



Docosahexaenoic acid (DHA), a fundamental fatty acid for the brain: New dietary sources



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ABSTRACT

Docosahexaenoic acid (C22: 6n-3, DHA) is a long-chain polyunsaturated fatty acid of marine origin fundamental for the formation and function of the nervous system, particularly the brain and the retina of humans. It has been proposed a remarkable role of DHA during human evolution, mainly on the growth and development of the brain. Currently, DHA is considered a critical nutrient during pregnancy and breastfeeding due their active participation in the development of the nervous system in early life. DHA and specifically one of its derivatives known as neuroprotectin D-1 (NPD-1), has neuroprotective properties against brain aging, neurodegenerative diseases and injury caused after brain ischemia-reperfusion episodes. This paper discusses the importance of DHA in the human brain given its relevance in the development of the tissue and as neuroprotective agent. It is also included a critical view about the ways to supply this noble fatty acid to the population.

1. Introduction

Strong and wealth information have been accumulated about the essentiality of n-6 and n-3 fatty acids since the first studies of George and Mildred Burr in the late 1920s, demonstrating the importance of lipids in the growth and development of the rat [1]. In the mid-1960s, Hansen et al., regarded the essentiality of linoleic (C18: 2n-6, LA) and alpha-linolenic (C18: 3n-3, ALA) fatty acids [2]. Later, the reports from Bang and Dyerberg demonstrated the cardio protective role of n-3 long-chain polyunsaturated fatty acids (C20-22; n-3, LCPUFA) from marine origin [3]. Then, research from Bazan and Joel identified that docosahexaenoic acid (C22: 6n-3, DHA) and arachidonic acid (C20: 4n-6, AA) are accreted in significant amounts into the brain tissue [4,5]. Up to day, multiple and robust experimental, clinical and epidemiological evidence have been established about the health and nutritional importance to humans of polyunsaturated fatty acids (PUFAs), especially those of long-chain (20 or more carbon atoms) [6,7]. It is in this context that in the last three decades, one of these fatty acids, DHA, has acquired special interest for researchers due to their unique physicochemical characteristics and from the biochemical and physiological effects resulting from the presence of the fatty acid at cellular

membranes [8]. DHA is of particular interest due to its highly unsaturated structure (six double bonds, being the fatty acid most unsaturated in our body) and cell location, which is mostly concentrated at the sn-2 position of phospholipids forming cell membranes, thus providing a great fluidity to these structures [9].

DHA is almost exclusively present in significant amount in diverse seafood (fish, shellfish, micro- and macroalgae and even some mammals). Precisely, it has been proposed that was the incorporation of these seafoods to the human nutrition which marked a significant turning point in human evolution [10], a process that was characterized by the increase in size and complexity of the brain tissue and by the development of mental, behavioral and motor skills with strong cognitive components [11]. Additionally to the evolutionary importance of DHA for our specie, its relevance is magnified during pregnancy and the early stage of childhood where the fatty acid plays a crucial role in brain and retinal development [12], and function, directly affecting the cognitive function [13] and the visual acuity of child [14]. Along with the benefits for brain and visual development, which transform DHA into an essential fatty acid in the perinatal period, most recently several studies have demonstrated a neuroprotective role for the fatty acid, specifically during aging and in neurodegenerative diseases and brain

Abbreviations: LA, linoleic acid; ALA, alpha-linolenic acid; PUFA, polyunsaturated fatty acids; LCPUFA, long-chain polyunsaturated fatty acids; DHA, docosahexaenoic acid; AA, arachidonic acid; NPD-1, neuroprotectin D-1; TNF- α , tumor necrosis factor-alpha; IL-1 β , interleukin 1-beta; EPA, eicosapentaenoic acid; IL-6, interleukine; PCBs, polychlorinated biphenyls

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ischemia-reperfusion episodes [15,16]. It is now accepted that neuroprotectin D-1 (NPD-1), a structural derivative of DHA, shows significant neuroprotective actions, particularly in the preservation of the structure and physiology of neurons and glial cells [17]. This paper reviews the current information, from different backgrounds, supporting the importance of DHA for humans, particularly in brain development and in the neuroprotective properties of the fatty acid, as well as how to get a sustainable way to increase the consumption of DHA by the population.

2. DHA, diet and evolution

The strong evidence supporting the crucial role of DHA in the evolution of our specie, mainly on the growth and physiology of the central nervous system [18,19], was obtained by studies from fossils indicating that the turning point in human evolution was precisely produced after early humans began the consumption of seafood, mainly fish, shellfish, some mammals and algae [20,21]. It is estimated that late archaic humans (*Neanderthals*) consumed protein from terrestrial hunted animals or from the remains left by other hunters such as, wolves, hyenas and big cats. These foods contained very low fat and were also very low in n-3 LCPUFA, in contrast to the significant consumption of marine-derived foods by the considered modern humans (from the second half of the upper Paleolithic period) [22], which supplied significant fat high in n-3 LCPUFA. The inclusion of seafood in the diet was coincident with the advent of the first cultures that produced ceramics, textiles and tools and practicing personal ornamentation and decoration of their cemeteries, skills which later led to the origin of people which formed the first Asian and Mediterranean civilizations [11]. Based on this background authors such as Crawford, Cunnane and others researchers [22–25], proposed a direct link between diet and brain size, specifically on how the usual inclusion of seafood in the diet allowed the expansion of the gray matter in the brain cortex. This evolutionary process characterized as encephalization was very limited and slow for *Australopithecus* and similar hominids but reached an exponential growth in the last 200,000 years, particularly in the stage where the *Homo erectus* was evolved to *Homo sapiens* [24]. Actually the human brain, compared with other species (42 species in total) [25], has the highest amount of DHA (35–40% of total polyunsaturated fatty acids) and this fatty acid is mainly located at the phospholipids forming the neuronal membranes and the retina [25,26]. A particular aspect of the growth of the human brain associated with the intake of food from marine origin, when compared to other species, is the size of the tissue with relation to the body size [24]. In contrast, in other studied mammals, the brain size decreases logarithmically with the increase of the body size [24–26].

The capacity of human brain for the biosynthesis of DHA from its precursor ALA is very low. It has been estimated that less than 1% of ALA consumed is converted into DHA [5], enzymatic process that occurs mainly in the liver [5]. This limitation introduces a metabolic inability to ensure an adequate supply of DHA to the brain, which was probably limiting at a particular time in our evolution [27]. This fact strengthens the hypothesis about the importance of the inclusion of seafood in the diet of our ancestors and its relation to the significant increase of the cortex and the total brain volume and mass, with the subsequent development of language and the first tools used by humans [26,27]. Living near the sea and/or lakes allowed hominids a wide access to a variety of seafood, less complex and easy to digest than terrestrial animal food and requiring less preparation for consumption compared to red meat, which probably ensured an adequate supply of high quality nutrients, mainly proteins, energy and n-3 LCPUFA [26].

3. DHA and brain development

As discussed above, DHA is the most abundant n-3 LCPUFA in the central and peripheral nervous system, representing the major proportion of PUFAs in brain and retina. This fatty acid is present in large

amounts in phospholipids of brain gray matter [28]. DHA takes an important role in neurogenesis and synaptogenesis, particularly in fetal development and during the first two years of life [26]. Fetal DHA accretion occurs actively along pregnancy but is most active during the third trimester as has been demonstrated after the supplementation of pregnant women with fish oil (200 mg DHA/day) where it was also observed that DHA supplementation during pregnancy limited the decline in maternal DHA status during the last trimester [29]. For this reason the nutritional status of DHA for the pre-gestational mother and during pregnancy and lactation represents a critical step for the brain and visual development of her child [30,31]. Neonates with higher concentrations of DHA in umbilical plasma phospholipids have longer gestational length in comparison to neonates with low concentration [32]. A study in women with less than 20 weeks of pregnancy who received 600 mg DHA/day, demonstrated a significant reduction of preterm delivery and low-weight birth with good tolerance to supplementation and no adverse effects [33]. As many other studies, this protocol demonstrated that DHA supplementation improves the nutritional status of the fatty acid both in the mother and her child because the efficient transfer of the fatty acid through the placenta [34], by the increase of DHA levels in maternal milk [35] and in the phospholipids of umbilical cord blood during lactation [36]. This supplementation should be most relevant for mothers having low ingestion of marine foods [37]. It was demonstrated that DHA supplementation during pregnancy increases the expression of fatty acid transport proteins and consequently increases the transport of n-3 LCPUFA through the placenta and to fetal the blood [38]. Pregnant women, who consumed fish oil rich in DHA (2.2 g DHA/day), from the 20th week of pregnancy until the partum, with no adverse effects, delivered children who showed significant better visual and coordination capacity [39]. Similar results were obtained after the supplementation of mothers with 500 mg DHA/day along the pregnancy, which was associated with high blood DHA levels and better cognitive development evaluated at 5.5 year-old of child [40]. When DHA supplementation also included 5-methyltetrahydrofolate supplementation (400 µg/day) cognitive benefits were prolonged until 6.5 year-old [41].

It has been demonstrated that high plasma levels of DHA in the mother and particularly in breast milk, directly correlate with the better growth and development of the brain and visual system in children [42,43]. Regarding this observation, a multivariate analysis showed that a lower intake of DHA during pregnancy increases the risk of reduced visual acuity in children [44,45]. Accordingly, it has been proposed that the intake of DHA during pregnancy would predict a better visual development in infants [44]. These findings have corroborated that supplementing mother's diet with DHA during pregnancy and lactation, or consumption of formula enriched in DHA, helps to increase the tissue levels of DHA in the infant with a better visual and neurological development [44], favouring in term child a better retinal development and function [46]. Conversely, during pregnancy and/or lactation a diet low in n-3 LCPUFA may have direct implications for the visual and neurological development of the child [47,48]. An example of this effect is that infants fed breast milk poor in DHA (less than 0.17% of total fatty acids, usually woman milk contains 0.3–0.4% DHA) show lower DHA levels in erythrocytes, reduced visual acuity and reduced language development at 14 months post-partum compared to infants fed breast milk containing 0.36% DHA [12,31]. A study in pregnant women who received supplementation of DHA (400 mg/day) from the sixteenth week of pregnancy until the delivery, showed a significant increase in visual acuity, particularly in newborns males, establishing that DHA supplementation possibly is the best predictor for this indicator of nervous system development [49]. Several studies have established a direct relationship between higher erythrocyte DHA levels (in mother and children) and the best visual and neuronal development of children [50–52], which in the long-term has benefits in the development of cognitive and motor skills in these children [53]. Even, perinatal supplementation with DHA reduced the risk of lower scores

on IQ in children from families with very low incomes [54,55]. DHA provided to newborn through formulas improves cortical maturation and visual function [56]. A study carried-out with children of six month-old who did not receive maternal milk and who were fed a formula containing egg yolk enriched with DHA (115 mg DHA/100 g food), showed at 12th month-old a significant increase of DHA level at erythrocyte phospholipids (34% increase) and a better visual development, evaluated as retinal and visual cortex maturation, observation that established the importance of a permanent and continuous supply of DHA during the first year of life [57]. Formulas that supply a minimum of 0.35% DHA favor a better brain development evaluated as Mental Development Index, especially until 4 month after delivery [58]. In addition to early dietary intake of DHA, dietary intake of AA is necessary for optimal brain and eye development in infants [59]. Child who did not receive maternal milk and that must be feed with formula, these formula must contain DHA and also AA, to avoid a lower brain development, especially visual, feeding which can be prolonged until 39 months after birth, [60] an even until 4 year-old [61]. A study in newborns (n = 181) of 1–9day-old randomly distributed in four groups according to the DHA levels of formulas (DHA 0%; 0.32%; 0.64%; 0.96%, with AA 0.64% in all formulas) fed until 12th month, demonstrated that only formulas containing DHA (in any of the three doses) produced a better cognitive development compared to control group (0% DHA) [13]. A significant higher capacity of memory and problem solving were observed when preterm child with a birth weight < 1500 g were fed with human milk supplemented in DHA (32 mg/day) and AA (31 mg/day) until discharge from the hospital i.e. during about 9 weeks [62]. Higher mental development index scores were also reported from preterm child with a birth weight over 2000 g who received formula enriched in DHA (0.05 g/100 g) and AA (0.1 g/100 g) [63]. Another study demonstrated that the supplementation of 420 full term newborn children with n-3 LCPUFA (daily, 250 mg of DHA and 60 mg of eicosapentaenoic acid; C20: 5n-3, EPA) from their birth until six months, showed a significant accretion in the DHA content of erythrocyte phospholipids and an early development of language and communication skills [64]. A follow-up study of preterm children (n = 107) who received non supplemented and supplemented formula with 0.5% DHA from their birth until nine months, demonstrated that girls showed significant benefits in their capacity of alphabetization, verbal and total intellectual coefficient and high scores in memory trials [65]. Preterm child fed formula supplied with DHA, compared with preterm child fed formula containing no DHA, showed visual acuity and development similar to preterm child fed maternal milk [66]. A recent study, which included information from 28 countries, found that the levels of DHA in breast milk contribute significantly to achieve better performance on match tests in children from low-income families and superior to the results obtained with children from high-income families and/or increased spending on education and who were not breastfed [67].

It has been shown that the presence of certain polymorphisms in genes encoding Δ -5 and Δ -6 desaturases, enzymes responsible for the formation of n-3 LCPUFA from the precursor ALA, are associated to significant changes in the levels of these fatty acids, particularly DHA [68]. For example, the presence of rs 174575 polymorphism in the gene encoding Δ -6 desaturase enzyme in children allows higher tissue concentration of DHA and higher scores on IQ tests [69], a situation that would indicate the importance of gene variations in the metabolism of n-3 LCPUFA and a consequent beneficial effect on brain development. Children of 9 month-old supplemented with n-3 LCPUFA with high DHA content (supplied from fish oil) showed significant cognitive benefits when were subjected to “free-play-tests” (evaluation of attention) and better arterial pressure at the end of infancy, indicating that n-3 LCPUFA consumption, especially DHA, may positively influence brain development and that cognitive and cardiovascular benefits should be closely related [70]. Pre-scholar 4 year-old child daily supplemented with DHA (400 mg) for 4 months have significant higher blood DHA

levels which was positively correlated with increment of punctuation obtained for vocabulary and comprehension tests [71]. Autochthonous Australian children (n = 409) from 3 to 13 year-old daily supplemented with 750 mg DHA and 60 mg EPA for a period of 20 weeks exhibited a significant increment of the scholar performance, especially those children between 7 and 12 year-old [72]. The supplementation of fish oil rich in DHA of 7–12 year-old children having low scholar performance, bad conduct and attention-deficit/hyperactivity disorder, was also associated with a better lecture capacity and learning and an improved conduct with their parents, associated with an increased in DHA level of erythrocyte phospholipids which is a valuable biomarker of DHA tissue status [73]. However not absolute compliance about DHA has established. The *Scientific Opinion on the essential composition of infant and follow-on formulae, 2014*, of the European Food Safety Authority (EFSA) Panel notes that “...there is no convincing evidence that the addition of DHA to infant and follow-on formulae has benefits beyond infancy on any functional outcomes”. However, the proposal of the Panel to add DHA to infant formulae and follow-on formula is based on its structural role in the nervous tissue and the retina and its involvement in normal brain and visual development, the need of the developing brain to accumulate large amounts of DHA in the first two years of life and the consideration that the intake of pre-formed DHA generally results in an erythrocyte DHA status more closely resembling that of a breast-fed infant than is achieved with ALA alone [74].

4. DHA and neuroprotection

DHA is a fundamental fatty acid not only for the neuronal structure, but also for the neuronal signaling [15]. This fatty acid has been most recently identified as a neuroprotective agent against cerebral aging, neurodegenerative diseases and cerebrovascular diseases, especially in the injury produced by ischemia-reperfusion episodes [15,17]. Postmenopausal women having low blood n-3 LCPUFA levels, especially DHA, and high levels of saturated, monounsaturated and *trans* fatty acids, show increased risk to develop cerebrovascular stroke [75]. With regard to the neuroprotective effects of DHA supplementation, possible mechanisms for these effects have been proposed being the following the most relevant; DHA may: *i*) maintain the integrity and function of the neuronal membranes; *ii*) preserve neuronal signaling pathways and; *iii*) significantly reduce neuronal death [8,15,17]. The exact mechanisms why DHA may exerts these neuroprotective effects are not yet fully understood. However, it has been postulated that the DHA-derivative neuroprotectin D-1 (NPD-1) would be primarily the responsible for the neurological benefits associated to DHA protection [76]. Under normal conditions DHA is mainly located at the phospholipids of the neuronal membranes and not in the neuronal cytoplasm [77]. However, under adverse conditions, such as cerebral inflammation and/or ischemia-reperfusion episodes, DHA is released from membrane phospholipids to the cytoplasm by the action of the enzyme phospholipase A2, being subsequently transformed into NPD-1 by the 15-lipoxygenase enzyme [78]. It has been observed an increase in the in situ formation of the NPD-1 in rats subjected to experimental brain ischemia-reperfusion episodes, and wherein the further administration of NPD-1 (400 ng per 48 h) generates a significant cerebral protection against the injury caused by ischemia–reperfusion episodes [79]. Experimentally the formation of NPD-1 is stimulated by various factors, highlighting: *i*) the increase in oxidative stress induced by H₂O₂; *ii*) the presence of tumor necrosis factor-alpha (TNF- α) and interleukin 1-beta (IL-1 β) and; *iii*) during brain ischemia-reperfusion episodes [78,80,81]. Several follow-up studies in humans have shown that a high consumption of fish, especially oily fish (which are important sources of DHA), is inversely associated with the risk of stroke [82–85]. Nevertheless, there are controversial studies about the relationship between fish consumption and risk of stroke and cerebral infarction. In a population-based case-control study, the risk of stroke (OR:1.95, 95% CI) and cerebral infarction (OR: 1.98, 95% CI) was greater in those with the highest

quintile of fish consumption [86]. On the other side a population-based study in women, those with higher fish intake showed 16% lower risk of stroke in comparison with women in the lowest quintile of fish consumption, especially lean fish consumption. It is concluded that fish consumption was not associated with the risk of cerebral infarction [87].

Currently, there is strong evidence that oxidative stress is produced during brain aging and neurodegenerative diseases, generating a significant peroxidation of n-3 LCPUFA in neuronal membranes [88,89]. Therefore, the uncontrolled oxidation of DHA (and possibly of AA) would be relevant in the origin of the damage at the neuronal membrane level [90]. Among neurodegenerative diseases, Alzheimer's disease particularly shows the best evidence of the benefits produced by DHA at the molecular level, probably through the formation of NPD-1 [91]. This metabolite has the ability to: *i*) reduce the generation of pro-inflammatory cytokines; *ii*) reduce the formation of β -amyloid peptide, a cytotoxic structure considered neurotoxic and oxidative stress promoter which disrupts synaptogenesis and induces neuronal apoptosis; *iii*) stimulate the expression of anti-apoptotic genes, and; *iv*) reduce the expression of pro-apoptotic genes [92,93]. Most recently it was described that NPD-1 favors the production of a disintegrin alpha-secretase, a metalloprotease with neurogenic and neurotrophic properties and that can inhibit the generation of β -amyloid peptide, a neurotoxic molecule directly associated with the neurological damage of Alzheimer's disease [81,94]. Related to the intake of DHA and neuroprotection, it has been observed that individuals who have frequent consumption of fatty fish and/or nutritional supplements containing n-3 LCPUFA, show a lower risk for developing neurodegenerative diseases compared to those individuals having low intake of these fatty acids [95,96]. Healthy older Australians (n = 391, 60–90 year-old, women 53.7% and men 46.3%) having normal cognitive skills, who received daily 1720 mg DHA and 600 mg EPA for a period of 18 months, compared with a similar control group who received a placebo of olive oil capsules, showed a significant improvement in cognitive performance (measured with standardized trials) and in some general indicators of good health such as blood glucose and lipid profile [97].

The relationship between DHA and Alzheimer's disease generated research interest about the neuroprotective actions of the fatty acid [98]. Framingham Heart Study established that increasing blood DHA levels may reduce by 47% the risk to develop Alzheimer's disease [99]. However this is only a correlational observation that needs more analytical exploration. A recent study of 40 older Alzheimer's patients utilizing ¹³C-labelled DHA, demonstrated an important alteration of DHA metabolism, which reaffirmed the strong relationship of DHA with the process of neurological aging [100]. OmegaAD study carried-out with subjects diagnosed mild or moderate Alzheimer's disease (n = 204, 74 ± 9 year-old) who consumed daily a supplement of 1.7 g DHA + 0.6 g EPA during 6 months, showed a consistent reduction in depressive symptoms [101] together with an increase in appetite and weight of patients [102]. Furthermore, a significant reduction of the expression levels of genes involved in inflammation and neurodegenerative processes was observed in mononuclear leukocytes of Alzheimer's disease patients in correlation with DHA and EPA increase in plasma [103]. The same researchers also observed, derived from the supplementation with DHA, a reduction of the levels of interleukine (IL)-6, IL-1 β and the secretion of the lipopolysaccharide-induced granulocyte colony stimulant factor, reducing inflammation which characterized Alzheimer's disease, showing the importance of the n-3 LCPUFA in the eventual prevention and/or treatment of the disease [104]. With regard to β -amyloid peptide, the same protocol of supplementation with DHA increased the plasma levels of transthyretin, a molecule that by binding to the β -amyloid peptide may reduce the accumulation of the toxic peptide at the brain with a direct effect in the reduction of the progression of the pathology [105]. Strengthening these results, it has been demonstrated that consumption of 2.3 g/day of a n-3 fatty preparation rich in DHA (1.7 g/day), for a period of six

months, increases the presence of DHA at the cerebrospinal liquid, suggesting the existence of an active transfer of DHA across the hemato-encephalic blood barrier [106]. There is evidence about a relationship between APOE lipoprotein and Alzheimer's disease [107]. There are three isoforms of the APOE lipoprotein (APOE- ϵ 2, ϵ 3, ϵ 4) and subjects having the ϵ 4 allele show a higher risk to develop Alzheimer Disease. Supplementation of APOE ϵ 4 carrier subjects with DHA in high doses before the onset of Alzheimer's disease has shown to diminish its incidence [107].

Dietary supplementation with DHA (240 mg) and AA (240 mg) of older men with diagnosed cognitive impairment proved to improve the cognitive dysfunction associated to organic brain disease or due to aging [108]. In a randomized, double-blind, placebo-controlled trial the supplementation of older subjects (86 ± 6 year-old, n = 25) with mild cognitive impairment with DHA (supplied as phospholipids) plus melatonin and tryptophan during 12 weeks produced a significant improvement of cognitive and olfactory skills [109]. A study conducted in 50 subjects, older than 65 years having light cognitive deterioration, who randomly received a supplement rich in EPA (1.65 g EPA + 0.16 g DHA, n = 17), or rich in DHA (1.55 g DHA + 0.40 g EPA, n = 15) or ALA (2.2 g, n = 15), demonstrated that only those who consumed higher amount of DHA (second group) exhibited a reduction of depressive symptoms and risk to develop dementia [110]. Another study with two groups of older subjects with mild cognitive decline (n = 36) who received a supplement of fish oil rich in DHA or placebo during 12 months, demonstrated that fish oil, which was well-tolerated by the experimental group, significantly increased cognitive functions especially memory capabilities [111]. According to this information and strengthening the importance of DHA in preserving cognitive functions, a study with 1111 postmenopausal women showed that erythrocyte phospholipid DHA levels are directly correlated with hippocampal and whole brain volume, as evaluated by nuclear magnetic resonance, suggesting that a low level of omega-3 index (EPA + DHA) in blood cells may signal increased risk of hippocampal atrophy [112]. Other study with healthy subjects (55 ± 8 year-old), who consumed 999 mg DHA during 24 weeks, showed a significant improving of learning and memory, thus supporting the role of DHA in the protection of brain aging [113]. It is interesting to evaluate how DHA is provided as supplement. A study with healthy subjects (n = 45) being 61–72 year-old who consumed during 12 weeks DHA from krill oil phospholipids (mainly as phosphatidylcholine) resulted in a significant increase in cognitive and memory skills associated with a better brain blood flow measured as blood oxyhemoglobin levels [114]. Also, a study with women having 60–80 year-old who consumed DHA (800 mg/day) mixed with lutein (12 mg/day) demonstrated a significant improvement of memory and rate of learning compared to the non supplemented women group [115]. DHA has not only effect in aging, as recently demonstrated by a study in a group of adult young people 18–45 year-old, n = 176) who consumed a supplement of 1.16 g DHA/day, and gained a significant improvement in memory capacity and time of reaction to resolving problems [116]. In addition to this study, two other studies observed important effect of DHA over the modulation of the hemodynamic brain response. In the first study 22 healthy subjects consumed during 12 weeks 1 g of fish oil rich in DHA or 1 g of fish oil rich in EPA or 1 g of olive oil. Only the group who consumed DHA exhibited a significant increase of total hemoglobin and oxygenated hemoglobin, which is indicative of an increase of the brain blood flow, when they were subjected to different trials to evaluate cognitive skills [117]. The second study also demonstrated that consumption of fish oil rich in DHA (1–2 g/day) by young individuals 18–29 year-old, n = 65) allowed a significant increase in the concentrations of total oxyhemoglobin and hemoglobin, effect that is indicative of an increase of the brain blood flow during the cognitive tasks to which the subjects were submitted, indicating that DHA may modulate the brain hemodynamic [118]. DHA may also participate in the preservation of cognitive skills during aging, having a role in the visual protection. A study with

healthy subjects ($n = 74$, 45–77 year-old) who daily consumed during 90 days 252 mg DHA, 60 mg EPA and 90 mg Vitamin E, demonstrated a significant improvement of the visual capacity of the right eye [119].

Maybe one of the most interesting actions referred to DHA is those observed in students submitted to stressing situations (such as examination). A daily consumption of 1.5–1.8 g of DHA significantly reduces hostility and by 30% plasma levels of epinephrine [120]. Another interesting observation is that patients with schizophrenia show low level of DHA in plasma and erythrocyte phospholipids [121]. Patients with schizophrenia who received EPA during 12 weeks complementary to the pharmacological treatment of the disease showed increased DHA in erythrocyte phospholipids. However this increase was less than expected and was accompanied by an increase in ALA levels, which can be explained by a high metabolic use of DHA and/or a defect in biosynthesis or in incorporation of the DHA into membrane phospholipids [122]. A recent study where patients with schizophrenia received EPA together with vitamins C and E along with the pharmacological treatment, showed a reduction of the psychotic episodes and a significant increase of DHA plasma levels demonstrating a relationship between DHA metabolism and the antioxidant status of patients provided by vitamins C and E [123]. Phenylketonuric patients ($n = 21$, 9–25 year-old) daily supplemented with DHA (10 mg/kg) normalized erythrocyte DHA levels and improved clinical signals of fine motricity [124]. Another aspect about DHA and its neuroprotective effects has been demonstrated in subjects with autism, to whom supplementing with DHA (40 mg/capsule) and AA (40 mg/capsule) with daily doses of 6 capsules during 16 weeks produced a better regulation of brain signals and important improvement in the social integration of these patients [125]. An interesting aspect of DHA supplementation was observed in epileptic patients who received capsules containing 1000 mg fish oil (171 mg EPA, 112 mg DHA). It was observed a reduction of phospholipid degradation in brain neurons, a high level of phospholipids in membrane of neuronal vesicles and a general improvement of brain metabolism [126]. Positive effect of DHA supplementation as neuroprotector was also observed in episodes of headache. A dietary intervention of 56 individuals demonstrated that consumption of food rich in n-3 LCPUFA, especially DHA, reduced episodes of headache and significantly increased plasma levels of metabolic products of EPA (18-hydroxy-eicosapentaenoic acid) and DHA (17-hydroxy-docosahexaenoic acid) [127]. The almost exclusive effect of DHA compared to EPA and AA is aborded in two recent communications. In a study performed in C57BL/6 J mice with hypoxia-ischemia brain injury, researchers assayed the effect of DHA-enriched triglycerides or EPA-enriched triglycerides in neuroprotection. They found that the supplementation with DHA but not with EPA administrated in same doses (0.375 g n-3 /kg/dose) had a protective effect against hypoxia-ischemia brain injury, reducing oxidative damage and improving neurological outcomes assessed at 24 h and 8 weeks after brain injury [128]. Supplementing murine hippocampal HT22 nerve cells with DHA or AA, it was found that DHA supplementation had an indirect antioxidant effect in HT22 cells, increasing the expression of genes encoding for antioxidant proteins. However supplementation with AA did not showed any antioxidant effect and also worsened the composition of fatty acids of the cells increasing AA level and decreasing EPA and DHA levels [129].

5. Intake of n-3 PUFA and n-3 LCPUFA and nutritional relevance of DHA

Despite the several studies that supported the nutritional and metabolic significance of n-3 PUFA, until the early 1980s there were some doubts about the real importance of these fatty acids, particularly about the essentiality of ALA, the precursor of n-3 LCPUFA. This doubt was dispelled after the first report on deficiency of ALA recorded in 1982, related to the case of a 6 year-old girl who had undergone surgical resection of part of her small intestine, who received total parenteral

nutrition (75.9% LA and 0.66% ALA). After five months receiving parenteral nutrition, the girl presented neurological disorders, particularly numbness in his extremities, paresthesia and difficulty for walking, leg pain and blurred vision. However, when the parenteral formula was replaced for a formula with higher content of ALA (42.4% LA and 6.9% ALA), the neurological disorders were totally reversed [130]. Based on this case report, Holman and coworkers stated that ALA was an essential fatty acid and the minimum dose for preventing the symptoms caused by ALA deficiency was estimated to be in the range of 0.5–0.6% of total energy intake [130]. Subsequently, a study done with institutionalized elderly patients who received formula based on corn oil (61% LA and 0.5% ALA) through nasogastric feeding, showed they did not develop the neurological alterations observed in the 6 year-old girl, but developed dermatological disorders, particularly dermatitis and flaky skin, together with very low levels of circulating EPA and DHA. However, when 0.3% ALA was added to the formula, the skin symptoms were resolved in four weeks along with normalization in plasma levels of EPA and DHA [131]. Based on these results, researchers argued that for older adults the lowest daily intake of ALA should be 0.2–0.3% of the energy/day and for EPA plus DHA should be 0.1–0.2% of the energy/day, indicating that in absence of EPA and DHA the endogenous biosynthesis of these fatty acids from ALA is significantly increased [132]. Based on these data and in relation to the importance of DHA in the nervous system, particularly the brain and retina, currently there is relative agreement that humans are only able to transform 1% of ingested ALA into DHA, this conversion being more efficient and critical during the first years of life [133]. After the delivery, breast milk is the only food that provides all the essential nutrients for the newborn, with the contribution of necessary n-3 and n-6 LCPUFA to ensure optimal brain development, thus acquiring particular importance the maternal nutrition during pregnancy and lactation [134–136]. Fig. 1 shows the metabolic steps for the biosynthesis of DHA and other fatty acids intermediates.

Tissue DHA levels of women are higher than men, probably due to increased capacity to synthesize DHA from ALA due to oestrogenic stimuli of desaturase and elongase enzymes [137]. The relative content of DHA in human milk varies significantly in different populations, being values from 0.1% to up 1% of the total fatty acids present in human milk, variation primarily explained by the eating of fish or other seafood or from land animals that have been fed fishmeal and/or fish oil, such as chickens, turkeys and pigs [31,138]. It is noteworthy that in the past three decades the levels of DHA in breast milk has been significantly reduced in the general western population due, mainly, to the low consumption of foods considered good sources of DHA, among which are fatty fish (or blue fish), being tuna, mackerel, codfish, salmon, sardine and anchovy, the most important [139]. During pregnancy the minimal recommended ingestion of DHA according Food and

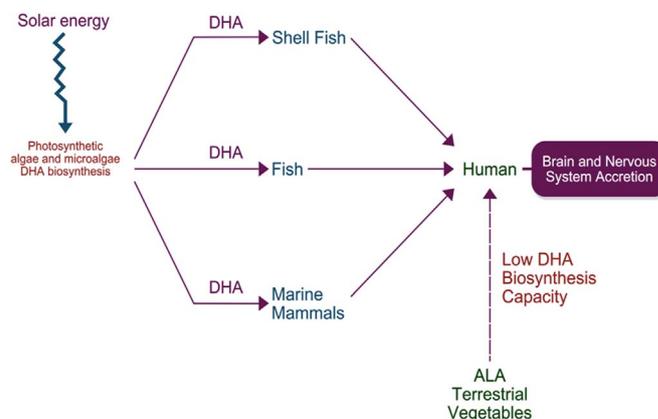


Fig. 1. Metabolic pathways for the formation of n-6 and n-3 fatty acids from precursors in humans.

Agriculture Organization (FAO) is 200 mg/day [140], but there is no universal consensus because it is difficult to obtain this ingestion due the multiple reasons discussed in the next section. However, when pregnant women are advised or received dietary counseling about the physiological relevance of DHA, it is possible to observe a significant increase in the consumption of those foods and/or supplements that provide such important n-3 LCPUFA [141]. Dietary counseling about the benefits of fish consumption during pregnancy, increases the consume n-3 LCPUFA in the diet [142]. Food and Agriculture Organization (FAO) and many researchers have established that the benefits of fish consumption rich in n-3 LCPUFA may overcome the possible negative effects of low metal or other organic contaminants eventually present in these foods [143,144]. A weekly consumption of two portions of fish rich in DHA, such as salmon, tuna, anchovy or mackerel, may contribute significantly to reach the minimal recommendation for the fatty acid during pregnancy, also increasing DHA levels of umbilical blood [145,146]. Low ingestion of foods which naturally provide DHA (such as fatty or blue fish) during pregnancy is critical to obtain enough levels of the fatty acid which is reflected in low levels of DHA in umbilical blood [147]. *Trans* fatty acid ingestion may also interfere with the availability of n-3 LCPUFA, such as DHA, to the mother and her child [148]. Policies to develop strategies to increase DHA consumption to the population, especially to pregnant and nursing women are actually of high priority. The ratio n6/n3 PUFAs is also important for the cognition and brain development. Higher levels of n-3 PUFAs in colostrum are positively associated with infant mental development, as well as a higher ratio n-3/n-6 PUFAs [149]. Furthermore, levels of LA in colostrum are negatively associated with child cognitive scores at ages 2 and 3 years, independently of breastfeeding duration [150].

Many models for these strategies can be designed. As an example, pregnant women who consumed DHA-rich eggs (135 mg DHA/egg), compared to pregnant women that similarly consumed non enriched-eggs (18 mg DHA/egg), showed higher erythrocyte and umbilical cord DHA levels [151]. Pregnant women who consumed a cereal-bar enriched with DHA (300 mg DHA/bar) delivered babies showing increased visual acuity until four month post partum [152], also showing a significant better capacity to resolve problems [153] and an improved organization of their dream [154]. Recommendations to increase fish consumption are counteracted by the fear about metal contamination of these foods, indeed many health professional recommended to avoid or reduce fish consumption, especially to pregnant women. Fish availability to some population is also difficulted by the high price compared to other foods, therefore policies to increase fish consumption result in low success. An effort to increase DHA availability to pregnant and nursing women was developed in Chile. A product named “Purita Mamá”, a powder milk substitute formula which is freely distributed to pregnant women who are controlled at the National Health Service, was supplemented with microencapsulated fish oil, allowing the providing of 60 mg DHA by portion (200 mL) of the reconstituted product. Despite some organoleptic problems, because the reconstituted product developed some uncomfortable smell that it is not accepted for some mothers, the formula has proved to increase DHA content of breast milk [155]. New version of the product which has been currently developed is expected to overcome this organoleptic limitation, transforming the formula in an inexpensive way (the product is distributed by the National Health Service with no cost for mothers) to increase DHA availability for the Chilean pregnant and nursing population [156,157].

6. Dietary sources of DHA

Traditionally the best way to ingest DHA is through the consumption of sea foods. However some concerns have been aroused because of the presence of heavy metals (mercury, lead, cadmium) and organic material (polychlorinated biphenyls, PCBs) in some fish, especially those captured at the north hemisphere [158,159]. Health professionals (Medical and Nutritionist) now currently recommend pregnant women

to reduce or avoid the consumption of fish and shellfish due this possible and not even present contamination. However, other alternatives are available to nutritionally supply DHA [160]. Fish oil is another alternative to obtain DHA and also EPA. These oils, which are obtained by cooking and pressing pelagic fish (mackerel, sardine, anchovy, etc.) can be highly deodorized, stabilized to oxidation and stripped from contaminants and even of cholesterol. These highly refined oils can be consumed as capsules or micro-encapsulated and most recently nano-encapsulated, allowing its incorporation to a wide variety of food matrices (milk, juices, cereals, etc.) [161]. Fish oil can be also separated into its forming fatty acids through chemical or enzymatic hydrolysis and by molecular distillation and concentrated in DHA (up to 80%). The fatty acid can be transformed into ethyl ester or transformed again into triglycerides with high concentration of DHA. Some algae and microalgae are a renewable alternative to obtain DHA. Species such as *Cryptocodinium*, *Mortierella* and *Schizochytrium* can be artificially cultivated in industrial bioreactors producing oils high in triglycerides containing DHA [162]. These cellular products, which are odorless and free from contaminants, are now available to be incorporated into different food matrices, especially to infant formulas. Phospholipids from marine origin containing DHA are another alternative [163]. Krill (*Euphausia superba*), a small crustacean of the Antarctic zooplankton is captured to obtain meal and oil. The oil has a high content of phospholipids rich in DHA [164] and it also contains astaxanthin, a carotenoid with strong antioxidant and anti-inflammatory properties [165]. However, in the near future the capture of krill will be restricted because ecological demands. Krill is the main food of whales and other marine animals. Marine phospholipids can also be obtained from fish meal or from the remains of processed fish from aquaculture. This novel procedure, not yet industrially available, consists in the treatment of fish meal or the remains of farmed fish with industrial proteases. These enzymes destroy the proteins of cellular membranes allowing the release of phospholipids which are the main lipid components of these structures, which can be further separated by solvents and purified at different degrees [166]. Another dietary source of DHA is hen egg yolk which its DHA content depends on the hen's diet. Considering that eating eggs is more affordable than eating fish, hen egg yolk with phospholipids enriched in DHA may be a good option to increase DHA consumption in general population [167,168]. In plasma, DHA is present in nonesterified form and esterified to lysophosphatidylcholine pools in similar amounts. Even though there is a preferential uptake by the brain of DHA esterified in lysophosphatidylcholine, this is not the major mechanism by which DHA is incorporated into the brain. Under normal conditions, nonesterified DHA is the major plasma pool supplying the brain with DHA [169,170]. Fig. 2 summarizes the supply of the DHA to humans through the food chain.

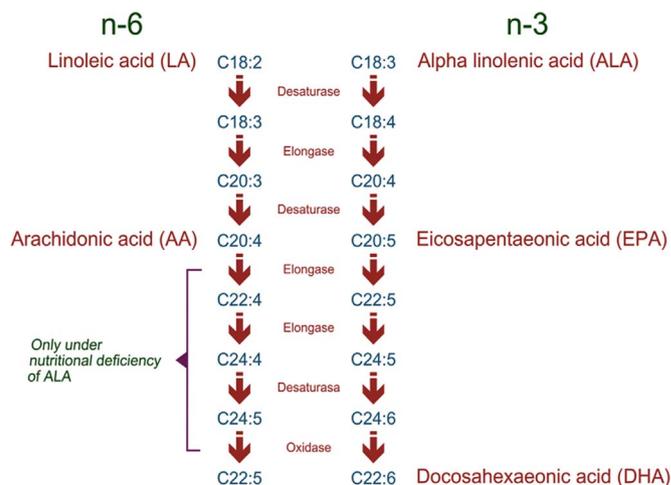


Fig. 2. Natural origin of DHA in the food chain.

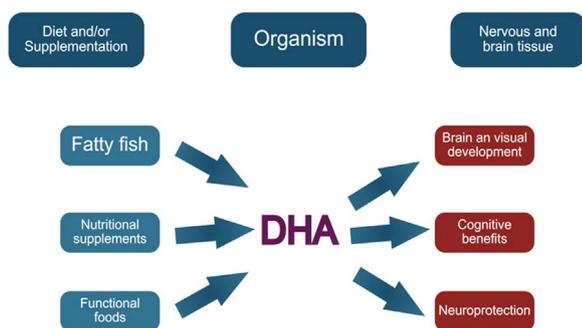


Fig. 3. DHA intake and/or supplementation and beneficial effects.

7. Conclusions and perspectives

The important physical-chemical features of DHA, i.e., high unsaturation and very low melting point ($-20\text{ }^{\circ}\text{C}$) give the fatty acid their biological functions as major structural component of membrane of neuronal and glial cells, acquiring both a structural and a functional role in these brain cells. In recent decades, many studies have reported the important biochemical and nutritional functions of this fatty acid, particularly in the brain, highlighting the possible involvement of DHA in the evolution of the human brain that sets us apart from other primates. The adequate supply of DHA throughout life, particularly during pregnancy, lactation and adulthood, is essential to promote proper brain development in utero and early life and preservation of brain tissue during aging. During pregnancy and lactation period DHA consumed from sea foods or from supplementation would produce important benefits in newborns, especially in cognitive and visual functions [9,31,48,136], whereas a high intake of DHA during aging may help to prevent cognitive decline [8,60,65], possibly through NDP-1 or other derivatives of DHA having an important neuroprotective function [17,76]. Additionally, several recent studies have established the importance of an adequate supply of DHA in infants and children and the benefits seen in school performance [171–173]. DHA and AA are both fundamental for brain development and function. Nevertheless, a low n-6/n-3 PUFAs ratio is critical for brain development and structural integrity, being the balance in the brain between n-6 and n-3 PUFA close to 1:1 [139]. A diet with high intake of LA compromises DHA accretion in the developing brain increasing brain levels of AA. On the contrary, higher dietary DHA intake favors brain DHA accretion [174]. A dietary approach to diminish n-6/n-3 PUFAs ratio could be lowering dietary intake of LA. In a randomized cross-over study in men, the replacement of vegetable oils high in LA with oils low in LA, while maintaining constant ALA, reduced AA:EPA ratio, however, LA intake was not associate with AA nor DHA levels in plasma phospholipids [175]. At the same time, increasing dietary ALA consumption has a positive effect in plasma and erythrocyte levels of EPA but not showing a concomitant increment in DHA levels in plasma and erythrocytes [176].

The low supply of DHA that today is provided by western diet has stimulated the development of foods and/or nutraceuticals of different presentations and content of DHA, the effort directed to obtain ingredients feasible to be utilized as sources of DHA achieving the incorporation of the fatty acid into diverse alimentary matrices [177]. As discussed above, nowadays are available marine oils rich in DHA or derived from krill or microalgae [178–180]. Micro- and nano-encapsulation of oils rich in DHA has allowed the incorporation of this fatty acid into various food matrices, particularly milk and dairy products, juices, bread, cookies, etc [177]. These new technological developments emerge as viable alternatives to increase the consumption of this indispensable nutrient in the population, particularly to those individual most vulnerable, such as pregnant women, nursing mothers, children, and senior groups [169]. An outline of the different forms to

incorporate DHA into our body and the benefits derived from such supplementation is shown in Fig. 3.

Author contributions

Rodrigo Valenzuela, Francisca Echeverría, María Hernandez-Rodas and Alfonso Valenzuela collected different manuscripts regarding the work of review, and wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

Declaration of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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