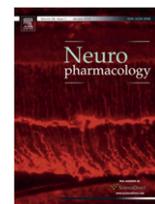


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Invited review

Cognitive enhancement by omega-3 fatty acids from child-hood to old age: Findings from animal and clinical studies

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ABSTRACT

Omega-(*n*-3) polyunsaturated fatty acids (PUFAs), including docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are major components of neuronal membranes and have a wide range of functions, from modulating synaptic plasticity and neurochemistry, to neuroimmune-modulation and neuroprotection. Thus, it is not surprising that *n*-3 PUFA are widely acknowledged to have cognitive-enhancing effects. Although clinical evidence is somewhat conflicting, probably in large part due to methodological issues, animal studies have consistently demonstrated that *n*-3 PUFA are indispensable for proper brain development, may enhance cognitive function in healthy, adult individuals and attenuate cognitive impairment in aging and age-related disorders, such as dementia. This review discusses and integrates up to date evidence from clinical and animal studies investigating the cognitive-enhancing effects of *n*-3 PUFA during development, child- and adult-hood, as well as old-age with associated neurodegenerative diseases, such as Alzheimer's disease. Furthermore, we cover the major underlying biochemical and neurophysiological mechanisms by which *n*-3 PUFA mediate these effects on cognition.

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1. Introduction

The global health burden of human mental and neurological disorders has surpassed that of both cardiovascular disease and cancer (Collins et al., 2011). At the same time, the availability of omega-(*n*-3) polyunsaturated fatty acids (PUFAs) in the Western diet has dramatically decreased during the last several decades (Bazan et al., 2011). Increasing evidence has linked deficiency in dietary intake of *n*-3 PUFAs to the burden of human mental and neurological disorders (Zhang et al., 2011) but the role of these

lipids in brain function is still incompletely understood. One aspect of brain function that has been extensively studied in relation to *n*-3 PUFA is cognition. Although supported by somewhat conflicting clinical evidence, it is thought that deficiency of *n*-3 PUFA has detrimental effects on cognitive brain development, while conversely, the dietary supplementation of *n*-3 PUFA may be beneficial (Karr et al., 2011). Furthermore, evidence from clinical and animal studies suggests that *n*-3 PUFA may have therapeutic value for cognitive impairment associated with normal aging and neurodegenerative disorders such as Alzheimer's disease (AD) (Zhang et al., 2011; Karr et al., 2011). With the increasing age of Western population and rise of neurodegenerative disorders, there is an urgent need for effective, mild therapy to prevent, delay or cure these disorders. Thus, it is timely, to determine whether the claimed benefits of otherwise safe and side-effect free *n*-3 PUFA can be substantiated by recent high-quality evidence. In this review, evidence from animal and clinical studies investigating the role of *n*-3 PUFA in cognitive function and enhancement in the life stages of development, child-and adulthood, and aging, will be discussed. The mechanisms by which *n*-3 PUFA are thought to mediate their effects are covered as well.

Abbreviations: PUFAs, Polyunsaturated fatty acids; *n*-3, omega-3; *n*-6, omega-6; LA, C18:2, *n*-6: linoleic-acid; AA, C20:4, *n*-6: arachidonic acid; DPA, C22:5, *n*-6: Docosapentaenoic acid; ALA, C18:3, *n*-3: Alpha-linolenic acid; EPA, C20:5, *n*-3: Eicosapentaenoic acid; DPA, C22:5, *n*-3: Docosapentaenoic acid; DHA, C22:6, *n*-3: Docosahexaenoic acid; AD, Alzheimer's disease; RCT, randomized controlled clinical trial.

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2. Major PUFA and their transport to the brain

Omega-3 and omega-(*n*)-6 fatty acids are vital for the brain, constituting about 30–35% of total brain fatty acids. In the mammalian brain, lipids constitute 50–60% of the dry weight, and the major brain lipid class is phospholipids (Youdim et al., 2000). In phospholipids, the most abundant *n*-3 and *n*-6 PUFA are respectively docosahexaenoic acid (DHA; C22:6, *n*-3) and arachidonic acid (AA; C20:4, *n*-6) (Youdim et al., 2000). See Fig. 1 for a simple diagram of brain fatty acid composition. Importantly, *n*-3 similar to *n*-6 PUFAs are dietary essential, as the body cannot synthesize them itself, and are critical for the development and function of mammalian brain and, in case of DHA, also retina (Youdim et al., 2000). DHA, the major *n*-3 PUFA in phospholipids and eicosapentaenoic acid (EPA, C20:5, *n*-3), another important membrane *n*-3 PUFA, although far less abundant, are long chain *n*-3 PUFA that can either be directly obtained from dietary sources, in particular fish oils (fatty fish), or enzymatically derived from alpha-linolenic acid (ALA, C18:3, *n*-3), which is an *n*-3 fatty acid derived from vegetable sources, such as flax, soy, rapeseeds and walnuts. Dietary intakes of α -linolenic acid among Western adults are typically in the range of 0.5–2 g per day. In Western society, intakes of fish or fish oils are also typically low. The major *n*-6 PUFA is arachidonic acid, which can be derived directly from meat and dairy products, or enzymatically, from linoleic acid (LA, C18:2, *n*-6) in vegetable sources. In Western society, the dietary intake of LA is typically 5–20 times higher than that of ALA (Calder, 2012; Youdim et al., 2000). See Fig. 2 for a simple overview of PUFA metabolism.

The importance of PUFA in neuronal health and function is suggested by the rigid maintenance of a unique membrane fatty acid composition with high levels of palmitate and the polyunsaturated fatty acids (PUFA), including DHA and arachidonic acid (AA), but very low levels of other PUFA, including EPA, α -linolenic acid (ALA) and linolenic acid (LA) (Youdim et al., 2000). In order to maintain relatively constant levels of PUFA, the brain relies on PUFA uptake from plasma, which may be supplied from the diet and/or the liver (Demar et al., 2005). Recent efforts have shed light on the means of transport by which PUFA are transported to the brain, which until recently was a matter of controversy and thought to involve lipoproteins or plasma proteins such as albumen. Using the *in situ* brain perfusion technique, Ouellet et al. (2009) demonstrated that both EPA and DHA in free form are rapidly transported through the blood brain barrier (BBB) as freely diffusible lipophilic drugs.

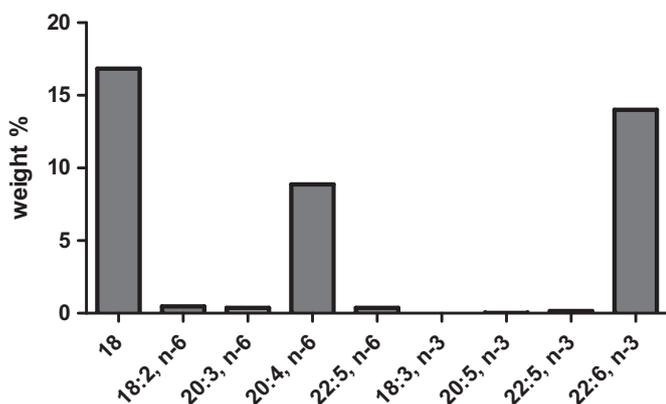


Fig. 1. Brain lipid profile (rat). Brain lipid profile, adapted from Palsdottir et al. (2012). C18:0: stearic acid; C18:2, *n*-6: linoleic-acid (LA); C20:3, *n*-6: Dihomo-gamma-linolenic acid (DGLA); C20:4, *n*-6: arachidonic acid (AA); C22:5, *n*-6: Docosapentaenoic acid (DPA); C18:3, *n*-3: Alpha-linolenic acid (ALA); C20:5, *n*-3: Eicosapentaenoic acid (EPA); C22:5, *n*-3: Docosapentaenoic acid (DPA); C22:6, *n*-3: Docosahexaenoic acid (DHA).

Since different PUFA appear to be equally well absorbed in the brain, this cannot explain the unique profile of fatty acids in neuronal membranes. For instance, brain phospholipids are much more (about 300 times) enriched in DHA than EPA (Chen et al., 2011). Much of this unbalanced distribution of membrane PUFA has to do with the unequal partitioning of these fatty acids between degradation and esterification processes. For instance, while α -linolenic acid (ALA) can rapidly diffuse from plasma to brain, it was demonstrated in adult rats fed a diet enriched in both DHA and α -linolenic acid (ALA) that the majority of ALA in the adult brain is recycled through β -oxidation; the remaining is incorporated unchanged into phospholipids (10%) and triglycerides (2%) or as newly synthesized DHA ($\leq 0.2\%$) (Demar et al., 2005). Similarly, more than 86% of uptake plasma linolenic acid (LA) is β -oxidized in the brain and only a small fraction ($<1\%$) is used to synthesize arachidonic acid (AA) (DeMar et al., 2006). A similar fate was reported for EPA. For instance, Chen et al. (2011) reported that of intracerebroventricularly (i.c.v) infused radiolabeled EPA, after 4 days, only 9% of intact EPA was recovered, the remaining was β -oxidized or lost by de-esterification from phospholipids. Yet AA (Green et al., 2010) and DHA (DeMar et al., 2004) are much better retained in the brain, making a case for direct intake of these long-chain PUFA rather than their shorter-chained precursors, if one were to ensure more efficient accumulation of these PUFA in the brain. It should be noted that in rats fed inadequate amounts of DHA, conversion of α -linolenic acid (ALA) to DHA in the liver is upregulated, thereby establishing normal brain levels of DHA, as long as ALA is provided (Demar et al., 2005). Thus, in cases of liver damage, failure or age-related impairment of liver function, one may need dietary intake of DHA to establish normal levels of DHA in the brain. Furthermore, neuronal membrane *n*-3 PUFA content may decrease with age and neurodegenerative diseases (Yehuda et al., 2002).

While the lipid composition of neurons is rigid, it can be modified to a certain degree by dietary alterations. For instance, increased intake of EPA over the course of 6–8 weeks can significantly increase brain content of EPA and metabolite DPA (C22:5, *n*-3) in mice, even though these are trace components of neuronal membranes (Luchtman et al., 2012; Meng et al., 2010). Prolonged intake of DHA will modestly increase brain DHA content, with a reciprocal decrease in brain *n*-6 content, in particular DPA (C22:5, *n*-6) (Bousquet et al., 2008). Conversely, complete long term dietary *n*-3 PUFA deprivation will deplete the brain of DHA, but with a reciprocal increase in brain *n*-6 content, in particular docosapentaenoic acid (DPA) (C22:5, *n*-6). In this way, the degree of unsaturation is more or less maintained, but the function of these fatty acids can be drastically different. For instance, high levels of DPA (*n*-6) instead of DHA will lead to cognitive impairment, even though these molecules differ only by a single double bond (Fedorova and Salem, 2006). Because of the increased amount of *n*-6 fatty acids in Western diet (increased use of cereals, vegetable oils and meats), the ratio of *n*-6 to *n*-3 PUFA has increased substantially during the last 150 years to 15:1–17:1 (reviewed in Simopoulos (2002)), with potentially far reaching consequences for brain function and mental/neurological health.

3. Function of N-3 PUFA

Once in the brain, PUFAs (including arachidonic acid (AA) and DHA) are converted by an acyl-CoA synthetase in acyl-CoAs and are then esterified into glycerophospholipids at the sn-2 (stereospecific numbered) position via the acyl-CoA transferase. The effects of PUFA on cellular function are complex but, summarized, include but are not limited to: 1) modulating the structure and function of lipid rafts and neuronal membranes, including raft and membrane-

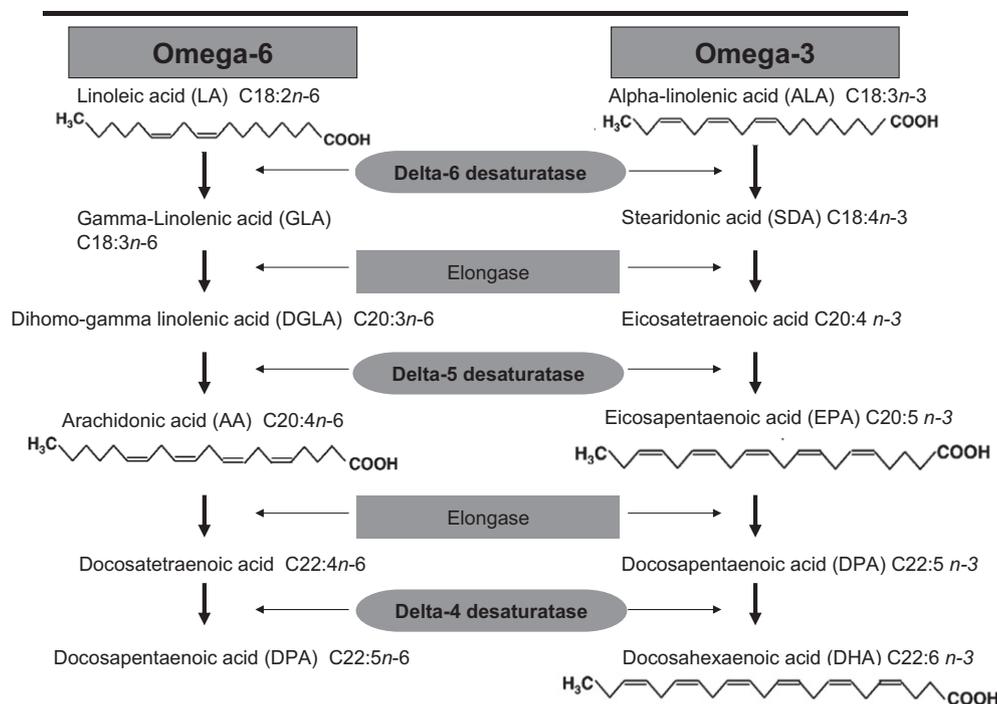


Fig. 2. Overview PUFA metabolism. Schematic representation of the chemical structure and conversion of omega-6 PUFA linoleic acid (C18:2, *n*-6) and omega-3 PUFA alpha-linolenic acid (C18:3, *n*-3) to their longer-chain metabolites. The elongase and desaturase enzymes required for the metabolism as well as the intermediate fatty acids are shown as well.

incorporated proteins, such as channels, receptors and signaling proteins; 2) acting as direct ligands to transcription factors of genes that play a role in a wide variety of processes, including fatty acid metabolism, neurogenesis and synaptogenesis, differentiation, inflammation and oxidative stress; 3) acting as precursors to biosynthesis of lipid mediators which are involved in regulation of many cell and tissue responses, particularly aspects of inflammation. Dietary intake of PUFA and the ratio of ingested *n*-6:*n*-3 PUFA can in turn affect how PUFA affect all these cellular functions (Calder, 2011).

3.1. Membrane fluidity

Long-chain esterified PUFA play an important role in modulating the structure of neuronal membranes. The lipid bilayer tends to exist at an optimum transition point between gel and liquid crystal, and the maintenance of this state, often referred to as fluidity, is of physiological importance and can be strongly influenced by fatty acid composition. Transmembrane and peripheral proteins of various shapes, molecular masses and charges (receptors, ion channels, and membrane bound-enzymes) regulate important cellular functions but their position, integration and functioning are all affected by membrane fluidity and thus fatty acid composition. Especially the double bonds in PUFA, which are exclusively in the *cis* conformation, have strong influence and the replacement of even a single double bond in these PUFAs is sufficient to exert a profound effect on the physical properties of the membrane (Frisardi et al., 2011; Youdim et al., 2000). Fatty acid composition of neuronal membranes can be directly altered by dietary modifications. Membrane fluidity also changes with age and neurodegenerative diseases, for various reasons, including increased content of cholesterol and ROS (Yehuda et al., 2002). ROS can lead to chemical cross-linking of membrane proteins and lipids and a reduction in membrane unsaturation. This not only decreases membrane

fluidity, but also results in inhibition of membrane-bound enzymes, ion-channels, and receptors (Frisardi et al., 2011). Replenishment of PUFA by means of dietary intake may compensate for these neurodegenerative changes (Yehuda et al., 2002), although the risk of peroxidability of PUFA is a matter of debate.

3.2. Gene expression related to synaptic function, neurochemistry and inflammation

PUFAs are known to mediate widespread effects on gene expression in the CNS (Calder, 2011; Kuperstein et al., 2008, 2005; Kitajka et al., 2004; Rojas et al., 2002), either as free ligands, or through their eicosanoid metabolites. Putative targets of *n*-3 PUFA in regulating gene expression include ligand-activated transcription factors, including the retinoic acid receptors (RARs), retinoid X receptors (RXRs), and peroxisome proliferator-activated receptors (PPARs). Retinoid signaling pathways have been implicated in regulating synaptic plasticity and learning and memory in rodents (Dyall et al., 2010; Dyall and Michael-Titus, 2008; Kitajka et al., 2004). Another important transcription factor involved with *n*-3 PUFA is cAMP response element binding protein (CREB), which is important for synaptic integrity and synaptic transmission and largely belonging to the CREB1 transcription pathway. CREB1-dependent gene expression plays an important role in learning and memory in mammals and is involved in hippocampal long term potentiation (LTP), the neurophysiological process underlying learning and memory (Sidhu et al., 2011; Cao et al., 2009). Brain-derived neurotrophic factor (BDNF) is a major neurotrophic factor transcribed by CREB1 and plays an important role in neuronal survival (Rao et al., 2007). Through these effects on gene expression, PUFA play a role in neurogenesis and synaptogenesis (reviewed in Su, 2010). PPAR γ , which PUFA can bind to, is able to directly downregulate inflammatory gene expression, but it also interferes with the activation of NF κ B, a major inflammatory

transcription factor responsible for the induction of pro-inflammatory cytokines and enzymes such as COX-2 and iNOS (Calder, 2011; Bordet et al., 2006; Bernardo and Minghetti, 2006). Anti-inflammatory effects may counter inflammation-induced cognitive impairment in aging and neurodegenerative disorders (further discussed in last section).

3.3. Synaptic function and neurochemistry

PUFA have widespread effects on synaptic function, integrity and neurochemistry, as touched upon above. The exact mechanisms are unknown, but likely involve a complex interplay of synergistic effects on neuronal membrane structure and function, gene expression but also metabolism to eicosanoids/docosanoids. Research over the past decade has demonstrated that quantitative changes in *n*-3 fatty acids in the body are often paralleled by quantitative changes of the monoamine concentration in the brain, in particular the frontal cortex, nucleus accumbens and the striatum, but also the hippocampus (Chalon, 2006; Zimmer et al., 1998; Chalon et al., 1998; Delion et al., 1997, 1994). Changes in PUFA intake, for instance by dietary depletion during pre- and post-natal life, but also throughout adulthood correlated with neurochemical alterations, including down-regulation of the vesicular monoamine transporter (VMAT-2) and a depletion of VMAT-associated vesicles in the hippocampus (Chalon, 2006; Kuperstein et al., 2008). A depletion of pre-synaptic vesicles can explain the neurotransmitter depletion in conditions of *n*-3 PUFA deficiency. Some of these neurochemical effects of *n*-3 PUFA depletion can be restored by dietary repletion if done early in neonatal life (Chalon, 2006). Furthermore, supplementation with *n*-3 PUFA, including EPA, has substantial effects on monoamine and metabolite levels in the brain of adult rodents (e.g. Luchtman et al., 2012; Meng et al., 2010).

3.4. Eicosanoid production and inflammation

A major way by which the ratio of *n*-3 and *n*-6 PUFA influence brain function is by affecting eicosanoid and docosanoid

production. It is beyond the scope of this chapter to discuss the eicosanoids derived from PUFA in detail, as there are excellent reviews on this complex topic (e.g. Calder, 2011, 2010; Frisardi et al., 2011; Russo, 2009; Serhan et al., 2008; Farooqui et al., 2007; Youdim et al., 2000). This section summarizes some of the most important aspects. Eicosanoids, which include prostanoids (prostaglandins, tromboxanes, prostacyclins) and leukotrienes are fast acting bioactive molecules that are key mediators and regulators of inflammation and immunity and are generated from 20-carbon PUFAs, including arachidonic acid (AA) and EPA, by the metabolic processes summarized in Fig. 3. Aside inflammation and immunity, eicosanoids also play a role in sleep, synaptic plasticity and neurotransmission. Through these widespread effects, eicosanoids could potentially affect cognition, although the exact relationship between these fast acting molecules and mental functions is unclear (Tassoni et al., 2008).

An important aspect of eicosanoids is to distinguish those derived from *n*-6 PUFA and those derived from *n*-3 PUFA, as they tend to have differential affects on immunity. Essentially, *n*-6 derived eicosanoids have more potent and more pro-inflammatory effects than *n*-3 derived eicosanoids. Dietary modification can influence the production of eicosanoids. For instance, PGE₂ production is increased by arachidonic acid (AA) feeding and decreased by EPA or DHA feeding. COX-2 metabolism and other aspects of the AA-metabolic cascade, which leads to PGE₂ production, have been associated with cognitive impairment (Rao et al., 2011). However, the role of AA-derived eicosanoids as solely pro-inflammatory is an over-simplification as PGE₂ was shown to have both pro- and anti-inflammatory effects. Furthermore, another eicosanoid derived from AA, lipoxin A₄, is anti-inflammatory. Lipoxins, but also EPA and DHA-derived resolvins play an important role in the resolution process of inflammation, which is concerned with specific mechanisms to promote return to homeostasis (Serhan et al., 2008). DHA can also give rise to neuroprotectins (docosanoids), biosynthesized via a lipoxygenase-mediated pathway, one of which, neuroprotectin D1 (NPD1), documented by Bazan (2009), is particular potent, can be induced

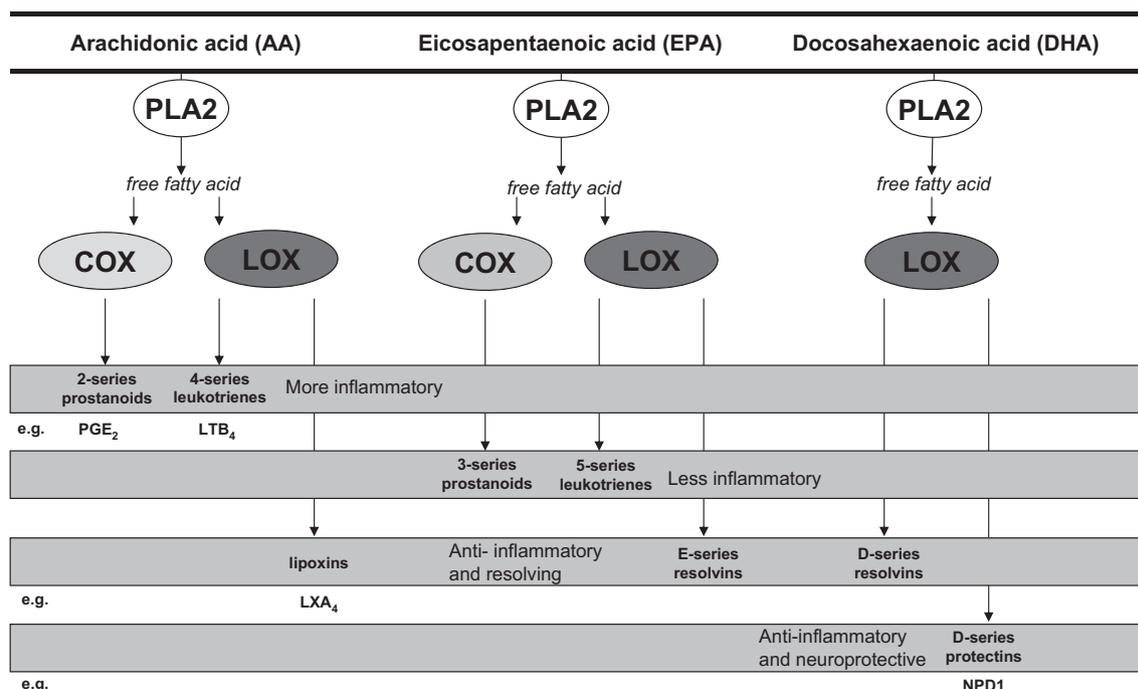


Fig. 3. Overview of eicosanoid/docosanoid metabolism. Simplified diagram of eicosanoid/docosanoid synthesis from AA, EPA and DHA. PGE₂: prostaglandin E₂; LTB₄: leukotriene B₄; LXA₄: lipoxin A₄; NPD1: Neuroprotectin D1.

by toxic conditions, such as oxidative stress, and plays a role in resolving inflammation and promoting cell survival. NPD1 can inhibit NF κ B and COX-2-mediated inflammation as well as attenuate bcl-2 mediated apoptosis. This protection may be more relevant to aging and neurodegenerative diseases.

4. *n*-3 PUFA and cognitive development

While the preceding sections do not cover the full extent of PUFA action in the brain, it should provide ample evidence to appreciate that PUFA could improve cognition by multiple, possibly synergistic mechanisms and that given the low side-effect profile, they could be ideal candidates for cognition-enhancing interventions. The following sections evaluate up to date evidence on the effects of *n*-3 PUFA depletion and/or supplementation on cognition in young, adult or aged humans and laboratory animals.

4.1. Evaluation of infant studies

Since initial reports by [Clandinin et al. \(1980\)](#) of the rapid accumulation of PUFA, in particular arachidonic acid (AA) and DHA, in human during the last trimester of pregnancy, the potential roles of DHA and AA in infant growth, development, and health have been the subject of intense research for several decades ([Hoffman et al., 2009](#)). Initial animal studies with rhesus monkeys fed normal amounts of *n*-6 PUFA (linoleic acid, LA), for normal growth, but inadequate amounts of *n*-3 PUFA (α -linolenic acid, ALA), revealed retarded neurodevelopment in these animals ([Carlson and Neuringer, 1999](#)). A large number of studies have confirmed these findings in rodents, as discussed in detail in Section 4.2 (and Table 1).

The increasing interest in the role of PUFAs in infant nutrition was enhanced by observations that plasma and erythrocyte lipid contents, as well as post-mortem brain content of these fatty acids, primarily DHA, were lower in infants fed formula (prior to 2002) than in breast-fed infants ([Sanders and Naismith, 1979](#) and reviewed in [Hoffman et al., 2009](#); [Heird and Lapillonne, 2005](#); [Auestad et al., 2003](#); [Carlson and Neuringer, 1999](#)). This is a particular concern in developing countries where access to animal sources rich in long-chain PUFA such as arachidonic acid (AA) and DHA are limited ([Huffman et al., 2011](#)). Aside the third trimester of pregnancy, significant amounts of PUFA accumulate in the brain (and retina) during the first postpartum year, making this period of life (the brain “growth spurt”) potentially susceptible to the effects of possible deficiencies. While breastmilk is considered the “gold standard” model for infant formula, and recommended as the sole source of nutrition for the vast majority of infants for approximately the first 6 months of life, its PUFA and in particular DHA content, was found to vary. While AA content is relatively consistent across populations worldwide, a study by [Brenna et al. \(2007\)](#) showed a greater than 20-fold range of means of DHA content across populations worldwide, from 0.06% to 1.4%. Interestingly, the reliance of human and other mammalian brains on DHA appears to be the result of evolution, as neurons first evolved in an aquatic environment where high levels of DHA were readily available ([Lassek and Gaulin, 2011](#)).

Yet the true benefit of maternal supplementation with *n*-3 PUFA (during pregnancy as well as lactation) or formulas containing supplemented *n*-3 PUFA is debated in literature reviewing the clinical trials conducted to evaluate the effect of supplementation on neurodevelopmental aspects such as psychomotor ability, cognitive and visual function in both term and preterm infants ([Dziechciarz et al., 2010](#); [Hoffman et al., 2009](#); [Simmer et al., 2008a, b](#); [Cheatham et al., 2006](#); [McCann and Ames, 2005](#); [Heird and Lapillonne, 2005](#); [Carlson and Neuringer, 1999](#)). While beneficial

effects of *n*-3 PUFA supplementation are reported, there is also strong inconsistency in findings. Randomized controlled clinical trials (RCTs) often used standardize tests such as the Bayley Scales of Infant Development, which assess global abilities of infants from birth to 42 months of age, and the Fagan Test of Infant Intelligence (FTII), which focuses on novelty-preference ([Heird and Lapillonne, 2005](#)). Inconsistency in findings from studies utilizing these tests is largely attributable to numerous study design heterogeneity and variation in factors such as levels of PUFA added to the formula and ratio of *n*-6 to *n*-3 content; duration of formula feeding and at what stage of development supplementation occurred (e.g. during pregnancy or lactation, or both); ages and numbers of infants evaluated; and sources and quality of PUFA; and, importantly, variation in PUFA requirements between infants, the latter of which is a largely unexplored area ([Dziechciarz et al., 2010](#); [Heird and Lapillonne, 2005](#)). Furthermore, lack of significant effect of *n*-3 PUFA supplementation on cognitive performance may also be due to adequate baseline maternal and infant nutrition, as most studies are conducted in developed countries ([Dziechciarz et al., 2010](#)).

Background interacting genetic and environmental factors other than diet are also known to affect infant cognitive development, and thus may have contributed to the differences in outcomes among the studies ([Hoffman et al., 2009](#)). [Cheatham et al. \(2006\)](#) made an excellent point stating that global assessments of early cognitive function (e.g., the Bayley Scales of Infant Development) may not be sensitive enough to detect the potentially specific effects of PUFA supplementation on certain aspects of cognitive functions. Various cognitive functions develop independently, at different stages, each of which could be affected differently by nutritional interventions. For instance, [Cheatham et al. \(2006\)](#) describe research that shows that specific cognitive outcomes (e.g., problem solving, attention, and processing speed in infancy; distractibility in toddlers; and attention in preschool and school-aged children) are related to DHA intake or the amount of DHA in circulating cells or plasma lipids. Despite researchers' best efforts, these results combined show that valid research on the true benefits of *n*-3 PUFA supplementation during the brain growth spurt of the developing infant is still in its “infancy”. [Cheatham et al. \(2006\)](#) advise on the use of a more systemic developmental cognitive neuroscientific approach targeted to more specific aspects of cognitive functioning.

4.2. Evidence from animal studies

Due to the urgency of the topic, effects of *n*-3 PUFA deficiency and supplementation on cognitive development have been extensively researched in basic experimental studies with rodents ([Fedorova and Salem, 2006](#)). A significant advantage of animal studies is that more experimental control over factors such as maternal or pup nutritional status can be achieved, without the complicating background aspects of demographic status. Furthermore, factors that potentially confound a valid measure of cognitive performance, such as anxiety, can be more easily controlled. Table 1 presents an overview of rodent research on the developmental effects of *n*-3 PUFA deficiency/supplementation. Dietary depletion of *n*-3 PUFA, in particular DHA, is most optimally achieved pre-natally in rodents, or over successive generations, as *n*-3 (essentially, DHA) levels are tenaciously retained by the adult brain ([Chalon, 2006](#); [Fedorova and Salem, 2006, Table 1](#)). However, significant loss of brain DHA can also be achieved in a one-generation model in which both rats and mice are artificially reared by methods using a bottle–nipple system to hand feed pups with artificial milk containing carefully dosed *n*-3 content, allowing total control over *n*-3 intake ([Fedorova and Salem, 2006, Table 1](#)). As long as *n*-6 PUFA are provided, dietary restriction of only *n*-3 PUFA does not grossly affect development or growth, but

Table 1
Effects of developmental *n*-3 supplementation/deficiency on cognition and hippocampal LTP.

Species	Test	Dosing/Exp/design essentials	Effect of <i>n</i> -3 deficiency/supplementation	References
Long Evans rat	Barnes circular maze	Pregnant dams fed <i>n</i> -3 adequate/deficient diet; then male newborns were lactated and weaned on same (pelleted) diet for 8 weeks.	<ul style="list-style-type: none"> Spatial memory training (escape latency and errors) and reversal was impaired, but normal 2-week retention. 	Fedorova et al. (2009)
Mice	Barnes circular maze	Mouse pups were artificially reared with <i>n</i> -3 adequate/deficient artificial milk, then weaned on same (pelleted) diets for 7 weeks.	<ul style="list-style-type: none"> Spatial memory training (escape latency and errors) impaired, but 10-day retention or working memory not impaired. 	Fedorova et al. (2007)
Sprague–Dawley rat	Morris water maze	Pregnant dams fed normal, normal + fish oil supplemented, or <i>n</i> -3 deficient diet; then male offspring was weaned on same diets but half of deficient animals fed fish oil from post-natal day 60.	<ul style="list-style-type: none"> Spatial and working memory (escape latency) best in rats fed normal diet + fish oil. <i>N</i>-3 deficiency did impair performance but this could be partially recovered by adding fish oil 	Chung et al. (2008)
Long Evans rat	Morris water maze	Dams were fed <i>n</i> -3 adequate diet, then male offspring either dam-fed, or artificially reared (hand-fed) on <i>n</i> -3 deficient or adequate diet, then weaned on these diets for 9 weeks.	<ul style="list-style-type: none"> Spatial memory performance during training (escape latency) as well as retention test (probe trial) impaired in <i>n</i>-3 deficient animals. 	Lim et al. (2005)
Long Evans rat	Morris water maze	Dams were fed <i>n</i> -3 adequate diet, then males either dam-fed, or artificially reared (hand-fed) on <i>n</i> -3 deficient diet or diets with DHA, DPA (<i>n</i> -6) or both.	<ul style="list-style-type: none"> On the Morris water maze learning trials (escape latency) as well as retention test (probe trial), DPA fed rats were as impaired as <i>n</i>-3 deficient rats, while DHA or DHA/DPA fed rats had similar performance as controls. 	Lim et al. (2005)
Wistar rat	Passive avoidance	Newborn Wistar male rats were lactated by dams fed normal diet or <i>n</i> -3 deficient diet, but the latter were repleted with DHA and AA during lactation in an additional group.	<ul style="list-style-type: none"> Passive avoidance learning was impaired in rats fed from <i>n</i>-3 deficient dams, but repletion with DHA restored this impairment. 	García-Calatayud et al. (2005)
Long Evans rat	Morris water maze	Female rats were fed <i>n</i> -3 adequate or deficient diets through three generations and the male F3 offspring provided with <i>n</i> -3 adequate diets at birth and weaning, but also 7 weeks. Behavioral testing was done at 9 and 13 weeks.	<ul style="list-style-type: none"> <i>N</i>-3 deficiency significantly impaired Morris water maze learning (escape latency) and retention (probe trial), but repletion with <i>n</i>-3 adequate diet at birth and weaning restored this impairment at both 9 and 13 weeks of testing. Repletion at adult age (7 weeks) recovered performance only when testing was done at 13 weeks. 	Moriguchi and Salem (2003)
Long-Evans rat	Morris water maze	Two groups of dams were fed <i>n</i> -3 adequate or deficient diet and male off-spring (F2 as well as F3) weaned on these diets and tested at 8–9 weeks.	<ul style="list-style-type: none"> Both the 2nd and especially 3rd generation offspring of <i>n</i>-3 inadequate fed dams were impaired on the spatial memory training (escape latency). In the retention test (probe trial), only 3rd generation offspring was impaired. 	Moriguchi et al. (2000)
Long-Evans rat	Olfactory-test and Morris water maze	Two groups of dams were fed <i>n</i> -3 adequate or deficient diet and F2 males weaned to these diets and tested at 8–9 weeks.	<ul style="list-style-type: none"> Olfactory-based learning as well as Morris water maze learning (escape latency) were impaired in <i>n</i>-3 deficient animals. 	Greiner et al. (1999)
Sprague–Dawley	Passive-avoidance	Female rats received diet supplemented with low or high dose of <i>n</i> -3 PUFA throughout pregnancy and lactation. Offspring was tested at post-natal day 21 and 90.	<ul style="list-style-type: none"> While supplementation at both doses benefited motor performance, there was no effect on learning and memory performance. 	Coluccia et al. (2009)
Swiss OF1 mice	Morris water maze/Active avoidance	Female mice were fed either a normal (palm oil) or <i>n</i> -3 enriched diet during pregnancy and lactation and offspring were weaned and maintained on these diets. Rats were tested at young (7–11 weeks), mature (9–10 months), and old (17–19 months) age.	<ul style="list-style-type: none"> Supplementation with <i>n</i>-3 PUFA did not affect spatial leaning (escape latency) in any group, but spatial memory in the retention test (probe trial) was improved in mature mice. In the avoidance test, <i>n</i>-3 PUFA caused a decrement in performance in mature and old mice, while in juvenile mice, an improvement was seen. 	Carrié et al. (2000)
Long-Evans rats	Morris water maze	Rat pups were artificially reared from day 5–18 on milk substitutes containing adequate LA and ALA, but with low/high AA or DHA added, then weaned and maintained on these diets for 6 weeks.	<ul style="list-style-type: none"> Performance on spatial learning and memory (swimming distance) was not different among the diet groups. 	Wainwright et al. (1999)
C57Bl/6 mice	LTP	Pregnant mice were fed with an <i>n</i> -3 fatty acid adequate (control) or deficient diet throughout pregnancy and the lactation period, then LTP induced in brain slices of 18 day old pups.	<ul style="list-style-type: none"> LTP was impaired in hippocampal slices of DHA deficient pups. 	Cao et al. (2009)

leads to a decrease of the brain levels of DHA, region specifically (frontal cortex and hippocampus are susceptible), with a reciprocal increases in the *n*-6 PUFA docosapentaenoic acid (DPA, 22:5, *n*-6) in the nervous system (Chung et al., 2008; Levant et al., 2007; Chalon, 2006; Fedorova and Salem, 2006; Delion et al., 1997). DPA, however, cannot replace DHA, for the adequate development of cognition, despite the fact that these molecules only differ by a single double bond at the *n*-3 position (Fedorova and Salem, 2006; Lim et al., 2005).

Cognitive assessments are often done with maze-tests, such as the Morris water maze and the Barnes's circular maze, but also Radial Arm Maze, Olfactory Discrimination and Passive/Active avoidance learning (Fedorova and Salem, 2006). The Morris water maze is a very commonly used test, as it measures hippocampus-dependent deficits in spatial memory (Sharma et al., 2010). A disadvantage of the Morris water maze is that the stress, evoked by the water, may interfere with memory performance. Thus, compounds that reduce stress, such as *n*-3 PUFA could affect memory performance indirectly, rather than having a direct cognitive-enhancing effect. Conversely, deficiency of *n*-3 PUFA would have a stronger effect in the water maze. For instance, Fedorova et al. (2009, 2007) found a less strong memory deficit by *n*-3 deficiency in the Barnes Maze than is generally reported in the water maze (see Table 1). Thus, the advantage of animal studies here once again is that one has the luxury of choosing the most appropriate test, with least possibility of confounding effects of anxiety. An obvious disadvantage of these studies, however, could be the lack of translatability to the vastly more complex human cognition and testing paradigms. Furthermore, animal studies are often biased towards the male sex, but studies suggest that there could be important sex difference in effects of *n*-3 intake on cognition (Lassek and Gaulin, 2011).

As indicated in Table 1, an impressive amount of evidence indicates that deficiency of *n*-3 PUFA, in particular DHA, during the critical developmental stages described above significantly impairs maze performance (Fedorova et al., 2009, 2007; Xiao et al., 2006; Lim et al., 2005; García-Calatayud et al., 2005; Moriguchi et al., 2000; Greiner et al., 1999), which can be restored by repletion of the *n*-3 PUFA in the diet at birth or weaning (García-Calatayud et al., 2005; Moriguchi and Salem, 2003) and young adult hood (Chung et al., 2008; Moriguchi and Salem, 2003). DHA cannot be replaced by its closest long-chain equivalent; *n*-6 PUFA docosapentaenoic acid (DPA, C22:5, *n*-6), as pups fed a diet rich in LA and DPA but low in DHA had impaired spatial memory performance compared to rats fed a diet rich in DHA (Lim et al., 2005). While dietary depletion of *n*-3 PUFA cause detrimental effects on brain development, supplementation with *n*-3 PUFA of a normal non-depleted diet during the early stages of development also has demonstrated benefits on cognitive performance (Chung et al., 2008; Carrié et al., 2000), although lack of effect on cognition has been found as well (Coluccia et al., 2009; Wainwright et al., 1999; Carrié et al., 2000). In general, the strongest effects of *n*-3 PUFA supplementation are observed in conditions of deficiency. No evidence has been demonstrated that deficiency or supplementation with EPA alone has effects of cognitive development; DHA appears to be the critical PUFA for cognitive enhancement, at least during development.

Indirect clues about the developmental effects of *n*-3 PUFA deficiency/supplementation on cognition can also be obtained from neurophysiological studies. In the fetal rat brain, the bulk of neurogenesis (when neuron precursors have ceased mitosis and mature into cells with a definitive neuronal phenotype) is between E14 and E17. During this period and especially shortly therefore (second week post-natally), synaptogenesis is initiated. Green et al. (1999) elegantly demonstrated that these periods of neurogenesis and synaptogenesis coincide with large increases in brain fatty acid

accumulation, including DHA, in phospholipids, but DHA steeply increased prior to the period of synaptogenesis while other fatty acids reached plateau, implying its important role during this critical period of development. Thus, depletion of DHA during these critical periods of brain growth could catastrophically interfere with neurogenesis and synaptogenesis (Coti Bertrand et al., 2006).

Indeed, a number of recent *in-vitro* and *in-vivo* studies have directly related DHA deficiency or supplementation to neurogenesis (Kawakita et al., 2006) and major aspects of synapse development and function, including neurite outgrowth (Robson et al., 2010; Cao et al., 2009; Calderon and Kim, 2004); neuron size (Ahmad et al., 2002); the expression of pre- and post-synaptic proteins involved in vesicle trafficking and recycling processes, as well as synaptic transmission, including synaptic puncta (synapsins (a family of neuron-specific phosphoproteins associated with the membranes of synaptic vesicles) associated with synaptic vesicles), glutamate receptors and other proteins (Sidhu et al., 2011; Cao et al., 2009; Cansev and Wurtman, 2007). The importance of DHA was dramatically illustrated by Cao et al. (2009), who found that gestational DHA-deprivation in fetal hippocampi inhibited neurite outgrowth and synaptogenesis in cultured hippocampal neurons, however *in-vitro* supplementation of 1 μ M DHA (approximates physiological concentration) but not arachidonic acid (AA), oleic acid or docosapentaenoic acid (DPA, *n*-6) to these neurons could restore the neurite outgrowth and synaptogenesis. The same group also found that DHA metabolite DEA (N-docosahexaenoyl ethanolamide) contributes significantly to hippocampal neuronal development, including hippocampal neurite growth and synaptogenesis, at significantly lower concentrations than DHA itself (Kim et al., 2011). Interestingly, in neonatal brains, EPA rather than DHA was shown to play an important role in myelinogenesis (Salvati et al., 2008).

N-3 PUFAs are well known to promote the expression and activity of neurotrophins, which play a role in neuronal growth, differentiation and survival. One of these neurotrophic factors, brain-derived neurotrophic factor (BDNF) has been linked to DHA status in the brain. For instance, 15 weeks of *n*-3 PUFA deprivation in young rats reduced DHA in the frontal cortex, along with BDNF mRNA and protein (Rao et al., 2007). We previously observed in our lab that EPA administration to fully differentiated neuroblastoma cells increases cell viability, through modulation of BDNF receptors (Kou et al., 2008).

Increasing evidence has demonstrated that *n*-3 PUFA, including DHA and EPA, or their combination, modulate LTP. In terms of development, Cao et al. (2009) found that maternal dietary depletion of DHA impairs LTP in offspring mice. Other findings pertain more to adult or aged rodents, or rodents exposed to a neurotoxic challenge, as will be discussed below. These studies show that *n*-3 PUFA can influence one of the most important and extensively described neurophysiological process underlying learning and memory.

5. *N*-3 PUFA in young and adult individuals

The majority of research has focused on the developmental effects of dietary intake of *n*-3 PUFA as well as its effects on age-related cognitive decline and dementia, but few studies have thoroughly examined the cognitive impact of *n*-3 PUFA during the stage of life in between, during young or advanced, healthy adulthood.

5.1. Evidence from studies on children and young adults

However, a few elegant studies have been done. For instance, in a neuroimaging study, McNamara et al. (2010) showed in healthy

boys (8–10 years old) that only 8 weeks of supplementation with 400 mg (low, normal dose) or 1200 mg (high dose) of DHA per day dose dependently increased the erythrocyte membrane DHA composition, which was positively correlated with functional activity in the dorsolateral prefrontal cortex and inversely correlated with reaction time during performance on an attention task. Fontani et al. (2005) who compared the effect of daily supplementation of fish oil (4 g) for 35 days in 33 healthy individuals (aged 22–51) with placebo (16 participants) reported beneficial effects of *n*-3 PUFA on reaction time in attention tasks, as well as an improvement in mood assessments.

Not all positive effects have been found. In Kennedy et al. (2009), 90 healthy, cognitive intact 10–12 year old children were fed either 400 mg or 1000 mg of DHA per day for 8 weeks, but reported no reliable beneficial effects of these diets on cognitive performance. Karr et al. (2012) assessed the effects of a four week fish oil (480 mg DHA/720 mg EPA) treatment in healthy college-aged individuals on cognitive performance, but reported limited cognitive benefits, although acknowledged that the dose of fish oil may have been sub-therapeutic and sample size too small. Surprisingly, de Groot et al. (2007) reported that higher plasma DHA levels were associated with a slower learning curve in healthy well-nourished non-pregnant woman. This is in contrast with a study by Lassek and Gaulin (2011), who showed that cognitive performance in young females benefits from DHA intake, although the latter conclusion was based on 24-h dietary recall surveys (further discussed below). Mazereeuw et al. (2012) performed a meta-analysis of several randomized double-blind placebo-controlled studies on the cognitive benefits of *n*-3 PUFA supplementation in both cognitively impaired and intact subjects and concluded that there were no substantial beneficial effects in healthy subjects. Overall, few studies have examined the cognitive effects of *n*-3 PUFA through childhood, young adulthood, and middle age (Karr et al., 2011) and the inconsistency in findings has likely to do with the methodological complexities involved in working with human samples (and issues such as complex demographic backgrounds, variation in DHA and/or EPA dosing and duration, sample size, etc).

5.1.1. Psychiatric conditions could interfere with cognition

One important aspect of adult cognitive performance that should be mentioned is the possible presence of underlying psychiatric disorders, such as attention-deficit disorders, dyslexia, autism and depression, which may be present in children and adults and could potentially affect cognitive performance. Studies suggest that plasma *n*-3 and also *n*-6 fatty acids of children with such deficits is lower than healthy individuals, possibly due to prenatal PUFA deficits, either due to due to neural deficits in PUFA accrual, or deficient maternal PUFA intake (Schuchardt et al., 2010; Sinn et al., 2010; McNamara and Carlson, 2006). Conversely, various clinical trials suggest that supplementation with PUFA may be beneficial in managing these disorders, including cognitive symptoms, although inconsistency between findings is reported as well (Sinn et al., 2012; Schuchardt et al., 2010; Sinn et al., 2010; McNamara and Carlson, 2006).

While it has been suggested that EPA may be more effective than DHA in managing these disorders, for reasons not completely understood (Jazayeri et al., 2008; Song and Zhao, 2007; Peet and Stokes, 2005), others reported similar effects on mood between EPA and DHA or even an advantage of DHA compared to EPA (Sinn et al., 2012). Milte et al. (2012) observed DHA to be more effective than EPA in improving cognition in ADHD-afflicted subjects. The EPA vs DHA debate on mental function is an important one and largely underinvestigated. One unresolved question in comparing EPA and DHA is to what extent EPAs contribution to improved cognition and/or mood is due to conversion to DHA.

5.1.2. Sex difference in the relationship between dietary PUFA and cognitive performance

Often ignored as well in scientific studies of the relationship between *n*-3 PUFA and cognition is gender differences. Females must provide DHA for the growth of the unusually large brains of their offspring from maternal fat stored during childhood, so their need for DHA is particularly great. Lassek and Gaulin (2011) demonstrated in a sample of 4000 American children aged 6–16 from the Third National Health and Nutrition Examination Survey that females also have a two-fold greater cognitive benefit from DHA intake than males. Furthermore, they found that only in females, *n*-6 PUFA contributed negatively to cognitive performance. These results were particularly remarkable as 33 other fatty acids and nutrients were not related to cognitive outcome measures. A disadvantage of the study was that the authors estimated dietary intake of nutrients by 24 h dietary recall, which may not be an accurate measure of long-term nutrient and fatty acid intake (this issue is further discussed in Section 6.1). Nonetheless, this study does point out the importance of considering gender differences in the relation between *n*-3 PUFA intake and cognition.

5.2. Findings from animal studies

While in (young) adults, brain development has been established, neuroplasticity is ongoing and a limited number of experiments with animal studies (Table 2) have shown that *n*-3 PUFA supplementation in child- and adult-hood can modulate synaptic plasticity (LTP) and neurochemistry, as well as cognitive performance.

Pan et al. (2011) measured the effect of increasing doses of DHA and found that appropriate doses (150 or 300 mg/kg/d) significantly improved learning and memory in the Morris water maze, but that a higher intake (600 mg/kg/d) increased memory impairment. Unique about this study is that the researchers used a control diet that was not deficient in *n*-3 PUFA, thus demonstrating a true supplementation effect of DHA. This in contrast with Lim and Suzuki (2000), who also demonstrated a memory-enhancing effect of DHA, but only compared to controls that were fed a diet deficient in *n*-3 PUFA, so the observed effect was not a true supplementation effect, and merely an off-set of DHA-deficiency effect. Similarly, Gamoh et al. (1999) and Moriguchi and Salem (2003) found that DHA can improve memory performance in young rats in the Radial Arm Maze and Morris water maze respectively, but these rats were depleted with *n*-3 PUFA over three generations. Nonetheless, it shows that post-developmental DHA supplementation can restore effects of depletion. Similar to Pan et al. (2011), it would be of interest to study more the effect of *n*-3 supplementation in rats fed *n*-3 adequate diets, to determine the true memory-enhancing potential of *n*-3 PUFA in healthy animals.

Thus, we have conducted a number of experiments demonstrating the effects of feeding a purified form of EPA (mostly 0.8%) for 7–8 weeks on animal cognition and neurochemistry in adult rats (Taepavarapruk and Song, 2010; Song et al., 2009; Song and Horrobin, 2004) and mice (Luchtman et al., 2012) fed standard *n*-3 adequate rodent chow (Table 2). Remarkably, in none of these studies, EPA by itself modified learning and memory performance in the water maze (Luchtman et al., 2012; Song et al., 2009; Song and Horrobin, 2004), however, it did significantly modulate neurochemistry, including hippocampal dopamine (DA), noradrenaline (NA) and metabolites, and attenuated toxin-induced impairments of memory (see below). Although brain fatty acid concentration was not measured in most studies, in Luchtman et al. (2012), it was found that the EPA diet in mice only increased brain EPA and its direct metabolite DPA (C22:5, *n*-3) rather than DHA. We also found in an unpublished study that EPA can enhance LTP

Table 2
Effects of (young) adult *n*-3 supplementation on cognition and hippocampal LTP.

Species	Test	Dosing/exp/design essentials	Effect of <i>n</i> -3 supplementation	References
Sprague–Dawley	Morris water maze	Two-month old rats were fed a diet supplemented with appropriate (150–300 mg/kg) or high doses (600 mg/kg) of DHA for 1 month.	<ul style="list-style-type: none"> The appropriate doses of DHA resulted in improved spatial learning performance (escape latency) as well as retention (probe trial), but high dose impaired performance. 	Pan et al. (2011)
Crlj:CD-1 mice	Maze	Three-week old mice were fed a diet supplemented with DHA ethyl ester (DHA-EE) plus palm oil (rich in palmitic and oleic acid, but no <i>n</i> -3 PUFA) for 5 months.	<ul style="list-style-type: none"> Compared to mice fed standard diet with added palm oil (control diet), <i>n</i>-3 supplemented rats performed better in the maze. Note that the control diet was deficient in <i>n</i>-3 PUFA. 	Lim and Suzuki (2000)
Wistar rats	Radial Arm Maze	Rats were depleted with <i>n</i> -3 PUFA over three generations. Male offspring was weaned on depleted diet and at 5-weeks fed the diet supplemented with DHA for 10 weeks.	<ul style="list-style-type: none"> DHA supplementation improved reference but not working memory errors in the maze. 	Gamoh et al. (1999)
Long Evans rat	Morris water maze	Female rats were fed <i>n</i> -3 adequate or deficient diets through three generations and the male F3 offspring provided with <i>n</i> -3 adequate diets at birth and weaning, but also 7 weeks. Behavioral testing was done at 9 and 13 weeks.	<ul style="list-style-type: none"> <i>N</i>-3 deficiency significantly impaired Morris water maze learning (escape latency) and retention (probe trial), but repletion with <i>n</i>-3 adequate diet at birth and weaning restored this impairment at both 9 and 13 weeks of testing. Repletion at adult age (7 weeks) recovered performance only when testing was done at 13 weeks. 	Moriguchi and Salem (2003)
C57Bl6 mice	Morris water maze	Six week old male mice were fed a diet supplemented with EPA for 12 weeks, then tested.	<ul style="list-style-type: none"> EPA did not improve spatial learning (escape latency) or retention (probe trial) compared to control. 	Luchtman et al. (2012)
Sprague–Dawley rat	Morris water maze	Two-month old rats were fed regular rodent chow supplemented with EPA for 7 weeks and tested on the water maze.	<ul style="list-style-type: none"> The EPA diet did not significantly affect water maze performance (but it attenuated an olfactory-bulbectomy induced impairment of memory). 	Song et al. (2009)
Wistar rats	Morris water maze	Adult Wistar rats were fed regular rodent chow supplemented with EPA for 8 weeks and then tested on the water maze.	<ul style="list-style-type: none"> The EPA diet did not significantly affect water maze performance (but it attenuated an interleukin-1 (IL-1) induced impairment of maze performance). 	Song and Horrobin (2004)
Wistar rats	LTP	Three-week old rats received chow depleted of fish-oil, or this chow (from 5 weeks) with ethyl-EPA (E-EPA) added.	<ul style="list-style-type: none"> EPA administration increased LTP in the CA1 but not dentate gyrus region of the hippocampus. 	Kawashima et al. (2010)
Wistar rats	Morris water maze and LTP	Young (3–4 months) rats were fed standard rodent chow supplemented with ethyl-EPA (E-EPA) or DPA (<i>n</i> -3) for 8 weeks, then tested for memory and LTP.	<ul style="list-style-type: none"> EPA but not DPA enhanced a component of hippocampal LTP (mean population EPSP slope), but did not affect memory performance. 	Kelly et al. (2011)

induction and amplitude. A similar effect was observed by Kelly et al. (2011) after feeding 3–4 month old rats with EPA and its metabolite DPA. Kawashima et al. (2010) also found an EPA-induced enhancement of LTP in adult rats, but these rats were previously fed a diet deficient in EPA and DHA, so this was not a true supplementation effect (Table 2). These results demonstrate that EPA and not only DHA may have cognition enhancing effects.

Interestingly, Robson et al. (2010) compared the effects of arachidonic acid (AA), EPA and DHA on neurite outgrowth of dorsal root ganglion (DRG) cells from rats of different ages (post-natal day 3 (P3), and 9 (P9), adult stage (2–4 months) and 18–20-month-old) and reported that all three fatty acids and especially DHA enhanced neurite outgrowth, at all these life stages. The effects were comparable to that of compounds such as nerve growth factor (NGF) and all-trans retinoic acid (ATRA), which have well-documented neurotrophic properties and roles in neuronal differentiation. While not direct evidence for cognitive enhance, it could

be an indirect support for the use of *n*-3 PUFA to improve neuronal function.

6. Clinical findings in aged and demented individuals

Prevalence rates for all dementias, including AD, in the USA are 5–10% for population aged 65–85 and 25–45% for those older than 85. The rates will increase rapidly due to baby boomers now reaching 65. The seriousness of the problem is significant and thus the search for risk factors, therapeutic targets and potential opportunities for therapy have been intensively researched (Cole et al., 2009; Issa et al., 2006). Omega-3 PUFAs offer a desirable therapeutic opportunity due to their accessibility, low cost and generally safe and side-effect free long-term use (Cole et al., 2009). Furthermore, Alzheimer's disease is strongly correlated with decreases in omega-3 PUFA levels in the brain and peripheral tissues (Dyall and Michael-Titus, 2008).

6.1. Findings from observational studies

The large majority of studies are observational of nature. While informative, a common flaw in reported cross-sectional (e.g. Nurk et al., 2007; Kalmijn et al., 2004) and prospective (e.g. Devore et al., 2009; Van Gelder et al., 2007; Barberger-Gateau et al., 2007; Huang et al., 2005; Morris et al., 2005; Engelhart et al., 2002; Kalmijn et al., 1997) epidemiological studies is the limited reliability of dietary recall surveys and crude food frequency questionnaires to approximate blood levels of *n*-3 PUFA in relation to cognitive decline (Tan et al., 2012; Cole et al., 2009; O'Brien et al., 2009). While these studies (except Devore et al., 2009 and Engelhart et al., 2002) generally report a positive association between *n*-3 PUFA intake and lowered risk of cognitive decline, their validity can thus be questioned.

Cross-sectional (Tan et al., 2012; Lopez et al., 2011; Wang et al., 2008; Dullemeijer et al., 2007; Cherubini et al., 2007; Tully et al., 2003 (serum); Conquer et al., 2000) or prospective (Samieri et al., 2011; Whalley et al., 2008; Dullemeijer et al., 2007; Beydoun et al., 2007; Schaefer et al., 2006; Laurin et al., 2003; Heude et al., 2003) surveys that actually measured blood levels of *n*-3 PUFA rather than historical dietary patterns may offer more scientifically accurate insights and these studies generally support a protective effect of *n*-3 PUFA against cognitive decline. In these studies, those that measured fatty acid composition of phospholipids in red blood cells (RBCs) (as in Tan et al., 2012; Whalley et al., 2008; Heude et al., 2003) may be more valuable than those that measure fatty acids in plasma (as in Samieri et al., 2011; Lopez et al., 2011; Wang et al., 2008; Dullemeijer et al., 2007; Beydoun et al., 2007; Cherubini et al., 2007; Schaefer et al., 2006; Tully et al., 2003 (serum); Laurin et al., 2003; Conquer et al., 2000), as fatty acid composition in RBCs more reliably represents long term dietary exposure to *n*-3 PUFA. Tan et al. (2012) used cognitive testing as well as brain imagery and found that lower levels of RBC DHA and EPA in late middle age were associated with markers of accelerated structural and cognitive aging. Whalley et al. (2008) also found that RBC total *n*-3 PUFA and DHA concentrations were associated with benefits for cognition. Heude et al. (2003) reported that higher proportions of both saturated and total *n*-6 PUFAs in RBCs were associated with greater risk of cognitive decline, whereas a higher proportion of total *n*-3 fatty acids was associated with a lower risk of cognitive decline.

It should be noted that not all studies controlled for possession of the ApoE4 allele, which is a known genetic risk factor for AD. However, the relationship between *n*-3 PUFA and cognitive impairment is still complex, even when ApoE4 carriage is controlled for. For instance, those studies that did control for the allele (e.g. Whalley et al., 2008; Barberger-Gateau et al., 2007; Huang et al., 2005) reported less protection by *n*-3 PUFA in ApoE4 carriers. On the other hand, Samieri et al. (2011) and Van de Rest et al. (2008) (randomize clinical study) found a beneficial effect of *n*-3 PUFA (EPA and DHA) against cognitive impairment only in ApoE phenotype carriers. Lopez et al. (2011) and Schaefer et al. (2006) found that higher plasma levels and dietary intake of DHA was associated with significantly reduced odds of AD, regardless of ApoE phenotype. Yet Kröger et al. (2009), in a large-scale Canadian prospective study found no association between blood measures of total *n*-3 PUFAs, DHA or EPA with the incidence of dementia and AD and their results were corrected for ApoE4, or blood mercury content (which can be present in fish and neurotoxic).

6.2. Findings from clinical studies

A very limited number of clinical trials have been done to determine the cognition-enhancing effects of *n*-3 PUFA in healthy

elderly (Danthiir et al., 2011 (ongoing study with reported methodology); Dangour et al., 2010; Van de Rest et al., 2008), elderly mild (age-related) cognitive decline (Yurko-Mauro et al., 2010; Chiu et al., 2008) and samples with dementia or AD (Quinn et al., 2010; Chiu et al., 2008; Freund-Levi et al., 2006), or other groups (Andreeva et al., 2011).

In studies with healthy elderly, mild beneficial effects of *n*-3 PUFA have been observed. For instance, Dangour et al. (2010) observed a small benefit in the domain of attention in *n*-3 PUFA treated participants that also carried the ApoE4 phenotype. Yurko-Mauro et al. (2010) investigated the effects of daily DHA supplementation (900 mg) for only 24 weeks in 485 adults over 55 years old with mild age-related cognitive decline. They reported a doubling in plasma DHA values of treated participants, along with a significant increase in performance on episodic memory, but not executive function or working memory. In studies with demented patients, those that benefit the most from *n*-3 PUFA treatment are the milder cases. For instance, in a study by Freund-Levi et al. (2006), 174 AD patients, with mean age of 74, were subjected to a daily regimen of 1.7 g DHA and 0.6 g EPA for a total of 12 months, and tested on a number of standard cognitive assessments. While patients in the *n*-3 PUFA supplemented group showed mean 2.4- and 3.6-fold increases in the ratios of DHA and EPA respectively, compared to placebo, this did not result in a significant overall improvement on cognitive performance. However, in a subgroup with very mild cognitive dysfunction, a significant reduction in the cognitive decline rate was observed compared to placebo, suggesting that those with milder cognitive impairment may benefit from *n*-3 PUFA treatment. Quinn et al., 2010 suggested that intervention with *n*-3 PUFA prior to AD onset would be more beneficial. Boston et al. (2004) conducted a preliminary assay to investigate whether ethyl-EPA alone (500 mg twice daily) can improve cognitive function in 20 AD patients. They used a somewhat unusual design in which rate of cognitive decline during 12 weeks was used as a baseline period to compare rate of decline during the following 12 weeks during which treatment with EPA took place. There was little difference between treatment and baseline periods in the rate of cognitive decline, suggesting the EPA treatment was not effective. Aside the low sample size, it is possible that the treatment period was too short, although RBC fatty acid measures indicated significant increases in EPA and DPA (C22:5, *n*-3) content. As suggested above, it is likely that the patients were in a too advanced stage of disease for the treatment to have significant effect.

6.3. Findings from animal studies

Studies investigating the beneficial effects of *n*-3 PUFA on cognitive performance in aging or age-related diseases generally used aged rodents and standard models of neurodegenerative disorders (Table 3). The following sections address these types of studies individually.

6.3.1. Findings from studies with aged rodents

In terms of normal aging, literature on the effects of *n*-3 PUFA on cognitive function in normal, aged rats is sparse. Most studies challenged their animal subjects with inflammatory or neurotoxins to model neurodegeneration. However, Gamoh et al. (2001) showed that chronic administration of DHA in 100 weeks old rats that were previously *n*-3 PUFA deficient could enhance Radial arm maze performance, along with a reduction in hippocampal lipid peroxidation, a common age-related neurotoxic process. While this is not a true supplemental effect of *n*-3 PUFA at old age, it does show that even at a very old age, *n*-3 PUFA can restore/enhance cognitive performance. Effect of *n*-3 PUFA supplementation per se were

Table 3
Effects of *n*-3 supplementation on LTP and cognition in aged rats or rats with neurodegeneration.

Species	Test	Dosing/exp/design essentials	Model	Effect of <i>n</i> -3 supplementation	References
Wistar rats	Morris water maze and LTP	Aged (20–22 month rats) were fed standard rodent chow supplemented with ethyl-EPA (E-EPA) or DPA (<i>n</i> -3) for 8 weeks.	Aging	<ul style="list-style-type: none"> Both EPA and DPA attenuated an age-induced impairment of LTP as well as spatial learning (escape latency) with EPA having the strongest effect. 	Kelly et al. (2011)
Wistar rats	Radial Arm Maze	Rats were depleted with <i>n</i> -3 PUFA over three generations. Male offspring was weaned on depleted diet and at 100-weeks fed the diet supplemented with ethyl-DHA for 10 weeks.	Aging	<ul style="list-style-type: none"> Chronic administration of DHA significantly decreased the number of reference memory errors and working memory errors. 	Gamoh et al. (2001)
SAMP8 mice	T-Maze food shock avoidance	Ten month old mice received a standard diet supplemented with low or high dose of DHA for 8 weeks.	Aging	<ul style="list-style-type: none"> High dose of DHA improved acquisition and retention in the T-maze. 	Petursdottir et al. (2008)
Wistar rats	LTP	Aged (20–22 month rats) were fed standard rodent chow supplemented with ethyl-EPA (E-EPA) for 4 weeks, then infused with A β .	Aging and i.c.v. ^b A β ^d infusion	<ul style="list-style-type: none"> Aβ inhibited LTP significantly in aged rats and EPA improved this. 	Minogue et al. (2007) and Lynch et al. (2007)
Wistar rats	LTP	Adult rats were fed standard rodent chow supplemented with ethyl-EPA (E-EPA) for 4 weeks, then injected with LPS.	I.P. ^c injection of LPS	<ul style="list-style-type: none"> LPS significantly inhibited expression of LTP, but EPA improved this effect. 	Lonergan et al. (2004) and Kavanagh et al. (2004)
Wistar rats	Radial Arm Maze	Rats were made fish-oil deficient and 5-week old second generation rats fed TAK-085 (purified fish oil containing ethyl-EPA and ethyl-DHA) for 12 weeks. ^a	I.c.v. A β infusion	<ul style="list-style-type: none"> Aβ infusion impaired reference and working memory errors in fish-oil deficient rats, but TAK-085 supplementation improved performance. 	Hashimoto et al. (2011)
Wistar rats	Active-Avoidance	Rats were made fish-oil deficient through three generations. At 25 weeks, third generation rats were then supplemented with ethyl-DHA for 12 weeks, then A β infused. ^a	I.c.v. A β infusion	<ul style="list-style-type: none"> Avoidance learning was significantly impaired in Aβ infused rats, while DHA treated rats infused with Aβ performed even better than controls. 	Hashimoto et al. (2006, 2002)
Wistar rats	Radial Arm Maze	Rats were made fish-oil deficient through three generations. At 20 weeks, third generation rats were then supplemented with ethyl-DHA for 12 weeks, then A β infused. ^a	I.c.v. A β infusion	<ul style="list-style-type: none"> Aβ infusion impaired reference and working memory errors in fish-oil deficient rats, but DHA supplementation improved performance. 	Hashimoto et al. (2005a,b)
Wistar rats	Radial Arm Maze	Rats were made fish-oil deficient through three generations. At 5 weeks, third generation rats were supplemented with EPA for 12 weeks, then A β infused. ^a	I.c.v. A β infusion	<ul style="list-style-type: none"> Aβ infusion impaired reference and working memory errors in fish-oil deficient rats, but EPA supplementation improved performance. 	Hashimoto et al. (2009)
Long Evans rat	Radial Arm Maze	Adult rats were fed a regular rodent chow supplemented with ethyl-EPA (E-EPA) for 7 weeks, then infused with IL-1 β .	I.c.v. IL-1 β infusion	<ul style="list-style-type: none"> IL-1β d induced memory errors, which were prevented by EPA. 	Taepavarapruk and Song (2010)
Transgenic APP ^{swe} /PS1 ^{dE9} mice	Morris water maze	Adult mice (6 months) were fed several experimental diets containing varying <i>n</i> -6/ <i>n</i> -3-ratios, saturated and polyunsaturated fatty acid and cholesterol contents.	Accumulation of A β in transgenic mice	<ul style="list-style-type: none"> Only a marginally significant effect of the model was found on spatial learning (escape latency) and retention (probe trial). The diets, irrespective of <i>n</i>-3 content, did not reverse this effect. 	Oksman et al. (2006)

^a In these studies, the fish oil deficient diet did have *n*-3 PUFA, including ALA and EPA, but no DHA or AA.

^b I.c.v., intracerebroventricular.

^c I.p., intraperitoneal.

^d A β , Amyloid-beta.

investigated in the following studies: Petursdottir et al. (2008) fed senescence-accelerated prone 8 (SAMP8) mouse, which develop learning and memory impairments at 8–12 months of age, for 8 weeks with DHA and found that it improved cognitive decline in a maze test. Kelly et al. (2011) fed aged rats (20–22 months) standard rodent chow supplemented with ethyl-EPA or its metabolite DPA and found that both diets attenuated an age-induced impairment of LTP as well as spatial learning in the Morris water maze, with EPA having the strongest effect (Table 3).

Neurogenic and synaptogenic effects of DHA were found in neonatal rats (Sidhu et al., 2011; Cao et al., 2009; Cansev and Wurtman, 2007; Kawakita et al., 2006; Calderon and Kim, 2004) but Robson et al. (2010) also investigated whether these effects could be found in adult and aged rodents. In that process, the authors compared the effect of arachidonic acid (AA), EPA and DHA at four different life stages of rat dorsal root ganglia (DRG) cells and found that neurite-enhancing effects were still seen at the adult and old stage, and while both AA and EPA had

neurotrophic effects, DHA had the strongest effects. The same research group also demonstrated that treatment of aged rats with fish oil (EPA + DHA) may reverse a decrease in neurogenesis that is observed with age in the dentate gyrus of the hippocampus. This was paralleled by attenuation of a decrease in retinoid receptor expression, which may play an important role in neurogenesis (Dyall et al., 2010). In another study of the group, the group demonstrated that treatment of aged rats with fish oil reversed age-related decreases in the GluR2 and NR2B subunits of respectively *N*-methyl-D-aspartate (NMDA) receptors and the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors. Signaling through these receptors plays an important role in synaptic plasticity underlying learning and memory, such as LTP. Along these lines, Kelly et al. (2011) demonstrated that *n*-3 PUFA EPA and its metabolite DPA (C22:*n*-3), possibly through conversion to DHA, in aged rats can improve memory impairment and LTP.

6.3.2. Findings from studies with models of neurodegeneration

Studies that investigate the effect of *n*-3 PUFA against age or AD-related neurodegeneration (reviewed in Hooijmans et al., 2012; Hashimoto and Hossain, 2011; Dyall and Michael-Titus, 2008), generally use models of peripheral or central injection of pro-inflammatory agents, such as cytokines, or endotoxin, or use brain infusion of amyloid- β peptides, as a more specific model of AD, or mice transgenic for one or more mutations that induce AD. With some exceptions (e.g. Oksman et al., 2006), these studies have generally produced convincing evidence that DHA (Hashimoto et al., 2006, 2005a,b; 2002;), EPA (Hashimoto et al., 2009; Taepavaraprak and Song, 2010; Minogue et al., 2007; Lynch et al., 2007; Lonergan et al., 2004; Kavanagh et al., 2004) or their combination (Hashimoto et al., 2011) is effective at preventing cognitive impairment, inhibition of LTP, and neurodegeneration. Conversely, *n*-6 PUFA were demonstrated to aggravate it (Amtul et al., 2012; Ma et al., 2009). While these studies do not provide direct evidence of the mechanisms by which the *n*-3 PUFA protect, it is likely through a synergy of protective mechanisms explained in previous sections, including modulating membrane fluidity, gene expression and eicosanoid/docosanoid metabolism, which can in turn affect synaptic plasticity, neurochemistry, oxidative stress and inflammation.

7. Summary and concluding remarks

While methodology in animal studies can still be improved (Dyall, 2011), it is apparent when reviewing animal and clinical studies, that with increasing control over experimental design, remarkably potent beneficial effects of *n*-3 PUFA on cognitive function are observed, across all age-cohorts, but in particular in the developing (potentially *n*-3 deficient) and aged or cognitively impaired cohort. Thus, where *n*-3 PUFA are not sufficiently supplied, which may lead to cognitive impairment, or in elderly, cognitively impaired individuals, *n*-3 PUFA supplementation may help to restore cognitive function. On the other hand, in healthy (young and adult) individuals with adequate *n*-3 PUFA intake, evidence of cognition-enhancing effects by *n*-3 PUFA supplementation is less convincing, although less frequently studied as well. Findings from animal studies are quite striking in the sense that neuronal development and cognition appears dramatically impaired, *in-vivo* as well as *in-vitro* in a DHA-void or inadequate nutritional environment. These studies have in many cases also shown that nutritional repletion with DHA can restore some of these cognitive or neuronal deficits. These findings altogether support that omega-3 PUFA should be adequately supplied during human pregnancy and infancy.

Less consistency of *n*-3 PUFA effects on cognition is found in studies conducted in the somewhat ambiguous period between development and aging, possibly due to the large age-span and thus inherent sampling variation that applies to these studies. Some studies measure the effects of *n*-3 PUFA repletion in *n*-3 depleted (young) adult samples, whereas others report the effects of true supplementation in *n*-3 adequate populations. The conclusion from these studies is that repletion of the diet with *n*-3 PUFA in most cases restores *n*-3 deficiency induced cognitive impairment. This may be a valuable outcome for Western society, where intake of *n*-3 PUFA, including α -linolenic acid (ALA) and fish oils, tends to be low. The cognitive enhancing effects of *n*-3 PUFA supplementation per se (on a *n*-3 adequate nutritional background) is somewhat less convincing, as improvement in cognitive performance was observed, but also impairment after a high dose of DHA. In studies conducted with EPA supplementation, no effect on memory performance was observed, although the *n*-3 PUFA did modulate brain neurochemistry and LTP in these studies, suggesting that EPA, despite its trace contents in the brain, can potentially modulate neuronal function. A more direct comparison between EPA and DHA within studies would be valuable in drawing more definite conclusions about the potential cognitive enhancing effect of EPA compared to DHA. Important in such studies would be to address to what extent EPA becomes converted to DHA, as the latter may contribute to cognitive enhancement. Impaired performance due to supplementation of large amounts of *n*-3 PUFA could possibly be due to oxidation of the fatty acids administered, as highly unsaturated fatty acids are prone to oxidation and thus extra care should be taken when ingesting large amounts of PUFA-rich oils.

Findings from studies in aged rodents suggest that supplementation with EPA or DHA improves cognitive impairment. Similarly, studies with toxin-induced rodent models of neurodegeneration consistently report that EPA, DHA or their combination is beneficial in improving cognitive impairment, although in many of these studies, animals were first made *n*-3 PUFA deficient, which evoked the toxin-induced neurodegeneration. Thus, it is unclear from these studies whether neurodegeneration and cognitive impairment in *n*-3 adequate subjects could improve with *n*-3 PUFA supplementation. Clinical trials do offer perspective on such a question, although in a less experimentally controlled environment.

Clinical trials on the developmental effects of *n*-3 supplementation are abundant, but lack of experimental control and methodological variation across studies impedes their validity in judging the benefit of pre- and post-natal *n*-3 supplementation. Very little controlled clinical research has been done to measure the potential benefit of *n*-3 supplementation in healthy, cognitively intact young and adult individuals, but based on findings from animal research, there is promise for this large population to improve or restore cognition by *n*-3 PUFA, especially if a deficiency was suffered early in life. Individuals with cognitive impairment due to an underlying affective or psychiatric condition may particularly benefit from *n*-3 PUFA intake. Studies on gender differences suggest that females may benefit more from *n*-3 intake than males. More stringent, however, is the need for improved, safe and side-effect free interventions for preventing, delaying or treating the cognitive decline that is observed in the elderly, or worse, patients with dementia and AD. While the majority of observational research has suggested a relation between *n*-3 PUFA intake and reduced risk of cognitive decline, these studies, even if they are prospective of nature, have the least experimental control to draw valid conclusions about causality between dietary intake of *n*-3 PUFA and prospective risk of cognitive decline. The far more superior randomized clinical studies (RCTs) have only demonstrated a benefit in case of mild

cognitive impairment and early stages of AD, but relatively few studies have been conducted to date.

While greater experimental control can be achieved in animal studies and causality thus more validly and reliably inferred, these studies also need to be treated with caution. The vast majority of animal studies, for instance, use standard rodent memory tests, as the Morris water maze, to evaluate cognitive performance. While these studies have proven relevance to the integrity and function of brain structures critically involved in human cognition, such as the frontal cortex and hippocampus, outcomes from these tests are difficult to interpret in terms of the much more complex human cognitive performance. Furthermore, factors such as anxiety, which strongly interfere with cognition, are easier to measure and control for in animal studies than in human studies. Nonetheless, the consistency by which animal studies link *n*-3 PUFA to cognition warrant further clinical research.

A last remark that should be made is with regards to the type of *n*-3 PUFA formulas used in testing. What is obviously lacking in current research as is a systematic approach to elucidating whether DHA, EPA or their combination at different proportions have the most beneficial effects on cognition at all age cohorts. Research with EPA or that compares EPA and DHA directly is lacking, especially in the setting of cognitive performance/impairment. Furthermore, the role of *n*-6 PUFA in cognition is under-investigated. The authors stress the importance of an overall improved systematic approach towards the testing of PUFA in both animal as well as human studies.

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