life for the elderly and their caregivers. AD and neurodegenerative diseases in general are putting significant pressure on healthcare resources both in developed and developing countries. Once it is clinically diagnosed, there is little prospect of improving the prognosis of AD [1]. Hence, to reduce the risk of cognitive decline in the elderly, closer attention needs to be paid to the possible impact of lifestyle and other non-genetic, hence modifiable, risk factors that are present in younger adults. Diet quality is a lifestyle risk factor contributing to AD and chronic degenerative diseases in general. Among the dietary nutrients most closely associated with optimal function of the brain, the $\omega$-3 polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA; 22:6$\omega$3) is particularly important.

We review here the possible link between oily fish intake, DHA and declining cognitive function associated with aging, with emphasis on three types of human studies – epidemiological studies of fish or DHA intake, measurement of blood or brain DHA levels, and clinical trials of supplements containing DHA in aging-related cognitive decline, AD, or other forms of dementia. A recent review thoroughly evaluated the literature on observational studies of fish intake and clinical trials using DHA in AD [2] but did not discuss brain or plasma DHA in any detail. That report also did not explore the significant impact of apolipoprotein E (ApoE) on omega-3 fatty acids on the risk of AD. Animal studies linking DHA to AD will be described briefly but have recently been discussed elsewhere [3, 4] and are therefore not the focus of this review.

2. DECLINING COGNITIVE FUNCTION IN THE ELDERLY

2.1. Clinical Aspects
Dementia is defined as a clinical syndrome which associates a memory disorder and impairment in at least one other cognitive domain, both of which significantly interfere with social function or activities of daily living [5, 6]. AD is the most frequent cause of dementia, accounting for 50-60% of all cases [1]. It is characterized by a progressive decline in memory function with objective evidence of impaired episodic memory on neuropsychological testing. Aside from the presence of certain predisposing genetic polymorphisms, several clinical features can be used to support the diagnosis of probable AD. These include the presence of atrophy in the medial temporal lobe and/or hippocampus on brain imaging, biomarkers in cerebrospinal fluid (see Section 2.2 - Neuropathological Features), or specific abnormal patterns of brain glucose uptake seen with positron emitting tomography [6]. The diagnosis of AD is considered definite only when specific neuropathological criteria are met (see below) or when genetic evidence (mutation on chromosome 1, 14 or 21) is present in addition to the clinical criteria cited above [6].

Before the dementia stage, AD pathology may manifest through subtle cognitive decline greater than expected for an individual's age and education but with minimal impact on daily living. This transitory and still reversible stage is usually termed mild cognitive impairment (MCI), but there is as yet no clear consensus on its definition [7-10].

2.2 Neuropathological Features

Senile plaques and neurofibrillary tangles in the medial temporal lobe structures and cortical areas are the neuropathological hallmark of AD [1]. AD is characterized by the accumulation of various β-amyloid (Aβ) peptides resulting from the cleavage of the amyloid precursor protein, in particular peptides composed of 40 (Aβ40) and 42 (Aβ42) amino-acids. Aβ is produced constitutively during cell metabolism but under normal conditions, brain Aβ
does not accumulate. According to the amyloid cascade hypothesis [11], the central event in AD pathogenesis is an imbalance between Aβ production and clearance, with increased Aβ production in familial AD and decreased Aβ clearance in sporadic AD [1].

There are several pools of soluble and insoluble Aβ that are normally in equilibrium. Soluble forms of Aβ40 and Aβ42 have toxic effects and are thought to be responsible for the severity of the neurodegenerative process in AD [12]. Conversely, the level of insoluble Aβ, which is a major part of total amyloid load, distinguishes AD from controls but is not correlated with disease severity or number of amyloid plaques [12]. Soluble Aβ species constitute a fluctuating intra- and extra-cellular pool whereas insoluble Aβ is usually viewed as relatively stable.

The other characteristic lesion of AD is the presence of intra-cellular neurofibrillary tangles composed of abnormally hyperphosphorylated tau protein. Tau pathology is generally considered as a downstream event but may contribute to neuronal dysfunction and cognitive impairment [1]. AD pathology is also accompanied by synaptic dysfunction and neuronal apoptosis.

2.3 Risk Factors

Old age is the main risk factor for AD since the prevalence of AD increases almost exponentially from 1% at 60-64 years old to 25% or more at 85 years old and older [1]. The presence of certain predisposing genetic polymorphisms can also greatly increase the risk of AD. Mutations in the amyloid precursor protein, presenilin 1 and presenilin 2 genes have been identified in the familial form of AD, a rare autosomal dominant disorder with onset before 65 years old. In sporadic late-onset AD, presence of the ε4 allele of ApoE is a significant
predisposing factor which lowers the age at onset [13] (see also Section 6.2.2 - Apolipoprotein E).

Risk of AD is also associated with reduced brain ‘reserve capacity’, a parameter linked to lower education and insufficient activities that are cognitively and/socially stimulating, e.g. puzzles, crosswords or card games [1]. Risk factors for vascular disease also increase the risk for AD, including hypertension, dyslipidemia, atherosclerosis, smoking, diabetes, obesity [1]. Conversely, dietary anti-oxidants, B vitamins, and \( \omega-3 \) PUFA such as DHA may have a potentially protective effect [14-16].

3. BIOLOGICAL FUNCTIONS OF DHA

The biological functions of DHA have been the matter of a recent extensive review [4], so we will only briefly summarize putative mechanisms of action by which it might reduce the risk of cognitive decline or AD. In addition to specific functions in the brain, DHA has several non-specific properties which could also potentially contribute to its protective effect against the neurodegenerative process in AD.

3.1 Neuronal Membranes

DHA is a major component of the lipids in neuronal membranes where it clearly exerts a structural and functional role. The physical state of the neuronal membrane is critical in the control of transfer of the neuronal information, i.e. neither too rigid nor too fluid for the ionic exchange between the inner and outer walls of the membrane [17]. DHA also modulates properties of the hydrophobic core of the membrane bilayer, including conferring a high degree of flexibility and directly interacting with membrane proteins, thereby impacting on speed of signal transduction, neurotransmission, and formation of lipid rafts [18].
3.2 Amyloid β

Many in vitro and animal studies suggest a direct role of DHA on Aβ [3] (see also Section 7 - Animal Models). In various animal models and using a variety of experimental protocols, DHA exerts a protective effect against neuropathological signs of AD, including Aβ accumulation, synaptic marker loss, and hyperphosphorylation of tau [19]. The DHA-derived mediator, neuroprotectin D1 (NPD1), is formed in the brains of AD patients as well as in human brain cells in culture. In cytokine-stressed human neural cells, DHA attenuates Aβ secretion, an effect accompanied by the formation of NPD1. NPD1 promotes brain cell survival via the induction of anti-apoptotic and neuroprotective gene-expression programs that suppress Aβ42-induced neurotoxicity [20]. Enrichment of membranes with DHA protects neurons in culture against apoptosis when exposed to soluble Aβ [21]. This protective effect depends on the way the experiment is conducted; pre-treatment with eicosapentaenoic acid (EPA, 20:5ω3) and DHA can actually reduce the survival of neurons incubated with Aβ42, but this deleterious effect was not observed when EPA and DHA were added at the same time as the Aβ42 peptide [22].

In living humans, Aβ measurements can be performed in CSF or in plasma, the most convenient fluid to sample for clinical studies. Despite controversy regarding their ability to predict AD, plasma Aβ40 and Aβ42 concentrations and their ratio have been proposed as a measure of the impact of treatments in clinical AD trials [23, 24]. The possible efficacy of DHA in the treatment of AD could therefore potentially be evaluated by its ability to reduce the plasma level of soluble Aβ [12]. However, to our knowledge, Aβ concentration has not yet been measured in any of the few clinical trials assessing the impact of ω3 PUFA supplementation against cognitive decline or dementia in humans (see Section 5.3 - Clinical Trials).
3.3 Cardiovascular Properties

The protective effect of EPA and DHA in cardiovascular disease is well documented in humans [25], in particular through randomized controlled trials (RCT) [26-29]. Elevated plasma triacylglycerols (TAG) are a component of the metabolic syndrome which is linked to higher risk of cognitive decline, particularly in the presence of elevated inflammation [30]. More specifically, elevated plasma TAG are a risk factor for dementia, in particular of the vascular type [31, 32]. In obese mice, lowering TAG with gemfibrozil reversed cognitive impairment [33].

One of the main effects of dietary ω3 PUFA is to reduce elevated plasma TAG [29, 33-42]. The hypotryglyceridemic effect of ω3 PUFA is believed to be due mostly to their potent enhancement of lipolysis through activation of peroxisome proliferator-activated receptors (PPAR; see Section 3.6 - Nuclear Receptors) [39]. Therefore, if supplemental DHA or EPA were to be shown to have a protective effect against cognitive decline or AD, the mechanism might well involve TAG lowering.

3.4 Anti-inflammatory Properties

Inflammation plays a major role in the development and progression of AD. The accumulation of Aβ in AD is accompanied by an inflammatory response resulting from microglia activation and recruitment of astrocytes, inducing the expression of pro-inflammatory cytokines [24, 43]. In turn, cytokines stimulate Aβ synthesis and amyloid formation, setting off a vicious circle of exacerbation of inflammation [24]. Overproduction of reactive oxygen species by brain cytokines increases oxidative stress and hence the risk of
neurodegeneration [44]. Transient acute systemic inflammation also increases the vulnerability of the brain to neurodegenerative disease [45].

Long-chain PUFA are viewed as potent modulators of inflammation. Most of the mediators formed from EPA and DHA (leukotrienes, resolvins, NPD1) are anti-inflammatory, whereas those formed from the ω6 PUFA, arachidonic acid (ARA, 20:4ω6) are mostly pro-inflammatory [4, 46]. The anti-inflammatory effects of ω3 PUFA could therefore contribute to their protective effect against neurodegeneration.

Very few RCT have assessed the impact of DHA or EPA supplementation on peripheral inflammatory markers in disease, and the few that have been reported are of small sample size and have shown inconsistent results [37, 47-51]. In the OmegAD trial conducted in patients with mild to moderate AD receiving 1.7 g DHA and 0.6 g EPA daily, higher plasma concentrations of EPA and DHA were associated with reduced release of interleukin-1β, interleukin-6 and granulocyte colony-stimulating factor from peripheral blood mononuclear cells [52], results supporting a dampening effect of EPA and DHA on peripheral inflammatory markers.

3.5 Oxidative Stress

Oxidative stress results from an imbalance between formation and degradation of pro-oxidants or decreased cellular antioxidant protection mechanisms, and may result in increased cell damage and apoptosis. Excessive oxidative stress stimulates multiple signalling pathways in the central nervous system that then participate in pathophysiological processes leading to cell damage and, eventually, cell death [53]. The brain is particularly susceptible to oxidative stress because, on the one hand, it has a high content in easily peroxidizable long-chain PUFA, in particular DHA and ARA, and on the other hand, because
mitochondrial function to fuel the brain's normal energy requirements consumes a lot of glucose, a process associated with relatively high production of free radicals [54].

In AD, the accumulation of Aβ is associated with increased free radical production and increased lipid peroxidation in the brain [54]. In post-mortem brain samples from AD patients, oxidative damage can be observed in the form of oxidized lipids, proteins and DNA [55]. Important oxidative damage has also been observed in subjects with MCI, suggesting an early role of oxidative stress [56].

DHA administration exerts antioxidant activity as shown by increasing glutathione reductase activity and decreasing accumulation of lipid peroxide and reactive oxygen species in the cortex and hippocampus of AD model rats [57, 58]. Moreover, a transgenic mouse model of AD given an ω3 PUFA deficient diet exhibited increased oxidative damage [59]. Some animal models of ischemia-reperfusion brain injury show significant free radical scavenging capacity of DHA and protection against peroxidative damage of lipids and proteins in developing and adult brains, with attenuation of neuron loss and cognitive and locomotor deficits [18]. However, in a cellular model, DHA did not prevent the oxidative stress induced in vitro in neurons exposed to soluble Aβ peptides [21, 43].

The few human studies examining the impact of DHA supplementation on oxidative stress have yielded inconsistent results. In a double-blind controlled trial, 59 subjects were randomized to 4 g/d of purified EPA, DHA, or olive oil for 6 weeks while maintaining their usual diet. In one study, urinary F₂-isoprostanes, a measure of lipid peroxidation, were significantly decreased by dietary EPA or DHA in type 2 diabetic subjects [48]. Plasma 8-iso-PGF₂α was significantly decreased after 3 months of supplementation with ω3 PUFA [60], also suggesting inhibition of ARA metabolism to eicosanoids and/or a possible antioxidant effect of EPA and DHA. However, supplementation of healthy adult men with high dose of EPA and
DHA (total 5.3 g/d) may also increase lipid peroxidation, an effect not suppressed by high
dose vitamin E (900 IU/d) [61]. Therefore, DHA supplementation at low doses may be
protective against oxidative stress, whereas higher DHA doses promote lipid peroxidation
[62]. The DHA response to oxidative stress may also be linked to the production of NPD1
generated enzymatically from unesterified DHA. NPD1 may in turn inhibit oxidative stress or
have other neuroprotective functions at the site where the free DHA is produced [63, 64].
NPD1 production may be influenced by redox balance and availability of other antioxidants.

3.6 Nuclear Receptors

Changing fatty acid intake, particularly PUFA, is an important means of modulating
gene expression in various tissues [65, 66]. EPA and DHA interact with at least four families
of transcription factors – peroxisome proliferator-activated receptors (PPAR), liver retinoid X
receptors, hepatic nuclear factor-4α, and sterol regulatory-element-binding protein. EPA and
DHA also generate a large range of eicosanoids modulating transcription factor activity [67].
PPARs are ligand-inducible transcriptional factors with a central role in regulating gene
expression. PPARs belong to the class II superfamily of nuclear receptors which also includes
retinoic acid receptors (RAR). RXR, the receptor of 9-cis retinoic acid, is considered as the
common dimerisation partner of several other nuclear receptors in particular PPARs [68].

Among the three known isoforms of PPAR (α, β/δ and γ), PPARγ is the most studied,
partly because of its ability to inhibit inflammatory gene expression in brain cells [69, 70].
PUFA bind PPARs with a higher affinity than saturated or monounsaturated fatty acids and
induce gene transcription by interacting with distinct DNA promoter sequences in the target
genes predominantly in the form of PPAR/RXR heterodimers [71, 72]. Modulation of the
expression of genes targeted by PPAR may well also be affected by dietary intake of ω3 PUFA because of the high efficacy of ω3 PUFA as PPAR ligands [4, 73].

RAR and RXR play key roles in many aspects of brain development, including embryonic neurogenesis, morphological differentiation of catecholaminergic neurons, and activity-dependent plasticity [18]. RAR and RXR are also highly expressed in the hippocampus, which may be relevant to understanding the role of DHA in AD. Several studies show that DHA can specifically bind to and directly activate RXR, leading to the regulation of the expression of genes usually under the control of retinoic acid [74-76]. These results are potentially important for treatment or prevention of AD since the retinoic acid signalling pathway is involved in the maintenance of synaptic plasticity and memory in aged animals [77-80]. Indeed, a link between the metabolism of retinoids and late onset AD was recently proposed [81, 82].

4. EPIDEMIOLOGICAL STUDIES

4.1 Prospective Studies

We turn back now from the biological functions of DHA to its possible role in the link between fish, ω3 PUFA intake and AD. The strongest support for a causal association between low fish and/or low DHA intake in AD comes from prospective epidemiological studies conducted in France, the Netherlands, Scandinavia, Italy and the USA. Of the nine prospective studies that have been published, most show that higher intake of fish or long-chain ω3 PUFA decreases the risk of cognitive decline, dementia or AD in the elderly (Table 1).

The Rotterdam Study was the first to report that fish intake protected against the risk of AD [83]. However, at a length of just 2 years, the initial follow-up period of this study was
relatively short. In fact, in the subsequent 6 year follow-up of this cohort, no significant relationship was observed between fish intake and risk of AD or all cause dementia [84]. A lower risk of incident dementia or cognitive decline in those consuming more fish has nevertheless been reported in several other independent prospective cohort studies [85-90].

Only one prospective study has not shown a significant benefit of higher fish intake and risk of AD [91]. This study was based on a subsample of 488 participants representing about 25% of the main cohort in which both dietary DHA intakes were calculated and plasma DHA measurements made. A protective effect of higher plasma DHA was observed against the risk of all cause dementia but not for AD. This negative finding for AD *per se* may have been due to a lack of statistical power because the 50% risk reduction for AD in subjects consuming fish more than twice a week was almost statistically significant (95% CI 0.20-1.27; *P* =0.14).

Furthermore, several studies suggest that certain polymorphisms in apolipoprotein E (ApoE) may inhibit or prevent the beneficial effect of fish in reducing the risk of cognitive decline in the elderly (see Section 6.2.2 - Apolipoprotein E).

4.2 Cross-Sectional Studies

Three cross-sectional studies describe better cognitive performance on various neuropsychological tests in middle-aged [92] and older persons [93, 94] who regularly consumed fish. In these studies, consumption of fish other than fried fish was also associated with lower prevalence of subclinical infarcts and white matter abnormalities on brain magnetic resonance images, thereby indicating a lower risk of vascular dementia [95]. Despite the association between higher fish consumption and better cognitive performance reported in these cross-sectional studies, this type of study cannot ascertain causality, i.e. whether the observed dietary behaviour is the cause or the consequence of cognitive impairment. Indeed, dementia is accompanied by a progressive loss of autonomy in activities of daily living which
impairs the ability for shopping, preparing adequate meals and, eventually, eating well. Hence, the value of observational epidemiological studies in understanding the link between dietary intake of fish or DHA and risk of dementia or cognitive decline depends crucially on their design.

4.3 Fish Intake Compared to DHA Intake

DHA intake is normally a direct function of fish and seafood intake [91, 96, 97]. The two principal reasons for this direct association are that – (i) on a population basis, there is almost no other dietary source of DHA other than fish or seafood, and (ii) the conversion rate from α-linolenic acid and EPA to DHA through the desaturation-chain elongation system is very limited in humans and has essentially no impact on plasma DHA [98, 99]. Despite the clear association between fish and seafood intake and DHA intake, DHA intake is not as clearly linked to lower risk of AD as is fish intake [100]. A precise quantitative estimate of DHA intake is generally impossible from food frequency questionnaires because the number of fish items they provide is too limited and commonly not adequately validated for this purpose. Moreover, EPA and DHA intake has high day-to-day variability because fish and seafood intake is typically sporadic. Random within person error due to day-to-day fluctuation in dietary intake and measurement error tends to bias relative risks toward 1, i.e. to underestimate the strength of the possible association between the exposure (DHA intake) and the outcome (cognitive status) [101].

Moreover, consistency between fish and DHA intake in disease associations such as for AD depends on the power of the study to detect an association, which itself is strongly linked to the total number of subjects involved (not only AD cases). In spite of appropriate adjustments, other residual confounders in observational studies usually remain: (i) Fish
consumers differ from non-consumers in many ways that may themselves be associated with higher or lower risk of dementia e.g. education, income, lifestyle [93]. (ii) Fish is not eaten alone but is a component of “healthy” dietary pattern, e.g. the Mediterranean diet, which is associated with lower dementia risk [102]. Moreover, as noted earlier fried fish and lean fish may be less protective against AD than fatty fish [88, 95]. The recently identified interaction of AD risk with ApoE genotype means stratification of ApoE4 status is now also needed for proper interpretation of the association between fish or DHA intake and dementia (see Section 6.2.2 - Apolipoprotein E).

5. BIOLOGICAL STUDIES OF DHA IN AD AND OTHER FORMS OF COGNITIVE DECLINE

5.1 Blood DHA

Plasma DHA is usually proportional to fish and seafood intake, e.g. lower blood DHA occurs in those consuming low amounts of fish and higher plasma DHA is observed in those consuming more fish [91, 103]. Lower blood DHA is observed in those adhering to a diet strictly avoiding fish or seafood, i.e. in vegans [104]. Hence, the amount of DHA in human blood is normally significantly positively correlated to DHA intake, but at DHA intakes >2 g/d, DHA in plasma phospholipids approaches saturation and increases only marginally thereafter [99]. Since AD and other forms of cognitive decline are significantly associated with low fish intake and lower DHA intake (Table 1), one would normally expect to see that AD was associated with lower blood DHA (plasma and/or erythrocytes). We review here the evidence for this prediction from both prospective and cross-sectional studies.

5.1.1 Blood DHA - Prospective Studies
Five published prospective studies have reported plasma DHA in the elderly with cognitive decline after follow-up [105-107], or who converted to AD or other dementia [107, 108] (Table 2; Figure 1). Of these five prospective studies, only the one by Heude et al [106] reported significantly lower plasma DHA. Although mean plasma or erythrocyte DHA is not different from control values in these prospective studies, they nevertheless demonstrate a significant inverse association between baseline plasma or erythrocyte DHA and risk of cognitive decline or incident dementia [91, 105, 108, 109]. For instance, the largest prospective study to date was conducted in a subsample of the Three City study [108]. It showed that for each increase of one standard deviation in plasma DHA, risk of all-cause dementia was reduced by 24%. However, mean blood DHA in those converting to AD compared to those that did not convert was similar in both groups. In the Three City study [108], the association of higher blood DHA to lower risk of cognitive decline became non-significant in multivariate analyses, but the inverse association with plasma EPA remained.

The longest prospective study of the association between DHA intake, plasma DHA and cognitive decline in the elderly had a mean follow-up of 9.1 years. It reported that risk of all causes of dementia was significantly associated with both lower DHA intake and lower DHA in plasma phosphatidylcholine [91]. This study also showed a significantly reduced risk (~50%, p=0.05) for all-cause dementia in the highest quartile of plasma DHA [91]. However, the direct association between lower DHA intake and higher risk of all causes of dementia was present only when the lower three quartiles of DHA intake were combined and compared to the highest DHA intake. It was also only significant for all causes of dementia but not for AD alone. Apart from the large number of subjects studied, one of the strengths of this study was the assessment of DHA intake and plasma DHA in a specific class of blood phospholipids (phosphatidylcholine).
In the study by Beydoun et al. [105], which was based on the Atherosclerosis Risk in Communities study, higher plasma phospholipid or cholesteryl ester content of EPA and DHA was protective against decline in verbal fluency. However, no association was found with global cognitive decline or other neuropsychological tests in that study. In the placebo arm of the FACIT trial, higher long chain ω3 PUFA in plasma cholesteryl esters was associated with less cognitive decline over 3 years on tests of sensorimotor speed and complex speed but in contrast to Beydoun et al [105], was unrelated to changes in word fluency [109]. The largest prospective study reporting fatty acid analyses showed a significantly decreased risk of global cognitive decline with higher erythrocyte EPA, DHA and total ω3 fatty acids [106]. Hence, it is very important to make a distinction between static blood DHA measurements in those with or without cognitive decline, versus the more dynamic measure of blood DHA in relation to risk of cognitive decline.

5.1.2 Blood DHA - Cross-sectional Studies

There are more published reports of blood DHA in dementia, AD or other forms of cognitive decline that are based on cross-sectional studies than there are based on prospective studies. Nevertheless, relative to each study’s own controls, our post hoc analysis is that overall mean blood DHA values for AD or other forms of cognitive decline is not different from 100%, i.e. not different from blood DHA in healthy age-matched controls (Figure 2). If one outlying cross-sectional study [110] is deleted from this analysis, overall blood DHA values in AD and other forms of dementia relative to controls is actually numerically (but not statistically) lower in these pooled cross-sectional studies than in the prospective studies (compare Figures 1, 2). The lack of association of low blood DHA with AD in cross-sectional studies may also be due in part to modification of dietary intake during progression towards
dementia and the related difficulties that subsequently arise in activities of daily living such as shopping or preparing meals.

Three key points emerge from this published literature: First, compared to control values, blood levels of DHA (plasma or erythrocytes) are not different in AD or other forms of dementia, regardless of whether the studies are prospective or cross-sectional in design (Table 2, Figures 1,2). Second, without reference to changing cognitive function (preferably in a prospective study design), blood DHA values alone are not a very useful measure of cognitive status. Third, it remains to be established whether the overall similarity in plasma and erythrocyte DHA levels seen in AD as in healthy age-matched controls is a function of confounders or inadequate study design, e.g. masking due to genotype effects, small numbers of subjects in most cross-sectional studies, or mixing of data for cognitive decline of different etiology. Alternatively, they may also be a function of changes in DHA metabolism brought on by cognitive decline itself (see Section 6 - Linking Epidemiological and Biological Studies) [111].

5.2 Brain DHA

If low DHA intake increases the risk of deteriorating cognition in the elderly, to understand such a link, it is important to know whether low brain DHA occurs in those with clinical or pathological evidence of cognitive decline, particularly AD. Our analysis here is based on seven independent publications that in total report data for about 100 brain DHA analyses from AD patients and age-matched controls (Table 3). For the hippocampus, the three published studies all showed AD brain had 30-50% lower free DHA and also lower DHA in brain PE. However, in the parahippocampus, brain DHA was not different in AD compared to healthy, age-matched controls. Barring the results of one study [112], overall values for DHA in frontal cortex phospholipids were variable but, on average, very similar in AD
compared to healthy controls (Table 3; Figure 3). Phospholipids are the brain’s main repository of DHA but this pool may not be as important as free DHA could be in helping the brain resist the oxidative damage occurring in AD. Free DHA is more labile and although only one report gives values for free brain DHA (in the hippocampus), they were 50% lower in AD than in controls [20] (Table 3; Figure 3).

However, in these reports, when low brain DHA was observed, it was rarely specific for DHA: either ARA and/or adrenic acid (22:4ω6) were also decreased [112-114]. In some reports, lower DHA concentration was specifically due to a decrease in one of the brain phospholipid classes, most commonly plasmalogens but not in the % composition of DHA itself in the individual phospholipid classes [115]. In AD, increases of up to 30% in the DHA content of frontal cortex, superior and middle temporal and in the white matter of parietal and frontal cortex have also been reported [115-117]. Four fold higher adrenic acid has been observed for the gray matter of parietal and frontal cortex and in the parahippocampal region [115-117]. Thus, there is considerable disparity and non-specificity for DHA data in AD brain. There is actually more agreement about the importance of a significant decrease in the concentration of brain phospholipids in AD, particularly in plasmalogen PE, than there is about changes in brain DHA per se [111]. Hence, the changes in brain phospholipid (or free fatty acid) pool size during AD may be equally or more important than the possible changes in fatty acid composition of these lipid classes [118].

One difference between these studies that might help account for the disparity of brain DHA data is that different researchers have used different criteria to define their AD groups. PL and DHA content of brain tissue also varies markedly from region to region [119], especially from white to gray matter. Hence, imprecise dissection of brain tissue used in these analyses could also markedly skew the results. Nevertheless, one report shows lower DHA in both white and gray matter [112]. Possible differences in analytical methodology are not
always easy to identify but could also contribute to variation in reported DHA values between studies but should not be a factor within any given report.

Aside from methodological issues, homeostatic mechanisms slow down brain DHA loss during dietary $\omega$3 PUFA deprivation [120], so this might affect the rate of decline of brain DHA in the elderly or in response to processes precipitating AD. Animal studies suggest that plasma DHA varies much more than brain DHA and is much more influenced by dietary $\omega$3 PUFA intake. Hence, even if mild, chronic dietary $\omega$3 PUFA (Table 1) might help the AD brain resist losing DHA, at least until the stage at which neurodegeneration and lipid peroxidation were extensive.

The natural atrophy of the human brain as it ages [121] adds a complication to expression of brain fatty acid data because it affects the point of reference, whether it be tissue weight (dry or wet), phospholipid content or protein or DNA content (See Section 6.2.1 – DHA Metabolism During Healthy Aging). Nevertheless, in AD, DHA seems to be markedly lower in the hippocampus, a brain region intimately involved in memory formation and retrieval (Figure 3). It remains to be determined whether this relatively specific change in brain DHA is in part a function of inadequate DHA intake or specific pathological mechanisms more rapidly destroying DHA in the hippocampus than elsewhere in the brain.

5.3 Clinical Trials

Converging results from animal and prospective observational studies show a protective effect of dietary consumption of fish or $\omega$3 PUFA for risk of AD. However, to date, just a single RCT has been reported for the primary prevention of cognitive decline by EPA and DHA [122]. In that study, 302 cognitively healthy older participants were randomly assigned to receive either 1,800 mg/d EPA+DHA, 400 mg/d EPA+DHA, or placebo capsules.
for 26 weeks. There were no significant differential changes in any cognitive domain among the three groups at either 13 or 26 weeks intervention except slightly more decline in memory at 13 weeks in the 400 mg group than the placebo, a difference that might well be attributable to chance. An interaction with ApoE genotype was observed, i.e. ApoE4 carriers in both fish oil groups improved in the cognitive domain of attention after 26 weeks of intervention compared with placebo. However, such post-hoc analyses in small subgroups must be interpreted with caution, all the more since the interaction was not consistent with that found in observational studies [86, 88, 105].

In an exploratory preventive RCT [125], 49 healthy elderly women received a DHA supplement or placebo for 4 months with or without lutein. Multiple cognitive tests measuring verbal fluency, memory processing speed and accuracy were performed at randomization and 4 months later. DHA alone or in combination with lutein significantly improved verbal fluency, whereas rate of learning and delayed recall were improved only in the group receiving the DHA plus lutein supplement [125]. DHA supplementation caused no change on any other test. Higher DHA serum level was significantly associated with better verbal fluency. However, this small trial had several limitations, including a high rate of dropouts not taken into account by an intention-to-treat analysis (14%), and an important ceiling effect in most cognitive tests which could have prevented any improvement.

Several RCTs have focused on patients who already suffered from various stages or types of dementia or cognitive impairment. To date, the largest, published double-blind RCT in mild to moderate AD recently showed very modest cognitive benefits of a mixed dietary PUFA supplement containing both DHA (1.7 g/d) and EPA (0.6 g/d) compared to a corn oil placebo [123]. No overall difference in cognitive function assessed by the Mini-Mental Status Examination (MMSE) or the modified cognitive portion of the Alzheimer Disease Assessment Scale (ADAS-cog) was observed between the controls and the intervention group after the
first 6 months of active or placebo treatment. However, in a sub-group of 32 AD patients who had a relatively high level of cognitive function at the start of the study, (MMSE >27), MMSE scores declined significantly more slowly during the first 6 months of active treatment [123]. Interestingly, cognitive decline seemed to halt when the placebo group also received the ω3 supplementation during the 6 following months.

Again, such post-hoc analyses must be interpreted with caution since the randomization was not stratified by cognitive level and could not therefore ensure that the placebo and intervention groups were still comparable. Furthermore, after switching to the DHA+ EPA supplement, the reduced decline in cognitive performance in the com oil placebo group may have been due to redressing an imbalance in dietary ω6 to ω3 ratio. Hence the choice of placebo could be important in such studies.

Similar results were reported in a recent small RCT [124]. The placebo and the treatment groups were patients at varying stages of AD or cognitive impairment. They consumed a mono-therapy of 1080 mg of EPA and 720 mg of DHA or olive oil esters as the placebo for 6 months. ADAS-Cog test scores improved significantly in the EPA+DHA group compared to the placebo but only in those with mild cognitive impairment. This study also showed that higher % EPA in erythrocytes was significantly associated with better cognitive outcome, suggesting that DHA may not be the only ω3 PUFA linked to better cognition [124].

Three other small preliminary trials of supplements combining DHA and EPA or DHA and ARA in patients with various forms of dementia have also been reported, and all three showed that the supplement had no clear benefit for cognitive function [123]. In 20 patients suffering from dementia from thrombotic cerebrovascular disease showed an improvement in MMSE score with 720 mg DHA/d for 6 months [126]. Conversely, a small open-label pilot study on the efficacy of ethyl-EPA (500 mg twice daily over 12 wk) for the treatment of
patients with mild to moderate AD did not demonstrate any effect on cognition [127]. Finally, a double-blind RCT that evaluated the impact of 1.5 g/d of EPA+DHA for 12 weeks in 190 mildly depressed individuals did not observe any benefit on mood or cognitive function [128].

Hence, at best, a very modest clinical benefit of mixtures of PUFA including DHA has been reported but it is not yet clear whether DHA itself has any effect once aging-associated cognitive decline or AD is clinically evident. Several aspects of the clinical trials with DHA or other PUFA will need further study before conclusions can be drawn about the efficacy of such dietary interventions to improve various features of cognition. First, the breadth of doses of DHA used in these studies covered a 36 fold range - 120 mg/d to 4.3 g/d (see Table 4), a huge range that, importantly, produced no apparent dose-related effects on cognition. Second, two of these clinical trials raised the possibility that a beneficial effect of supplemental DHA may exist when cognitive decline is mild but that supplemental DHA may not necessarily be recommended when the decline is more severe, possibly because additional DHA might contribute to degenerative processes in the brain related to lipid peroxidation [123, 124]. Third, only one of these studies [122] separated the groups according to ApoE4 genotype, a factor which may well compromise the protective effect of dietary ω3 PUFA in lowering the risk of progression towards serious forms of cognitive decline (see Section 6.2.2 - Apolipoprotein E).

6. LINKING EPIDEMIOLOGICAL AND BIOLOGICAL STUDIES

A link is starting to emerge between lower fish intake, lower DHA and heightened risk of AD (Table 1). However, we agree with Fotuhi et al [2] that this link is still tentative. For instance, within the prospective studies, three studies evaluating a possible link between DHA intake from fish and seafood and risk for dementia and AD were not consistent (Table 1): The Zutphen study reported a protective effect of total DHA+EPA intake against cognitive decline
[129], an effect which was not found in the CHAP study (which only reported total ω3 fatty acid intake) [90]. Conversely, the latter showed a protective effect of DHA intake specifically against AD without reporting about other causes of dementia [89]. In a subsample of the Framingham study, the opposite was observed, i.e. DHA protected against all cause dementia but not AD [91]. Also, at least statistically, low fish intake seems to have a stronger association with risk of AD than does low DHA intake. Hence, it seems premature to conclude that the protective effect of fish against risk of AD can be equated to a protective role specifically of DHA.

Lower fish (or DHA) intake in AD would presumably lead to seeing lower blood DHA in AD but this is not observed (Figures 1,2). Brain DHA data for AD also vary considerably. Three studies do suggest that DHA may be lower in the hippocampus in AD (Figure 3) but this effect is not specific for DHA, either in the hippocampus or in other brain regions that have been studied. Currently, no published studies link blood or brain DHA to the neuropathology specific to AD but such a relationship would greatly strengthen a mechanistic link between low DHA and specific processes contributing to AD.

Hence, broadly, there are two possibilities: either there really is no biological link between risk of AD, low DHA intake, and low blood or low brain DHA or, owing to methodological or other experimental differences between studies, the moderately good link present in the epidemiological literature is simply not clearly revealed in the biological literature as it presently stands, perhaps because features of most of the studies mask or confound the variables under study. Some of the methodological challenges affecting the possible relationship between fish intake, DHA intake and risk of AD will be discussed first; biological reasons for the masking of such a possible relationship will be discussed subsequently (see Section 6.2 - Biological Issues).
6.1 Methodological Issues

The possible relationship between DHA intake and AD is vulnerable to the classical pitfalls of nutritional epidemiology affecting observational studies. One major limitation is that despite appropriate adjustments, residual confounders still contribute importantly to the variability observed. Higher DHA intake may also merely be a marker of a generally protective lifestyle including a healthy diet, cognitive and physical activities, and better management of vascular risk factors, all of which could contribute to maintain optimal brain functioning during aging but for which complete statistical adjustment cannot be achieved.

Observational studies also face the difficulty of estimating true quantities of dietary intake of specific nutrients due to cumulative sources of variability and error, i.e. day-to-day intra-individual variability, and poor reliability of most food frequency questionnaires, dietary recall, and food composition tables. Most published reports have not considered cooking methods but concentrations of EPA and DHA in plasma phospholipids may correlate more with consumption of non-fried but not with fried fish [97]. Moreover, it is impossible to differentiate the specific effects of a nutrient such as DHA from those of another one to which it is closely linked in the usual diet and which may also affect cognition (see Section 6.2.3 - Other Nutrients in Fish). Some of the lack of agreement between observational studies of fish intake or DHA levels and those using DHA supplementation is potentially attributable to artifacts caused by methodological differences, including different definitions of cognitive function or dementia, different analytical methods, and possibly even different definitions of ‘healthy’ controls.

Intervention studies including RCTs have their own pitfalls. Contrary to RCTs with drugs, administration of nutritional supplements does not start from zero intake of the nutrient in the supplement. Indeed, individuals are already exposed to varying dietary sources of EPA and DHA, which may confound the true effect of the supplementation. Differing background
DHA intakes, especially the very low DHA and EPA intakes in the USA, may also play a role in the lack of agreement between studies in different countries. In principle, randomization should correct for different basal dietary EPA and/or DHA exposure in the intervention and control groups. Nevertheless, as the target population for an RCT, individuals may be selected who are more or less prone to suffer from insufficient dietary intake of the supplement to be used, e.g. hospitalized elderly who are at higher risk of undernutrition [126]. These individuals might benefit more from the supplementation than healthy community dwellers such as those included in a large primary prevention trial [122]. As already shown for RCT of fish oil supplementation in cardiovascular disease [27], overly optimistic estimates of effect sizes, poor patient adherence to a long course supplementation, and higher than anticipated losses to follow-up may all reduce the power of a trial and hence its ability to demonstrate significant differences.

Indeed, even the placebo can create bias; olive oil might have some protective effects against cognitive decline [130], while a placebo based on ω6 fatty acids such as linoleic acid might increase the risk of dementia [86]. The true difference between the intervention and treatment groups would therefore be artificially minimized in the first case, but increased in the second. Of course, 240 mg olive oil is a very small quantity compared to the overall dietary intake of some populations but 1 g corn oil provides 0.6 g linoleic acid, which could contribute importantly to total intake of ω6 fatty acids.

Another major limitation of most intervention studies is their poor selection of individuals who could actually benefit from a dietary supplement because of their low spontaneous DHA intake. In the clinical trials with DHA, different time courses and different durations of DHA supplementation or composition of the supplements make the results difficult to interpret. The duration of the supplementation is generally limited to a few weeks or
months, which may be quite insufficient to reverse a chronic deficiency and the associated
cognitive dysfunction despite the rapid plateau in plasma levels achieved in approximately
one month after supplementation [103].

6.2 Biological Issues

If there really is a link between higher intake of DHA itself (or other ω3 fatty acids) and
lower risk of AD, then further research is needed to identify and understand the biological
processes that could mask this relationship and prevent the normal link between low DHA
intake and low plasma DHA from being seen in AD. DHA synthesis and β-oxidation are both
very low in humans [91] so, unlike in many animal models, in non-vegan humans, DHA intake
has a very important effect on whole body DHA homeostasis [99]. To our knowledge, no
study has yet examined the association of DHA and AD in vegans or other populations that
strictly eat no fish. The brain is rich in DHA and brain DHA levels are relatively hard to
manipulate after weaning, so it is somewhat more understandable that brain DHA levels could
be the same in AD as in healthy, age-matched controls. Lipid peroxidation and other
degenerative processes linked to inflammation that play a key role in AD may degrade and
deplete DHA but their quantitative impact on DHA levels would usually be small unless lipid
peroxidation was occurring on a massive scale. If lipid peroxidation did consume brain DHA,
one would expect DHA levels to decrease but not more so than the other long chain PUFA in
the brain (mostly ARA and adrenic acid).

Clinical depression also is a factor that may contribute to AD and two prospective
studies have reported higher plasma ARA:DHA in more depressed subjects [109].
Interestingly, higher plasma phospholipid EPA and DHA was protective against decline in
verbal fluency but only in the least depressed subjects; no such association was found with global cognitive decline or other tests in that study [106].

At DHA intakes up to 2 g/d, plasma phospholipid DHA concentrations increase in a dose-dependent, saturable manner in response to dietary DHA [103]. DHA kinetics in erythrocytes follow a similar pattern, but it takes a longer time to reach a steady state [103]. Peripheral DHA levels, in particular in plasma, may not adequately reflect DHA incorporation into neuron membranes. Red blood cells might be a better compartment to assess the link between habitual DHA intake and brain DHA. On the other hand, plasma DHA may be a more sensitive measure of short term dietary intake and release from membrane phospholipids after phospholipase cleavage. Plasma DHA might therefore better reflect the bioavailability of fatty acids as substrates for cyclooxygenases and lipoxygenases but be a poor predictor of the long-term effect of dietary DHA on brain aging.

Rapoport [131] estimates that the healthy young adult human brain turns over DHA at a rate of about 4.5 mg/d. If Rapoport’s calculation is correct, why the necessary daily dose of DHA to impede cognitive decline in AD varies so widely (from as little as 180 mg/d to almost 40 times that amount; Table 4) also needs to be understood. The turnover of DHA includes its use for membrane replacement, β-oxidation, synaptic integrity, gene regulation and synthesis of docosanoids and neuroprotectins but does not take into account factors such as DHA losses to lipid peroxidation which could contribute to a higher DHA requirement during aging or, especially, in AD [132]. Moreover, the amount of DHA that should be consumed to meet the healthy adult brain’s requirement for DHA is still not known.

Whether brain DHA turnover changes with healthy aging, the presence of an ApoE4 allele, or during cognitive decline remains to be determined. Whole body β-oxidation of a single dose of $^{13}$C-DHA in healthy, young adults is very low (<5% in a one month follow-up;
suggesting that, like the brain, the healthy, adult human body is not rapidly catabolizing large amounts of DHA relative to average DHA intakes common in North America (generally ≥100 mg/d; [134, 135]). Therefore, a better understanding of DHA metabolism during healthy aging, including its transport and β-oxidation, seems crucial to understanding the changes in DHA utilisation occurring during aging-related cognitive decline.

6.2.1. DHA Metabolism During Healthy Aging

There is no consensus for criteria defining healthy aging or healthy elderly controls. In research studies, mean ages of «elderly» groups can be as low as 50 y or as high as 75-80 y old. Identifying changes in ω3 PUFA metabolism that occur during healthy aging may help to determine how dietary DHA or EPA contributes to healthy aging and, by inference, how they might reduce the risk of cognitive decline and AD.

We compared the plasma DHA response to a fish oil supplement in healthy elderly and young adults [136]. Our subjects were screened for clinical evidence of cardiovascular disease, chronic inflammation, thyroid, liver and renal disease, malnutrition, and early indicators of diabetes. At baseline, both EPA and ARA were higher in elderly compared to young adults. During the fish oil supplementation phase, plasma DHA increased significantly higher and more rapidly in the elderly, reaching a plateau after 2 weeks of supplementation whereas in young, plasma DHA was still increasing after 3 weeks [136]. EPA and ARA remained higher in elderly compared to young throughout the fish oil supplementation. Other reports confirm the likelihood that baseline fasting plasma EPA and DHA are somewhat higher in the healthy elderly [137-139]. These results suggest that long chain PUFA metabolism is subtly altered during healthy aging, due possibly to changes in their β-oxidation or perhaps to lower capacity of organs such as the brain to utilize EPA, DHA and ARA.
We have also unpublished results using a unique tracer – uniformly carbon-13 (\(^{13}\)C)-labeled DHA - in young and elderly. Our preliminary results suggest that the incorporation of \(^{13}\)C-DHA into plasma TAG and free fatty acids is already different between young and healthy elderly subjects within the first four hours after its intake. In a follow-up of one month, overall \(\beta\)-oxidation of \(^{13}\)C-DHA was similar between young and elderly but four hours after the single oral dose of \(^{13}\)C-DHA, \(\beta\)-oxidation was significantly higher in the elderly, an effect corresponding possibly to the higher plasma incorporation of \(^{13}\)C-DHA 4 hours dosing. Therefore, our data suggest that \(\omega_3\) PUFA metabolism changes mildly but significantly during healthy aging. Whether this would have any impact on risk of cognitive decline is still unknown.

Not only do plasma \(\omega_3\) PUFA levels and response to a supplement change during healthy aging, but the brain also seems to undergo important changes in lipid composition. Between 20 y old and 100 y old, the healthy brain loses 20% of its wet weight, corresponding to a loss of 36% of its dry matter weight [121]. Lipid content is markedly affected by the brain atrophy associated with aging since cholesterol and phospholipids decrease by 47% and 42%, respectively [121]. Unfortunately, brain PUFA composition was not reported in this study. Nevertheless, it is clear that better criteria to define healthy elderly controls are needed, which may avoid some of the conflicting data reported in relation to PUFA and cognitive deterioration in the elderly.

### 6.2.2 Apolipoprotein E

One of the biological factors that may mask the relationship between DHA and lower risk for AD is polymorphisms in ApoE. Around 23% of the Caucasian population carries, i.e. has at least one copy, of the \(\varepsilon 4\) allele of ApoE [140], which is the genetic risk factor most
associated with late onset or sporadic AD. Premature cognitive decline in ApoE4 carriers occurs in midlife, a decade or more before the onset of MCI and/or AD [141]. Children aged 11 years old with higher general intelligence had higher erythrocyte ω3 PUFA, but the correlation was only significant for those without the ε4 allele of ApoE [142]. The same population was retested at 64 years old and their general intelligence was even more highly positively correlated with erythrocyte ω3 PUFA, but there was still no correlation in carriers of ApoE4 [142].

Two recent prospective studies indicate that ApoE4 carriers are not protected against dementia by higher fish intake [86, 88]. Nevertheless, one recent study evaluating the impact of fish oil on cognitive performance in the healthy elderly reported that after 26 weeks of supplementation with either 0.4 or 1.8 g/d of ω3 PUFA ApoE4 carriers had improved scores on tests of the cognitive domain of attention compared to the placebo [122]. Plasma EPA and DHA concentrations were given but no information was provided as to whether they changed differentially in relation to ApoE4 genotype. In AD patients in the OmegAD study, weight gain and appetite improved in EPA+DHA group but only in those not carrying the ApoE4 allele [147]. Hence, DHA and/or EPA supplementation might also work through mechanisms that could contribute towards improved nutritional status of AD patients. Here again, the interaction with genetic polymorphisms deserves further research.

Response of plasma DHA and plasma lipids to a fish oil supplement is influenced by the ApoE4 genotype [143-145]. We have preliminary results on incorporation of EPA and DHA into plasma lipids of ApoE4 carriers and non-carriers that clearly support this gene-by-diet interaction [146]. We analyzed the plasma fatty acid profiles of 8 carriers and 20 non-carriers of ApoE4, before and after 6 wk after a supplement of 3 g/d of EPA and DHA combined [146]. In contrast to what we anticipated at baseline, ApoE4 carriers had
significantly higher EPA and DHA in plasma TAG. Unlike in non-carriers of ApoE4, EPA in plasma free fatty acids in ApoE4 carriers did not change nor did DHA change in plasma TAG after the supplement. These data suggest that the ApoE4 interaction with PUFA metabolism is a nutrient-gene interaction which must be taken into account when assessing the risk for AD.

6.2.3. Other Nutrients in Fish

If there really is no link between low DHA and risk of AD but there is a link to low fish intake, other nutrients in fish that are currently drawing less attention than ω3 PUFA may actually be contributing to a protective effect against AD. For instance, fish protein may be protective against type 2 diabetes. In lean fish, protein is the most abundant nutrient [148]. Cod protein may affect glucose metabolism by improving insulin sensitivity [149] and may also lower plasma C-reactive protein [149] in insulin resistant men and women. Much epidemiological evidence suggests that type 2 diabetes is associated with a 2-3 fold increased relative risk for AD [150, 151] and since aging itself is linked to somewhat increased prevalence of insulin resistance, improving insulin sensitivity by intake of fish protein may help in the management of whole body glucose metabolism [151]. Conversely, in addition to ω3 PUFA, fish oil capsules may provide some protective lipid-soluble vitamins but contain no fish protein.

A possible role of EPA in the health of the brain aging may have been underestimated. Indeed, higher plasma EPA but not DHA was strongly linked to lower risk of incident dementia in the Three City study [108]. The EPA:DHA ratio varies considerably across fish species. Whereas most fishes contain about twice as much DHA as EPA, the reverse is observed in
Atlantic herring or shrimp [152]. Most studies used combinations of EPA and DHA which makes it difficult to discern whether they have specific roles in cognition [103].

Other micro-nutrients found in fish may also exert protective effects on the brain, i.e. selenium, vitamins A and D, or vitamin B_{12} [96]. Plasma selenium concentration has been reported to correlate to that of plasma EPA and DHA [153]. In addition to long chain ω3 PUFA, these nutrients might exert protective effects in the brain by various mechanisms, including the anti-oxidant properties of selenium, activation of vitamin D receptors and retinoic acid receptors in the brain by vitamins D [154] and A [155], respectively, and homocysteine-lowering effects of vitamin B_{12} [156]. It is therefore very difficult to disentangle their respective effects. These nutrients might act as confounders in the relationship between fish intake and risk of AD, i.e. they would explain a protective effect wrongly attributed to DHA. Alternatively, they may also have synergistic effects which would enhance the protective action of DHA against brain aging. For instance, selenium is an anti-oxidant that may help protect DHA against lipid peroxidation. Similarly, DHA activates PPARs, which are a partner of dimerisation of RXR retinoid receptors activated by vitamin A derivatives [157].

Supplements of ω3 PUFA could therefore be more efficacious if they included such synergistic nutrients. In that sense, the positive impact on several cognitive outcomes of a supplement of DHA and lutein, a carotenoid, deserves further attention [125]. Indeed, some fish species such as salmon eat small invertebrates rich in carotenoid pigments which give them their color, and which are precursors of vitamin A

6.2.4 Fish as a Component of a Healthier Diet

It may also be important to think in terms of dietary patterns rather than just the intake of specific nutrients or food types. For instance, in the Three City Study, when considered
alone, fish intake was not significantly associated with decreased risk of dementia but this association was present when fish was jointly considered with daily consumption of fruits and vegetables [86]. Fish consumers are generally higher consumers of fruits and vegetables which provide antioxidants that help minimize the risk of lipid peroxidation [93] of long-chain PUFA. The DHA content of fish species may also vary considerably according to where they are caught or fish farming conditions [96].

Grilled fish is a common component of a Mediterranean dietary pattern whose protective effect against AD was recently described [102]. Fish consumption is therefore widely viewed as a component of a healthy diet, one that also includes higher fruit and vegetable consumption that provide vitamin C, carotenoids, and polyphenols exerting potentially important anti-oxidant effects which could contribute to slowing down brain aging [93, 158-160]. A combination of both long-chain ω3 PUFA and anti-oxidants to protect the PUFA against peroxidation could therefore be more beneficial than either alone. Method of preparation of the fish may also have an important impact on the amount ω3 PUFA and possibly vitamins consumed.

6.2.5 Insufficient DHA or Excessive ω6 PUFA?

DHA status should not be considered independently of ω6 PUFA intake. Indeed, DHA and EPA compete with ARA for the sn-2 position on membrane phospholipids [103]. Is AD associated with low DHA intake or is it rather a question of too much ω6 PUFA intake? In the Three City study, higher plasma ARA:DHA was associated with increased risk of incident dementia whereas plasma concentrations of ARA or DHA alone were not [108]. Normal individuals could therefore potentially have a cognitive benefit from high intake of DHA and/or EPA but only if they correct an excessive intake of ω6 fatty acids [161]. A dietary trial in which
healthy adult men consumed a ratio of linoleic acid to α-linolenic acid of 4:1 or 10:1 showed that lowering linoleic acid intake was associated with higher plasma phospholipid EPA and lower plasma ARA:EPA, but did not modify ARA or DHA [162].

7. ANIMAL STUDIES

The transgenic mouse - Tg2576 - with the APPswe mutation is the main animal model used to study the dietary effects of ω3 PUFA on AD. These mice exhibit memory loss and Aβ deposition. In two studies, adult Tg2576 mice were given different levels of dietary ω3 PUFA (control diet, ω3 PUFA deficient diet, and the same ω3 PUFA deficient diet supplemented with DHA) for about 100 days [163, 164]. ω3 PUFA deficiency led to a loss of key pre- and postsynaptic markers essential for cognition. Indeed, ω3 PUFA deficiency decreased N-methyl-D-aspartate receptor subunits (pre-synaptic) in the cortex and hippocampus of mice and also decreased Ca2+/calmodulin-dependent protein kinase (postsynaptic) in the cortex. These effects were significantly higher in Tg2576 mice compared to the non-transgenic controls [163].

Reduction of dietary ω3 PUFA resulted in an 80-90% decrease of the p85 α subunit of phosphatidylinositol 3-kinase and the post-synaptic actin-regulating protein, drebrin, a change also observed in the brains of AD patients. ω3 PUFA deficiency also increased caspase-cleaved actin content and oxidative stress markers in the brain cortex [164]. Conversely, mice fed a ω3 PUFA deficient diet supplemented with DHA were partly protected against these effects and behavioral deficits. DHA-supplemented mice also exhibited improved spatial memory [163, 164]. A 40% decrease of the number and burden of amyloid plaques was observed in the same transgenic mouse model submitted to a high DHA diet (containing 0.6% DHA) compared to a low DHA diet (ω6:ω3 of 85:1, with 6% fat as safflower oil), but not control
DHA treatment also lowered the levels of Aβ42 but not Aβ40 in the cortex of these animals [165].

Conversely, in a double transgenic mouse model of AD expressing mutant genes for amyloid precursor protein and presenilin 1, a diet containing 4% fish oil with DHA as 5.7% of total fatty acids did not modify the hippocampal levels of either soluble or insoluble Aβ compared to mice receiving a standard control diet [161]. In that study, the high ω3 PUFA diet did not improve or protect the cognitive performance of the transgenic compared to non-transgenic mice. Brain levels of ω6 PUFA were strongly inversely correlated with cognitive impairment, whereas no correlations existed between brain ω3 PUFA levels and cognitive performance in transgenic or non-transgenic mice [161]. However, the small number of animals in this experiment (n = 8 in each group) may have hampered detection of a significant difference despite the decreases of 25% and 17%, respectively, in soluble Aβ40 and Aβ42 in the group receiving the DHA-enriched diet.

More recently, in the Tg-AD triple transgenic mouse model of AD that exhibits both Aβ and tau pathologies, soluble Aβ40 and Aβ42 levels were significantly reduced after 6 months of dietary supplementation with DHA. After 9 months of supplementation, the DHA diet significantly reduced levels of soluble Aβ40 but not Aβ42. Insoluble Aβ levels were unchanged by the diet. DHA also reduced the accumulation of tau and phosphorylated tau in the soluble fractions of protein extracted from the whole brain [166].

Other studies confirm the protective effect of DHA in non-transgenic animal models of AD. Indeed, in a model of cognitive impairment in which the rats received an intracerebral infusion of Aβ40, dietary DHA provided a beneficial effect against the impairment of learning ability [58, 167, 168]. In these studies, DHA also decreased Aβ40 accumulation after its infusion. Some but not all animal studies suggest that dietary ω3 PUFA depletion could
potentiate markers of AD in the rodent brain, i.e. such as Aβ accumulation, amyloid plaques and cognitive impairment. Nevertheless, some inconsistencies exist between studies, including differences in the effect of DHA on the levels of Aβ and the impact of ω3 PUFA supplementation on cognitive performances. Furthermore, few effects of DHA supplementation on tests of cognitive performance or behaviour have been reported in these AD models. A major limitation of studies in living humans is their inability to have access to brain samples. Moreover, post-mortem studies are based on small and highly selected clinical series. Animal studies are therefore complementary approaches to verify the hypotheses on the mechanisms of action of fatty acids in animal brains.

A good animal model of AD is of primary importance as it permits investigation of mechanisms of the pathology which cannot be explored in humans, even if these animals incompletely express clinical symptoms or markers of AD neuropathology. One lesser known animal model of AD is the primate, Microcebus Murinus (mouse lemur), which naturally exhibit age-related brain changes closer to those in humans than presently seen in rodent models. Neurodegeneration in the aged mouse lemur is characterized by brain atrophy, presence of amyloid plaques, cytoskeletal tau pathology and a loss of cholinergic neurons. Aged mouse lemurs lose their cognitive and social capacities and exhibit certain age-associated similarities with human AD, while animals with elderly normal brain aging maintain memory function and social interaction [169].

8. BRAIN GLUCOSE UPTAKE: AN EMERGING MECHANISM

Assuming that the difficulty linking plasma or brain DHA to cognitive decline in the elderly is mostly methodological, i.e. that higher fish intake really does reduce the risk of AD or other forms of cognitive decline in the elderly, the mechanism of such an important effect
still needs to be defined. An overview of the biological roles of DHA that have been well established during the past 30 years is given in the section - Biological Functions of DHA; each of these roles could in some way be implicated in protecting the brain against AD. We add here a mechanism that is emerging as potential link between low DHA and risk of cognitive decline in the elderly – that of control of brain glucose uptake.

During healthy aging, brain glucose uptake decreases significantly in specific cortical regions [170], an effect that is more pronounced in the elderly with deteriorating cognitive function such as AD [171]. Experimental data support the hypothesis that ω3 PUFA intake could affect neuronal activity by altering brain fuel supply and hence energy metabolism. The main energy requirement of the brain is for Na+/K+ pump activity in nerve terminals and for oxidative phosphorylation, both of which are decreased in ω3 PUFA deficient rats [172, 173]. Glucose utilization, glucose transporter 1 (GLUT1) immunostaining and the amount of the endothelial glucose transporter GLUT1 protein are all decreased in the cerebral cortex in a rat model of dietary ω3 PUFA deficiency characterized by lower brain DHA content [173, 174]. GLUT1 is located in endothelial cells of brain microvessels and at the endfoot processes of astrocytes and is therefore the gateway for glucose entry into the brain. In a more recent and preliminary study, rats fed a DHA-supplemented diet had increased GLUT1 expression [175]. These data suggest a possible role of DHA in the control of GLUT1 expression and glucose transport into the brain.

This emerging role of ω3 PUFA and/or DHA in brain energy metabolism could be linked to brain glucose hypometabolism, which seems to be the earliest identified change in AD, occurring 25-30 years before the clinical horizon [176]. It is therefore now possible to address the questions: could DHA promote brain glucose metabolism in the elderly, and could
ω3 PUFA intake be a useful dietary approach to correcting impaired cerebral glucose metabolism during aging thereby reducing the risk of aging-related cognitive decline and AD?

9. DISCUSSION

The effectiveness of strategies involving DHA to reduce the risk of AD depends on a good understanding of how low intake or tissue levels of DHA would increase the risk of AD or other forms of cognitive decline in the elderly. Since the extensive report of Maclean et al in 2005 [177], numerous important contributions have been made in this field. A fairly solid basis now exists for believing that low fish and DHA intake contribute significantly to the risk of AD. Lower blood DHA is also fairly convincingly linked to a higher risk of cognitive decline and AD. However, the overall literature on static measurements of either blood or brain DHA shows no statistical difference in AD compared to healthy controls. Thus blood DHA needs to be assessed in relation to cognitive status and, by itself, is not presently a very useful marker actual or impending cognitive decline. Clinical trials published to date that involve DHA supplementation have also not been that promising. Hence, we agree with the conclusion of previous reviews [2, 177] that more research is definitely needed. However, we would suggest that the focus could be less on whether low dietary DHA is a factor increasing the risk of cognitive decline or AD and could be more on establishing how DHA would be biologically linked to AD.

Such mechanistic research is essential if DHA or other PUFA are to find a useful role as nutritional supplements protecting against risk of AD or other forms of dementia. Well designed RCTs that address a specific mechanism of action of DHA are urgently needed that target individuals with low dietary DHA intake and raised risk of cognitive decline but who are not yet demented. It is not yet clear whether dietary DHA only has a preventive role, i.e. in risk reduction, or whether it could also be used to treat AD; if the latter, how early DHA
supplements should be started is uncertain because DHA is unlikely to correct neuronal loss. There is also a need to establish which categories of cognitive decline are likely to be responsive to higher intake of fish or DHA or other PUFA; i.e. AD, vascular dementia, or mixed or other forms of dementia. Consensus is also needed on definitions of AD and criteria to be used for subject selection so that independent studies can be compared more directly. The optimal duration of supplementation permitted a clinical benefit to be observed still needs to be defined, as do doses and an optimal EPA:DHA ratio that are effective but do not increase risk of brain damage due to lipid peroxidation.

In the absence of easily accessible and specific biomarkers of AD, exploratory trials are needed to assess the impact of various doses and proportions of EPA and DHA on intermediate biomarkers i.e. oxidative stress, inflammation, and Aβ concentration in older persons. Animal models may be able to help identify both mechanistic aspects of PUFA involvement in neurodegeneration and suitable biological markers of incipient cognitive decline.
Acknowledgements

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[166] Green KN, Martinez-Coria H, Khashwji H, Hall EB, Yurko-Mauro KA, Ellis L, and LaFerla FM, Dietary docosahexaenoic acid and docosapentaenoic acid ameliorate amyloid-
{beta} and tau pathology via a mechanism involving presenilin 1 levels. J Neurosci 2007;27:4385-4395.


Table 1

Prospective observational studies relating fish or dietary DHA consumption to risk of dementia or cognitive decline.

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Age (years)</th>
<th>Food or nutrient</th>
<th>Outcome</th>
<th>Results (multivariate models)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotterdam Study (The Netherlands)</td>
<td>5386</td>
<td>≥ 55</td>
<td>Fish</td>
<td>All cause dementia</td>
<td>Protective association of fish consumption (at least 18.5 g/d) with all-cause dementia and AD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total fat SFA</td>
<td>AD</td>
<td>Increased risk of vascular dementia with total fat and saturated fat</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VaD</td>
<td>Mean follow-up 2.1 years</td>
</tr>
<tr>
<td>PAQUID (France)</td>
<td>1416</td>
<td>≥ 68</td>
<td>Fish</td>
<td>All cause dementia</td>
<td>No association with any class of fatty acids</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AD</td>
<td>Mean follow-up 6.0 years</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>VaD</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Protective association of at least weekly fish consumption against all-cause dementia and AD</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7 years of follow-up</td>
</tr>
<tr>
<td>Chicago Health and Aging Project (USA)</td>
<td>815</td>
<td>65 - 94</td>
<td>Fish</td>
<td>AD</td>
<td>Protective association with fish consumption. total ω3 PUFA and DHA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total ω3 PUFA EPA, DHA</td>
<td>Mean follow-up 3.9 years</td>
<td>No association with EPA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chicago Health and Aging Project (USA)</td>
<td>3718</td>
<td>≥ 65</td>
<td>Fish</td>
<td>Cognitive decline over 6 years (global measure from 4 standardized tests)</td>
<td>At least weekly fish consumption associated with slower cognitive decline</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total ω3 PUFA EPA, DHA</td>
<td></td>
<td>No association with ω3 PUFA, EPA or DHA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular Health Cognition Study</td>
<td>2233</td>
<td>≥ 65</td>
<td>Fish</td>
<td>All cause dementia</td>
<td>Protective association with nonfried fish for AD only in ApoE4 negative (N=1570); NS when additionally adjusted for education and income</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>AD</td>
<td>No association in ApoE4 positive (N=474)</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Age</td>
<td>Major Exposure</td>
<td>Major Outcome</td>
<td>Findings</td>
</tr>
<tr>
<td>--------------------------------------------</td>
<td>--------------</td>
<td>-------</td>
<td>----------------</td>
<td>---------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Three City Study (France) [86]</td>
<td>8085</td>
<td>≥ 85</td>
<td>Fish</td>
<td>All-cause dementia</td>
<td>Fish associated with lower risk of all-cause dementia only in ApoE4 negative (N = 5944)</td>
</tr>
<tr>
<td>Zutphen Elderly Study [178]</td>
<td>342</td>
<td>69-89</td>
<td>Fish</td>
<td>Cognitive decline over 3 years in MMSE score</td>
<td>Fish inversely but not significantly associated with cognitive decline</td>
</tr>
<tr>
<td>Zutphen Elderly Study [129]</td>
<td>210</td>
<td>70-89</td>
<td>Fish</td>
<td>Cognitive decline over 5 years</td>
<td>Fish and EPA+DHA intake associated with less cognitive decline</td>
</tr>
<tr>
<td>Cardiovascular Risk Factors, Aging and Dementia Study (Finland) [87]</td>
<td>1449</td>
<td>60-80</td>
<td>Fish</td>
<td>Cognitive performance 21 years later</td>
<td>SFA associated with poorer cognitive function and increased risk of MCI</td>
</tr>
<tr>
<td>Framingham Heart Study [91]</td>
<td>488</td>
<td>76</td>
<td>Fish</td>
<td>All-cause dementia</td>
<td>No significant association</td>
</tr>
<tr>
<td>Atherosclerosis Risk in Communities Study [179]</td>
<td>7814</td>
<td>50-65</td>
<td>Total ω3 and ω6 PUFA</td>
<td>Cognitive decline over 9 years</td>
<td>Higher long-chain ω3 PUFA and balanced ω6/ω3 ratio associated with lower risk of cognitive decline, especially for verbal fluency and among hypertensives</td>
</tr>
</tbody>
</table>

PAQUID: Personnes Agées QUID
SFA: Saturated fat
MUFA: Mono-unsaturated fat
PUFA: Polyunsaturated fat
AD: Alzheimer disease
MCI: Mild Cognitive Impairment
Table 2

Blood docosahexaenoic acid (% of total fatty acids) in relation to cognitive status in the elderly.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Type of study, duration follow-up</th>
<th>Cognitive status</th>
<th>N</th>
<th>Age (y) Mean</th>
<th>DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>A. PLASMA PL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tilvis et al., [180]</td>
<td>Cross-sectional</td>
<td>AD</td>
<td>11</td>
<td>69</td>
<td>7.4 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VD</td>
<td>19</td>
<td>73</td>
<td>6.4 ± 0.4</td>
</tr>
<tr>
<td>Corrigan et al. [110]</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>49</td>
<td>74</td>
<td>5.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MID</td>
<td>6</td>
<td>81</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD</td>
<td>36</td>
<td>81</td>
<td>4.4 ± 0.9</td>
</tr>
<tr>
<td>Conquer et al. [181]</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>19</td>
<td>77</td>
<td>4.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIND</td>
<td>27</td>
<td>83</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD</td>
<td>13</td>
<td>83</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Laurin et al.[107]</td>
<td>Cross-sectional</td>
<td>Control - all</td>
<td>79</td>
<td>77</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ApoE4 non-carriers</td>
<td>65</td>
<td>N/A</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ApoE4 carriers</td>
<td>13</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIND</td>
<td>43</td>
<td>79</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Demented - all</td>
<td>52</td>
<td>81</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ApoE4 non-carriers</td>
<td>28</td>
<td>N/A</td>
<td>2.4 ± 0.7</td>
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<tr>
<td></td>
<td></td>
<td>ApoE4 carriers</td>
<td>24</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Laurin et al. [107]</td>
<td>Prospective, 5 y</td>
<td>Control</td>
<td>52</td>
<td>-</td>
<td>2.0 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIND</td>
<td>16</td>
<td>-</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Demented</td>
<td>11</td>
<td>-</td>
<td>2.7 ± 1.2</td>
</tr>
<tr>
<td>Manzato et al., [182]</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>98</td>
<td>72</td>
<td>3.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIND</td>
<td>93</td>
<td>82</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>Beydoun et al. [105]</td>
<td>Prospective, 6 y</td>
<td>Control</td>
<td>2111</td>
<td>56</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD</td>
<td>140</td>
<td>57</td>
<td>3.0 ± 0.9</td>
</tr>
<tr>
<td><strong>B. PLASMA PC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conquer et al. [181]</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>19</td>
<td>77</td>
<td>4.9 ± 0.4</td>
</tr>
</tbody>
</table>
### C. PLASMA CE

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Condition</th>
<th>CIND</th>
<th>AD</th>
<th>CD</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schaefer et al.</td>
<td>Prospective, 9 y</td>
<td>CD</td>
<td>899</td>
<td>76</td>
<td></td>
<td>&gt;4.2% = lower risk for CD</td>
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<tr>
<td>Tilvis et al.</td>
<td>Cross-sectional</td>
<td>AD</td>
<td>11</td>
<td>69</td>
<td></td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>VD</td>
<td>19</td>
<td>73</td>
<td></td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>Corrigan et al.</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>49</td>
<td>74</td>
<td></td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MID</td>
<td>6</td>
<td>81</td>
<td></td>
<td>2.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD</td>
<td>36</td>
<td>81</td>
<td></td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td>Tully et al.</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>45</td>
<td>69</td>
<td></td>
<td>1.2 ± 0.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD¹</td>
<td>42</td>
<td>76</td>
<td></td>
<td>0.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD²</td>
<td>42</td>
<td>75</td>
<td></td>
<td>0.7 ± 0.7</td>
</tr>
<tr>
<td>Beydoun et al.</td>
<td>Prospective, 6 y</td>
<td>Control</td>
<td>2111</td>
<td>56</td>
<td></td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD</td>
<td>140</td>
<td>57</td>
<td></td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>Dullemeyer et al.</td>
<td>Prospective, 3 y</td>
<td>Control</td>
<td>404</td>
<td>60</td>
<td></td>
<td>every doubling in ω3 PUFA proportion ↓ rate (9-12%) in speed-related cognitive domains</td>
</tr>
<tr>
<td>Dullemeyer et al.</td>
<td>Cross-sectional</td>
<td>807</td>
<td>60</td>
<td></td>
<td>No association with any of the 5 cognitive domains</td>
<td></td>
</tr>
</tbody>
</table>

### D. PLASMA TL

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Condition</th>
<th>CIND</th>
<th>AD</th>
<th>Dementia</th>
<th>Value</th>
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</thead>
<tbody>
<tr>
<td>Cherubini et al.</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>725</td>
<td>74</td>
<td>57</td>
<td>2.3 (2.2 - 2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CIND</td>
<td>153</td>
<td>81</td>
<td>85</td>
<td>2.2 (2.1 - 2.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dementia</td>
<td>57</td>
<td>85</td>
<td>85</td>
<td>2.0 (1.8 - 2.2)</td>
</tr>
<tr>
<td>Samieri et al.</td>
<td>Prospective, 4 y</td>
<td>Control</td>
<td>1149</td>
<td>74</td>
<td></td>
<td>2.4 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dementia</td>
<td>65</td>
<td>78</td>
<td></td>
<td>2.2 ± 0.8</td>
</tr>
</tbody>
</table>

### E. ERYTHROCYTE TL
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Group</th>
<th>AD</th>
<th>VD</th>
<th>VD 1st Quartile</th>
<th>VD 4th Quartile</th>
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<tbody>
<tr>
<td>Tilvis et al. [180]</td>
<td>Cross-sectional</td>
<td>AD</td>
<td>11</td>
<td>69</td>
<td>10.6 ± 1.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>VD</td>
<td>19</td>
<td>73</td>
<td>5.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Corrigan et al. [110]</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>49</td>
<td>74</td>
<td>3.2 ± 2.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MID</td>
<td>6</td>
<td>81</td>
<td>5.6 ± 1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD</td>
<td>36</td>
<td>81</td>
<td>4.4 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Heude et al. [106]</td>
<td>Prospective, 4 y</td>
<td>Control</td>
<td>219</td>
<td>69</td>
<td>6.3 ± 1.1</td>
<td></td>
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<td>CD</td>
<td>27</td>
<td>69</td>
<td>5.9 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Boston et al. [127]</td>
<td>Cross-sectional</td>
<td>Control</td>
<td>10</td>
<td>-</td>
<td>41.2 ± 20.9³</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>AD</td>
<td>22</td>
<td>81</td>
<td>35.3 ± 21.5³</td>
<td></td>
</tr>
<tr>
<td>Wang et al. [185]</td>
<td>Cross-sectional</td>
<td>CD</td>
<td>13</td>
<td>77</td>
<td>5.4 ± 1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AD</td>
<td>10</td>
<td>75</td>
<td>4.2 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

AD: Alzheimer's disease; ApoE4: Apolipoprotein E ε4; CE: cholesteryl esters; CIND: cognitive impaired non demented; CD: cognitive decline; MID: multi-infarct dementia; PC: phosphatidylcholine; PUFA: polyunsaturated fatty acids; TPL: total phospholipids; TL: total lipids; VD: Vascular dementia

¹ 1st quartile of AD
² 4th quartile of AD
³ µg/g
Table 3

Docosahexaenoic acid in brain lipids in the healthy elderly compared to Alzheimer’s disease (AD).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cognitive status</th>
<th>N</th>
<th>Age (y)</th>
<th>Total PL</th>
<th>Free</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PE</td>
<td>PC</td>
</tr>
</tbody>
</table>

**FRONTAL CORTEX**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cognitive status</th>
<th>N</th>
<th>Age (y)</th>
<th>Docosahexaenoic Acid³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brooksbank et al. [186]</td>
<td>Control 6</td>
<td>73</td>
<td>25.9</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>AD 6</td>
<td>75</td>
<td>25.9</td>
<td>2.5</td>
</tr>
<tr>
<td>Soderberg et al. [112]¹</td>
<td>Control 8-10</td>
<td>n/a</td>
<td>23.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>AD 8-10</td>
<td>n/a</td>
<td>12.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Soderberg et al. [112]²</td>
<td>Control 8-10</td>
<td>n/a</td>
<td>4.6</td>
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<tr>
<td></td>
<td>AD 8-10</td>
<td>n/a</td>
<td>2.0</td>
<td>TR</td>
</tr>
<tr>
<td>Guan et al. [115]</td>
<td>Control 13</td>
<td>72</td>
<td>2129³</td>
<td>491³</td>
</tr>
<tr>
<td></td>
<td>AD 15</td>
<td>80</td>
<td>2512</td>
<td>361</td>
</tr>
<tr>
<td>Skinner et al. [117]¹</td>
<td>Control 10</td>
<td>68</td>
<td>18.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AD 9</td>
<td>79</td>
<td>17.3</td>
<td></td>
</tr>
<tr>
<td>Skinner et al. [117]²</td>
<td>Control 8</td>
<td>68</td>
<td>3.6</td>
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</tr>
<tr>
<td></td>
<td>AD 7</td>
<td>79</td>
<td>4.6</td>
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**PARAHIPPOCAMPUS**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cognitive status</th>
<th>N</th>
<th>Age (y)</th>
<th>Docosahexaenoic Acid³</th>
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<tbody>
<tr>
<td>Corrigan et al. [113]</td>
<td>Control 6</td>
<td>73</td>
<td>20.7</td>
<td>1.6</td>
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<tr>
<td></td>
<td>AD 8</td>
<td>77</td>
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<td>Skinner et al. [117]¹</td>
<td>Control 10</td>
<td>68</td>
<td>16.9</td>
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<td></td>
<td>AD 12</td>
<td>79</td>
<td>16.6</td>
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<tr>
<td>Skinner et al. [117]²</td>
<td>Control 8</td>
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</tr>
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<td>AD 7</td>
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<td>5.0</td>
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**HIPPOCAMPUS**

<table>
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<th>Reference</th>
<th>Cognitive status</th>
<th>N</th>
<th>Age (y)</th>
<th>Docosahexaenoic Acid³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soderberg et al. [112]</td>
<td>Control 8-10</td>
<td>n/a</td>
<td>16.9</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>AD 8-10</td>
<td>n/a</td>
<td>7.9</td>
<td>0.6</td>
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<tr>
<td>Prasad et al. [116]</td>
<td>Control 9</td>
<td>78</td>
<td>1017³</td>
<td>121³</td>
</tr>
<tr>
<td></td>
<td>AD 9</td>
<td>78</td>
<td>557</td>
<td>84</td>
</tr>
<tr>
<td>Lukiw et al., [20]</td>
<td>Control 6</td>
<td>69</td>
<td>55³</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AD 6</td>
<td>70</td>
<td>25³</td>
<td></td>
</tr>
</tbody>
</table>

AD: Alzheimer’s disease; n/a: not available; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PL: phospholipids; TR: Trace
a Unless otherwise noted, docosahexaenoic acid values are % of total fatty acids
b nmol/g wet tissue
c estimated from Figure 5 of Lukiw et al., [20], nmol/mg protein
1 Gray matter
2 White matter
Table 4

Impact of ω3 PUFA supplementation in the elderly with dementia or declining cognitive function.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cognitive status</th>
<th>Age (y)</th>
<th>Supplement</th>
<th>Dose (g)</th>
<th>Duration (mo)</th>
<th>Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terano et al.</td>
<td>DTCD 10</td>
<td>83</td>
<td>None</td>
<td></td>
<td>12</td>
<td>6% lower scores</td>
</tr>
<tr>
<td>[126]</td>
<td>DTCD 10</td>
<td>83</td>
<td>DHA</td>
<td>4.3</td>
<td>12</td>
<td>17% higher scores</td>
</tr>
<tr>
<td>Suzuki et al.</td>
<td>Control 8</td>
<td>78</td>
<td>DHA + EPA</td>
<td>0.6 + 0.5</td>
<td>6</td>
<td>75% of subjects improved&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>[187]</td>
<td>CD 22</td>
<td>78</td>
<td>DHA + EPA</td>
<td>0.6 + 0.5</td>
<td>6</td>
<td>55% of subjects improved&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Kotani et al.</td>
<td>MCI 9</td>
<td>70</td>
<td>P (olive oil)</td>
<td>0.24</td>
<td>3</td>
<td>No change</td>
</tr>
<tr>
<td>[188]</td>
<td>MCI 12</td>
<td>67</td>
<td>DHA + ARA</td>
<td>0.24</td>
<td>3</td>
<td>8 - 15% higher scores&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>AD 8</td>
<td>67</td>
<td>DHA + ARA</td>
<td>0.24</td>
<td>3</td>
<td>No change</td>
</tr>
<tr>
<td>Freund-Levi et</td>
<td>AD 85</td>
<td>73</td>
<td>P (corn oil)/</td>
<td>4.0/</td>
<td>6/6</td>
<td>No change&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>al. [123]</td>
<td>DHA + EPA</td>
<td>1.7 + 0.6</td>
<td></td>
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<tr>
<td>Chiu et al.</td>
<td>AD 89</td>
<td>73</td>
<td>DHA + EPA</td>
<td>1.7 + 0.6</td>
<td>12</td>
<td>No change&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>[124]</td>
<td>CIND 14</td>
<td>70-81</td>
<td>DHA + EPA</td>
<td>0.7 + 1.1</td>
<td>6</td>
<td>No change significant improvement in ADAS-cog test compared to P</td>
</tr>
<tr>
<td></td>
<td>CIND 9</td>
<td>70-81</td>
<td>P (olive oil)</td>
<td>1.8</td>
<td>6</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>AD 10</td>
<td>70-81</td>
<td>DHA + EPA</td>
<td>0.7 + 1.1</td>
<td>6</td>
<td>No change</td>
</tr>
<tr>
<td></td>
<td>AD 13</td>
<td>70-81</td>
<td>P (olive oil)</td>
<td>1.8</td>
<td>6</td>
<td>No change</td>
</tr>
<tr>
<td>Boston et al.</td>
<td>AD 19</td>
<td>81</td>
<td>Ethyl-EPA</td>
<td>1</td>
<td>3</td>
<td>No change</td>
</tr>
<tr>
<td>[127]</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

AD: Alzheimer's disease; ARA: arachidonic acid; CD: cognitive decline; DHA: docosahexaenoic acid; DTCD dementia from thrombotic cerebrovascular disease EPA: eicosapentaenoic acid; MCI: mildly cognitive impaired; MMD: mild to moderate dementia; NS: not significant; P: placebo.

<sup>a</sup> % of subjects with improved cognitive score

<sup>b</sup> attention and memory tests

<sup>c</sup> Except in subjects with scores >27 on the Mini-mental state exam

<sup>d</sup> In unknown proportions
FIGURE LEGENDS

Figure 1

Blood docosahexaenoic acid (DHA; % control) as calculated from the values reported in the published prospective studies listed in Table 2. As a % of each study's own controls, overall mean plasma DHA value for the two data sets showing all causes of dementia including AD was 114 ± 22 (mean±SD; black bars). For the four data sets reporting other forms of cognitive decline excluding dementia or AD, it was 100 ± 5 (gray bars). As shown in the original studies, only the data of Heude et al [106] reported DHA significantly different from each study’s own control values (100%; * p<0.05). Note that two other published prospective studies were excluded from this analysis since they had no control group per se [91, 109]. CE: plasma cholesteryl esters, PL: plasma phospholipids, RBC: red blood cell total lipids, TFA: plasma total fatty acids.

Figure 2

Blood docosahexaenoic acid (DHA; % control) in all causes of dementia or dementia excluding Alzheimer's disease (AD) as calculated from the values reported in the published prospective studies listed in Table 2. DHA values significantly different from each study’s own control values (100%) as reported in the original study are identified by an asterisk (* p<0.05). As a % of control, overall mean blood DHA value for the nine data sets reporting all cause dementia including AD was 93 ± 31 (mean±SD; black bars). For the nine data sets reporting other forms of cognitive decline except AD, it was 141±131 (gray bars). If the data of Corrigan et al (1991) [110] are considered to be an «outlier», the overall means for all causes of dementia including AD become 79 ± 20 and 82 ± 20 for other forms of cognitive decline. Hence, even within studies, these cross-sectional studies on various forms of cognitive
decline show wide variability in blood DHA relative to controls. Note that two other published cross-sectional studies were excluded from this analysis since they had no control group per se \cite{185,189}. CE: plasma cholesteryl esters, PC: plasma phosphatidylcholine, PL: plasma phospholipids, RBC: Red blood cell total lipids, TFA: plasma total fatty acids.

Figure 3.

Docosahexaenoic acid (DHA) in phosphatidylethanolamine (PE), phosphatidylcholine (PC), total phopholipids (TPL), or as free DHA from Alzheimer’s disease brain samples. In two studies, gray matter (A) was distinguished from white matter (B). Data are shown as % control as calculated from the original data reported in Table 3 \textit{(*p<0.05, as reported in the original study). Compared to controls (100%), lower DHA was most consistently seen in hippocampus (3 studies), was not significantly different from control in parahippocampus (2 studies) or frontal cortex (4 studies), which had the most variability.}
All-Cause Dementia  Other Cognitive Decline

RBC [106]  
CE [105]  
PL [105]  
PL [107]  
TFA [108]  
PL [107]

Blood DHA (% control)
DHA in Frontal Cortex

DHA in Parahippocampus

DHA in Hippocampus

Free DHA

[112]
[115]
[117]
[186]

[113]
[117]

[112]
[116]
[20]