Dietary DHA and health: cognitive function ageing

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Abstract

DHA is a key nutritional n-3 PUFA and needs to be supplied by the human diet. DHA is found in significant amounts in the retinal and neuronal cell membranes due to its high fluidity. Indeed, DHA is selectively concentrated in the synaptic and retinal membranes. DHA is deemed to display anti-inflammatory properties and to reduce the risk of CVD. Consumption of larger amounts of DHA appears to reduce the risk of depression, bipolar disorder, schizophrenia and mood disorders. Conversely, it has been shown that loss of DHA from the nerve cell membrane leads to dysfunction of the central nervous system in the form of anxiety, irritability, susceptibility to stress, dysexzia, impaired memory and cognitive functions, and extended reaction times. DHA plays an important role in ensuring a healthy ageing, by thwarting macular degeneration, Alzheimer's disease, and other brain disorders at the same time as enhancing memory and strengthening neuroprotection in general. A reduced level of DHA is associated with cognitive decline during ageing. Different mechanisms for this fundamental DHA role have been put forward. Namely, neuroprotectin D1, a DHA derivative, may support brain cell survival and repair through neurotrophic, anti-apoptotic, and anti-inflammatory signalling. Many of the effects of DHA on the neurological system may be related to signalling connections, thus leading to the study of the related signalolipidomics. Therefore, the present review will focus on the influence of DHA deficiency upon ageing, with specific emphasis upon neurological disorders related to cognitive function and mental health.

Key words: DHA; Marine sources; Ageing; Cognitive disorders; Mental health

Introduction

In developed countries, population ageing is a major demographic trend and will remain so in the next decades. Accordingly, health issues concerning the elderly have increased in importance and have entailed an ever-growing level of economic costs. Among these health issues, loss of memory and alterations in behaviour associated with declining brain function have a large impact on society and the economy. These changes with ageing are also key symptoms of degenerative brain diseases, such as Alzheimer's disease (AD) and other dementia forms. Furthermore, there are many forms of chronic debilitating brain disorders besides dementias. It has been claimed that in the next years the impact of the wide array of brain disorders will possibly surpass that of CVD and cancer taken together. Therefore, it is of paramount importance to achieve a deeper knowledge of the conditions for optimal brain function and cognition. It is important to point out that prevention is more effective than treatment in curbing the societal and economic costs. Taking this into account, nutrition may have a very significant role for this objective.

In fact, there are aspects associated with nutrition that affect the risk of cognitive function decline and neural and psychiatric outcomes.

DHA, one of the most important marine n-3 PUFA, may have a strong influence on brain health. Indeed, consumption of larger amounts of n-3 PUFA, particularly DHA, appears to reduce the risk of depression, including postpartum depression, bipolar disorder (manic depression), schizophrenia, and mood and behaviour disorders. It has also been hypothesised a connection between DHA in the diet and in the nerve cell membrane and the risk of dysfunction of the central nervous system in the form of anxiety, irritability, susceptibility to stress, dysexzia, stereotypic behaviour, aggressiveness, reduced learning capacity, impaired memory and cognitive functions, and extended reaction times.

The present review will focus on the role of DHA in the nervous system and cognitive function as well as in the prevention of cognitive decline associated with ageing. The state-of-the-art in these scientific areas of research will be analysed taking into account the DHA chemical form (Fig. 1), that is, the wider chemical structure where DHA is bound.
and phospholipid (PL)) and its effects on DHA bioaccessibility and bioavailability.

**DHA and its role in cognitive ageing: evidence discussion**

Ageing and the cognitive function decline associated with it pose a great challenge to societies in developed countries. The loss of cognitive abilities may vary immensely in kind and degree and may affect not only elderly, but also middle-aged individuals. In the most serious situations, pathologies are identified. As aforementioned, there are many forms of chronic debilitating brain disorders, nutrition being a possible key to the prevention and mitigation of some of their effects. DHA plays an important role in ensuring healthy ageing, by possibly thwarting macular degeneration, AD and Parkinson’s disease, and other brain disorders at the same time as enhancing memory and strengthening neuroprotection in general. A reduced level of DHA in the blood is associated with cognitive decline during ageing (10). An overview of the various studies concerning the impact of DHA on AD (and other cognitive decline situations) as well as on healthy individuals is presented in Table 1.

There are several important studies correlating dietary DHA and cognitive function ageing effects. These studies relate to different human populations that can be healthy or presenting mild cognitive impairment (MCI)/AD/other cognitive function disorders.

Some interesting studies, either observational or randomised controlled trials (RCT), have been carried out with healthy populations (11–13). For instance, in a community-dwelling cohort, levels of α-linolenic acid (ALA), EPA and DHA were assessed in serum PL of volunteers not taking fish oil supplements (11,14). It was found out that only the associations between serum PL DHA and non-verbal reasoning and working memory remained after adjustment for participant education and vocabulary. Moreover, DHA increased cognitive performance in an RCT involving mentally healthy individuals older than 55 years (13,15). Daily supplementation of 900 mg of algal (Schizochytrium sp.) DHA for 24 weeks was associated with significantly lower paired associative learning errors than the placebo case. Similar results were attained by an RCT study (12) on executive functions and neuroimaging in a group of healthy subjects whose age ranged between 50 and 75 years. The authors registered a benefit in executive function including verbal fluency. They also found alterations in white matter microstructural integrity (interpreted as beneficial) as well as increases in gray matter volume in the frontal, temporal, parietal and limbic areas (12). In a large cohort of Chinese adults (average age of 65 years; part of the Singapore Longitudinal Aging Studies (SLAS)), daily consumption of fish oil supplements was associated with higher Mini-Mental State examination scores and a lower risk of cognitive decline over a 1·5-year period (16).

All these studies involving healthy subjects have some drawbacks. In fact, while the study by Witte et al. (12) involved a very small population (n = 65), the SLAS did not control the level of DHA intake. Therefore, both studies’ conclusions are weakened by these shortcomings. The study by Yurko-Mauro et al. (13) seems better designed and more robust than others and clearly points to positive effects of 0·9 g DHA/d. However, the study by Velho et al. (17) did not find an effect of any PUFA on cognitive function. Hence, though studies on healthy elderly seem to point to a beneficial net effect of DHA on cognitive ageing, evidence is still far from convincing, further studies being required.

**Fig. 1.** Chemical structure of the different chemical forms in which DHA may be found. PL, phospholipid; R’, choline, serine, ethanolamine, etc.; EE, ethyl ester.
Table 1. Overview of some significant intervention and observational studies concerning the effects of DHA on the cognitive decline due to ageing

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Study length (months)</th>
<th>Subjects (n)</th>
<th>Age (years)</th>
<th>DHA intake (g/d)</th>
<th>DHA source/form</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observational study</td>
<td>–</td>
<td>280</td>
<td>35–54</td>
<td>–</td>
<td>–</td>
<td>Positive association between DHA and non-verbal reasoning and working memory in healthy volunteers</td>
<td>(11)</td>
</tr>
<tr>
<td>Prospective cohort study</td>
<td>18</td>
<td>1475</td>
<td>≥55</td>
<td>–</td>
<td>Fish oil/TAG</td>
<td>Daily consumption of fish oil supplements was associated with higher Mini-Mental State examination scores and lower cognitive decline</td>
<td>(16)</td>
</tr>
<tr>
<td>Cross-sectional and prospective study</td>
<td>5–12</td>
<td>187</td>
<td>&gt;65</td>
<td>–</td>
<td>–</td>
<td>The exact effect of n-3 PUFA intake on cognitive function of elderly was unclear, warranting further study</td>
<td>(17)</td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td>6</td>
<td>65</td>
<td>50–75</td>
<td>–</td>
<td>Fish oil/TAG</td>
<td>DHA (and other n-3 PUFA) was beneficial in executive function including verbal fluency in healthy subjects</td>
<td>(12)</td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td>6</td>
<td>485</td>
<td>≥55</td>
<td>0.9</td>
<td>Algal/TAG</td>
<td>DHA associated with significantly lower paired associative learning errors in healthy subjects</td>
<td>(13)</td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td>6</td>
<td>23</td>
<td>55–90</td>
<td>0.7</td>
<td>Fish oil/TAG</td>
<td>DHA improved Alzheimer’s Disease Assessment Scale score in subjects with mild cognitive impairment</td>
<td>(18)</td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td>3</td>
<td>21</td>
<td>68.1 (so 6-3)</td>
<td>0.2</td>
<td>–</td>
<td>DHA improved immediate memory and attention score in subjects with mild cognitive impairment</td>
<td>(19)</td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td>12</td>
<td>36</td>
<td>≥60</td>
<td>1.3</td>
<td>Fish oil</td>
<td>DHA provided benefit for several measures of memory function in subjects with mild cognitive impairment</td>
<td>(20)</td>
</tr>
<tr>
<td>Observational study</td>
<td>31</td>
<td>186</td>
<td>65–84</td>
<td>–</td>
<td>–</td>
<td>Only high DHA and other n-3 PUFA intake evidenced a borderline non-significant trend for a protective effect against the development of mild cognitive impairment</td>
<td>(23)</td>
</tr>
<tr>
<td>Observational study</td>
<td>48</td>
<td>397</td>
<td>55–90</td>
<td>–</td>
<td>Fish oil</td>
<td>Although a causal effect of fish oil supplement use on cognition cannot be concluded from results, they highlight the need for future research</td>
<td>(22)</td>
</tr>
<tr>
<td>Observational study</td>
<td>–</td>
<td>5395</td>
<td>≥55</td>
<td>–</td>
<td>–</td>
<td>DHA was not associated with AD risk</td>
<td>(29)</td>
</tr>
<tr>
<td>Observational study</td>
<td>–</td>
<td>815</td>
<td>65–94</td>
<td>–</td>
<td>–</td>
<td>DHA was associated with reduced risk of AD</td>
<td>(25)</td>
</tr>
<tr>
<td>Observational study</td>
<td>–</td>
<td>899</td>
<td>55–88</td>
<td>–</td>
<td>–</td>
<td>Top quartile of plasma phosphatidylcholine DHA was associated with reduced risk of AD</td>
<td>(26)</td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td>6</td>
<td>23</td>
<td>55–90</td>
<td>0.7</td>
<td>Fish oil/TAG</td>
<td>DHA produced no difference in Alzheimer’s Disease Assessment Scale score in AD subjects</td>
<td>(18)</td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td>3</td>
<td>8</td>
<td>67.0 (so 6-3)</td>
<td>0.2</td>
<td>–</td>
<td>DHA did not improve immediate memory and attention score in AD patients</td>
<td>(19)</td>
</tr>
<tr>
<td>Randomised controlled trial</td>
<td>18</td>
<td>402</td>
<td>76 (so 8-7)</td>
<td>2.0</td>
<td>Algal/TAG</td>
<td>DHA provided benefit for cognitive score in ApoE4 allele-negative AD patients</td>
<td>(32)</td>
</tr>
</tbody>
</table>

AD, Alzheimer’s disease.
Nutrition Research Reviews

reviewed(21). Namely, the Memory Improvement After DHA also been carried out. The evidence has been recently results(22,23), but they were observational studies where high supplementation trials show no significant on the protection against the development of MCI(25). These latter studies oppose the view that benefits of DHA are easier to detect during ageing whenever there is some MCI or memory complaint or possibly if an individual is under the influence of some physical or mental stressors(21).

A critical appraisal of these studies relating to MCI raises doubts about the beneficial action of DHA on MCI onset and development. The more positive results were attained in studies with small populations (thirty-six or lower)18-20. The studies with larger populations (186 or higher) did not show significant results(22-23), but they were observational studies where high DHA intakes were not tested by a significant share of the subject set. Accordingly, the protective role of DHA in MCI is still dubious.

Nevertheless, the effects of DHA on cognitive ageing, MCI and dementia other than AD have been more supported by evidence than those on AD. Whereas, according to some authors, DHA improved cognitive abilities in individuals with MCI, the effects on AD patients were not obvious(10,18,19). Indeed, it has been mentioned that once AD is clinically evident, supplementation trials show no significant effect of DHA on AD(24). Nevertheless, several prospective observational studies clearly point to a protective effect of higher DHA intake against risk of AD(25,26). Hence, prevention is more effective than treatment. This assessment of the observational studies has been shared by different review papers(24,27,28). On the other hand, there are other observational studies that did not find any association between DHA intake and AD risk(29).

A meta-analysis reviewing the association of n-3 PUFA and DHA with AD incidence found no significant evidence(20). However, in some populations, such as the Dutch(29), fish consumption and DHA intake are quite low(31), thus entailing statistical problems given the very low number of subjects with a DHA intake high enough to reduce AD incidence. Furthermore, another interesting study(32), the Alzheimer’s Disease Cooperative Study, found out that DHA did not produce any benefit in the primary outcomes, but observed a benefit for cognitive score in ApoE4 allele-negative patients. Indeed, AD patients in this group had a significantly lower decline in the Alzheimer’s Disease Assessment Scale score over 18 months with a daily dosage of 2g of DHA.

A comparison between the studies concerning DHA and AD (Table 1) shows that some studies do not have a representative population sample(29,39) and, as such, their significance is quite weakened. The Dutch study(29) seems much more solid and representative. The other observational studies are more modest and show beneficial DHA effects on AD that were not found in the Dutch study(25,26). The RCT study by Quinn et al(32) may harbinger a new generation of studies that are supported by a priori genetic analysis. This will provide much more insight. Meanwhile, evidence connecting DHA intake and containment of AD progression after its onset is very insufficient.

Whether healthy or MCI or AD subjects, the assessed studies do not provide incontrovertible outcomes. It is possible that the beneficial effects of DHA concern solely AD and MCI prevention and be entirely absent once clinical conditions, especially if severe (AD), are already present. But, results do not allow for such conclusion. Perhaps, more importantly, future studies should always separate population groups in accordance to their genes, since some causal links may only occur in specific genotypes. Studies encompassing larger populations and longer periods are also warranted.

DHA and its role in cognitive ageing: dose–response and mechanisms

The calibration of the DHA dosages for achieving a significant response is another issue that requires new studies. Some of the daily DHA dosages are quite high. For instance, in order to achieve 2 g/d of DHA, a daily meal of 130 g of Atlantic mackerel or 120 g of Atlantic salmon may be required (Table 2). Therefore, it would be difficult to achieve such high DHA intakes without supplements. Moreover, in future RCT, the issue of DHA bioavailability (see the ‘Dietary sources of DHA, bioaccessibility and bioavailability’ section) should be taken into account – for instance, the same DHA dosage given to different individuals can lead to different levels of bioavailable DHA as a result of changes in the functioning of the digestive system due to age and disease – and a better selection of DHA supplements (including chemical binding form) should be ensured.

For those studies involving AD patients, it has been observed that though DHA intake is low, brain DHA levels are frequently similar to the controls, thus suggesting that low DHA intake leads to low plasma DHA, but does not necessarily decrease brain DHA(29). Accordingly, these authors have claimed that animal models involving dietary n-3 PUFA deficiency in order to deplete brain DHA may not be adequate in AD research. Moreover, it has been claimed that the fatty acid (FA) profile of plasma total lipids is not an appropriate measure of DHA status in AD because it seems to mask lower DHA in plasma PL offset by higher DHA in plasma cholesteryl esters(33,34). Hence, it is of paramount importance to analyse DHA in each lipid class. AD has been associated with changes in plasmalogens choline as well as in the amount of DHA found in different PL(35).

In the mechanistic analysis of the link between DHA and cognitive function, it should be noted that DHA is by far the main n-3 PUFA present in the brain – its content within brain FA is 12–15%(36) – where it is predominantly located in neuronal membranes of the grey matter, especially in synapses(24). In addition, the brain FA-binding protein preferentially binds DHA (and other n-3 PUFA)(37), leading to higher levels of DHA incorporation in the molecular structures of the membranes(38).
DHA and health: cognitive function ageing

DHA is supplied to the central nervous system by the liver, where DHA attained from food is taken up and distributed to other organs(339). Besides, though there is evidence suggesting the expression and functional role of FA transporters at the blood–brain barrier(40), DHA can reach the brain by simple diffusion through this barrier(411). On the other hand, the dietary level of α-linolenic acid (ALA; 18 : 3 n-3), a precursor of DHA, does not correlate well with the level of DHA in the human body, making it advisable, for instance, to supplement the nursing mother’s diet with DHA(422). Furthermore, it should be remarked that plasma or erythrocyte DHA does not correlate well with DHA in the brain cells(24,45–45).

DHA is highly enriched in the PL of the synaptic plasma membrane and synaptic vesicles(46). Regarding this issue, it is worth analysing the pathways leading to the synthesis of some important PL. Phosphatidylcholine (PC), a fundamental brain PL, is synthesised through the Kennedy pathway(47) from three precursors: choline, a pyrimidine, and, typically, a PUFA (either DHA or other PUFA). Phosphatidylethanolamine (PE) may be synthesised from a PUFA and a pyrimidine. These precursors act by enhancing the substrate saturation of enzymes that bring about the incorporation of the precursors in PC and phosphatidylethanolamine(480). In accordance with this, it has been reported that synaptic proteins and PL are increased in gerbil brain by joint administration of uridine and DHA(48). Furthermore, it was found that continuous supply of DHA, but not arachidonic acid (20 : 4 n-6), may lead to an increase in brain phosphatide and synaptic protein levels according to animal models(49). Phosphatidylserine is also very important and abundant in the human brain and typically contains significant amounts of DHA(50). It is known that throughout childhood development DHA is accumulated within the brain PL, PC and phosphatidylethanolamine(51). DHA may also generate compounds bear resemblance to eicosanoids and are deemed as potential mediators of the biochemical processes in the central nervous system(53). DHA may also generate as potential mediators of the biochemical processes in the central nervous system(53). DHA may also generate compounds bear resemblance to eicosanoids and are deemed as potential mediators of the biochemical processes in the central nervous system(53). DHA may also generate as potential mediators of the biochemical processes in the central nervous system(53). DHA may also generate compounds bear resemblance to eicosanoids and are deemed as potential mediators of the biochemical processes in the central nervous system(53). DHA may also generate as potential mediators of the biochemical processes in the central nervous system(53).

Table 2. Average DHA content (mg/100 g) in different marine sources, not subjected to any culinary process(112,113,130–132)

<table>
<thead>
<tr>
<th>Category</th>
<th>Product</th>
<th>DHA content (mg/100 g)</th>
<th>DHA richness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bivalves</td>
<td>Common cockle</td>
<td>215</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Grooved carpet shell</td>
<td>55</td>
<td>Poor</td>
</tr>
<tr>
<td>Cephalopods</td>
<td>Common cuttlefish</td>
<td>38</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Common octopus</td>
<td>129</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>European squid</td>
<td>417</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Flying squid</td>
<td>225</td>
<td>Poor</td>
</tr>
<tr>
<td>Crustaceans</td>
<td>Norway lobster</td>
<td>77</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Red shrimp</td>
<td>28</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Rose shrimp</td>
<td>29</td>
<td>Poor</td>
</tr>
<tr>
<td>Fish</td>
<td>Alfonsino</td>
<td>48</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Atlantic cod</td>
<td>42</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Atlantic mackerel</td>
<td>1580</td>
<td>Rich</td>
</tr>
<tr>
<td></td>
<td>Atlantic salmon</td>
<td>1773</td>
<td>Rich</td>
</tr>
<tr>
<td></td>
<td>Auxiliary seabream</td>
<td>327</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Black scabbardfish</td>
<td>171</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Blackspot seabream</td>
<td>490</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Chub mackerel</td>
<td>2128</td>
<td>Rich</td>
</tr>
<tr>
<td></td>
<td>Common sole</td>
<td>29</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>European conger</td>
<td>425</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>European eel</td>
<td>3447</td>
<td>Rich</td>
</tr>
<tr>
<td></td>
<td>European hake</td>
<td>155</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>European plaice</td>
<td>153</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Gilthead seabream</td>
<td>1207</td>
<td>Rich</td>
</tr>
<tr>
<td></td>
<td>Greater forkbeard</td>
<td>28</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Horse mackerel</td>
<td>363</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Ling</td>
<td>21</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Meagre</td>
<td>147</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Monkfish</td>
<td>38</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Northern bluefin tuna</td>
<td>420</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Rainbow trout</td>
<td>387</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Red porgy</td>
<td>45</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Rubberlip grunt</td>
<td>79</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Sardine</td>
<td>1169</td>
<td>Rich</td>
</tr>
<tr>
<td></td>
<td>Sea bass</td>
<td>599</td>
<td>Rich</td>
</tr>
<tr>
<td></td>
<td>Silver scabbardfish</td>
<td>460</td>
<td>Medium</td>
</tr>
<tr>
<td></td>
<td>Smooth hound</td>
<td>51</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Swordfish</td>
<td>829</td>
<td>Rich</td>
</tr>
<tr>
<td></td>
<td>Thornback ray</td>
<td>44</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Wreckfish</td>
<td>418</td>
<td>Medium</td>
</tr>
<tr>
<td>Microalgae</td>
<td>Amphidinium sp. S1*</td>
<td>677</td>
<td>Poor†</td>
</tr>
<tr>
<td></td>
<td>Isochrysis galbana NIVA-4/91†</td>
<td>1580</td>
<td>Medium†</td>
</tr>
<tr>
<td></td>
<td>Prorocentrum triestinum S2†</td>
<td>752</td>
<td>Poor†</td>
</tr>
<tr>
<td></td>
<td>Thraustochytrium aureum</td>
<td>6590</td>
<td>Rich†</td>
</tr>
<tr>
<td>Seaweeds</td>
<td>Ascosphillum nodosum*</td>
<td>40</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Fucus spiralis*</td>
<td>83</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Fucus vesiculosus*</td>
<td>91</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Laminaria digitata*</td>
<td>16</td>
<td>Poor</td>
</tr>
<tr>
<td></td>
<td>Pelvetia canaliculata†</td>
<td>127</td>
<td>Poor</td>
</tr>
</tbody>
</table>
* For microalgae and seaweeds, DHA contents are given in mg/100 g DM.† For microalgae and seaweeds, richness was assessed assuming 20% DM as is usually the case in seafood.
modulation of kinase-mediated Bcl-2 gene family phosphorylation is affected. The activation of inflammatory signalling mediators (for instance, the PG-synthesising arachidonic FA enzyme cyclooxygenase-2) is repressed. Finally, the expression of proapoptotic signalling is also repressed.

Different mechanisms for the DHA role as a protective agent against cognitive decline have been put forward. Namely, NPD1 may support brain cell survival and repair through neurotrophic, anti-apoptotic and anti-inflammatory signalling. Indeed, many of the effects of DHA on the neurological system may be related to signalling connections, thus leading to the study of the related signalolipidomics. However, the action of NPD1 as a possible modulating agent of transport mediated by ApoE and its effect on β-amyloid precursor protein (β-APP) processing, soluble amyloid precursor protein α fragment (sAPP-α) or amyloid-β peptide secretion, generation, and secretion during ageing, and in cytokine-, hypoxia- and oxidation-stressed human brain cell models of AD are not fully understood. DHA itself has been linked to these events \(^{(64,66,67)}\). It is still unsettled if, under those conditions, NPD1 is formed from DHA or if there are alternative mechanisms for DHA action \(^{(68)}\).

However, there are aspects of the NPD1 action that need to be better understood, such as, the impact on the biophysics and kinetics of the membrane-embedded secreta-secreta-mediated cleavage mechanisms of β-APP\(^{(66,68)}\). Moreover, the effect of NPD1 on specific secreta activities is a still unexplored field, which deserves more attention, given its importance to the design of more effective and selective amyloid-β peptide-lowering agents \(^{(68,69)}\).

The Alzheimer’s Disease Cooperative Study AD study \(^{(32)}\) also suggests other biochemical interactions of DHA, given the sensitivity of ApoE4 allele-negative patients to DHA. It is known that ApoE can interact with various receptors in the brain, in neurons, astrocytes and in capillary endothelial cells at the blood–brain barrier \(^{(70,71)}\). ApoE4 is a lipid transporter, which may limit DHA transport in the brain. A comparison between old ApoE4 carriers with ApoE4 allele-negative individuals (carrying ApoE2 or ApoE3 alleles) points to a shorter DHA whole-body half-life in the former after an oral dose of \(^{(15}^\circ\mathrm{C})\) DHA \(^{(72)}\). It has been reported that an accumulation of DHA in the blood is associated with lower concentrations in cerebral tissue of ApoE4 mice, taking ApoE2 animals as a reference \(^{(73)}\). Such an inverse relationship between plasma and brain DHA contents suggests that plasma levels \(^{(74)}\) may reflect defective distribution in the brain rather than being a good correlate of brain DHA content. So, it seems that ApoE4 leads to less DHA being transported into the brain, thereby causing a deleterious effect in AD \(^{(21)}\).

A further mechanism relating DHA dietary intake and cognitive function ageing may involve the role of DHA in inflammatory processes. Indeed, DHA and EPA are deemed to display some anti-inflammatory properties \(^{(75,76)}\), thereby offsetting the pro-inflammatory effects of n-6 PUFA \(^{(76)}\). For diseases having a recognised central role of inflammation to the pathology such as asthma or rheumatoid arthritis, DHA supplementation in the diet may be protective. The DHA-derived docosanoids are potent endogenous anti-inflammatory and pro-resolving chemical mediators \(^{(77)}\). They may reduce chronic inflammation by attenuating NF-kB, thereby modulating the expression of pro-inflammatory cytokines. On the other hand, abundant evidence indicates that inflammatory processes are active in AD \(^{(78)}\). Epidemiological studies indicate a lower prevalence of AD in individuals treated with non-steroidal anti-inflammatory drugs, but clinical trials have not yielded strong effects \(^{(79)}\). It is known that AD is related to the activation of microglia by different factors, including β-APP and pro-inflammatory cytokines \(^{(80)}\). Microglia increase the levels of some cytokines, such as IL-6, and TNF-α, which may generate deviations from the normal neuronal function \(^{(81)}\). 

Besides, DHA incorporation into the cell membranes modulates the efficiency of NUMEROUS membrane transporters and enzymes \(^{(82)}\). The incorporation of DHA INTO cell membranes is of great importance, since many essential cellular processes take place in and on membranes \(^{(83)}\). These processes are affected by the biochemical and biophysical properties of organelle membranes. Precisely, the lipid composition of these membranes influences the membrane properties, which, in turn, decisively exert an effect upon the activity of membrane-embedded proteins \(^{(84)}\). For instance, membrane thickness can affect the location of proteins.

DHA may also affect directly the physical properties of the membranes, which depend on PL that are known to have a large importance in the neural membranes. For instance, PL, such as glycerophospholipids and sphingolipids, and sterols are prominent lipid classes in the membranes, but there is a large diversity of other minor lipid components \(^{(85)}\). The physical properties of membranes are affected both by the head groups and the hydrocarbon chains of lipid molecules. These effects can be tremendous not only on the properties, but also on the processes occurring within the membranes, even with subtle changes in lipid composition \(^{(86)}\). For instance, while a hypothetical bilayer of PC with two chains of a SFA such as stearic acid \((18:0)\) displays a packed ordered state without any diffusion of lipid substances, a bilayer of PC with two DHA chains exhibits a more disordered state with freely moving lipid molecules \(^{(86,87)}\). Moreover, longer FA chains and a higher content of sphingolipids and sterols in the membrane correlate with an enhanced thickness \(^{(88)}\). It has also been observed that asymmetric distribution of glycerophospholipids and sphingolipids between the two leaflets of the neural membrane may lead to dynamic lipid substructures \(^{(40)}\). Therefore, the connections between DHA and the membrane physical properties are another important research field deserving further scientific studies.

Future research on the mechanistic aspects connecting DHA and AD as well as other cognitive ageing disorders should also identify and quantify relevant biomarkers in the plasma and cerebrospinal fluid, bridging the gap between docosanoids, cytokines and neuronal cell changes.

**DHA and the cognitive function**

The effects of DHA on cognitive ageing need an understanding of the multiple connections between DHA and the highest degrees of brain activity. Several studies have been conducted...
regarding this subject (Table 3). A deficient level of DHA is related with changes in the operation of cognitive function, namely, in ageing, hyperactivity, AD, schizophrenia and peroxisomal diseases. Conversely, higher dietary intake of DHA is linked to better brain health. Indeed, DHA is enriched in synaptic membranes, being able to change their fluidity as well as neurotransmitter and receptor densities. These mechanisms whereby DHA affects neural cells have already been described in previous section, but more studies on their details and the way that DHA positively affects cognitive function are warranted. There are several studies of a medical nature pointing to the positive effect of DHA on cognitive function, but the full understanding of the underlying biochemistry remains elusive.

Many studies relate to human cognitive function evolution as a result of ageing. For instance, a study on the effects of a 90 d DHA supplementation (252 mg/d) on cognitive function in a healthy ageing population did not find any significant impact. Besides, it has been argued that there is greater evidence for DHA playing a preventive rather than curative role in dementia. This role may be more important in unhealthy populations, for instance, in patients with type 2 diabetes. Namely, it is not clear if an adequate brain DHA level can be kept in obesity and insulin-resistant states. Indeed, it is quite possible that the DHA level becomes inadequate, given evidence of greater cognitive decline in individuals with insulin resistance. Moreover, it has been reported that reference memory-related learning ability is positively correlated with DHA-derived docosanoids in aged rats. The same study did not find a significant correlation for EPA-derived mediators. Moreover, dietary DHA improves the learning-related spatial memory of DHA-deficient rats.

There is a lack of robust evidence to evaluate the effect of DHA in diet on the cognitive performance of young healthy adults. Some of the trials that have been done seem to present experimental design shortcomings. For instance, a placebo control is absent, sample size is small, and duration is too short. On the other hand, a cross-sectional study on adults aged between 30 and 70 years old showed a positive association between DHA blood levels and scores on cognitive performance tests. Against this backdrop, a recent work involving RCT has shown that DHA supplementation has improved both memory and reaction time in healthy young adults. It should be remarked that the habitual diet of adults seems to present shortfalls. Some of the trials that have been done seem to present experimental design shortcomings. For instance, a placebo control is absent, sample size is small, and duration is too short.

<table>
<thead>
<tr>
<th>Table 3. Summary of the main studies concerning DHA and cognitive function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Type of study</strong></td>
</tr>
<tr>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>Randomized controlled trial</td>
</tr>
<tr>
<td>Experimental trial</td>
</tr>
<tr>
<td>Experimental trial</td>
</tr>
<tr>
<td>Experimental trial</td>
</tr>
<tr>
<td>Observational study</td>
</tr>
</tbody>
</table>

**Reference:**

(1) DHA, eicosapentaenoic acid. |
Dietary sources of DHA, bioaccessibility and bioavailability

The large importance of DHA makes this an essential FA in human nutrition. Diets should be formulated in order to ensure an adequate level of DHA supply. The main source of DHA is seafood, particularly marine fish and shellfish\(^{(103)}\). DHA is found in the flesh of both lean and oily fish, with much greater amounts in the latter, and in the liver of some lean fish species, such as cod. There is also fish oil prepared from these raw materials rich in DHA\(^{(70)}\). There are also non-marine sources of DHA. However, DHA contents are only comparable with lean fish in the case of some meat-processing by-products and especially enriched foods. An overview of the DHA content in different sources and their characteristics is presented in Tables 2 and 4. Since meat, cereals and milk are more important in the Western diet, DHA intake is low\(^{(104)}\). Indeed, for a total intake of approximately 100 mg DHA/d, fish and seafood products are the largest contributor with 69.9 mg/d, followed by meat products with 19.6 mg/d, and egg products with 5.1 mg/d.

DHA is present primarily as TAG and, to a lesser extent, as NEFA in fish and derived unrefined raw oils\(^{(105)}\). In krill oil, a third fraction is found, since a substantial percentage of n-3 PUFA (and DHA) is bound in PL\(^{(105)}\). Pharmaceutical-grade, third fraction is found, since a substantial percentage of products with 19\(\text{:}\)12 EPA is the largest contributor with 69\(\text{:}\)9 mg/d of approximately 100 mg DHA/d, found in the amounts in the latter, and in the liver of some lean materials rich in DHA\(^{(76)}\). There are also non-marine sources of DHA. These are typically in the 250–500 mg/100 g range\(^{(111,112)}\).

Olly fish, such as herring, salmon and sardine, are the richest sources of DHA\(^{(106)}\). According to these authors, of thirty-seven commonly consumed types of fish products, DHA is the main n-3 PUFA, being on average 65% of total n-3 PUFA\(^{(107)}\). It should be remarked that DHA content in fish usually varies with the overall n-3 PUFA content. Three main classes of fish products may be differentiated on the basis of DHA content: relatively poor DHA sources (black scabbardfish, catfish, hake, megrim, tilapia); moderately rich DHA sources (halibut, pollock); and very rich DHA sources (herring, mackerel, salmon, sardine), corresponding to the approximate ranges <300, 300–500, and >500 mg/100 g, respectively\(^{(100–111)}\).

For a more detailed presentation of DHA concentrations in different marine sources, Table 2 based on the Portuguese Institute for the Sea and Atmosphere (IPMA) extensive database\(^{(111,112)}\) and different papers\(^{(115)}\) can be consulted. The six highest DHA contents are found in the European eel, chub mackerel, Atlantic salmon, Atlantic mackerel, gilthead seabream (wild) and sardine, all exceeding 1000 mg/100 g\(^{(111,112)}\).

The American Heart Association’s recommended daily intake (RDI) is 500 mg EPA + DHA for individuals without CHD\(^{(114)}\). The European Food Safety Agency has advised 250 mg of EPA + DHA\(^{(115)}\) and reference values for the EPA + DHA RDI are typically in the 250–500 mg range\(^{(116)}\). Specifically for DHA, an RDI of 250 mg has been put forward by ANSES (Agence Nationale de Sécurité Sanitaire de l’Alimentation, de l’Environnement et du Travail)\(^{(117)}\). A single weekly meal of 150 g of chub mackerel, Atlantic salmon or sardine may be more than enough to meet this DHA RDI (250 mg/d). For seafood moderately rich in DHA, the consumption of two to three weekly meals of 150 g may also be enough.

The level of DHA in a portion of food that is eaten may be quite different from the bioaccessible level, that is, the DHA concentration that is released from the food matrix into the intestinal lumen after digestion and is available for absorption\(^{(118,119)}\). On the other hand, bioavailability is usually defined as the fraction of an oral dose of a substance that reaches the systemic circulation\(^{(120)}\). The bio-accessible content is always equal or higher than the bioavailable content\(^{(121)}\). Bioaccessibility is usually determined by in vitro simulations of human digestion\(^{(119,122)}\). For bioavailability, according to the definition given above, cell lines and transwell assays are used for mimicking the intestinal lining barrier\(^{(122)}\) and cell cultures simulating the relevant liver tissues may also be used\(^{(123)}\). Bioaccessibility and, as a consequence, bioavailability of DHA may depend on the chemical binding form (DHA bound in ethyl ester, TAG or PL) (Fig. 1), matrix effects (fat and other components content in food), and, in the case of DHA in supplements, galenic form (microencapsulation, emulsification, etc.)\(^{(105)}\).

### DHA biosynthesis routes

Besides dietary DHA and the bioaccessibility/bioavailability issues, DHA may be biosynthesised in the human body. However, for healthy and non-vegetarian humans, despite the

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**Table 4. Non-marine DHA dietary sources and their main characteristics, advantages and drawbacks**

<table>
<thead>
<tr>
<th>Category</th>
<th>Product</th>
<th>DHA content (mg/100 g)</th>
<th>Product characteristics, advantages and drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk*</td>
<td>Cows’ milk, basal diet</td>
<td>0–10</td>
<td>Readily available, but extremely low content</td>
</tr>
<tr>
<td></td>
<td>Cows’ milk, special diet</td>
<td>10–30</td>
<td>Available, very low content</td>
</tr>
<tr>
<td></td>
<td>Cows’ milk, enriched</td>
<td>30–50</td>
<td>Available, but still very low content</td>
</tr>
<tr>
<td>Eggs†</td>
<td>Chicken eggs, basal diet</td>
<td>20–40</td>
<td>Readily available, but very low content</td>
</tr>
<tr>
<td></td>
<td>Chicken eggs, enriched diet</td>
<td>90–180</td>
<td>Available, low content</td>
</tr>
<tr>
<td>Meat‡</td>
<td>Lamb, muscle</td>
<td>10–20</td>
<td>Readily available, but very low content</td>
</tr>
<tr>
<td></td>
<td>Pork, muscle</td>
<td>10–50</td>
<td>Readily available, but very low content</td>
</tr>
<tr>
<td></td>
<td>Beef</td>
<td>10–20</td>
<td>Readily available, but very low content</td>
</tr>
<tr>
<td></td>
<td>Rabbit, muscle</td>
<td>10–30</td>
<td>Readily available, but very low content</td>
</tr>
<tr>
<td></td>
<td>Chicken, basal diet</td>
<td>10–30</td>
<td>Readily available, but very low content</td>
</tr>
<tr>
<td>Animal by-products§</td>
<td>Chicken, linsseed diet</td>
<td>20–50</td>
<td>Readily available, but very low content</td>
</tr>
<tr>
<td></td>
<td>Pork, subcutaneous fat</td>
<td>60–320</td>
<td>Available, nutritionally unbalanced, low content</td>
</tr>
<tr>
<td></td>
<td>Pork, viscera</td>
<td>10–50</td>
<td>Available, but very low content</td>
</tr>
</tbody>
</table>

* Values from Fonollá et al\(^{(133)}\) and Klopf et al\(^{(134)}\).
† Values from Lemahieu et al\(^{(135)}\).
‡ Values from Woods & Fearon\(^{(136)}\) and Zotte & Szendrö\(^{(137)}\).
§ Values from Sobol et al\(^{(138)}\).
availability of the necessary enzymes, there is extremely limited synthesis of DHA in adults.\(^{124,125}\) Unless induced by several years of a vegetarian diet, the human enzymic machinery is very inefficient in converting, for instance, ALA to EPA and DHA. Even with a diet deficient in DHA, the brain cells’ ability to synthesise DHA from ALA is very low.\(^{120}\) One study indicates a very low share of plasma ALA, < 0.2%, deployed to the synthesis of DHA via EPA.\(^{124}\) Indeed, it has been claimed an extremely low level of conversion of the precursor ALA to EPA, < 5%\(^{127}\) and to DHA, < 0.05%\(^{128}\). Several enzymes are required to elongate and desaturate ALA or other shorter and less unsaturated \(n\)-3 PUFA into DHA. Research has found evidence suggesting that DHA formation may be regulated independently of other FA in the pathway and that DHA binding to PPAR\(\alpha\) suppresses transcription of the \(\Delta-6\) desaturase gene, thereby down-regulating conversion of ALA to DHA.\(^{129}\) Indeed, it should be noted that the rate-limiting step in DHA synthesis is precisely the desaturation of ALA by \(\Delta-6\) desaturase.

An overview of the possible routes for attaining DHA in the human organism, taking into account enzyme action, conversion rates, genetic factors, and dependence on the starting \(n\)-3 PUFA, is presented in Table 5.

Conclusions

DHA is mainly found in seafood, being rich sources of DHA such as marine fish and shellfish. Oily fish such as herring, salmon, sardine and tuna provide the highest amount of DHA per meal. DHA intake may be associated with several health endpoints ranging from inflammatory processes, asthma and rheumatoid arthritis to CVD and diabetes mellitus as well as to depression and cancer. Particularly, DHA has an important role in the nervous system, which is highlighted by its prominence in neural tissues. DHA may lead to the formation of docosanoids such as NeuroPs or NPD1. Namely, the action of NPD1 in the central nervous system is influential in different ways. It is known that NPD1 leads to homeostatic signalling in response to cellular and systemic imbalances. Nevertheless, much needs to be known about the mechanisms and roles of NPD1. For instance, NPD1 as a possible modulating agent of NPD1 in the central nervous system is influential in different ways. It is known that NPD1 leads to homeostatic signalling in response to cellular and systemic imbalances. Nevertheless, much needs to be known about the mechanisms and roles of NPD1. For instance, NPD1 as a possible modulating agent of transport mediated by ApoE and its effect on \(\beta\)-APP processing is not fully understood. In spite of this, there seems to be some protection against cognitive decline with ageing and even improved memory and reaction time in healthy young adults. Indeed, for ageing-related MCI, some studies suggest that DHA may improve cognitive abilities. Nonetheless, for healthy subjects or MCI and AD patients, the evidence is still not convincing. In this context, it is worthwhile noting that for ApoE4 allele-negative AD patients, DHA produced a benefit in the cognitive score. Future studies should take the DHA bioavailability issue into account in order to achieve better results. On the other hand, research should try to separate the role of DHA and of EPA through studies using DHA only instead of fish oil rich also in EPA. Moreover, DHA in each main lipid class should be quantified instead of global DHA. Finally, future RCT and observational studies should always take into account the genetic traits of the population, since some effects may only be detected in subgroups with specific alleles.
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The planning as well as the final reading and reviewing of the paper was carried out by all three authors. C. C. wrote three sections, C. A. wrote another three sections, and N. M. B. wrote the last section and coordinated.

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References


