n-3 Fatty Acid-Derived Lipid Mediators against Neurological Oxidative Stress and Neuroinflammation

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OVERVIEW

In neural membrane phospholipids, essential fatty acids, namely arachidonic acid (ARA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3), are exclusively located at the sn-2 position of glycerol moiety of phospholipids. A small amount of eicosapentaenoic acid (EPA, 20:5n-3) is also present at the sn-2 position of neural membrane phospholipids. ARA belongs to the n-6 and EPA and DHA belong to the n-3 family of essential fatty acids. A high intake of food enriched in vegetable oils elevates levels of enzymic and nonenzymic mediators of ARA metabolism. Enzymic lipid mediators of ARA metabolism include prostaglandins (PGs), leukotrienes (LTs), thromboxanes (TXs), lipoxins (LXs), 2-arachidonylglycerol (2-AG), and arachidonylethanolamide (AEA) (Farooqui, 2009) (Figure 7.1). Nonenzymically, ARA is metabolized to 4-hydroxynonenal (4-HNE), isoprostanes (IsoPs), isoketals (IsoKs), and isofurans (IsoFs). ARA-derived lipid mediators produce prooxidant, prothrombotic, proaggregatory, and pro-inflammatory effects. In contrast, diets enriched in EPA and DHA (fish and fish oil) generate different enzymic and nonenzymic lipid mediators (Farooqui, 2011). The enzymic lipid mediators of EPA metabolism include E-series resolvins, 3-series PGs, 5-series LTs, and 17, 18-epoxyeicosatetraenoic acid (17, 18-EEQ). Nonenzymic mediators of EPA metabolism are cyclopentenone-isoprostanes (A3/I3-IsoPs) (Brooks et al., 2008). Similarly, the enzymic lipid mediators of DHA metabolism include D-series resolvins, neuroprotectins (NTPs), and maresins (MaRs) (Figure 7.1). The nonenzymic lipid mediators of DHA metabolism include 4-hydroxyhexanal (4-HHE), neuroprostanes (NPs), neuroketals (NKs), and neurofurans (NFs). Enzymically-derived lipid mediators of EPA and DHA metabolism not only down-regulate pro-inflammatory cytokines but also produce antioxidant, anti-inflammatory, antiatherogenic, antiarrhythmic, hypolipidemic, and vasodilatory effects (Hong et al., 2003; Hong et al., 2008; Marcheselli et al., 2003; Serhan, 2005; Serhan et al., 2008; Serhan et al., 2009; Marcheselli et al., 2010; Farooqui, 2009; Farooqui, 2012a). The nonenzymic lipid mediators of EPA and DHA metabolism also produce prooxidant and pro-inflammatory effects (Farooqui, 2011). Accumulating evidence suggests that ARA- and DHA-derived lipid mediators compete with each other and modulate induction and regulation of neuroinflammation by controlling the duration and magnitude of acute inflammation and oxidative stress, as well as the return of the injury site to homeostasis in the process of catabasis (the decline of the disease state) (Serhan et al., 2008; Farooqui, 2009; Farooqui, 2012a). Another important function of ARA, EPA, and DHA-derived lipid mediators is their involvement in the signal transduction network, which conveys the message of extracellular signals from the cell surface to the nucleus to induce a biological response at the gene level (Fahrenkrog, 2006). It is also reported that levels of ARA-, EPA-, and DHA-derived lipid mediators in neural and non-neural tissues are partly regulated by diet. Accumulating evidence supports the view that levels of EPA, ARA, and DHA, and their lipid mediators, not only orchestrate and control the onset of neuroinflammation and oxidative stress by coupling lipid metabolism with neural membrane lipid organization, but also cooperate with the action of lipid-dependent enzymes to execute appropriate downstream actions and responses. The present day Western diet is deficient in EPA and
DHA, but has high amounts of ARA (Farooqui, 2009; Farooqui, 2012a). The purpose of this article is to describe the antioxidant and anti-inflammatory effects of EPA- and DHA-derived lipid mediators in the brain.

**DHA IN THE BRAIN**

DHA is an absolute requirement for the development of the human central nervous system (CNS) and the continuous maintenance of brain cell function. EPA and DHA play an important role throughout life, as critical modulators of neuronal function and regulation of neuroinflammation and oxidative stress-mediated mechanisms in the normal brain during aging and chronic neurological diseases. Inadequate levels of DHA in the brain during development and old age induce cognitive deficits such as memory loss and learning disability in experimental animals (Farooqui, 2009). Thus, inadequacy of EPA and DHA in neural membranes may contribute to cholinergic, dopaminergic, and glutamatergic receptor dysfunction in synapses associated with the hippocampal neurons, and growing evidence suggests that low levels of DHA in the brain are associated not only with neurotraumatic, neurodegenerative, and neuropsychiatric diseases, but also with peroxisomal disorders (Farooqui, 2009). Dietary intake of EPA antagonizes the synthesis of PGE$_2$ from ARA and reduces the IL-1β-mediated increase in levels of PGE$_2$ (Song et al., 2004; Song et al., 2009). Furthermore, expression of IL-10 is also blocked by ethyl-EPA treatment. Based on these results, it is proposed that ethyl-EPA treatment produces beneficial effects in those neuropsychiatric disorders in which inflammation and oxidative stress play a critical role (Song et al., 2004; Song et al., 2009; Farooqui, 2009).

DHA acts as a ligand for the PPAR-γ and the RXR receptors. In conjunction with these receptors, which act as transcription factors, DHA modulates various enzymes and processes involved in neuroprotection and inflammation.
neurochemical processes that maintain cellular homeostasis by modulating lipid metabolism, neuronal cell differentiation, and apoptosis (Farooqui, 2009). DHA not only downregulates protein kinase C, Ras, and NF-κB, but also activates the Jak/Stat pathway, and sustains phosphorylation of EGFR. DHA attenuates the transcription of NF-κB-dependent genes. Thereby, the COX-2/PGE2-dependent generation of pro-angiogenic vascular endothelial growth factor and levels of anti-apoptotic bcl-2 and bcl-X(L) are reduced. Eicosanoid-independent proapoptotic pathways include enhanced lipid peroxidation, modulation of mitochondrial calcium homeostasis, and enhanced production of reactive oxygen species (ROS), as well as activation of p38. In the brain, DHA also restores levels of cerebellar phospho (p)-AKT, phospho-extracellular regulated kinase (p-ERK) and phospho-c-Jun N-terminal kinase (p-JNK), supporting their role in downregulation of neuronal apoptosis (Sinha et al., 2009). DHA also quenches gene expression of cyclooxygenase-2 and other enzymes, thereby diminishing the formation of pro-inflammatory eicosanoids. Pre-administration of DHA increases the corticohippocampal glutathione levels and glutathione reductase activity and suppresses the increase in lipid peroxide and ROS levels in the cerebral cortex and hippocampus of Alzheimer’s disease (AD) (Dan, 2009). In addition, DHA scavenges for free radicals, which diminish inflammatory response and oxidation of lipoprotein particles, notably low density lipoproteins (LDLs). DHA also suppresses insulin/neutropholic factor signaling deficits, neuroinflammation, and oxidative damage that contribute to synaptic loss and neuronal dysfunction in old age and demented subjects (Cole and Frautschy, 2010). Finally, DHA increases brain levels of neuroprotective brain-derived neurotrophic factor and reduces the ARA and its oxidative metabolites. The cross-talk among these molecular processes has distinct neuroprotective effects not only through the stabilization of neural membranes, modulation of ion channels, and receptors, but also through inhibition of inflammatory processes and generation of anti-inflammatory lipid mediators (Farooqui et al., 2007; Farooqui, 2009).

Dietary intake of DHA results in incorporation of this fatty acid into ethanolamine and serine glycerophospholipids. Among ethanolamine glycerophospholipids, ethanolamine and choline plasmapolipids are closely associated not only with the stability of synapse and functioning of various receptors but also with membrane fluidity and permeability, and maintenance of electrophysiological characteristics (Farooqui, 2009). DHA-enriched phosphatidylserine (PtdSer) is an essential cofactor for the activation of several proteins including protein kinase C, Raf-1 kinase, and AKT, which translocate from cytoplasm to the membrane for their activation, supporting the view that translocation of these kinases may be target signaling events modulated by the DHA-mediated neuronal specific increase of PtdSer (Kim et al., 2010). PtdSer also modulates activities of diacylglycerol kinase, nitric oxide synthase, and Na+, K+-ATPase (Ikemoto et al., 2000). Collective evidence suggests that EPA- and DHA-induced changes in membrane properties may further affect the ability of membrane receptors to interact with their ligands or intracellular signaling molecules as well as modulate the effect of membrane bound enzymes (Farooqui, 2009; Farooqui, 2011). As stated above, compared to DHA, levels of EPA in brain tissue are quite low. This may be due to its rapid β-oxidation of EPA following its uptake by the brain tissue (Chen et al., 2009a). In rat hepatocytes L-carnitine, a long chain fatty acid mitochondrial matrix transporter, not only increases β-oxidation of EPA, but only marginally elevates the oxidation of ARA and alleviates competitive inhibition of ARA-dependent PGE2 synthesis and COX-2 expression by EPA. It is suggested that L-carnitine modulates the competition between ARA and EPA in PG synthesis in liver cells by enhancing oxidation of EPA. This suggests that the beneficial effects of n-3 PUFA, especially EPA, are modulated by cellular oxidation capacity (Du et al., 2010).

EPA-DERIVED LIPID MEDIATORS IN THE BRAIN

EPA-derived lipid mediators include the 3-series PGs and TXs, 5-series LTs, and E-series resolvins (Resolin E1 and E2 or RvE1 and RvE2). The oxidized metabolites of EPA mediate anti-inflammatory and anti proliferative effects. The oxidation of EPA by COX and LOX enzymes results in the production of 3-series PGs and TXs, and 5-series of LTs. These lipid mediators have different biological properties from the corresponding analogs generated by COXs and LOXs-mediated oxidation of ARA. For example, TXA3 is less active than TXA2 in aggregating platelets and constricting blood vessels (Calder, 2009). In addition to generating less active lipid mediators, EPA exerts its effects on other aspects of inflammation such as leukocyte chemotaxis and inflammatory cytokine production. Some of these effects are likely due to changes in nuclear factor-κB-mediated gene expression (e.g. adhesion molecule) in microglia, astrocytes, and in visceral inflammatory and immune cells. In contrast, recent studies on the effects of prostaglandins (PGE2 and PGE3) and leukotrienes (LTB4 and LTB5) on endothelial permeability and mononuclear adhesion and migration across endothelial cell cultures indicate that PGE3 produces more pronounced effects on
trans-endothelial Evans blue-albumin (EBA) permeability than PGE₂, and these effects are antagonized by EP₁ and EP₂ antagonists (Moreno, 2009). LTB₄ and LTB₅ produce a slight effect on EBA extravasation (Moreno, 2009; Yin et al., 2007). It is suggested that EPA and ARA compete for the same COX enzymes (Zhao et al., 2004; Phillis et al., 2006), but the rate of oxidation of EPA is only 10% of the ARA. However, EPA significantly inhibits COX-1-mediated oxidation of ARA (Wada et al., 2007; Schmitz and Ecker, 2008), but the oxidation of ARA by COX-2 is only modestly inhibited by EPA. Metabolism of EPA by 15-LOX-like enzyme results in the synthesis of resolvins of the E-series (Arita et al., 2006; Arita et al., 2007), including resolvin E₁ (RvE₁; (5S,12R,18R)-trihydroxy-6Z,8E,10E,14Z,16E-eicosapentaenoic acid) and resolvin E₂ (15S,18R-dihydroxy-EPE) (Figure 7.2). EPA is oxidized to 18R-hydroxyeicosapentaenoic acid (18R-HEPE) by endothelial cell cyclooxygenase-2 (COX-2). Aspirin acetylates COX-2 and the acetylated enzyme no longer catalyzes the synthesis of PGs, but can still convert EPA to 18R-HEPE. During cell—cell interactions, 18R-HEPE is released to neighboring leukocytes, which through the action of 5-LOX converts it to RvE₁ via a 5(6) epoxide containing intermediate. RvE₁ is present in human whole blood, and its levels can be increased by ingestion of aspirin (Arita et al., 2006; Arita et al., 2007). RvE₁ is transformed into several metabolic products, including 20-hydroxy-RvE₁, 20-carboxy-RvE₁, 19-hydroxy-RvE₁, 18-oxo-RvE₁, and 10,11-dihydro-RvE₁ by human PMNs and whole blood as well as in murine inflammatory exudates, lungs, spleen, kidney, and liver (Seki et al., 2010). Among these products, 20-carboxy-RvE₁, 18-oxo-RvE₁, and 10,11-dihydro-RvE₁ are essentially biologically inactive and may serve as inactive biomarkers of RvE₁ metabolism in vitro. In contrast, 20-hydroxylated product of RvE₁ has some of the activity of RvE₁, suggesting that more metabolites of RvE₁ are generated during inflammatory response.

In non-neural tissues, RvE₁ and RvE₂ induce potent anti-inflammation/pro-resolution effect in vivo (Arita et al., 2006) via specific G protein-coupled receptors.
EPA-DERIVED LIPID MEDIATORS IN THE BRAIN

DHA-derived Lipid Mediators in the Brain

Metabolism of DHA by 15-LOX-like enzyme results in the formation of D-series resolvins. This enzyme converts DHA into 17S-hydroperoxy-DHA (17S-H(p) DHA), which is converted into several bioactive compounds, including resolvin D1-D6 (RvD₁, RvD₂, RvD₃, RvD₄, RvD₅, and RvD₆). In addition, interactions with aspirin result in the formation of aspirin-triggered D-series resolvins (AT-Rv) through sequential oxygenation initiated by aspirin-acetylated COX-2. These lipid mediators not only antagonize the effects of PGs, LTs, and TXs, but also modulate leukocyte trafficking and down-regulate the expression of cytokines in glial cells. They possess potent anti-inflammatory, neuroprotective, and pro-resolving properties (Hong et al., 2003; Marcheselli et al., 2003; Serhan, 2005).

DHA-derived D-series Resolvins

In neural and non-neural cells, D-series resolvins act through resolin D receptors (resoDR₁) (Serhan et al., 2008). ResoDR₁ modulate potent anti-inflammatory and immunoregulatory activities. D-series resolvins not only block the production of pro-inflammatory mediators, but also regulate the trafficking of leukocyte cells at the sites of inflammation (Serhan et al., 2008; Hong et al., 2003) and inhibit the expression of cytokines leading to modulation of neuroinflammation. Studies on characterization of ResoDR₁ are urgently needed to progress understanding of this area of DHA-derived lipid mediators.

DHA-derived Protectins and Neuroprotectins

Synapses are specialized anatomical sites (structures) that facilitate communication between neurons. They are essential for transmitting, processing, and storing information from one cell to another in the brain. They are enriched in DHA-containing plasmalogens, a unique class of phospholipids characterized by the presence of a vinyl ether bond at the sn-1 position of the glycerol moiety. It is well known that loss of synapses in the hippocampal and cortical regions is accompanied by a decrease in plasmalogens in Alzheimer’s disease (AD) patients (Selkoe, 2002; Selkoe 2008; Ginsberg et al., 1995; Ginsberg et al., 1998; Han et al., 2001; Guan et al., 1999). The molecular mechanism associated with loss of synapse and decrease in plasmalogens is not yet fully understood. However, it is proposed that Aβ oligomers induce stimulation of plasmalogen-selective PLA2 (PlsEtn-PLA2) and may be responsible for the decrease in plasmalogen levels and synaptic loss in AD (Farooqui, 2010a; Farooqui, 2012a). Since plasmalogens are essential structural phospholipids of synapses and neurons, decreases in plasmalogen pools may likely be responsible for the loss of synapse and the shrinkage of neurons that precedes neurodegeneration in AD. Synaptic loss not only correlates with cognitive impairment in AD, but also with insufficient mitochondrial ATP (Terry et al., 1991; Selkoe, 2002; Dong et al., 2007). The biosynthetic conversion of DHA to NPD₁/ PD₁ involves the action of 15-LOX to form an epoxide intermediate followed by enzymic hydrolysis (Figure 7.3). Investigations into the stereochemistry of PD₁ synthesis have confirmed that PD₁ is a 10R,17S-dihydroxydocosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid (Hong et al., 2003; Marcheselli et al., 2003; Serhan et al., 2008). The reaction sequence of biosynthesis for PD₁ via the epoxide intermediate distinguishes it from the formation of the double dioxygenation product 105,17S-dihydroxy-DHA. PD₁ is more potent than DHA in neuroprotective action. In sharp contrast, PD₁ positional isomers, including 4S,17S-diHDHA or 7S,17S-diHDHA, display less potent and non-selective actions. The occurrence of PD₁ has also been reported in brain, where it is called neuroprotectin D₁ (10R, 17S-dihydroxydocosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid, NPD₁)}
(Hong et al., 2003). Tritium-labeled NPD$_1$ (3H-NPD$_1$) binds to ARPE-19 cells with high affinity (K$_d$ 31.3 ± 13.1 pmol/mg of cell protein). The stereo-specific NPD$_1$ interactions with these cells provide potent protection against oxidative-stress-mediated apoptosis. 3H-NPD$_1$/PD$_1$ also shows specific and selective high affinity binding with isolated human neutrophils (K$_d$ approximately 25 nM). Neither resolvin E nor lipoxin A$_4$ compete for 3H-NPD$_1$/PD$_1$-specific binding with human neutrophils. Collectively, these results indicate that stereo-selective specific binding of NPD$_1$/PD$_1$ with retinal pigment epithelial cells as well as human neutrophils may occur through specific receptors in both the immune and visual systems (Marcheselli et al., 2010). The isolation and characterization of 10(S),17(S)-dihydroxy-docosahexa-4Z,7Z,11E,13Z,15E,19Z-enoic acid, a main dihydroxy conjugated triene derived from the lipoxigenation of DHA has also been reported, but nothing has been published on its interactions in neural and non-neural cells. It is an isomer of protectin/neuroprotectin D$_1$ (PD$_1$/NPD$_1$) and has been named PDX (Chen et al., 2009b). This metabolite inhibits human blood platelet aggregation at submicromolar concentrations.

Like resolvins, the neuroprotectins not only block the infiltration of PMN (Serhan et al., 2006), but also down-regulate the expression of cytokines in the glial cells (Hong et al., 2003; Serhan et al., 2008). NPD$_1$ reduces retinal and corneal injury (Mukherjee et al., 2004) and produces neuroprotective effects in ischemic injury (Marcheselli et al., 2003). Similarly, NPD$_1$ promotes neural cell survival via the induction of anti-apoptotic and neuroprotective gene expression programs that suppress A€€\textsuperscript{32}-mediated neurotoxicity in AD (Lukiw et al., 2005; Bazan, 2009a,b). DHA and NPD$_1$ protect synapses and decrease the number of activated microglia in the hippocampal system (Pomponi et al., 2008). The molecular mechanisms associated with the above processes are not fully understood. However, it is becoming increasingly evident that NPD$_1$ not only inhibits IL-1β-stimulated expression of COX-2, but also regulates apoptotic signaling at the level of the mitochondria, inducing the release of cytochrome c and activating effector enzyme, caspase-3. In addition, in rats infused with Aβ, DHA and its oxidative metabolites attenuate elevation in levels of lipid peroxides and ROS in the cerebral cortex and the hippocampus, indicating that DHA and its metabolites facilitate neuroprotection by down-regulating age-related activity, an enzyme that liberates Aβ from soluble amyloid precursor protein-β (Lukiw et al., 2005). Furthermore, soluble amyloid precursor protein-β stimulates the synthesis of NPD$_1$ (Lukiw et al., 2005; Bazan, 2009a,b). It is also reported that DHA increases protein levels of a genetically implicated risk factor, SorLA/LR11, a neuronal sorting protein that regulates APP processing to decrease Aβ production in a dose-dependent manner (Ma et al., 2007). This observation anchors the growing connection between LR11 and causal mechanisms of AD pathogenesis (Dodson et al., 2008). Recently, variants of the LR11 gene (SORL1) have been shown to correlate with risk of sporadic AD in several populations, providing direct genetic evidence for a proximal role of LR11 in AD (Lee et al., 2007).

Collective evidence suggests that DHA and its oxidative metabolites limit the generation and accumulation of the Aβ peptide, which is closely associated with the pathogenesis of AD. DHA and its metabolites also suppress several signal transduction pathways induced by Aβ, including two major kinases that phosphorylate the microtubule-associated protein tau and promote neurofibrillary tangle pathology (Farooqui, 2009; Farooqui, 2010a) (Table 7.1).

It has recently been shown that in macrophages, DHA is also metabolized through a 14-LOX pathway resulting in generation of 7,14-dihydroxy-docosa-4Z,8,10,12,16Z,19Z-hexaenoic acid. This metabolite is called maresin (MaR1). This metabolite not only terminates PMN infiltration, but also stimulates macrophage phagocytosis. An isomer of MaR1, 7,14S-dihDHA acts less potently than MaR1. This suggests that in macrophages MaR1 and other DHA-derived metabolites may stereo-selectively regulate catabasis and facilitate arrival of tissues to homeostasis through the inhibition of leukotriene A$_4$ hydrolase, a bifunctional

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**TABLE 7.1** Effects of Resolvins and Neuroprotectins in Animal Models of Neural and Non-Neural Diseases

<table>
<thead>
<tr>
<th>Animal Model</th>
<th>Effect of Resolin/Neuroprotectin</th>
<th>Molecular Mechanism</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Stroke</td>
<td>Beneficial</td>
<td>Decrease in proapoptotic Bcl-2 expression and inhibition of caspase-3; induction of neuroprotective gene expression</td>
<td>Mukherjee et al., 2007; Bazan, 2009a,b</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>Beneficial</td>
<td>Decrease in proapoptotic Bcl-2 expression and inhibition of caspase-3; induction of neuroprotective gene expression</td>
<td>Mukherjee et al., 2007; Bazan, 2009a,b</td>
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zinc containing enzyme that contains epoxide hydrolase and aminopeptidase activities (Serhan et al., 2009; Farooqui, 2011).

In retina, epithelium-derived factor acts as an agonist and induces the synthesis of NPD₁, thus promoting NPD₁-mediated paracrine and autocrine signal transduction processes. Also, DHA and epithelium-derived factor not only synergistically activate NPD₁ generation and antiapoptotic protein expression, but also down-regulate proapoptotic Bcl-2 protein expression and activation of caspase 3 during oxidative stress (Mukherjee et al., 2007). NPD₁ also promotes AKT translocation and activation and interacts with the PPAR-gamma family of ligand-activated nuclear receptors, which may be involved in various aspects of neuroinflammation and neurodegeneration (Palacios-Pelaez et al., 2010; Niemoller and Bazan, 2010; Farooqui, 2010a,b). Receptors for NPD₁ have not been characterized in brain tissue, but their occurrence has been suggested (Hong et al., 2003; Marcheselli et al., 2003; Mukherjee et al., 2004). Thus, NPD₁-mediated regulation targets upstream events of brain cell apoptosis and modulation of neuroinflammatory signaling promotes the cellular homeostasis and restoration of brain damage through the above mentioned mechanisms. It is tempting to speculate that the generation of DHA-derived Rvs, NPD₁, MaR, and synthesis of ARA-derived lipoxins may be internal neuroprotective mechanisms that block neuroinflammation and apoptosis-mediated brain damage caused by neurotraumatic and neurodegenerative diseases (Serhan, 2005; Bazan, 2009a,b; Farooqui, 2010a).

Lipodomics, proteomics, and genomics techniques have been used to identify and determine levels of ARA, EPA, and DHA-derived lipid mediator (PGs, LTs, TXs, LXs, RvEs, RvD, and NPD₁) (Serhan et al., 2006; Ariel and Serhan, 2007; Serhan et al., 2008; Bazan, 2009a,b). This information can be used for developing diagnostic tests in cerebrospinal fluid (CSF) for acute neural trauma in neurodegenerative and neuropsychiatric patients. The use of proteomics techniques for characterizing EPA and DHA metabolizing enzymes in subcellular organelles of the human brain and CSF may provide new information on properties and therapeutic targets of neurological disorders. Rvs and RvDs synthesizing and catabolizing enzymes may not only modulate the levels of these lipid mediators in the normal and diseased brain, but may also regulate the onset and progression of chronic, acute, and psychiatric diseases. Collectively, these studies suggest that combining lipidomics, proteomics, and genomics techniques may greatly enhance the existing knowledge of molecular homeostasis that occurs between inflammation and oxidative stress inducing mediators (PGs, LTs, TXs) and inflammation and oxidative stress blocking lipid mediators (LXs, RvEs, RvD, and NPD₁) in neural trauma, neurodegenerative and neuropsychiatric diseases (Serhan et al., 2008; Bazan, 2009a,b; Farooqui, 2010a).

Effect of EPA and DHA in Neurological Disorders

As stated above, diet influences and modulates brain function. Diets high in saturated fat and n-6 fatty acids not only increase insulin resistance (Tschoop and Thomas, 2006), but also negatively affect cognitive processing and increase the risk of neurological disorders (Luchsinger et al., 2002; Greenwood and Winocur, 2005; Convit et al., 2003). Consumption of EPA and DHA modulates neural membrane fluidity and permeability and improves spatial learning by regulating synaptic and cognitive function (Farooqui, 2009; Gomez-Pinilla, 2008). High intake of fruits, vegetables, fish, and whole grains (such as is typically found in a Mediterranean-type diet) along with phytochemicals (resveratrol and other polyphenols) produces beneficial effects on the human brain (Engelhart et al., 2002; Scarmeas et al., 2009; Farooqui, 2012b). The Western diet has extremely high levels of ARA, with an ARA to DHA ratio of about 20:1. The Paleolithic diet, on which human beings have evolved and lived for most of their existence, had a ratio of 2–1:1, and was high in fiber, rich in fruits, vegetables, lean meat, and fish (Simopoulos, 2002; Simopoulos, 2008; Cordain et al., 2005). The high intake of ARA-enriched food in the modern Western diet not only elevates levels of PGs, LTs, and TXs, but also upregulates the expression of pro-inflammatory genes including genes for cytokines (TNF-α, and IL-1β) enzymes (secretory phospholipase A₂, cyclooxygenase-2, and nitric oxide synthase). These genes and enzymes initiate and maintain neuroinflammation. In contrast, consumption of an EPA- and DHA-enriched diet produces anti-inflammatory effects that are partly supported by repression of genes that code for pro-inflammatory cytokines. Since the Western diet is low in DHA and high in ARA, it is linked to many chronic visceral diseases as well as neurodegenerative and neuropsychiatric disorders (Simopoulos, 2002; Simopoulos, 2008; Cordain et al., 2005; Farooqui, 2009). It is reported that intake of EPA and DHA is associated with reduced risk of age-related cognitive decline (Farooqui, 2009; Gorby et al., 2010). DHA is highly concentrated in the brain and enhances synaptic activities in neuronal cells. DHA attenuates neuronal cell death after ischemic injury not only by modulating neural membrane biophysical properties, but also by maintaining integrity in functions between presynaptic and postsynaptic areas.
These processes result in better stabilization of intracellular ion balance in ischemic injury. Additionally, EPA and DHA prevent apoptotic cell death in the brain by generating resolvins and neuroprotectins and inducing antiapoptotic activities such as decreasing responses to ROS, upregulating antiapoptotic protein expression, down-regulating apoptotic protein expression, and maintaining mitochondrial integrity and function (Farooqui, 2009; Mayurasakorn et al., 2011) (Figure 7.4).

DHA is enriched in synaptic fractions and there is a good correlation between low tissue levels of EPA and DHA and increased risk of depression, schizophrenia, memory loss, and a higher chance of developing AD (Farooqui, 2009). Furthermore, low dietary intake of EPA and DHA produces cognitive decline in AD patients. Based on these observations, it is suggested that enrichment of EPA and DHA in the diet may improve inflammation and oxidative stress in neurotraumatic and neurodegenerative diseases, not only through the effects of EPA and DHA on physicochemical properties of neural cell membranes, but also through modulation of genes and generation of RvDs and NPD (Farooqui, 2009; Farooqui, 2011). It should be noted that, to date, studies on the dietary intervention of DHA and EPA have so far failed (Quinn et al., 2010). The cause of failure is not fully understood. It is likely that short-term use of dietary DHA alone may not stop cognitive decline in AD, but long-term

**FIGURE 7.4** Regulation of inflammation, oxidative stress, and apoptotic cell death by neuroprotectins under pathological conditions (ischemic injury and Alzheimer’s disease) in the brain. NMDA-R, N-methyl-D-aspartate receptor; Glu, glutamate; PtdCho, phosphatidylcholines; lyso-PtdCho, lyso-phosphatidylcholine; cPLA₂, cytosolic phospholipase A₂; ARA, arachidonic acid; PAF, platelet activating factor; COX-2, cyclooxygenase2; 5-LOX, 5-lipoxygenase; 15-LOX, 15-lipoxygenase; PtdEtn, plasmalogen; PtdEtn-PLA₂, plasmalogen-selective phospholipase A₂; sPLA₂, secretory phospholipase A₂; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin 1beta; IL-6, interleukin-6; NF-κB, nuclear factor-κB; sAPP, soluble amyloid precursor protein; ADAM10, alpha-secretase; BACE1 or beta-site APP cleaving enzyme, β-secretase. Adapted from Farooqui, 2012a.
supplementation of EPA and DHA from childhood through to old age may not only restore signal transduction processes associated with behavioral deficits and learning activity, but may also produce several neuroendocrinological and immunological effects on brain tissue, which may be beneficial in ischemia and AD (Farooqui, 2010a). Thus, double blind, large AD patient, and multicenter studies are needed to test the efficacy of EPA and DHA.

CONCLUSION

Phospholipid-derived lipid mediators are important endogenous regulators of neural cell proliferation, differentiation, oxidative stress, inflammation, and apoptosis. They originate from enzymatic and nonenzymatic degradation of glycerophospholipids, ARA-derived lipid mediators including PGs, LTs, TXs, and LXs. These mediators induce and modulate proliferation, differentiation, oxidative stress, and neuroinflammation. LXs produce anti-inflammatory effects. The lipid mediators of EPA and DHA metabolism are RvEs, RvDs, NPDp, and MaR. These mediators produce antioxidant, anti-inflammatory, and antiapoptotic effects in the brain tissue. Regular intake of EPA and DHA may produce beneficial effects in ischemic injury and in AD.

References

REFERENCES


Abstract
Two families of essential fatty acids are known to occur in neural membrane phospholipids. Eicosapentaenoic and docosahexaenoic acid belong to the n-3 fatty acid family, whereas arachidonic acid belongs to the n-6 fatty acid family. ARA-derived lipid mediators produce oxidative stress and neuroinflammation, whereas eicosapentaenoic acid and docosahexaenoic acid-derived lipid mediators regulate immune systems by downregulating signal transduction processes associated with oxidative stress, neuroinflammation and neurodegeneration. Lipid mediators derived from eicosapentaenoic acid and docosahexaenoic acid retard excessive oxidative stress and neuroinflammation caused by ARA-derived lipid mediators. Aspirin initiates and induces resolution by triggering biosynthesis of specific epimers of docosahexaenoic acid-derived lipid mediators. Potent actions of eicosapentaenoic acid- and docosahexaenoic acid-derived lipid mediators in animal models of chronic human disease indicate that down-regulation of resolution pathways and their antioxidant effects may protect from many neurological diseases.

Keywords: Eicosapentaenoic acid; docosahexaenoic acid; arachidonic acid; prostaglandins; leukotrienes; thromboxanes; resolvins; neuroprotectins; inflammation; oxidative stress
The Impact of Omega-3 Fatty Acids on Quality of Life

Ondine van de Rest and Lisette CPGM de Groot

INTRODUCTION

Current evidence indicates that a low dietary intake as well as low levels of omega-3 fatty acids, such as eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3), may be associated with mental health problems, which are among the leading causes of impaired quality of life (QoL) in old age. A possible role for omega-3 fatty acids in mental health is supported by multiple plausible biochemical pathways, which are described by Parletta et al. (2013) and include the role of omega-3 fatty acids as structural components of the brain cell membranes, in increased brain phospholipid synthesis, increased neurite growth, improved membrane fluidity, more effective neurotransmission, its effects on eicosanoid synthesis, its anti-inflammatory and vasodilatory properties, and its role in protection against neuronal loss and neurodegeneration (Parletta et al., 2013).

QoL has been defined by the World Health Organization (WHO) as an individual’s perception of their position in life in the context of culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns (The WHOQOL Group, 1995). It is a broad ranging concept affected in a complex way by a person’s physical health, psychological state, level of independence, social relationships, and relationship to salient features of their environment (WHO, 1993). Depression, anxiety, and the presence of diseases such as cancer, cardiac disease, and diabetes are highly associated with a reduced QoL (Ali et al., 2010; Baumeister et al., 2011). Therefore, studies investigating these associations have mostly been performed in diseased populations and much less so in the general population. However, improving QoL is becoming an increasingly important outcome in research into the elderly population. The fact that we are living longer is a good thing for many people, particularly if these years can be lived well. QoL is hampered, however, by inevitable age-related deterioration of physical and mental health and by an increased risk of developing chronic diseases. These age-related problems are likely to affect the QoL of individuals in later years, as well as adding pressure to infrastructures such as health and social care services. For studies in health promotion, generic outcomes are even more relevant than disorder-specific outcomes. Such data are particularly needed since caregivers and professionals may wish to improve the patient’s QoL, especially for depression, dementia or cognitive impairment for which pharmacological treatment is often ineffective. In the current chapter, first the assessment of QoL will be addressed, and subsequently the chapter provides an overview of the current observational and clinical trial evidence with respect to the impact of omega-3 fatty acids on QoL.

ASSESSMENT OF QOL

There are several valid and reliable questionnaires that can be used to assess QoL. The most commonly used questionnaire is the Short-Form 36 Health Survey (SF-36) and another generic QoL questionnaire is the WHO QoL questionnaire (WHOQOL). These questionnaires are generic health-related QoL surveys, because they can be used across age (≥18 years), disease, and treatment groups, as opposed to disease-specific health surveys, which focus on a particular condition or disease. Disease-specific QoL measures are not included in the current chapter because this would introduce too much heterogeneity to the results and detract from the focus, which is on the general, particularly the elderly population.
The SF-36 consists of 36 items, grouped into eight scales; each 0–100 scale measuring a different aspect of health (Ware and Sherbourne, 1992). Two summary scores can be calculated: a psychometrically-based physical component summary score (PCS) and a mental component summary score (MCS). Physical health is assessed with scales on physical functioning, role limitations due to physical health, bodily pain, and general health. The mental health score is calculated with scales on vitality (energy/fatigue), role limitations due to emotional well-being, social functioning, and mental health. The reference period is the past four weeks for the scales that have a recall period. Higher scale scores represent better self-reported health and the scales have a good validity for measuring physical and mental health constructs (McHorney et al., 1993). There are also two shortened versions; the SF-12 and the SF-8, which measure the same eight health domains (Ware et al., 1996; Ware et al., 2001). The WHOQOL is a 100-item QoL instrument developed by the WHO to facilitate cross-cultural comparisons in QoL research (The WHOQOL Group, 1998b). The WHOQOL instrument focuses on the individual’s own views of their well-being. The WHOQOL-BREF was developed to enable a brief, but accurate, assessment of QoL in routine clinical work, epidemiological studies, and clinical trials (The WHOQOL Group, 1998a). The questionnaire comprises 26 items, including two general items and 24 items covering four domains: (1) physical health; (2) psychological health; (3) social relationships; and (4) environment. The scores of the two general items range from 1 to 5 and the domain scores range from 7 to 35, 6 to 30, 3 to 15 and 8 to 40 for the domains 1 through 4 consecutively. The total score range of the WHOQOL-BREF is 26–130 with higher scores indicating a favorable condition. The reference period is the previous two weeks and questions have a 5-point Likert scale. Studies have shown good content validity, discriminant validity and test–retest reliability (Nelson and Lotfy, 1999; Trompenaars et al., 2005).

### CURRENT EVIDENCE ON OMEGA-3 FATTY ACIDS AND QoL

**Evidence from Observational Studies**

Two observational studies have assessed the association between omega-3 polyunsaturated fatty acids (PUFAs) and QoL (Table 8.1) (Crowe et al., 2007; Silvers and Scott, 2002). Silvers and Scott (2002) observed a significant positive association between fish intake and self-reported scores on the mental health part of the SF-36 in 4,644 New Zealand adults (Silvers and Scott, 2002). Conversely, the association between fish consumption and physical health was negative (P for trend = 0.045). The second study was also cross-sectional and based on data from the same population-based survey performed in New Zealand. This time fatty acid composition of serum phospholipids was used as an indicator of omega-3 status, which resulted in a smaller sample size (n = 2,416) than in the first analysis. A significant positive trend (P for trend = 0.009) was observed across quintiles of the proportion of EPA in relation to self-reported physical health.

### Table 8.1 Summary of Observational Studies Assessing the Association Between Omega-3 Fatty Acids and QoL

<table>
<thead>
<tr>
<th>Author Year</th>
<th>Study Population (n)</th>
<th>Design</th>
<th>Nutritional Status</th>
<th>Measure (s) of QoL</th>
<th>Covariate(s)</th>
<th>Results</th>
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<td>Positive association between the proportion of EPA and the ratio of EPA:AA and self-reported physical well-being; P for trend = 0.009 and 0.012, respectively. No associations between the proportion of EPA or DHA with mental health. However, significant association between the EPA:AA ratio and self-reported mental health (P for trend = 0.044).</td>
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<td>Age, annual household income, smoking status, AUDIT category, eating patterns</td>
<td>Significant association between more fish intake and better self-reported mental health status (P for trend = 0.005) and between more fish intake and impaired physical functioning (P = 0.045).</td>
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QoL: Quality of life; SF-36: Short Form-36 Health Survey; BMI: Body mass index; AUDIT: Alcohol Use Disorders Identification Test; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AA: arachidonic acid; FFQ: Food Frequency Questionnaire.

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Evidence from Clinical Trials

Three intervention studies have been performed and these are summarized in Table 8.2. The first randomized trial was performed in healthy individuals \( (n = 302, \text{mean age } 70 \text{ years}) \), who were supplemented with either \( 1,800 \text{ mg EPA + DHA (EPA:DHA ratio } = 3:2; \approx 1093 \text{ mg EPA + 847 mg DHA), 400 mg EPA + DHA (226 mg EPA + 176 mg DHA) or placebo capsules for a period of 6 months (van de Rest et al., 2009). No treatment effects were found on total QoL or any of the separate domains as measured with the WHOQOL-BREF. Another trial was performed in 46 depressed women aged 66–96 years and living in a nursing home in Italy (Rondanelli et al., 2010). These women were supplemented with 2.5 g omega-3 fatty acids (1.67 g DHA + 0.83 g EPA) or placebo over 8 weeks. A significant difference between the omega-3 and the placebo group was found on the physical function score (difference (95% CI) 21.1 (10.8, 31.3), \( P < 0.001 \)) and also on the mental function score (difference (95% CI) 25.2 (16.3, 34.1), \( P < 0.001 \)) of the SF-36. The results on the total QoL score were not reported. A third trial was performed in 50 elderly subjects (\( > 50 \) years) with mild cognitive impairment who were randomly allocated to daily supplements containing DHA-rich oil (1.55 g DHA + 0.40 g EPA), EPA-rich oil (1.67 g EPA + 0.16 g DHA), or 2.2 g LA for 6 months.

With only two observational and three intervention trials, the number of studies that investigated omega-3 fatty acids in relation to QoL is very limited. Some results from the observational studies are favorable, but not consistently so, for mental and physical health components. The results only showed an effect of omega-3 fatty acid supplementation in the trial that included depressed individuals.
8. THE IMPACT OF OMEGA-3 FATTY ACIDS ON QUALITY OF LIFE

Dementia, depression, and coronary heart disease constitute the main causes of impaired QoL in the Netherlands (RIVM, 2006). It could well be that QoL in general, non-diseased populations is already close to optimal and that further improvement could not be achieved with a higher omega-3 fatty acid intake or supplementation. The total body of evidence from observational, as well as clinical studies on omega-3 and depression, one of the strongest determinants of QoL, is much larger and will be addressed in another chapter. However, the results observed in these studies support the current results for QoL, i.e. the evidence provides some support of a beneficial effect of omega-3 fatty acids in depressed individuals, but not in individuals without a diagnosis of depression (Appleton et al., 2010). If true, it can be concluded that extra omega-3 fatty acids are of little added value to mental health in a generally healthy population, but they could have a role as an adjuvant treatment for depression or dementia (Yehuda et al., 1996) and as such, also improve QoL.

In the field of omega-3 fatty acids and QoL, four studies used the SF-36 and one study used the WHOQOL-BREF questionnaire. Both questionnaires have shown good internal consistency, excellent discriminant validity, and good sensitivity (McHorney et al., 1993; Nelson and Lotfy, 1999; Trompenaars et al., 2005). Although the SF-36 is generally the most widely used QoL questionnaire, the WHOQOL includes a strong mental health component and emphasizes the perception of the individual. Because of the implications for an association between omega-3 PUFAs and mental well-being, the WHOQOL questionnaire is also a very suitable questionnaire to be used in future studies in the field. Despite the availability of several valid and reliable QoL instruments, it is a pity that they have seldomly been applied in efficacy trials that address mental well-being (Scholzel-Dorenbos et al., 2007).

The aspects included in QoL questionnaires have been generated by systematic qualitative and quantitative research among different cultures and populations and the psychometric properties have been validated in several studies. However, because mood state, physical pain or disease, and QoL are strongly correlated, QoL is a subjective concept to measure as it depends on self-reporting and on an individual’s perception of status. This possible lack of objectivity has to be taken into account.

Dietary DHA can be incorporated into the brain not only during development, but also later in life. The half-life of DHA in the brain is about 21 days, so studies should last at least 3–4 weeks before the additional DHA is incorporated into the brain (Connor et al., 1990). Furthermore, the underlying mechanism that is targeted should also be taken into account, since different plausible mechanisms have been proposed and could be classified as either short term (i.e. weeks to months) or long term (i.e. months to years). The three intervention studies had a study duration of 8 weeks or 26 weeks and a beneficial effect was only found in the shortest 8-week study. However, this result probably has more to do with the sensitive nature of the subjects studied in that particular study, i.e. depressed patients, rather than with the study duration.

CONCLUSION AND RECOMMENDATIONS

Based on the current evidence we conclude that the total body of evidence is too limited to draw a firm conclusion with respect to the impact of a higher intake of omega-3 fatty acids, either via diet or supplementation, on QoL. The results from the few available observational studies are partly favorable, but the results from the trials are not, except in depressed participants.

Because causality cannot be inferred from cross-sectional studies, which comprised two of the five studies performed, at least some prospective studies should be performed before a valid conclusion about the role of omega-3 fatty acids in QoL can be drawn. Moreover, intervention studies of sufficient study duration are warranted, and particularly in subjects with an impaired QoL and/or impaired omega-3 fatty acid status, in order to further develop our understanding of the effects of omega-3 fatty acids on QoL.

References


McHorney, C.A., Ware Jr., J.E., Raczek, A.E., 1993. The MOS 36-Item Short-Form Health Survey (SF-36). II. Psychometric and clinical validity of several valid and reliable QoL instruments, it is a pity that they have seldomly been applied in efficacy trials that address mental well-being (Scholzel-Dorenbos et al., 2007).
tests of validity in measuring physical and mental health constructs. Med. Care. 31 (3), 247–263.
Abstract
Improving quality of life (QoL) is becoming an increasingly important outcome in research into the elderly population and for studies in health promotion; generic outcomes are, next to disorder-specific outcomes, also very important. Current evidence indicates that low dietary intake and a low status of omega-3 fatty acids may be associated with mental health problems, which are among the leading causes of impaired QoL in old age. Some studies have also been performed that directly investigated the relationship between omega-3 fatty acids and QoL. In the current chapter these studies will be summarized and discussed. However, because the total number of studies is very limited (only two observational studies and only three intervention studies), no firm conclusions can yet be drawn. More research is warranted, for which some suggestions are provided in this chapter.

Keywords: omega-3 fatty acids; n-3 fatty acids; fish oil; quality of life; QoL
CHAPTER

13

Fish Oil Supplementation Prevents Age-Related Memory Decline: Involvement of Nuclear Hormone Receptors

S. Alfos

INTRODUCTION

Public health, economic development, and medicine gradually extend average life expectancy and the number of individuals older than 60 in the world is projected to exceed 1 billion in the year 2030, which will represent 16.5% of the total population (Kowal et al., 2010). Concern arises regarding the quality of life available to the elderly, not only in terms of physical well-being, but also in terms of mental well-being. Indeed, beyond Alzheimer’s disease, the prevalence of which increases with age, most of the elderly will exhibit subtle deficits in memory that are unrelated to neuropathologies. These mild memory deficits associated with normative aging are nonetheless disturbing for those affected (Erickson and Barnes, 2003). Therefore, cognitive decline in the elderly is a major socio-economic and healthcare concern.

In order to reduce the effects of normal aging on memory performance and hence increase the mental well-being of the elderly, closer attention should be paid to the possible impact of modifiable lifestyle factors such as the quality of diet. Indeed, nutrition is one of the major determinants of successful aging and may affect mental health (Dauncey, 2009). Among the dietary nutrients most closely associated with optimal brain function, long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs) are particularly important (Gomez-Pinilla, 2008; Parletta et al., 2013). Increasing the intake of n-3 PUFAs, particularly the LC n-3 PUFAs, may be a strategy to delay the onset of memory decline in the elderly.

EFFECTS OF AGING ON INCORPORATION OF DOCOSAHEXAENOIC ACID IN BRAIN PHOSPHOLIPIDS

The principal LC n-3 PUFA found in the adult mammalian brain is docosahexaenoic acid (22:6n-3, DHA) comprising 10–20% of total fatty acid composition, whereas eicosapentaenoic acid (EPA, 20:5n-3) and docosapentaenoic acid (DPA, 22:5n-3) represent less than 1% of total fatty acid composition (McNamara and Carlson, 2006). Studies in adult rodents and primates demonstrate that DHA concentrations differ between brain regions (Carrie et al., 2000a; Diau et al., 2005). In rodents, DHA concentration is higher in the frontal cortex and hippocampus (16–22% of total fatty acids) and lower in other brain regions such as the striatum (14% of total fatty acids) (Xiao et al., 2005). Most DHA accumulates in brain structures during prenatal development and the early post-natal period (Green et al., 1999; Innis, 2007; Martinez, 1992; McNamara and Carlson, 2006). In brain membranes, DHA is preferentially incorporated into the stereospecifically numbered-2 (sn-2) position of the phospholipids phosphatidylethanolamine (PE) and phosphatidylserine, and to a smaller proportion, into phosphatidylcholine (PC) (Rapoport, 1999). Fish oil supplementation can rapidly modify brain phospholipid composition as shown by a mass spectroscopy analysis. In this study, an oral gavage of fish oil (38% DHA + 48% EPA) in two-month-old rats, for a period of one month only, significantly increased the proportion of DHA-containing.
Brain aging is associated with significant changes in the levels of major phospholipid classes. Depending on the animals’ age and diet and also on the brain region studied, the results sometimes differ from one study to another. Globally, aging is associated with a significant progressive decrease in PE and PS in the prefrontal cortex and the hippocampus in rats between 18 to 32 months of age compared to 2–3-month-old rats (Babenko and Semenova, 2010; Dyall et al., 2007; Favrelière et al., 2000; 2003). In rodents, most of the studies also show a decrease in the levels of DHA in the brain during aging (Giusto et al., 2002; Labrousse et al., 2012; Little et al., 2007). More specifically, aging alters DHA concentrations in specific brain phospholipid classes. Therefore, during aging, a reduction in DHA levels in PE, PS, and PC is observed in the cortex, the hippocampus, and the cerebellum, associated with an increase in monounsaturated fatty acids (Barcelo-Coblijn et al., 2003a; Dyall et al., 2007; Favrelière et al., 2000; Latour et al., 2013; Little et al., 2007; Lopez et al., 1995). This age-related DHA decrease can be reversed by 2–3 months of fish oil supplementation (Barcelo-Coblijn et al., 2003a; Dyall et al., 2007; Favrelière et al., 2000; Latour et al., 2013; Little et al., 2007). Moreover, the accumulation in the brain, mostly in the PC, of orally administered radiolabeled DHA is reduced with increasing age from 2- to 10-week-old rats (Graf et al., 2010). These results suggest a reduced capacity of the aging brain to incorporate DHA in phospholipids.

The situation in humans seems more complex with limited alterations in brain DHA levels (Carver et al., 2001; Söderberg et al., 1991) or a slight decrease in the orbitofrontal cortex (McNamara et al., 2008) during normal aging. However, several studies have clearly shown a decrease in DHA amount in the PE in the hippocampus and the cortex of patients with Alzheimer’s disease (review in Cunnane et al., 2009).

The reduced DHA level in brain phospholipids may be due to a reduction in the activity of the enzymes allowing the incorporation of DHA into brain phospholipids (Andre et al., 2006; Giusto et al., 2002), but other changes in DHA metabolism during aging may contribute to a decrease in brain DHA volume.

In mammals, DHA can be synthesized from its nutritionally essential plant-derived precursor, α-linolenic acid (α-LNA, 18:3n-3), through a series of elongation and desaturation steps in the endoplasmic reticulum followed by peroxisomal β-oxidation (Sprecher, 2000). Although the liver is the major site of DHA synthesis, it can also be synthesized locally in the brain but with much less efficiency (Rapoport et al., 2010). Indeed, primary cultures of rat hippocampal neurons and astrocytes were able to synthesize and incorporate DHA from radiolabeled α-LNA, but the amounts produced were considerably less than those incorporated when the culture was incubated with radiolabeled DHA (Kaduce et al., 2008; Williard et al., 2001). Human and rodent studies using stable isotopes have concluded that less than 1% of dietary α-LNA is used to form longer chain n-3 fatty acids (Plourde and Cunnane, 2007). However, it now seems clear that in rats on an adequate α-LNA containing diet, the liver is capable of synthesizing sufficient DHA from circulating α-LNA to maintain a normal brain DHA level in the absence of dietary EPA or DHA (Rapoport and Igarashi, 2009). Nevertheless, DHA may also be obtained directly from dietary sources, particularly from fatty fish, but also from n-3 PUFA enriched foods or from fish oil containing both EPA and DHA in the form of nutritional supplements (Gebauer et al., 2006; Whelan and Rust, 2006). Different fish oils provide different ratios of EPA/DHA but also differ in their digestibility and oxidation properties (Tou et al., 2011). In humans, intravenously injected radiolabeled DHA rapidly entered the brain and accumulated preferentially in the gray matter in the neocortex (Uhmah et al., 2009). Studies in mice using radiolabeled DHA and EPA suggest that these compounds cross the blood–brain barrier to accumulate in the brain by a simple diffusion process (Ouellet et al., 2009). Most reports comparing DHA versus α-LNA intake suggest that the maximal concentration in the brain can only be achieved by including DHA in the diet (Arsenault et al., 2012; Brenna et al., 2009; Talahalli et al., 2010). Thus, it is questionable whether α-LNA can be as efficient as DHA in maintaining brain DHA levels and neurobiological functions (Barcelo-Coblijn and Murphy, 2009).

Several studies suggest that aging alters n-3 PUFA metabolism. It has been shown that hepatic delta-6-desaturase activity, the first and rate limiting enzyme in the conversion of α-LNA to LC n-3 PUFA, decreases during aging in rat and mouse (Bourre and Piciotti, 1992; Bourre et al., 1990; Dinh et al., 1993; Hrelia et al., 1989). The brain obtains DHA synthesized in the liver directly from the plasma unesterified fatty acid pool (Chen et al., 2008) and an age-related decrease in hepatic DHA biosynthesis may change plasma fatty acid concentrations. A recent study shows that the liver synthesis secretion rate of DHA is reduced in 20- and 30-month-old rats compared with 10-month-old rats.
DHA is Involved in Learning and Memory

One of the classical approaches to studying the role of a nutrient in cognitive functions and more specifically in memory is to induce a dietary deficiency of this nutrient in animals. However this is not easy to achieve for the DHA, since the adult mammalian brain tissue is predominantly composed of lipids and the brain has homeostatic mechanisms which delay pathological effects of reduced n-3 PUFA intake by increasing DHA half-life from 33 to 90 days in post-weaning n-PUFA deficient rats (DeMar et al., 2004). For these reasons, in general, brain DHA deficiency is induced by feeding an n-3 fatty acid deficient diet in utero (via the maternal intake) and during one to three generations, thus even leading to an 80% decrease in brain DHA levels (Moriguchi et al., 2000). In order to study the role of n-3 PUFAs in memory, several studies have investigated the effects of n-3 PUFA deficiency on animal behavior using different tasks. Most of these studies demonstrate that n-3 PUFA deficiency impairs cognitive functions and mainly learning and memory capacities (review in Fedorova and Salem, 2006). For example, using a multi-generational deficiency model, it has been shown that n-3 PUFA deprivation in rats results in reduced performance in learning and reference memory tasks evaluated in the Morris water maze (Moriguchi et al., 2000). Moreover, these authors have also shown that in n-3 PUFA deficient rats repleted with α-LNA and DHA, the degree of brain DHA level and spatial performance recovery in the Morris water maze depended upon the duration of dietary repletion (Moriguchi and Salem, 2003). These results demonstrate a positive correlation between brain DHA content and spatial memory performance in deficient or repleted animals.

Other studies have addressed the effects of dietary DHA supplementation in young animals without n-3 PUFA deprivation. Young (2-month-old) adult rats receiving fish oil by gavage during brain development and for 80 days of adulthood had enhanced performance in reference and working memory, as evaluated in the Morris water maze, and displayed an increase in DHA content in the hippocampus (Chung et al., 2008a). This study indicates that DHA may modulate memory capacities in animals without n-3 deprivation and that a link exists between DHA levels in the hippocampus and memory performance. The relationship between the duration of DHA intake and maze-learning ability was also studied. Dietary intake of DHA (2 g DHA-ethyl ester/100 g diet) improved maze-learning ability in mice after 1 month of feeding and was maintained for up to 3 months, whereas the increased DHA level in the brain was apparent for 2 weeks after feeding but not after 1 week (Lim and Suzuki, 2001). These results suggest that a sufficient level of brain DHA must be reached to induce an improvement in learning abilities. Tanabe et al. (2004) reported that chronic administration by gavage of fish oil in 5-week-old rats for 12 weeks improved reference and working memory using an eight-arm radial maze paradigm. The beneficial effect of fish oil supplementation on memory was associated with an increase of c-Fos positive neurons in the hippocampal CA1 region (Tanabe et al., 2004). Recent studies have also investigated the effect of DHA specifically in the adult hippocampus by using an electrophysiological approach. DHA supplementation (1% DHA from algal source in the diet) in mice from 6 to 12 months of age increased DHA content in total phospholipids by 29% in the hippocampus and enhanced hippocampal synaptic
transmission following brief high-frequency stimulation (Connor et al., 2012). Another study compared the effect of α-LNA or DHA on the electrophysiological properties of the entorhinal cortex neurons, a brain region linked with the hippocampus and involved in memory function. Mice exposed from 4 to 14.5 months of age to a diet enriched in DHA (1.14 mmol/kg/day) displayed an increase in DHA concentration in the cortex (+34%), whereas the DHA-increasing effect of an α-LNA diet (1.5 mmol/kg/day) was lower (+23%), when compared with control animals (Arsenault et al., 2012).

Moreover, only the DHA diet was able to increase the passive electrical properties of entorhinal cortex neurons. These authors suggest that α-LNA is partly converted to DHA, as reflected by the increased DHA concentrations in the cortex, but insufficiently to modulate the electrophysiological properties of entorhinal cortex neurons.

A study in humans has investigated the potential association between cognitive performance and serum phospholipid levels of DHA, EPA, and ALA in healthy middle-aged adults (Muldoon et al., 2010). The results indicated that DHA was associated with cognitive functioning, as well as with better scores on tests of nonverbal reasoning, mental flexibility, working memory, and vocabulary. In contrast, EPA and ALA were unrelated to any measure of cognitive performance. In a recent randomized controlled trial, 176 healthy adults aged between 18–45 years of age with a low intake of DHA were supplemented with 1.16 g DHA per day over a 6-month period and cognitive performance was assessed using a computerized cognitive test battery (Stonehouse et al., 2013). Reaction time of working memory in men and episodic memory in women were improved in the DHA supplemented group when compared with the placebo group.

Altogether these data suggest that brain DHA plays an important role in the maintenance of learning and memory performance in adults and that DHA may improve memory performance by enhancing synaptic plasticity in the hippocampus.

Since DHA levels decrease with age and as this nutrient plays a major role in brain function, mainly in learning and memory, several studies, both in animals and humans, have investigated the effects of LC n-3 PUFA supplementation, mainly in the form of DHA or fish oil, on memory performance during aging. Old age is the main risk factor for Alzheimer’s disease since its prevalence increases with age. Numerous studies have investigated the effects of LC n-3 PUFA supplementation in Alzheimer’s disease patients or in animal models of this pathology (review in Boudrault et al., 2009; Cole et al., 2009; Cunnane et al., 2009; Huang, 2010). A recent meta-analysis showed that in experimental animal models of Alzheimer’s disease, an LC n-3 PUFA supplementation improved cognitive function and reduced the amount of brain amyloid-β (Hooijmans et al., 2012).

However, in humans, randomized controlled trials (RCTs) have failed to demonstrate a benefit of LC n-3 PUFA supplementation in patients with Alzheimer’s disease, except in subjects with the mild form of the disease or mild cognitive impairments (MCI), supporting the idea of a preventive effect of LC n-3 PUFAs rather than a potential treatment modality (Solfrizzi et al., 2010). Thus, in a goal of nutritional prevention of Alzheimer’s disease, the LC n-3 PUFA supplementation must be given before irreversible damage has accumulated in the brain of Alzheimer’s disease patients. For this reason, in this review we only reported studies investigating the effects of LC n-3 PUFA supplementation during normal aging in humans, i.e. subjects with MCI or healthy elderly, or in animals, excluding experimental Alzheimer’s disease models.

Animal Studies

Several studies have investigated the effects of LC n-3 PUFA supplementation on learning and memory in rodents during aging. They mostly differ depending on the animal species, the route of administration, the dose and source of the LC n-3 PUFA, the duration of the supplementation, and the behavioral test used to evaluate memory. The great strength in animal models compared to human clinical trials or epidemiological studies is that they afford the opportunity to control experimental variables such as the composition of the diet and the environment.

The few studies that have investigated the effects of fish oil containing both EPA and DHA, in various ratios, in very old animals (20–25 months) when compared with young ones (1–5 months) on memory performance during aging give conflicting results. Two studies conducted in 2-year-old rats have not evidenced any effect of a fish oil supplementation over periods of either 1 or 4 months on learning and memory capacities (Barcelo-Coblijn et al., 2003a; Sergeant et al., 2011). Barcelo-Coblijn et al. (2003a) have shown that 2-year-old rats receiving a fish oil enriched diet (11% DHA + 3% EPA) for one month did not perform better in the Morris water maze learning test than the 2-month-old control rats. However, these authors only evaluated the learning ability of the rats to find the hidden platform in the Morris water maze over a
5-day period, but the memory performance using the classical probe test without platform was not evaluated. More recently, when using rats of the same age as in the Barcelo-Coblijn study but supplemented with fish oil (12% DHA + 18% EPA) for a longer period (4 months), Sergeant et al. (2011) did not observe any beneficial effect of fish oil supplementation on spatial learning or reference memory performance in very old rats (28 months) when compared with 6-month-old rats. Altogether these results suggest that dietary fish oil supplementation given at a late stage of life is not effective in the prevention of age-related memory decline. In a recent study, Labrousse et al. (2012) investigated the preventive effects of a two-month fish oil enriched diet on age-related memory decline in 20-month-old mice. The results of this study have shown that spatial working memory deficits evaluated in the Y-maze were reduced in aged mice fed with the fish oil enriched diet (10% EPA and 7% DHA) when compared with aged mice receiving a DHA-free diet (Labrousse et al., 2012). The effect of life-long consumption of fish oil was studied by Carrière et al. (2000b). Pregnant OF1 mice were fed on diets enriched with or without fish oil (7% DHA + 12% EPA), offspring were maintained on these diets after weaning, and behavioral tests were performed at 7–10 weeks, 9–10 months, and 17–19 months of age. A beneficial effect of fish oil supplementation on spatial reference memory performance, evaluated by the retention of platform location in the Morris water maze, was only observed in the group of mice aged 9–10 months, but not in younger or older mice. In an active avoidance paradigm, mice aged 7–10 weeks that were supplemented with fish oil performed better during the first training session than age-matched controls, whereas the opposite effect was observed in the oldest supplemented mice. This group displayed worse avoidance behavior, suggesting a negative effect of fish oil supplementation on learning performance on this task. Since PUFAs are readily oxidizable, the authors hypothesized that long-term fish oil supplementation may result in the formation of oxidation products in the brain leading to decreased memory performance (Carrière et al., 2000b).

In an as yet unpublished study we have investigated the potential preventive effect of fish oil on memory decline during aging when the supplementation is given at middle-age. Our results show that fish oil supplementation (18% EPA and 12% DHA) in the diet for 5 months in middle-aged rats (from 13 to 18 months of age) improved spatial working memory, but not reference memory, when evaluated by a delayed matching-to-place paradigm in the Morris water maze (Alfos et al., unpublished data). Two other studies have recently been conducted on a non-human primate, the gray mouse lemur (Languille et al., 2012; Vinot et al., 2011). Languille et al. (2012) observed that 14 weeks of fish oil supplementation (6 mg EPA and 30 mg DHA per day) in 6- to 9-year-old female mouse lemurs did not significantly improve spatial memory performance in the Barnes maze task, even if the fish oil diet supplemented animals tended to find the target compartment more frequently than the control animals when tested after a 24-hour delay. These results are in contrast to those by Vinot et al. (2011), whose study findings showed that the same fish oil supplementation (6 mg EPA and 30 mg DHA per day) for 5 months in 2-year-old mouse lemurs improved spatial memory performance in the Barnes maze task. It should be noted that for mouse lemurs, the median survival time is 4.9 years and the maximum lifespan is 8–10 years. Thus, some of the animals used in the study of Languille et al. were old and others were middle-aged. This difference in age may contribute to the great variability of the results obtained, leading to a non-significant effect of fish oil in this study, whereas in the study by Vinot et al. the animals were younger. These results suggest that fish oil supplementation in aged animals may have beneficial effects on the prevention of memory decline if it is given at middle-age rather than later in life.

As fish oil is composed of both EPA and DHA at p0.05 various concentrations, it is difficult to distinguish the specific effects of DHA or EPA in supplementation studies. For this reason and because DHA is the main LC n-3 PUFA in the brain, some authors have investigated the specific effect of DHA alone, and more rarely of EPA alone, on learning and memory performance in aged animals. Gamoh et al. (2001) demonstrated, in 25-month-old rats fed a fish oil deficient diet for three generations, that peroral administration of DHA 300 mg/kg/day, in the form of ethyl ester for 10 weeks, improved working and reference memory, as evaluated in a partially baited eight-arm radial maze. This effect of DHA supplementation on memory performance was associated with an increase of DHA levels in the cortex but not in the hippocampus and a decrease in lipid peroxide levels in the hippocampus (Gamoh et al., 2001). The effect of a DHA-fortified oil fraction extracted from a green alga, Chlorella vulgaris, containing 20% DHA and only 3% EPA of total fatty acids, was also investigated in middle-aged mice (Sugimoto et al., 2002). The authors showed that DHA-fortified oil fraction supplementation (2 g/100 g diet) for 2 months in 9-month-old mice increased brain levels of DHA and EPA, and improved working memory, but not reference memory, in a partially baited eight-arm radial maze, compared to control mice receiving a standard diet. Two studies compared the dose effect of DHA supplementation on memory in
aged animals. In the first study, Lim and Suzuki (2000) investigated the effect of different doses of DHA on maze-learning ability in young (3-week-old) and middle-aged (14-month-old) mice. Mice were fed for 5 months with diets enriched with different sources of DHA, ethyl ester, or/and PC from eggs. Thus, the experimental diets contained different amounts of DHA: 0.9 g/100 g fatty acids in the egg-PC group, 13.8 g/100 g in the egg-PC + DHA-ethyl ester group, and 23.7 g/100 g in the DHA-ethyl ester group. Globally, old mice supplemented with DHA, regardless of form or dose, took less time to reach the maze exit and strayed fewer times in the blind alleys of the maze, showing an increased learning ability. Jiang et al. (2009) also investigated the effects of two oral gavage doses of DHA, 50 mg or 100 mg DHA/kg/day, for 7 weeks, on the cognitive ability of 15-month-old female mice assessed by step-through and passageway water maze tests. The results showed that 7-week DHA supplementation in middle-aged mice significantly improved learning and memory compared to control mice and also demonstrated a dose effect of the DHA on memory performance. More recently, Kelly et al. (2011) studied the effect on memory performance in aged rats of two specific LC n-3 PUFAs, EPA and DPA, which is an intermediate product in the biosynthesis of DHA from EPA (Sprecher, 2000). Compared with aged control animals, 20–22-month-old rats supplemented with EPA or DPA (200 mg/kg/day) for 56 days performed better in a spatial learning task using the Morris water maze (Kelly et al., 2011). Moreover, the EPA supplementation, but not the DPA, induced an increase in DHA level in the cortical tissue of aged rats. The animal studies described above globally demonstrate that fish oil supplementation in aged animals has beneficial effects on the prevention of memory decline if it is given at middle-age rather than later in life. Moreover this result could be more specifically attributed to DHA since a DHA supplementation alone leads to an improvement of memory in aged animals. However, in the fish oil containing both EPA and DHA, the EPA can be efficiently converted to DHA in the liver (Gao et al., 2009). The beneficial effect of fish oil supplementation on memory observed in animals during aging must be confirmed in humans by RCTs.

**Human Studies**

Several epidemiological studies have investigated the link between n-3 PUFA intake or fish consumption and cognitive decline in the elderly (Fotuhi et al., 2009). Most of these studies suggest that an EPA + DHA intake in the form of fatty fish can reduce the risk of cognitive decline in elderly subjects with cognitive impairment no dementia (CIND) i.e. subjects with MCI or memory complaint without a dementia diagnosis of Alzheimer’s disease. In the Etude du Vieillissement Artériel (EVA) study, erythrocyte membrane lipid composition was used as a measure of omega-3 fatty acid intake in 246 healthy men and women whose cognitive function was assessed by the Mini-Mental State Examination (MMSE). The results showed that the proportion of total n-3 PUFAs in the erythrocyte membrane was inversely associated with the risk of cognitive decline (Heude et al., 2003). In the Atherosclerosis Risk in Communities study based on 2,251 subjects, the risk of global cognitive decline increased with elevated plasma palmitic acid, and arachidonic acid (AA) and higher n-3 PUFA in the plasma reduced the risk of decline in verbal fluency (Beydoun et al., 2007). Moreover, lower red blood cell DHA levels were associated with smaller brain volumes and reduced performance on tests of visual memory, executive function, and abstract thinking in persons free of clinical dementia (Tan et al., 2012). Two other studies also showed that cognitive test scores in elderly subjects were higher in fish oil supplement users than in non-users and this was associated, in one study, with an increase in erythrocyte membrane n-3 fatty acid content in fish oil supplement users (Gao et al., 2011; Whalley et al., 2004). Other cross-sectional or longitudinal population-based studies have shown that fatty fish intake was associated with a reduced risk of impaired cognitive function in non-demented subjects (Kalmijn et al., 2004; Kesse-Guyot et al., 2011; Morris et al., 2005; Roberts et al., 2010; van Gelder et al., 2007). In the Zutphen Elderly Study, the authors estimated that an average difference in daily DHA + EPA intake of approximately 380 mg is associated with a 1.1 point reduction in cognitive decline over 5 years (van Gelder et al., 2007).

These beneficial effects of fatty fish consumption observed in epidemiological studies, associated with the results obtained in animals studies, have encouraged the set-up of RCTs to investigate the protective properties of DHA and other n-3 PUFAs on cognitive performance in the elderly. A recent meta-analysis has examined the neuropsychological benefits of n-3 PUFAs in randomized double-blind placebo-controlled studies in healthy, CIND or Alzheimer’s disease subjects (Mazereeuw et al., 2012). This meta-analysis of studies published up to September 2011 combined 10 RCTs in subjects over 65 years: among them two RCTs were specifically conducted on Alzheimer’s disease subjects, three in healthy elderly, four in subjects with MCI or memory complaint and one in both Alzheimer’s disease and MCI subjects. The authors of this meta-analysis concluded that n-3 PUFA
supplementation has no benefit on global cognitive performance in Alzheimer’s disease patients as measured by the MMSE or the Alzheimer’s Disease Assessment Scale-Cognitive Subscale (ADAS-cog). There was no global effect of n-3 PUFAs on composite memory but when examined by domain, the meta-analysis indicated that the effects of n-3 PUFAs were limited to CIND subjects for immediate recall, attention, and processing speed. No effect was observed in healthy elderly subjects or those with Alzheimer’s disease even when cognitive subdomains were assessed. In the five studies including CIND subjects only, DHA supplementation had a beneficial effect mostly in verbal fluency (Johnson et al., 2008; Sinn et al., 2012), immediate and delayed verbal recognition memory, but not on working memory (Yurko-Mauro et al., 2010), immediate memory and attention (Kotani et al., 2006), or immediate and delayed verbal recall and learning abilities (Vakhapova et al., 2010). It should be noted that these studies differ greatly in duration (from 12 weeks to 108 weeks), in the dose of n-3 PUFA intake (from 240 to 1550 mg per day), and in the form of n-3 PUFA supplementation (DHA alone, DHA-PS or DHA + EPA). From this previous meta-analysis new RCTs have been published partly confirming its conclusions. In a small crossover placebo-controlled trial, 40 healthy subjects aged 51–72 years receiving 3 g per day of fish oil for 5 weeks (EPA 1500 mg and DHA 1050 mg) displayed better performance in a working memory test (Nilsson et al., 2012). In another small RCT conducted on seventy 45- to 80-year-old healthy subjects who received 252 mg DHA per day over a 90-day period, no beneficial effect of the supplementation on the cognitive function was observed (Stough et al., 2012). Geleijnse et al. (2012) conducted a large double-blind placebo-controlled trial (Alpha Omega Trial) including 2,911 coronary patients aged 60 to 80 years old consuming margarines (400 mg/day of EPA-DHA and/or 2 g/day of α-LNA) but observed no effect on global cognitive functions measured by the MMSE after 40 months of n-3 PUFA consumption. These two negative studies are in agreement with the meta-analysis of Mazereeuw et al. (2012) showing no effect of n-3 PUFA supplementation either in Alzheimer’s disease or CIND subjects when cognitive functions were only evaluated by the MMSE test, nor in elderly healthy subjects. More recently, an RCT conducted on 36 low socioeconomic-status elderly subjects between 61 to 71 years of age with MCI receiving a daily capsule of 430 mg of DHA and 150 mg of EPA for 12 months showed an improvement in short-term and working memory, immediate verbal memory and delayed recall capability in the fish oil group (Lee et al., 2013). This last study confirms the beneficial effect of fish oil supplementation in MCI subjects.

There are now nine RCTs that have assessed the effect of long-chain n-3 PUFA supplementation on cognitive function in elderly subjects with MCI. Results of these studies indicate that a high LC n-3 PUFA intake (>200 mg/day of DHA) slows down cognitive decline among elderly subjects with MCI without dementia. Such an effect was not observed in patients with Alzheimer’s disease, suggesting that LC n-3 PUFAs are less efficient on memory performance in the later course of the disease. Thus increasing the intake of LC n-3 PUFAs may be a strategy to maintain memory performance in the elderly. Other RCTs that are still in progress should finally ascertain the efficacy of LC n-3 PUFA supplementation alone or in combination with other nutrients in the prevention of cognitive decline in the elderly (Fotuhi et al., 2009).

The results obtained on animals, together with epidemiological and RCT evidence, support the idea that LC n-3 PUFAs given in the form of fish oil may have a role in the prevention of age-related memory decline in subjects with mild cognitive impairment. The question still to be addressed is how LC n-3 PUFAs, and mainly DHA, act on neurobiological processes to maintain cognitive function during aging.

DHA IMPROVES SYNAPTIC PLASTICITY DURING AGING: INVOLVEMENT OF RETINOID X RECEPTORS AND PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS

LC n-3 PUFAs may have multiple and complex mechanisms of action in the cells. Among them: 1) they could modulate the structure and the function of neuronal membranes by modifying the composition of phospholipids and/or by acting on membrane-incorporated proteins such as G-protein coupled surface receptors; 2) they can also be metabolized into lipid mediators which are involved in the regulation of many tissue functions (Calder, 2012; Schmitz and Ecker, 2008). Moreover LC n-3 PUFAs can act as direct ligands of transcription factors that modulate gene expression (Afman and Muller, 2012). Indeed, animal experiments and human studies have shown that LC n-3 PUFAs regulated gene expression in various tissues. Some studies on the effect of fish oil supplementation on gene expression have been conducted in humans by using microarray technology in peripheral blood mononuclear cells (PBMC) that are easily obtainable. In elderly subjects aged between 66 and 80 years, fish oil supplementation for 26 weeks changed the expression of 1,040 genes in PBMC leading to a more anti-inflammatory and anti-atherogenic gene expression profile (Bouwens et al., 2009). More recently, it
has been shown that 6-month fish oil supplementation in elderly subjects with Alzheimer’s disease induced up- or down-regulation of several genes in PBMC (Vedin et al., 2012). Further studies in animals have demonstrated that fish oil supplementation in mice and rats modulated the expression of several genes in the liver and the brain, and more specifically in the hippocampus, both in adult (Barcelo-Coblijn et al., 2003b; Berger et al., 2002) and old animals (Barcelo-Coblijn et al., 2003a; Kitajka et al., 2002; Puskas et al., 2003).

Through these effects on gene expression, LC n-3 PUFAs may modulate the expression level of synaptic plasticity-related genes that sustain learning and memory processes impaired during aging (Blalock et al., 2003; Lee and Silva, 2009). Indeed, EPA or DHA supplementation in aged rats (20–22 months old) for 2 months reversed the age-related decrease in long-term potentiation (LTP) in the hippocampus (Martin et al., 2002; McGahon et al., 1999). Moreover, Kelly et al. (2011) have shown that the beneficial effect of EPA or DPA (200 mg/kg/day) supplementation for 56 days on a spatial learning task in rats aged 20–22 months old was associated with an improvement of synaptic plasticity since DPA and EPA supplementation can attenuate the age-related decrease in LTP in the hippocampus. In adult (12-week-old) fat-1 transgenic mice with high endogenous DHA levels compared to wild-type mice, expression of synaptic plasticity-related genes such as synapsin-1, glutamate receptor subunit GluR1, neuregulin (GAP-43) and post-synaptic density protein-95 (PSD-95) were up-regulated in the hippocampus and water maze memory performance was improved (He et al., 2009). In the aged (24-month-old) rat brain, the decrease in the levels of GluR2 and NR2B glutamate receptor subunits involved in synaptic plasticity was reversed after a 12-week fish oil supplementation (160 mg EPA and 110 mg DHA/kg per day) (Dyall et al., 2007). In an as yet unpublished study we have shown that a 5-month fish oil supplementation (18% EPA and 12% DHA) in the diet of middle-aged rats (from 13 to 18 months old) restored GAP-43 mRNA levels in the hippocampus to that of adult rats and induced an increase in hippocampal RC3 mRNA expression (Alfos et al., unpublished data). This effect of fish oil supplementation on synaptic plasticity was associated with an improvement of spatial working memory in aged rats (Alfos et al., unpublished data). Altogether, these results suggest that LC n-3 PUFAs might improve or maintain memory performance by, partly, modulating the expression of synaptic plasticity-associated genes in the aging brain, thus reinforcing the strength of connections between neurons.

LC n-3 PUFAs regulate gene expression by binding to a variety of nuclear receptors including those belonging to the steroid/thyroid hormone nuclear receptor superfamily (Vanden Heuvel, 2012). Indeed, it was initially reported in the 1990s that EPA, DHA, and some of their derivatives act as endogenous ligands of the peroxisome proliferator-activated receptors (PPARs) (Krey et al., 1997). More recently, DHA has also been shown to bind the retinoid X receptors (RXRs), primarily involved in the transduction of the retinoid signaling pathway (de Urquiza et al., 2000; Lengqvist et al., 2004). RXRs can also be activated by 9-cis retinoic acid (9-cis RA), one of the active metabolites of vitamin A, and this receptor is the obligate common dimerization partner of numerous nuclear receptors, including PPARs, retinoic acid receptors (RARs, activated by all-trans retinoic acid), the vitamin D receptor (VDR), thyroid hormone receptors (TRs) (Desvergne, 2007; Lefebvre et al., 2010). Therefore, RXRs are a master regulator of multiple distinct biological pathways (Dawson and Xia, 2012). Nevertheless, there is a debate regarding the physiological ligands of RXRs (Wolf, 2006), as 9-cis RA is very difficult to detect in vivo because of its instability and its very low concentration (Kane, 2012), the RXRs are a converging point in mediating the physiological effects of retinoids and LC n-3 PUFAs either by directly binding retinoids and DHA or by acting as a heterodimerization partner of RARs and PPARs.

It is now well established that these nuclear hormone receptors act as transcription factors which, upon activation by their ligands, modulate the transcription of several target genes by binding on specific sequences on DNA (Chambon, 1996; Gronemeyer et al., 2004). Several isotypes of these receptors are widely expressed in various regions of the adult brain and notably in the hippocampus, both in rodents and humans, suggesting a role of these receptors in brain functions (Krezel et al., 1999; Moreno et al., 2004; Rioux and Arnold, 2005; Zetterstrom et al., 1999). In the adult brain, these nuclear receptors modulate the transcription of target genes involved in synaptic plasticity processes, suggesting that these nuclear hormone receptors play major roles in learning and memory (Lane and Bailey, 2005; McCaffery et al., 2006; van Neerven et al., 2008). Fatty acids can modulate the expression of some nuclear receptors such as PPARs, RARs, and RXRs. Indeed, Hajjar et al. (2012) have shown that 10-week fish oil consumption in young rats improved spatial learning and memory in the Morris water maze and increased the expression of PPARβ and PPARγ in the hippocampus. Moreover, we have also shown that a high-fat diet in rats induced a decreased expression of RARβ and PPARγ mRNA and an increased expression of RXRβ/γ associated with changes in the expression level of RC3 and GAP-43, both in the striatum and hippocampus (Buaud et al., 2010).
Using a gene microarray approach, Blalock et al. (2003) have shown that RXR\(\gamma\) gene expression was one of the several genes down-regulated in the CA1 region of the hippocampus in aged (24-month-old) and middle-aged (14-month-old) rats. This age-related down-regulation of RXR\(\gamma\) gene expression was positively correlated with impaired memory performance evaluated in the Morris water maze task and the object memory task. Moreover, it has recently been demonstrated in mice that the reduced despair behavior and improved working memory in mice treated with DHA implicated more specifically the RXR\(\delta\) isoform (Wietrzych-Schindler et al., 2011). These results highlight the major role of RXR\(\gamma\) in the maintenance of memory processes.

We have also previously shown that aging in rodents induced a decrease in the expression of some nuclear hormone receptors, mainly RAR\(\beta\) and RXR\(\delta/\gamma\) in the brain and more particularly in the hippocampus, which was associated with a decreased expression of some of their target genes involved in synaptic plasticity such as neurogranin (RC3) and GAP-43 (Boucheron et al., 2006; Enderlin et al., 1997; Feart et al., 2005). This hypo-expression of nuclear receptors and target genes involved in synaptic plasticity in the aging brain can be reversed by RA administration or vitamin A supplementation which also reverses the age-related memory deficit (Etchamendy et al., 2001; Mingaud et al., 2008). As for the retinoids, we can postulate that an age-related decrease in the brain level of DHA leads to a hypo-activation of fatty acid nuclear receptors and of their target genes which can be reversed by LC n-3 PUFA supplementation. In fact, we have recently demonstrated that a 5-month fish oil enriched diet in middle-aged rats (13 to 18 months old) suppressed the age-related decrease in RXR\(\gamma\) mRNA expression and increased RAR\(\beta\) mRNA expression in the hippocampus (Alfos et al., unpublished data). This effect of fish oil supplementation on RXR expression was concomitantly associated with an increase in GAP-43 and RC3 mRNA, together with an improvement of spatial working memory in aged rats (Alfos et al., unpublished data). Dyall et al. (2007) also observed that the age-related decrease in RXR\(\alpha\), RXR\(\beta\) and PPAR-\(\gamma\) protein levels in the rat forebrain were reversed by 12 weeks of fish oil supplementation.

In order to activate nuclear receptors or to be converted in biologically active derivatives such as neuroprotectin D1 (NPD1) in the cells, DHA must either be directly captured from the plasma in the unesterified form or released from membrane phospholipids by Ca\(^{2+}\)-independent phospholipase A2 (iPLA2) (Green et al., 2008; Rapoport et al., 2011). As reported above, aging in rats is associated with a reduced unesterified DHA concentration in the plasma (Gao et al., 2013). Moreover, the iPLA2 mRNA expression decreased in the rat hippocampus during aging whereas the mRNA expression of cPLA2, involved in AA release from membrane phospholipids, was unchanged (Aid and Bosetti, 2007). These data, in combination with those showing a decrease of DHA in brain phospholipids, suggest that the brain intracellular level of DHA may be reduced during aging, thus leading to a hypo-activation of nuclear receptors which can be reversed by fish oil supplementation.

Taken together, these data suggest that one of the mechanisms supporting the effects of LC n-3 PUFAs on synaptic plasticity that underlies memory processes may be the regulation of gene expression in the brain via nuclear hormone receptors and mainly PPARs and RXRs which are able to bind LC n-3 PUFAs and their derivatives. Aging is associated with a decrease in some of these nuclear receptors in the brain, which may reflect a reduction in intracellular DHA level, leading potentially to impaired gene expression. Therefore, LC n-3 PUFAs contained in fish oil may act as positive nutritional modulators that activate RXRs and PPARs and therefore restore normal gene expression and signaling pathways in the brain.

**SUMMARY AND CONCLUDING REMARKS**

An analysis of the data from animal studies indicates that aging is associated with a reduction in DHA levels in specific classes of phospholipids in brain regions involved in memory such as the hippocampus and the cortex. These animal study findings are in contrast to those from studies in humans, which found a slight or no decrease in brain DHA contents during normal aging and a clear decrease in DHA levels in the hippocampus and the cortex in Alzheimer’s disease. These contrasting results in human post-mortem studies may be linked to variations in the age and cognitive status of the subjects studied, but also to variations in life-long consumption of fatty acids among subjects that may influence brain fatty acid content. In animal studies, environmental factors and, in particular the diet, are easily controllable and standardized, thus allowing for fewer variations in the results. The reduced DHA content in brain phospholipids during aging could be the result of a reduction in the activity of the enzymes specifically incorporating DHA in these phospholipids. But an altered metabolism of LC n-3 PUFAs in the liver, as brain DHA is obtained from the unesterified fatty acid pool of the plasma which is maintained by liver synthesis, could also contribute to the decrease in brain DHA content. In order to confirm that intracellular DHA bioavailability is reduced in the
Considering the major role of DHA and, to a greater extent, LC n-3 PUFAs in the maintenance of synaptic plasticity processes that underlie learning and memory, a reduction in brain DHA level during aging may contribute to memory impairment in the elderly. Therefore LC n-3 PUFA supplementation in the form of fish oil could be one of several valuable strategies to prevent age-related memory decline. Indeed, an analysis of the data presented in this review shows that, in most animal and human studies, a fish oil supplementation has a beneficial effect on the prevention of memory decline during aging. However, in humans, RCTs demonstrate that fish oil supplementation was only able to slow down memory decline in subjects with middle cognitive impairments but not in patients with Alzheimer’s disease. These results indicate that LC n-3 PUFAs act as preventive nutrients on cognitive decline during aging but are less efficient when pathophysiological changes may have accumulated to an irreversible degree.

Although LC n-3 PUFAs are major constituents of the brain and play a major role in its functioning, other nutrients that are also involved in the maintenance of normal brain functions (review in Bourre, 2006a; 2006b) could also be effective in the improvement of memory during aging. Indeed, studies in rodents have demonstrated that food supplements containing DHA and EPA, in association with other nutrients such as B vitamins, vitamin E, choline, uridine, and phospholipids, increased the number of dendritic spines in the hippocampus, increased the levels of synaptic proteins, enhanced synaptogenesis and improved memory (de Wilde et al., 2011; Wurtman et al., 2010). However, in humans, as for the single LC n-3 PUFA supplementation, the combined supplementation of LC n-3 PUFAs with other nutrients for 12 to 24 weeks improved memory performance (delayed verbal recall) only in the patients with very mild Alzheimer’s disease (Scheltens et al., 2010; 2012). Several other RCT studies using LC n-3 PUFA with a combination of other nutrients that are ongoing or in preparation will broaden our understanding of the nutritional prevention of memory decline in elderly healthy or MCI subjects (Mi et al., 2013).

Several mechanisms of action could be involved in explaining the beneficial effect of EPA and DHA on the prevention of memory decline during aging. Here we postulate that LC n-3 PUFAs act on synaptic plasticity via nuclear hormone receptors that regulate gene expression. Indeed LC n-3 PUFAs and their derivatives are ligands of PPARs and RXRs that are involved in the maintenance of learning and memory by regulating genes involved in synaptic plasticity processes. Animal studies suggest that LC n-3 PUFA supplementation during aging could restore hippocampal RXR levels, and mainly RXRγ, that in turn up-regulates synaptic plasticity-associated target genes leading to a reduction in age-related memory decline. Recent study results highlight the major role of RXRγ in the maintenance of adult brain function. Indeed it has been shown that RXRγ is involved in learning and memory processes (Wietrzych-Schindler et al., 2011) and in remyelination processes after brain injury (Huang et al., 2011). It is now well accepted that RXRs play unique modulatory and integrative roles as a converging point in mediating the physiological effects of retinoids and LC n-3 PUFAs either by directly binding retinoids and DHA or by acting as a heterodimerization partner of RARs and PPARs. These data suggest that the beneficial effect of LC n-3 PUFAs on gene expression and memory could be in part mediated by the RXRs, and more particularly RXRγ, by acting as a lipid sensor in the cell or by integrating other nutritional signals.

Besides their action on the transcriptional regulation of synaptic plasticity-related genes, nuclear hormone receptors are known to regulate the expression of genes involved in numerous functions in the adult brain that can also be modulated by LC n-3 PUFAs such as inflammation and neurogenesis. Indeed, LC n-3 PUFAs have anti-inflammatory properties by modulating the expression of cytokines and are able to reduce the naturally occurring neuro-inflammation in the aging brain (Calder, 2011; Laye, 2010). PPAR and RXR agonists modulate neuro-inflammation processes mainly by inhibiting pro-inflammatory gene expression (Chung et al., 2008a; Moraes et al., 2006; Xu et al., 2005). LC n-3 PUFAs have also been shown to promote hippocampal neurogenesis both in adult and aged animals (Dyall et al., 2010; He et al., 2009; Kawakita et al., 2006). Several results suggest that nuclear hormone receptors such as PPARs and RARs/RXRs play major roles in neurogenesis by modulating the survival and differentiation of newly proliferated cells (Bonnet et al., 2008; Yu et al., 2012). Other studies are needed to determine the complex mechanisms of action of LC n-3 PUFAs on memory processes during aging.

As previously discussed, it is now well recognized that vitamin A and its derivatives, the retinoids, play major roles in the synaptic plasticity process and in learning and memory in the adult brain. More specifically, results from our laboratory have demonstrated that pharmacological activation of retinoid signaling by short-term treatment with RA, the active metabolite of vitamin A, or life-long nutritional vitamin A supplementation, have beneficial effects on hippocampal plasticity and restore impaired memory performance in aged mice (Etchamendy et al., 2001;
Mingaud et al., 2008). In the same way our recent results in middle-aged rats supplemented for 5 months with fish oil show a beneficial effect of LC n-3 PUFAs on synaptic plasticity markers associated with an improvement of spatial working memory (Alfos et al., unpublished data). Moreover, RXRs can both bind DHA and 9-cis retinoic acid and are the obligate heterodimerization partner of RARs. These data suggest that the potential synergistic effects of a combined DHA and retinoid partnership on nuclear receptor signaling pathways, synaptic plasticity, and memory should be investigated in aged animals.

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13. FISH OIL SUPPLEMENTATION PREVENTS AGE-RELATED MEMORY DECLINE: INVOLVEMENT OF NUCLEAR HORMONE RECEPTORS


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Abstract
Normal brain aging is associated with multiple morphological and neurochemical changes leading to cognitive decline. Long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFAs) and mainly docosahexaenoic acid (DHA), are major components of neuronal membranes and play a critical role in maintaining learning ability and memory in adults. DHA levels in brain phospholipids decrease with age as a result of altered LC n-3 PUFA metabolizing enzyme activities. Increasing the intake of LC n-3 PUFA with a fish oil-enriched diet may be a strategy to delay the onset of memory decline in the elderly. Our analysis of the literature presented in this review indicates that fish oil supplementation has beneficial effects on the prevention of memory decline both in humans and animals if it is given at middle-age rather than late in life when pathophysiological changes are irreversible. Here we postulate that LC n-3 PUFA by binding to retinoid X receptors (RXRs) and peroxisome proliferator-activated receptors (PPARs) modulate synaptic plasticity-associated gene expression and therefore maintain memory performance during aging.

Keywords: brain aging; memory; n-3 polyunsaturated fatty acids; LC n-3 PUFA; DHA; retinoic acid nuclear receptors; RARs; retinoid X receptors; RXRs; peroxisome proliferator-activated receptors; PPARs; synaptic plasticity
CHAPTER 16

The Effectiveness of Fish Oil as a Treatment for ADHD

Modhi Ali S. Alshammari and Ronald Ross Watson

A REVIEW OF THE LITERATURE

The history of attention deficit hyperactivity disorder (ADHD) began in 1902 when a British doctor named George Frederick Still diagnosed cases in the United Kingdom of impulsive behavior in some young patients who were exhibiting challenging behaviors. For the past 50 years, researchers in many different fields (e.g., education, psychology, medicine, and nutrition) have investigated the nature of ADHD in attempts to find an effective treatment for it. Recently, ADHD has been defined as a psychiatric disorder in the American Psychiatric Association’s Diagnostic and Statistical Manual-IV (DSM-IV, 2000). There are two indices of symptoms by which a diagnosis of ADHD can be made: First, ADHD can be diagnosed using a minimum of six of the following inattention symptoms: (a) not paying close attention to detail, (b) having trouble maintaining attention on tasks, (c) not listening when spoken to directly, (d) not following through on instructions and failing to finish schoolwork, (e) having trouble organizing activities, (f) avoiding activities that require a high amount of sustained mental effort, (g) losing things needed for tasks and activities, (h) becoming distracted easily, and/or (i) being forgetful in daily activities. Second, ADHD can also be identified if six of the following hyperactivity-impulsivity symptoms are displayed: (a) fidgeting with hands or feet or squirming in seat, (b) getting up from seat when remaining in seat is expected, (c) running about or climbing when and where it is not appropriate, (d) having trouble playing or doing free time activities quietly, (e) acting as if driven by a motor, (f) talking excessively, (g) answering questions before they have been finished, (h) having trouble waiting one’s turn, and/or (i) interrupting or intruding upon others (DSM-IV, 2000).

Upon reviewing both sets of symptoms (i.e., inattention and hyperactivity-impulsivity) found in the DSM-IV (2000), the authors of the current study identified two facts about how researchers have been dealing with ADHD. First, this disorder is an educational concern (i.e., identified and treated in schools), and second, its diagnostic criteria describe childhood or adolescent (i.e., school age) behavioral problems. Reviewing the educational literature showed that ADHD is one of the recognized learning disabilities, and researchers in the field of special education (SE) have conducted an enormous amount of research into assisting young people with ADHD in achieving better success in school. However, in the field of SE educators have been highly influenced by the medical model, in which a physician identifies a problem in their patient with the goal of fixing it. Following this model, teachers have been struggling to identify the problems or weaknesses in young people with ADHD and then trying to fix these deficiencies. The exact causes of ADHD are still unknown, yet both physicians and teachers have been working to design treatments that might help reduce its symptoms.

In the past two decades, ADHD has been a real issue for families, teachers, physicians, and researchers. For instance, Schwing (2009) stated that ADHD has been the most prevalent childhood disorder, with an estimated incidence as high as 18% (Schwing, 2009). Furman (2005) described ADHD as the most common neurobehavioral condition of childhood (Furman, 2005). Glöckner-Rist et al. (2013) stated that ADHD is characterized by symptoms of inattention, hyperactivity, and impulsivity. As shown in the DSM-IV (2000) criteria, ADHD is not a disease per se, but rather a group of symptoms representing a final common behavioral pathway for a gamut of emotional, psychological, and/or...
learning problems (Furman, 2005). Although the exact cause of ADHD remains unknown, many risk factors have been implicated. In a review conducted by the State University of New York Upstate Medical University, Banerjee et al. (2007) concluded that in addition to genetic predispositions, there were many environmental factors that could contribute to a person’s development of ADHD, such as exposure to alcohol before birth, the mother smoking during pregnancy, pregnancy and delivery complications, and low birth weight. One critique that could be made about the conclusion of Banerjee et al. is that those environmental factors could also lead to a number of other problems and therefore might not be an adequate causal explanation for the development of ADHD. Making bold statements about the cause and effect relationship between those types of factors and ADHD may not be appropriate because it may mislead researchers.

The authors of the current study concluded that all of the current treatments for ADHD (educational, pharmaceutical, and nutritional) have been geared towards reducing the symptoms, as there is no known cure for the disorder. Consequently, we believed that after a patient stopped their medication, symptoms would return. However, prolonged exposure to the medications used for treating ADHD comes with side effects. These include, but are not limited to, decreased appetite, sleeping difficulties, and behavioral or verbal tics, although these are not common. In addition, different patients react differently to the same medication. This means that there is no one-size-fits-all dosage, which creates problems for the healthcare professional writing the prescription. Varying doses must be tried until the optimum dosage is found that reduces symptoms in the patient without producing substantial side effects.

As a result, researchers in all fields have been using different approaches to find a treatment for the symptoms of ADHD that minimizes side effects and maximizes the positive effects of the treatment. The various approaches include social, educational, pharmaceutical, and psychotherapeutic interventions. Although the educational approach to understanding ADHD and its treatment is the most researchable one in the literature, since so many people receiving ADHD diagnoses have been students, the authors of the current study will focus on the medical approach. The rationale for this is that the medical approach reflects the research interests, clarifies the problem, and answers the research questions relating to the current study. It is of interest to the authors of the current study that researchers in the nutrition and SE fields have found that, although there are no specific foods that have been found to cause ADHD, some nutritional deficiencies may worsen ADHD symptoms. Two nutritional deficiencies that have been the subject of research linked to ADHD symptoms relate to essential fatty acids and iron (Yehuda et al., 2011). One of the most promising findings was emphasized by Colter et al. (2008), who found that patients with ADHD had significantly lower levels of docosahexaenoic acid (DHA, 22:6n-3), which is an omega-3 fatty acid that is a primary structural component of the human brain (Guesnet and Alessandri, 2011).

Purpose

The purpose of this study was to investigate the effect of fish oil (i.e., omega-3 and omega-6 fatty acids) in patients with ADHD by exploring two major areas: (a) the effect of the substances in fish oil on the level of fatty acid in the blood of patients with ADHD, and (b) the effect of consuming fish oil on the behavioral/physical symptoms experienced by patients diagnosed with ADHD.

Analyzing the Studies

A total of 15 studies were selected: Antalis et al. (2006); Bulut et al. (2007); Colter et al. (2008); Germano et al. (2007); Gustafsson et al. (2010); Huss et al. (2010); Johnson et al. (2009); Kirby et al. (2010a, b); Manor et al. (2012); Perera et al. (2012); Raz et al. (2009); Sinn, 2007; Vaisman et al. (2008) and Yehuda et al. (2011). Table 16.1 outlines the purpose, sample, design, and results of each of these 15 studies. All of the studies are arranged alphabetically by the researchers’ surnames and numbered from 1 to 15 for ease of cross referencing to the other tables (i.e., Tables 16.2–16.5).

Findings

Based on the 15 selected studies, the authors of the current study calculated the number of studies conducted during each year (i.e., from 2006 to March 2013) to observe how researchers’ interests might have varied from year to year. We demonstrated those trends with a simple chronological graph included in this review (see Figure 16.1). The graph shows that researchers were the most interested in investigating this research problem in 2010.

The results that were found in the 15 studies ranged from significant improvements in patients after administering the treatments to some positive outcomes. Also, because the 15 studies were conducted using different research problems, sampling procedures, research designs, and measurement systems, drawing an overall conclusion regarding results would have
<table>
<thead>
<tr>
<th>Study</th>
<th>Purpose</th>
<th>Participants</th>
<th>Design</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Antalis et al. (2006)</td>
<td>To assess the frequency of skin/thirst symptoms in patients with ADHD To compare the level of blood fatty acids to those of patients in the control group</td>
<td>35 Patients with ADHD 112 Patients without ADHD</td>
<td>Case-control</td>
<td>Patients with ADHD had higher skin/thirst symptoms Saturated fatty acids were higher in patients with ADHD</td>
</tr>
<tr>
<td>2. Bulut et al. (2007)</td>
<td>To examine the association between malondialdehyde and ADHD</td>
<td>20 Patients</td>
<td>Case-control</td>
<td>Malondialdehyde was significantly higher in patients with ADHD</td>
</tr>
<tr>
<td>3. Colter et al. (2008)</td>
<td>To determine the relationships between EFA and specific ADHD behaviors</td>
<td>11 Patients with ADHD 12 Patients without ADHD</td>
<td>Case-control</td>
<td>Lower omega-3 correlated with higher score on several Conners’ behavioral scales</td>
</tr>
<tr>
<td>4. Germano et al. (2007)</td>
<td>To investigate the effects of EPA and DHA on patients’ attention levels To investigate the correlation between omega-3 supplement and AA/EPA ratio</td>
<td>31 Patients with ADHD 36 Patients without ADHD</td>
<td>Regional pilot study</td>
<td>Decrease in the hyperactivity score mean values Decrease in AA/EPA ratio</td>
</tr>
<tr>
<td>5. Gustafsson et al. (2010)</td>
<td>To measure efficacy of EPA in patients with ADHD</td>
<td>92 Patients with ADHD</td>
<td>Randomized controlled trial</td>
<td>Improvement in Conners’ Parent/Teacher Rating Scales</td>
</tr>
<tr>
<td>6. Huss et al. (2010)</td>
<td>To evaluate the nutritional effects of polyunsaturated fatty acids in combination with magnesium and zinc in patients with ADHD</td>
<td>810 Patients</td>
<td>Observational longitudinal study</td>
<td>Reduction in symptoms of ADHD</td>
</tr>
<tr>
<td>7. Johnson et al. (2009)</td>
<td>To assess whether supplementation with omega-3/6 fatty acids was effective in reducing ADHD symptoms</td>
<td>75 Patients with ADHD</td>
<td>Randomized placebo-controlled trial</td>
<td>Improvement in Clinical Global Impression results No significant difference in Attention Deficit Hyperactivity Disorder Rating Scale-IV Scores In the end 47% showed improvement in ADHD symptoms</td>
</tr>
<tr>
<td>8. Kirby et al. (2010a)</td>
<td>To examine the relationship between polyunsaturated fatty acid status in cheek cells with behavior reports and cognitive performance</td>
<td>411 Patients</td>
<td>Randomized controlled trial</td>
<td>Showed correlation between fatty acid levels and parents’ and teachers’ scores</td>
</tr>
<tr>
<td>9. Kirby et al. (2010b)</td>
<td>To assess the effects of omega-3 supplementation on cognitive test performance and behavior ratings in a typically developing population</td>
<td>348 Patients</td>
<td>Randomized controlled trial</td>
<td>Reduction in hyperactivity/impulsivity using SNAP and teacher SDQ Placebo showed better results in overall SDQ teachers’ scores</td>
</tr>
<tr>
<td>10. Manor et al. (2012)</td>
<td>To study the efficacy of phosphatidylserine omega-3 in reducing ADHD symptoms</td>
<td>150 Patients with ADHD</td>
<td>Randomized controlled trial</td>
<td>Improvement in the Conners’ Parents Scale and CHQ, but not in SDQ scale</td>
</tr>
<tr>
<td>11. Perera et al. (2012)</td>
<td>To assess the effectiveness of combined omega-3 and omega-6 supplementation in patients with ADHD</td>
<td>98 Patients with ADHD</td>
<td>Double-blind placebo-controlled study</td>
<td>No change after 3 months Showed reduction in symptoms after 6 months</td>
</tr>
<tr>
<td>12. Raz et al. (2009)</td>
<td>To test the influence of short term essential fatty acids on ADHD</td>
<td>78 Patients</td>
<td>Double-blind placebo-controlled study</td>
<td>Significant improvement was shown in the experimental group.</td>
</tr>
<tr>
<td>13. Sinn (2007)</td>
<td>To determine fatty acid deficiency symptom levels and their relationship with items on Conners’ ADHD index, and whether FADS predicted degree of response to polyunsaturated fatty acid supplementation</td>
<td>Study 1, in a general population (N = 547) Study 2, in patients with ADHD (N = 104)</td>
<td>Cross-sectional correlational</td>
<td>No improvement was shown in FADS in the polyunsaturated fatty acids group compared to the placebo group</td>
</tr>
</tbody>
</table>

**TABLE 16.1** Summary of the 15 Studies in Which Fish Oil was Used in Patients with ADHD
been impossible, and categorizing similar studies together in sub-groups was required. The authors of the current study identified two major sub-groups: (a) nine studies investigated the effect of fish oil on ADHD behavioral/physical symptoms and (b) seven studies investigated the level of fatty acid in the blood of patients with ADHD.

### The Effect of Fish Oil on the Behavioral/Physical Symptoms of ADHD

Three out of the nine researchers in this category (Manor et al., 2012; Germano et al., 2007; Kirby et al., 2010b) used an omega-3 supplement with the participants. Manor et al. (2012) studied the effect of omega-3 in reducing the symptoms of ADHD. The participants were 150 patients, whose ages ranged from 6 to 13 years old. All participants had been diagnosed using the criteria for ADHD as presented in the DSM-IV (2000). The patients were divided into two groups, with one receiving phosphatidylserine (PS) omega-3, and the other receiving a placebo. Changes in participants’ ADHD symptoms were measured using three measurements (i.e., Conners’ Teacher/Parent Rating Scale [CRS-T], the Strengths and Difficulties Questionnaire [SDQ], and the Patient Health Questionnaire [CHQ]). The findings of Manor et al. were as follows: (a) in the CRS-T, there was an improvement in the PS omega-3 group’s score; however, there were no differences when comparing the PS omega-3 group to the placebo group; (b) results of CRS-T and CHQ showed that the PS omega-3 group performed better than the placebo group; and (c) there were no observed differences between the two groups for any of the SDQ subscales. The authors of the current study criticized the study by Manor et al. regarding one aspect, which was that switching the study from a double-blind study to open-label in the middle of the treatment reduced the time for comparing the two groups to 15 weeks, and might have negatively affected the results.

In contrast to the study by Manor et al. (2012), Kirby et al. (2010b) included more participants (i.e., 348 patients) in their study, whose ages ranged from 8 to 10 years old. However, the Kirby et al. study was similar to that of Manor et al. in that they were both investigating the effect of omega-3 supplementation on children with ADHD. In the study by Kirby et al., the participants were divided into two groups, with 171 participants receiving omega-3 and 177 receiving placebo. For 16 weeks, the study was a comparison between the two groups; however, the researchers included an extra 8 weeks as an open-label study, in which all patients in both groups were given omega-3. To measure the effects of the supplement, the researchers used parent-teacher questionnaires (i.e., Swanson Nolan and Pelham [SNAP] and SDQ). The researchers found a reduction in hyperactivity/impulsivity when comparing SNAP results and the teachers’ SDQ results to baseline after 16 weeks. However, the overall SDQ teachers’ scores were better in the placebo group. The authors of the current study criticized Kirby et al. regarding two aspects of the study: (a) the researchers reported a variety of outcomes in tables without further explanation; and (b) the researchers focused on the results found after 16 weeks but generalized the data for 24 weeks, which might mislead the reader.

### TABLE 16.1 (Continued)

<table>
<thead>
<tr>
<th>Study</th>
<th>Purpose</th>
<th>Participants</th>
<th>Design</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>14. Vaisman et al. (2008)</td>
<td>To investigate the incorporation of long-chain omega-3 fatty acids provided as phospholipids or fish oils into blood and compare it with placebo</td>
<td>83 Patients with ADHD</td>
<td>Randomized controlled trial</td>
<td>The TOVA score showed an increase in the phospholipid and fish oil groups, but not in the placebo group</td>
</tr>
<tr>
<td>15. Yehuda et al. (2011)</td>
<td>To address the effects of a mixture of EFA on sleep-deprived ADHD patients</td>
<td>78 Patients with ADHD</td>
<td>Controlled clinical trial</td>
<td>Showed improvement in quality of life and quality of sleep</td>
</tr>
</tbody>
</table>

**Note:** Studies were arranged alphabetically by the researchers’ surnames and numbered from 1 to 15; the purpose, participants, design, and intervention were provided as a summary of each study.
In contrast to the studies of Manor et al. (2012) and Kirby et al. (2010b), Germano et al. (2007) conducted their study with a very small sample size (i.e., only 16 patients with ADHD) as a one-group pretest-posttest design. Also, in the Germano et al. study, the treatment period lasted only 8 weeks. However, similar to the Manor et al. study, Germano et al. used Conners’ scale to measure the differences between the pre- and posttests. Germano et al. included two parts in their study: first, they investigated the effect of eicosapentaenoic acid (EPA) and DHA on patients’ attention levels. The second part of their study will be discussed in the section below that examines findings on fatty acid levels in the blood of patients with ADHD.

All patients in the Germano et al. study received omega-3 supplements for 8 weeks, as the study included only one group. The effects of the supplement were measured using Conners’ scale, and the researchers found that hyperactivity and inattention scores decreased compared to baseline. The small sample size of 16 patients, using only one group, and implementing the treatment for only 8 weeks might have negatively influenced the results from the Germano et al. study.

In conclusion, after analyzing the three studies (Manor et al., 2012; Germano et al., 2007; Kirby et al., 2010b), the authors of the current study believed that some evidence had been found across the three studies showing promising effects of using omega-3 to treat patients with ADHD. However, more investigations in this area are needed. Future researchers may want to consider using direct observation as a method of measuring patients’ behaviors, as we believe this might be a superior method to questionnaires, which rely to some degree on the feelings and opinions of teachers and parents. Also, treatment periods in future studies need to be longer so that clear results about the effectiveness of omega-3 can be obtained.

Four out of the nine researchers in this category (Johnson et al., 2009; Perera et al., 2012; Raz et al., 2009; Yehuda et al., 2011) used omega-3 and omega-6 as a supplement with the participants. In the Perera et al., 2012 study, the researchers investigated the effect of omega-3 and omega-6 as a treatment for patients with ADHD in behavioral and educational settings. The researchers included 94 patients aged between 6 and 12 years, who had been treated with a methylphenidate and standard behavioral therapy for 6 months or more (Perera et al., 2012). The total sample was divided into two groups (i.e., 48 patients were allocated to the experimental group and 46 patients were allocated to the control group). The researchers did not find any differences between the two groups after 3 months of treatment. Parera et al. measured the patients’ behaviors by using an 11-item checklist, which was completed by the patients’ parents. After 6 months of treatment, Parera et al. found that the combination of omega-3 and omega-6 was effective in reducing most of the symptoms of ADHD compared to patients who took the placebo (i.e., the control group). The authors of the current study criticized the Parera et al. study regarding one critical aspect, which was that all patients included in the study had been taking medication and attending behavioral therapy sessions before, and continued to do so during, and after the study was conducted. Therefore, we believed that these uncontrolled variables might have affected the validity of the treatment results.

The 2009 study by Johnson et al. included 75 patients aged between 8 and 18 years of age, none of whom received any outside treatment for ADHD during the study. The researchers in this study investigated the effects of using omega-3 and omega-6 in adolescents with ADHD. In this study, 78% of the patients had at least one comorbid diagnosis such as anxiety, autism or a learning disability (Johnson et al., 2009). The participants were divided into two groups, with one group receiving an active supplement and the other receiving a placebo for the first period of the study (i.e., 3 months), and then both groups receiving an active supplement for another 3 months (i.e., period 2). Two measurements were used to assess outcomes: the ADHD Rating Scale-IV and the Clinical Global Impression (CGI). Therefore, the Johnson et al. study was more reliable than that of Parera et al. because it used two measurement systems. Johnson et al. found that the results of the ADHD Rating Scale-IV were not significant for the group who took an active supplement compared to the placebo group. However, when using the CGI measurement results, the researchers found that the active group showed a significant improvement compared to the placebo group. The improvement in CGI results was to a near-normal range. The authors of the current study criticized the Johnson et al. study for failing to mention whether the patients received any other type of medication or school intervention to treat comorbid diagnoses. If so, this might have affected the results of the study, and may have explained the positive findings.

Approaching the effects of using omega-3 and omega-6 in patients with ADHD from a different direction, Yehuda et al. (2011) investigated the effect of an essential fatty acid (EFA) mixture in sleep-deprived ADHD children (Yehuda et al., 2011). Similar to the studies by Parera et al. and Johnson et al., Yehuda et al. included a reasonable sample size of 78 patients diagnosed with ADHD, who ranged from 9 to 12 years of age. In the study, there were 40 patients who received omega-3 and omega-6 (i.e., the experimental
One of the most interesting studies in this category was the investigation by Raz et al. (2009), because of its well-developed measurement systems. The researchers used Test of Variables of Attention (TOVA), which is a computer-based test. However, the Raz et al. study was similar to those of Perera et al. (2012), Johnson et al. (2009), and Yehuda et al. (2011) in investigating the effects of omega-3 and omega-6 in patients with ADHD. Raz et al. included 78 patients who were diagnosed with ADHD and who had not taken any medication or EFAs for the past 3 months. The patients were aged between 7 and 13 years of age. To measure the effects of the supplement, the researchers used parent and teacher questionnaires. They used TOVA to measure the attentiveness of the patients. The researchers found a significant difference in the active (i.e., experimental) group compared to the placebo (i.e., control) group. The authors of the current study criticized Raz et al. for the short treatment period used in the study, which only lasted 7 weeks.

In conclusion, after this portion of the analysis, the authors of the current study believed that major evidence had been found across the four studies, which could be seen as showing fairly effective results of using omega-3 and omega-6 to reduce ADHD symptoms. However, as found in our review of the omega-3 research, more investigations are needed in this area. Future researchers might use direct observation or TOVA as methods of measuring patients’ behaviors, as we believe that relying solely on questionnaires is not always effective. The treatment periods in this category also need to be longer so that clear results can be found. Overall, and on the basis of the selected studies, the authors of the current study believed that the effectiveness of using omega-3 and omega-6 was clearer than using only omega-3 in patients with ADHD.

One of the strengths of this study was that the patients in the sample had been diagnosed with ADHD by two different psychologists. The findings were measured using a questionnaire, which was completed only by the patients. Asking the patients to self-complete the questionnaire was unique within this study category, and was not used in either the Parera et al. or the Johnson et al. studies. The researchers found a significant improvement in the quality of life of the participants who took the supplement, specifically in the quality of participants’ sleep. The authors of the current study criticized the Yehuda et al. study for using only a self-reported questionnaire, as this might have biased the results.

Two out of the nine researchers in this category (Huss et al., 2010; Sinn, 2007) used this supplement with participants. In Sinn’s 2007 study, the goal was to find the relationship between fatty acid deficiency symptoms (FADS) in patients and the patients’ scores on Conners’ ADHD index. Sinn’s first study analyzed the results of 347 patients from the general population. Using Conners’ ADHD index and a background questionnaire completed by the parents as a measurement scale in this study, Sinn found that Conners’ index scores were positively correlated with the total FADS (Sinn, 2007). This means that an increase in the Conners’ index scores of the participants was accompanied by an increase in participants’ FADS. Sinn’s second study was a randomized, controlled, double-blind study that investigated the effect of a polyunsaturated fatty acid (PUFA) supplement, including 400 g fish oil, on the FADS of a group with ADHD after 15 weeks. In this study, 451 patients with ADHD aged 7 to 12 were included. After supplementation with PUFA or placebo oil, the researcher used Conners’ rating scale to measure the outcomes. The researcher found that after 15 and 30 weeks of taking the supplement, there were no improvements in the FADS of the control group compared to the placebo group. However, there were overall improvements in all groups (i.e., active and placebo). The authors of the current study criticized Sinn’s study for using patients who were not officially diagnosed with ADHD.

While Sinn (2007) used Conners’ scales to measure the outcomes, Huss et al. (2010) used different instruments (SNAP and SDQ) to investigate the effect of an omega-3/-6-zinc-magnesium combination on the ADHD symptoms of participants. Huss et al.’s study was the largest in terms of participants with 810 patients, 94.7% of whom received the recommended dose of four capsules of the supplement per day. The researchers found that the four items related to emotional problems from the SDQ scale decreased in participants compared to baseline. Sleep problems also decreased. The authors of the current study criticized Huss et al. for not using a control group, which made the effectiveness of the treatment unclear.

In conclusion, after analyzing these two studies, the authors of the current study believed that some evidence had been found in support of the effectiveness of the supplements. However, when we compared the findings in this category with those from the previous categories, we noted that the findings from the studies that used omega-3 and omega-6 showed greater improvements in outcomes. One of the limitations that prevented us from making any bold statements about the findings from this category was the fact that there were only two studies investigating this research problem. Therefore, there is a need for additional research in this area.
Fatty Acid Levels in Blood of Patients with ADHD

OMEGA 3

Four out of the seven researchers in this category (Germano et al., 2007; Gustafsson et al., 2010; Kirby et al., 2010a; Vaisman et al., 2008) used omega-3 as a supplement with participants. Vaisman et al. investigated the incorporation of omega-3 and fish oil into the blood. The researchers included 83 patients aged between 8 and 13 years. The researchers tested fatty acid levels in the plasma of the participants after receiving a placebo, omega-3 or fish oil. They found that TOVA scores differed between the three groups, with the highest scores being found in the omega-3 group, followed by the fish oil group, and the lowest scores being found in the placebo group.

Using a different measurement system, Kirby et al. (2010a) investigated levels of fatty acid in patients and compared it to patients’ learning and behavior styles. The researchers included 411 patients in the study, aged between 8 and 10 years, with participants being selected from the general population. The fatty acid results were collected from a cheek cell sample and compared to results from a variety of questionnaires. These questionnaires were designed to identify symptoms of ADHD in the participants. The researchers found a correlation between fatty acid levels and parent-teacher questionnaire results. They also found a positive association between omega-3 fatty acid levels and pro-social behavior (Kirby et al., 2010a).

Gustafsson et al. (2010) measured the level of fatty acid directly from patients’ blood samples. In this study, 92 patients aged between 7 and 12 years old were included. The patients received omega-3 or a placebo for 15 weeks. The researchers analyzed the fatty acid in the serum phospholipid and red blood cell membranes. They found that EPA improved Conners’ Teacher Rating Scales (CTRS), but not Conners’ total score (Gustafsson et al., 2010). Similarly, Germano et al. (2007) investigated the correlation between an omega-3 supplement and arachidonic acid (AA)/EPA ratios to measure the change in AA/EPA. In this study, there were 31 patients with ADHD and 36 non-ADHD diagnosed controls. All participants received an omega-3 supplement for 8 weeks. The results were obtained using the fatty acid content and AA/EPA ratio in the blood. The researchers found that before the supplementation, participants with ADHD had higher AA/EPA ratios, which decreased in this group after supplementation.

In conclusion, after analyzing the studies on fatty acid levels, the authors of the current study believed that all the studies were consistent in that they all attempted to measure/monitor the fatty acid in patients; however, each one used a different approach. While some evidence was found across the four studies, we believe more research is needed in this area and that it should be conducted using similar methods.

NO SUPPLEMENTS WERE GIVEN

Three out of the seven researchers in this category (Antalis et al., 2006; Bulut et al., 2007; Colter et al., 2008) did not use supplements with participants. Antalis et al. (2006) investigated the frequency of skin/thirst symptoms in patients with ADHD, and compared the level of blood fatty acid to the control group. The researchers included 35 patients with ADHD and 112 controls, all of whom were college students. The researchers also used Conners’ Adult ADHD Rating Scale (CAARS) and blood analysis on both groups. In the first analysis they found that patients with ADHD had higher results on the skin/thirst symptoms and on all CAARS subscales. In the second analysis, they found that the omega-3 level was lower in patients with ADHD; however, omega-6 was higher in those with ADHD.

With a very small sample size of 23, Colter et al. (2008) conducted a study in which they investigated the difference in fatty acid levels between patients with ADHD and a control group. The researchers wanted to examine the differences in dietary intake between 11 patients with ADHD and 12 controls (aged 10 to 16 years). The researchers used blood samples to measure fatty acid levels. They also asked participants to record their diets for 7 days. They found that the ADHD group had higher dietary intake of protein and saturated fatty acid, but not of omega-3, EPA or DHA. The ADHD group also had lower DHA and omega-3 blood levels compared to the control group.

Bulut et al. (2007) examined the levels of malondialdehyde (MDA) in ADHD patients. In this study, the researchers included 20 patients with ADHD and 21 healthy volunteers. The measurement in this study was different from those used in other blood studies, because the researchers measured the level of MDA in the plasma. The researchers’ rationale was that MDA is the end product of fatty acid oxidation. They found that patients with ADHD had higher levels of MDA than the control group, which means that the oxidation level in ADHD patients is higher.

In conclusion, after analyzing these three studies, the authors of the current study believed that enough evidence had been found across the three studies to indicate promising findings. However, more research is needed to confirm those findings and to arrive at a clear conclusion.

CONCLUSION

The purpose of this study was to investigate the effects of fish oil (i.e., omega-3 and omega-6 fatty acid)
### TABLE 16.2 Analyses of the Patients and Geographical Regions Across All 15 Studies

<table>
<thead>
<tr>
<th>Participants</th>
<th>Age as Categorized by School Levels</th>
<th>Gender</th>
<th>Patients Included</th>
<th>Geographical Regions</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>P</td>
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<td>M</td>
<td>H</td>
</tr>
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<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>3.</td>
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<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>4.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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</tr>
<tr>
<td>5.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>6.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>7.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>8.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>9.</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td>10.</td>
<td>✓</td>
<td>✓</td>
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<td>✓</td>
</tr>
<tr>
<td>11.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>12.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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</tr>
<tr>
<td>13.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>14.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>15.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>T</td>
<td>4</td>
<td>13</td>
<td>6</td>
<td>3</td>
</tr>
</tbody>
</table>

Note: S = Study (i.e., studies were numbered from 1 to 15 alphabetically by the researchers’ surnames [See Table 16.1]); T = Total; P = Pre-school (i.e., from 3 to 6 years old); E = Elementary (i.e., from 7 to 12 years old); M = Middle school (i.e., from 13 to 15 years old); H = High school (i.e., from 16 to 18 years old); C = College (i.e., from 19 to 25 years old); Un. = Unreported; B = Boys; G = Girls; AD. = ADHD-diagnosed; Nor. = Normal; To. = Total included patients; AM = America (i.e., the United States of America and Canada); EU = Europe (i.e., the United Kingdom, Sweden, Germany, and Italy); ME = Middle East (i.e., Israel and Turkey); AU = Australia; "✓" = Yes.
### TABLE 16.3 Analyses of the Overall Examined Variables and Research Designs Across All 15 Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>n-3</th>
<th>n-3/ n-6 FA/</th>
<th>n-3</th>
<th>n-3/ n-6 FA/</th>
<th>General Research Design</th>
<th>Duration of Treatment (Weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<td>0–15</td>
</tr>
<tr>
<td>2.</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>OL</td>
<td>0–15</td>
</tr>
<tr>
<td>3.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>CC</td>
<td>0–15</td>
</tr>
<tr>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>CS</td>
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<tr>
<td>5.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Regional pilot study</td>
<td>15–30</td>
</tr>
<tr>
<td>6.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>15–30</td>
</tr>
<tr>
<td>7.</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>15–30</td>
</tr>
<tr>
<td>8.</td>
<td>✓</td>
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<td>✓</td>
<td>✓</td>
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<td>15–30</td>
</tr>
<tr>
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<td>✓</td>
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<td>✓</td>
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</tr>
<tr>
<td>10.</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>15–30</td>
</tr>
<tr>
<td>11.</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>15–30</td>
</tr>
<tr>
<td>12.</td>
<td>✓</td>
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<td>✓</td>
<td>✓</td>
<td></td>
<td>15–30</td>
</tr>
<tr>
<td>13.</td>
<td>✓</td>
<td>✓</td>
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<td>✓</td>
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</tr>
<tr>
<td>14.</td>
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<td>✓</td>
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<td>15–30</td>
</tr>
<tr>
<td>T</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>0–15</td>
<td>15–30</td>
</tr>
</tbody>
</table>

Note: S = Study (i.e., studies were arranged alphabetically by the researchers’ surnames and numbered from 1 to 15 [See Table 16.1]); T = Total; n-3 = Omega 3; n-6 = Omega 6; FA + = Fatty acid with other supplements; No = Nothing has been given; CT = Controlled trial (i.e., Randomized controlled trial, Randomized placebo-controlled trial, Controlled clinical trial, Double-blind placebo-controlled study); OL = Observational and longitudinal study; CC = Case-control; CS = Cross-sectional correlation; RP = Regional pilot study; “✓” = Yes.
# TABLE 16.4 Analyses of the Measurement Systems and the Overall Outcomes Across the Nine Studies About Behavioral Symptoms of ADHD

<table>
<thead>
<tr>
<th>S</th>
<th>SNAP</th>
<th>SDQ</th>
<th>Conners'</th>
<th>CL/QU</th>
<th>TOVA</th>
<th>CGI</th>
<th>RS</th>
<th>Overall Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sub.</td>
<td>P. / T.</td>
<td>Sub.</td>
<td>P. / T.</td>
<td>Sub.</td>
<td>P. / T.</td>
<td>Sub.</td>
<td>Dr.</td>
</tr>
<tr>
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</tr>
<tr>
<td>4.</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>10.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>11.</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>12.</td>
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<tr>
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<tr>
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<td></td>
<td></td>
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<td></td>
</tr>
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<td>2</td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

11% 22% 11% 33% 44% 11% 22% 11% 11% 11% 22% 77%

Note: S = Study (i.e., studies were arranged alphabetically by the researchers’ surnames [See Table 16.1]); T = Total; SNAP = Swanson Nolan and Pelham; SDQ = Strengths and Difficulties Questionnaire; Conners’ = (Conners’ Adult ADHD Rating Scales, Conners’ ADHD index, Conners’ Teacher Rating Scale, and Conners’ Abbreviated Parent-Teacher); CL/QU = Checklist/Questionnaire; CGI = Clinical Global Impression; RS = Attention Deficit Hyperactivity Disorder Rating Scale; Sub. = Subject/participants (i.e., adults and children); P. / T. = Parents and/or teachers; TOVA = Test of Variables of Attention; Dr. = trained doctors (i.e., pediatricians or patients’ psychiatrists); PO = positive outcomes have been found; SPO = some positive outcomes have been found; “√” = Yes; “----” = Not included (i.e., those are the studies that focus on the blood of the ADHD patients [See Table 16.5]).
### TABLE 16.5
Analyses of the Measurement Systems and the Overall Outcomes Across the Seven Studies About the Fatty Acid Levels in the Blood of Patients with ADHD

<table>
<thead>
<tr>
<th>Study</th>
<th>Measuring the Fatty Acid Levels in the Blood of the ADHD Patients Using:</th>
<th>Overall Outcomes</th>
</tr>
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<td></td>
<td>Blood Test</td>
<td>Cheek Cell</td>
</tr>
<tr>
<td>1.</td>
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</tr>
<tr>
<td>2.</td>
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<td>Yes</td>
</tr>
<tr>
<td>3.</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>4.</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>5.</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>6.</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>7.</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>8.</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Note: S = Study (i.e., studies were arranged alphabetically by the researchers' surnames [See Table 16.1]); T = Total; AA/EPA = Arachidonic acid/eicosapentaenoic acid; PO = Positive outcomes have been found; \(\checkmark\) = Yes; \(\_\_\_\_\_\_\_\_\没人该= No included (i.e., these are the studies that focus on the behavioral/physical symptoms [See Table 16.4]).
in patients with ADHD by exploring two major areas: (a) the effects of fish oil supplements on the levels of fatty acid in ADHD patients’ blood; and (b) the effects of using fish oil on the behavioral/physical symptoms of ADHD. The major limitations of the current study were that we were restricted only to literature that was written in the English language, and to that which was available online. Therefore, we might have missed out on potentially significant research and findings that were written in different languages or published only in print. However, with respect to the existing limitations, the authors of the current study identified gaps in the literature as follows.

For the 7-year span of research that we analyzed (i.e., from 2006 to March 2013), only 15 studies were chosen for the current study. The 15 studies were conducted in four geographical regions: (a) two in America (i.e., the United States of America and Canada); (b) seven in Europe (i.e., the United Kingdom, Sweden, Germany, and Italy); (c) five in the Middle East (i.e., Israel and Turkey); and (d) one in Australia. The total sample size across all 15 studies was 2819, of which 86% were elementary school students. Therefore, we encourage future researchers to include pre-school children as well as older students and adults in their studies (for more information about gaps in the literature regarding sampling procedures, see Table 16.2).

In the treatments procedures, in most of the studies we reviewed (i.e., 26%), the researchers used omega-3/omega-6 as a supplement to affect the behavioral/physical symptoms of patients with ADHD. However, in most reviewed studies (i.e., 26%), the researchers used omega-3 as a supplement to affect fatty acid levels in the blood of patients with ADHD. Therefore, the authors of the current study encourage future researchers to be consistent in examining all supplements (for more information about gaps in the literature regarding treatment procedures, see Table 16.3).

One of the most challenging issues we found across all 15 studies was the variation of measurement systems. For instance, the measurements that the researchers used to record behavioral/physical symptoms of ADHD varied from study to study. Therefore, the authors of the current study encourage future researchers to use the most reliable and valid tests so that future reviewers can draw clear and complete conclusions (for more information about gaps in the literature regarding measurement systems, see Tables 16.4 and 16.5).

Final Thought

ADHD presents a very challenging and complicated problem. However, by organizing cooperative research teams in which researchers from different fields can work together, we might find a solution for this problem and a way to help individuals diagnosed with ADHD to experience higher levels of academic/vocational success and a better quality of life.

References


Gusnet, P., Alessandri, J.M., 2011. Docosahexaenoic acid (DHA) and the developing central nervous system (CNS)—Implications for dietary recommendations. Biochimie. 93 (1), 7–12.


<table>
<thead>
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<th>REFERENCES</th>
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Abstract

The purpose of this study was to investigate the effect of the substances in fish oil (i.e., omega-3, omega-6, fatty acid) on patients with attention-deficit hyperactivity disorder (ADHD) by exploring two major areas: (a) the level of fatty acid in the blood of patients with diagnoses of ADHD, and (b) the effect of fish oil on patients’ behavioral/physical symptoms of ADHD. Fifteen studies were selected, analyzed, and synthesized using systematic procedures. The publication dates of the studies ranged from 2006 to March 2013, and the selected studies were conducted in four different geographical regions. The total sample size across all 15 studies was 2819, of which 86% were elementary school students. As treatment procedures, in most of the reviewed studies (i.e., 26%), n-3/n-6 was used as a supplement to affect the behavioral/physical symptoms of ADHD and n-3 was used as a supplement to affect levels of fatty acid in the blood of patients with ADHD. The measurement systems varied from study to study. In conducting this study, we have identified gaps in the literature, and we hope that this work will be of value to future researchers.

Keywords: Omega-3; omega-6; fatty acid; fish oil; ADHD
Dietary Omega-3 Sources during Pregnancy and the Developing Brain: Lessons from Studies in Rats

Caroline E. Childs and Philip C. Calder

INTRODUCTION

The most common dietary omega-3 fatty acid is the essential fatty acid α-linolenic acid (ALA; 18:3n-3), with good dietary sources of this including plant seeds, seed oils (especially flaxseed oil, also known as linseed oil), and some nuts. ALA is a substrate for the production of long-chain omega-3 polyunsaturated fatty acids (PUFAs) including eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (DPA; 22:5n-3), and docosahexaenoic acid (DHA; 22:6n-3) (Leonard et al., 2004, Figure 24.1). Alternatively, these long-chain omega-3 PUFAs can be consumed directly from food sources such as oily fish. Animal studies have demonstrated that consuming a diet deficient in omega-3 fatty acids during pregnancy and lactation results in neurological abnormalities in offspring, such as impairments of cognitive and visual function (Brenna, 2011; Lauritzen et al., 2001) and that these impairments are associated with a reduction in brain DHA content.

DHA is a major component of the brain and retina, and it accumulates within the fetal rat brain during pregnancy and during the post-natal period (Green et al., 1999), peaking at around 6 weeks of age (Childs et al., 2011). In humans, transfer of DHA to the developing fetus occurs predominantly in the last 10 weeks of pregnancy, with the majority of this DHA accumulated within fetal adipose tissue in order to support brain and retinal development during the first months of post-natal life (Haggarty, 2004).

There is significant interest in whether dietary provision of pre-formed DHA is advantageous in maximizing brain DHA content compared to a diet replete in its precursor ALA. It has been hypothesized that direct provision of DHA will prevent any limitations in either the maternal or fetal endogenous synthesis pathways from adversely affecting brain development. The series of desaturase and elongase enzymes which generates DHA from ALA (Leonard et al., 2004; Figure 24.1) is also involved in the metabolism of the omega-6 PUFA linoleic acid (LA; 18:2n-6) into its longer-chain, more unsaturated derivatives (e.g. arachidonic acid, AA, 20:4n-6). In Western diets, consumption of LA is about ten times that of ALA (Blasbalg et al., 2011; Burdge and Calder, 2006), suggesting that synthesis of omega-6 PUFA will predominate over that of omega-3 PUFA, and that the ability to synthesize DHA from ALA may be limited. Indeed, studies with stable isotopes in humans have indicated that human infants convert just 1% of ALA into DHA, and that this capacity is significantly lower in adults (Brenna et al., 2009). If pre-formed dietary DHA is required for infant development, this has significant public health implications, as the current recommended intake of EPA + DHA for adults in the UK is a minimum of 450 mg/day, with oily fish as the major source of these two fatty acids, yet it is estimated that only 27% of UK adults and less than 10% of UK children and adolescents habitually eat oily fish (Scientific Advisory Committee on Nutrition, 2004).

The use of studies of rat pregnancy to inform knowledge and understanding of human pregnancy has both limitations and advantages. The developmental maturity of rats and humans is significantly different at the time of birth. In terms of relative size, the rat reaches a proportion of adult size comparable to that of a newborn human at 12 days post-partum,
indicating that the rat is significantly less developmentally mature at birth compared to a human infant (Quinn, 2005). In terms of brain development, comparative studies indicate that the rat cerebral cortex corresponds to that of a newborn infant at day 12–13 postpartum (Romijn et al., 1991). This suggests that rat models using interventions during pregnancy may be considered suitable models for early prenatal brain development in humans, and only studies which include interventions which continue to at least day 12 post-partum (i.e. during lactation) can be hypothesized as a model of interventions during full-term human gestation. While there are many differences between human and rat pregnancies, including the much larger litter size among rats, with a corresponding increased relative lactation burden, and shorter duration of lactation, rat models allow for far more stringent and/or extreme dietary manipulation than is possible in humans, which is essential for hypothesis testing and mechanistic studies. Use of a rat model, for example, allows for the possibility of timed interventions from the moment of, or even before, conception, and greater access to developing tissues than would ever be possible in human studies.

Here, we review available data from rat studies which have provided supplementary dietary ALA or long-chain omega-3 PUFA during pregnancy and/or lactation, which include measures of brain fatty acid composition. Five papers which provided supplementary ALA were identified, nineteen which provided long-chain omega-3 PUFA, and six which directly compared the efficacy of ALA and long-chain omega-3 PUFA. Data from studies where rats were provided with omega-3 PUFA-deficient diets were not included, as these have been reviewed in detail elsewhere (Brenna, 2011; Lauritzen et al., 2001).

ALA SUPPLEMENTATION AND BRAIN FATTY ACID COMPOSITION

Based on the data from the five papers which provided dietary ALA in excess of the amount typically included in laboratory rat food, it was clear that the duration of the nutritional intervention was key to whether brain fatty acid composition was influenced (Table 24.1). In the studies where dietary interventions were provided throughout pregnancy, and brain samples were collected from offspring at birth, data indicate that increased dietary ALA can significantly increase brain long-chain omega-3 PUFA status, with a higher brain content of EPA (Cheon et al., 2000), DPA (Fernandes et al., 2011; Guesnet et al., 1997; Lenzi Almeida et al., 2011) and DHA (Cheon et al., 2000; Fernandes et al., 2011; Guesnet et al., 1997; Lenzi Almeida et al., 2011) compared to controls. An effect of diet on long-chain omega-6 PUFA status was also observed, with lower AA (Cheon et al., 2000; Fernandes et al., 2011; Lenzi Almeida et al., 2011), 22:4n-6 (Cheon et al., 2000; Lenzi Almeida et al., 2011), and 22:5n-6 (Guesnet et al., 1997) content (Table 24.1).

Only one study provided information on the longevity of any effect by providing ALA for a limited period and taking samples after animals returned to a control diet (Rao et al., 2007). In this study, rats were provided with additional ALA only during pregnancy. In offspring post-weaning, no significant differences in brain fatty acid composition were apparent compared to controls, although it should be noted that only male pups were considered in the analysis. The study by Cheon et al. (2000) indicated that there were subtle sex differences in the offspring response to maternal dietary ALA, as female pups at birth did not display the reductions in AA and 22:4n-6 seen in males, and had a higher ALA, EPA, and DHA at weaning compared to controls.
<table>
<thead>
<tr>
<th>Study</th>
<th>Rat Model</th>
<th>Dietary Supplement</th>
<th>Duration of Intervention</th>
<th>Brain Samples Analyzed</th>
<th>Effects on Brain Fatty Acid Composition</th>
<th>Effects Reported on Brain Functional Outcome Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fernandes et al., 2011</strong></td>
<td>Wistar</td>
<td>Control (7% w/w soybean oil)</td>
<td>Diets to males and females from weaning, rats mated and diet continued throughout pregnancy, lactation, and post-weaning</td>
<td>Total fatty acids in hippocampus at birth</td>
<td>Flaxseed vs. modified control (% total fatty acids): 18:1n-9, LA, DPA, DHA ↓ 18:0, ALA, AA, EPA</td>
<td>At day 30 post-partum: Flaxseed vs. control: Time taken to complete Morris water maze test (spatial memory) on day 1–3 and day 5 Flaxseed vs. control/modified control: Time spent in correct quadrant of Morris water maze when platform removed (spatial memory storage)</td>
</tr>
<tr>
<td><strong>Lenzi Almeida et al., 2011</strong></td>
<td>Wistar</td>
<td>2.5% w/w Flaxseed 0.7% soybean oil + casein 1% Soybean oil + modified casein</td>
<td>Diets to males and females from weaning, rats mated and diet continued throughout pregnancy</td>
<td>2hours post-partum, whole brain</td>
<td>Flaxseed vs. casein (% total fatty acids): ↑ 11:0, 15:0, 17:1n-9, 18:1n-9, trans-LA, LA, 18:3n-6, 20:3n-6, 20:3n-3, 22:2n-9, 24:0, DPA, DHA ↓ 14:1n-9, 15:1n-9, 16:1n-9, 18:0, trans 18:1n-9, 22:1n-9, AA, EPA, 22:4n-6, 24:1n-9 Flaxseed vs. modified casein (% total fatty acids): 15:0, 17:1n-9, 18:1n-9, LA, 20:3n-6, 20:3n-3, 22:2n-9, DPA, DHA ↓ 12:0, 13:0, 16:1n-9, 18:0, trans 18:1n-9, ALA, AA, EPA, 22:4n-6, 24:1n-9</td>
<td>Brain weight and relative brain weight in flaxseed diet significantly higher than casein or modified casein diet</td>
</tr>
<tr>
<td><strong>Rao et al., 2007</strong></td>
<td>Wistar</td>
<td>Control (18% protein, 7% soybean oil w/w) Treatment I (12% protein, 7% soybean oil w/w) Treatment II (3% flax oil, 4% soybean oil w/w)</td>
<td>During pregnancy, returned to control diet during lactation</td>
<td>Brain membranes of male pups at weaning</td>
<td>No significant differences between any group at weaning</td>
<td>Treatment II vs. control: absolute brain weight in male pups ↑ relative brain weight in male pups</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Study</th>
<th>Rat Model</th>
<th>Dietary Supplement</th>
<th>Duration of Intervention</th>
<th>Brain Samples Analyzed</th>
<th>Effects on Brain Fatty Acid Composition</th>
<th>Effects Reported on Brain Functional Outcome Measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cheon et al., 2000</td>
<td>Liquid diets providing 35% energy from fat, with varying LA:ALA ratio</td>
<td>One week prior to mating – lactation and weaning diet</td>
<td>Male and female pups at 0, 3, 8, and 16 weeks old</td>
<td>Male pups, LA1 vs. LA5 (% total fatty acids): LA (8, 16), ALA (0, 8, 16), EPA (0, 8, 16), DHA (0) LA (3), AA (0, 3, 8, 16), 22:4n-6 (0, 3, 8, 16) Female pups, LA1 vs. LA5 (% total fatty acids): LA (16), ALA (0,3,8,16), EPA (0,3,8,16), DHA (0,3) LA (3,8), AA (3,8), 22:4n-6 (8,16)</td>
<td>No significant differences between LA1 and LA5 in Morris water maze or Open Field test</td>
<td></td>
</tr>
<tr>
<td>Guesnet et al., 1997</td>
<td>Wistar 5% fat content w/w with varying ALA content per 100 g diet: 5 mg 100 mg (0.22% energy) 200 mg (0.45% energy) 400 mg (0.9% energy) 800 mg (1.8% energy)</td>
<td>2 weeks prior to mating and throughout pregnancy and lactation</td>
<td>Brain phospholipids of pups at day 1, 3, 7, and 14 post-partum</td>
<td>In one-day-old pups, ALA vs. control (% total fatty acids): Dose dependent ↑ in DHA content Dose dependent ↓ in 22:5n-6 content. DPA at 400 mg and 800 mg DHA content plateaued on day 7 and 14 post-partum among pups fed &gt;200 mg / 100 g diet, while 22:5n-6 reached lowest levels</td>
<td>N/A</td>
<td></td>
</tr>
</tbody>
</table>
In terms of neurological outcome measures, supplementary ALA had inconsistent effects. For example, both higher absolute brain weight (Lenzi Almeida et al., 2011) and lower absolute brain weight (Rao et al., 2007) have been reported compared to controls. However, both these studies identified that there was increased brain weight relative to body weight, indicating an influence of dietary ALA on the pattern of fetal growth. No consistent effects on cognitive function with increased dietary ALA were observed, but available data are limited to just two studies. Fernandes et al. (2011) reported that performance in the Morris water maze was significantly greater among offspring of ALA-supplemented dams, while Cheon et al. (2000) reported no differences in Morris water maze or Open Field Test performance between offspring of dams fed a control or an ALA-rich diet.

Twenty papers were identified where long-chain omega-3 PUFAs were provided to rats during pregnancy and brain fatty acid composition of offspring was assessed (Table 24.2). Of these, nine included interventions during pregnancy only (Childs et al., 2011; Glozman et al., 1999; Ikeno et al., 2009; Innis and de la Presa Owens, 2001; Martin et al., 2004; Roy et al., 2012; Sable et al., 2012; Schiefermeier and Yavin, 2002), four during pregnancy and lactation (Amusquivar et al., 2000; Högyes et al., 2003; Sable et al., 2012; Suganuma et al., 2010), seven during pregnancy, lactation, and the post-weaning period (Haubner et al., 2002; 2007; Ozias et al., 2007; Saste et al., 1998; Stockard et al., 2000; Trevizol et al., 2013; Yonekubo et al., 1993) and two during lactation only (Amusquivar et al., 2000; Yeh et al., 1993).

Providing additional dietary long-chain omega-3 PUFA during pregnancy significantly increased brain DHA content (Childs et al., 2011; Glozman et al., 1999; Ikeno et al., 2009; Innis and de la Presa Owens, 2001; Martin et al., 2004; Roy et al., 2012; Schiefermeier and Yavin, 2002; Trevizol et al., 2013), and in one study was also associated with increased brain EPA content (Ikeno et al., 2009). The long-chain omega-6 PUFA content of brain was significantly lower among rats fed a diet containing long-chain omega-3 PUFA, with lower AA (Ikeno et al., 2009; Innis and de la Presa Owens, 2001; Martin et al., 2004; Roy et al., 2012; Trevizol et al., 2013), and in one study was also associated with increased brain EPA content (Ikeno et al., 2009). The long-chain omega-6 PUFA content of brain was significantly lower among rats fed a diet containing long-chain omega-3 PUFA, with lower AA (Ikeno et al., 2009; Innis and de la Presa Owens, 2001; Martin et al., 2004; Roy et al., 2012; Trevizol et al., 2013), and in one study was also associated with increased brain EPA content (Ikeno et al., 2009).
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<tr>
<td>Trevizol et al., 2013</td>
<td>Wistar</td>
<td>3 g/kg p.o. soybean oil, fish oil, hydrogenated vegetable fat</td>
<td>Six weeks prior to conception, throughout pregnancy and lactation, provided to male pups until 90 days old</td>
<td>Male pups aged 90 days: total lipid from cortex, hippocampus, and striatum</td>
<td>Fish oil vs. soybean oil (% total fatty acids): 17:1n-7 (hippocampus, striatum), LA (striatum), DPA (cortex, striatum), DHA (cortex, hippocampus, striatum).</td>
<td>Amphetamine (AMPH) -induced mania: Exploratory activities after AMPH in all groups except the fish oil treated rats.</td>
</tr>
<tr>
<td>Sable et al., 2012</td>
<td>Wistar</td>
<td>7% w/w content: Control (NF/B) 7% w/w soybean oil, omega-3 supplemented (NF/BD/O) 2.5%, soybean oil, 4.5% MaxEPA fish oil (0.5% EPA, 0.3% DHA)</td>
<td>Throughout pregnancy; Throughout pregnancy and lactation</td>
<td>Total brain membranes, day 22 post-partum.</td>
<td>Omega-3 supplemented vs. control throughout pregnancy and lactation (g/100 g): DHA AA Omega-3 supplemented vs. control during pregnancy only: No significant differences in brain membrane composition</td>
<td>No effect of omega-3 supplementation on brain-derived nerve growth factor or nerve growth factor levels</td>
</tr>
<tr>
<td>Childs et al., 2011</td>
<td>Wistar</td>
<td>13% w/w fat content Soybean oil Sunflower oil Salmon oil</td>
<td>Pregnancy</td>
<td>Brain PE, day 20 gestation, 3, 6, 9, 12 weeks post-partum</td>
<td>Salmon vs. soybean/sunflower (% total fatty acids).</td>
<td>N/A</td>
</tr>
<tr>
<td>Roy et al., 2012</td>
<td>Wistar</td>
<td>Omega-3 supplementation used alongside B12 deficiency. 7% w/w content: Vit B12 deficient Vit B12 deficient + omega-3 High folate, B12 deficient High folate, B12 deficient + omega-3</td>
<td>Throughout pregnancy</td>
<td>Total brain membranes, day 22 gestation</td>
<td>Vit B12 deficient + omega-3 vs. Vit B12 deficient (g/100 g): DHA AA High folate, vit B12 deficient + omega-3 vs. High folate, vit B12 deficient (g/100 g): DHA AA</td>
<td>No effect on relative brain weight. Vit B12 deficient + omega-3 vs. Vit B12 deficient: Brain malondialdehyde</td>
</tr>
</tbody>
</table>
Suganuma et al., 2010
Wistar
Total fat content not specified.
Control (soybean) DHA-enriched (soybean + fish oil)

Day 7 gestation / C0 post-natal day 14
Post-natal day 7, total brain phospholipids
DHA vs. control (% fatty acids):

mDHA k AA

At post-natal day 7 all rats subjected to cerebral hypoxic-ischemia
DHA vs. control:
TUNEL-positive cells (neurons) at day 10
Capsase-3 immunoreactivity (marker of neurons undergoing apoptotic cell death) at day 8, 10, and 14.
Western blot analysis, oxidative DNA injury

Ikeno et al., 2009
Sprague-Dawley
Soybean oil diet (5.8% w/w) DHA-enriched diet (5.5% w/w)

Day 7 gestation / C0 delivery. IUGR induced at day 14 gestation
Day 1 and 7 post-delivery, brain phospholipids
IUGR DHA-enriched vs. IUGR soybean oil (% phospholipid), day 1:
EPA, DHA
IUGR DHA-enriched vs. IUGR soybean oil (% phospholipid), day 7:
DHA AA

Haubner et al., 2007
Not specified
10% w/w fat content:
0% DHA
0.3% w/w DHA
0.7% w/w DHA
3% w/w DHA

Day 2 gestation, throughout pregnancy, lactation, and weaning
Myelin fatty acid composition of whole brain at post-natal day 24
3% DHA vs. 0% DHA (g/100 g):
LA, 20:3n-6, DPA, DHA
k AA, 22:5n-6

Auditory startle latency at post-natal day 15:
Significantly longer latency on 0.7% and 3% DHA diets vs. control

Ozias et al., 2007
Long-Evans
7% w/w soybean oil
7% w/w sunflower oil
6.8% w/w sunflower oil + 0.2% w/w DHA

From mating and throughout weaning for phospholipids
Total brain phospholipids
DHA vs. control (% phospholipid): No significant differences in DHA content at weaning of 1st, 3rd or 4th litter offspring. 2nd litter:
Soybean DHASCO vs. Soybean DHASCO vs. sunflower DHASCO

Joshi et al., 2004
Wistar
All 7% w/w fat content
I: 18% protein
II: 12% protein, folic acid deficient
III: 12% protein, folic acid supplemented
IV: 12% protein, fish oil supplemented

Day 1 gestation / C0 delivery
Offspring at delivery:

Group 4 vs. Group 1:
Brain Phospholipids:
Soybean + DHASCO vs. Soybean N/A
No significant differences in DHA content at weaning at 1st, 3rd or 4th litter offspring.

N/A

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<tr>
<td>Martin et al., 2004</td>
<td>Wistar</td>
<td>10% w/w fat content (low protein, 10% corn oil, 10% DHA-TG)</td>
<td>Conception — Delivery</td>
<td>Forebrain phospholipids (PE, PC, PS/PI) at birth</td>
<td>Low protein + DHA vs. low protein (weight %): DHA (PE, PC, PS/PI) ↓ AA (PE, PC)</td>
<td>N/A</td>
</tr>
<tr>
<td>Högnes et al., 2003</td>
<td>Wistar</td>
<td>Supplement formula (5% w/w containing DHA, EPA and AA)</td>
<td>1 week prior to conception – end of lactation</td>
<td>Phospholipids (PE, PS, PC, PI) of neural membranes at day 12 post-delivery</td>
<td>Supplement vs. placebo (% fatty acids): 18:1n-9 (PE), AA (PC), DHA (PE, PS, PC, PI)</td>
<td>At day 14 post-delivery pups were exposed to toxic brain stimulation. Long-chain PUFA supplemented rats lost fewer cholinergic cells and neurons compared to controls and had a lower degree of cholinergic fibre degeneration.</td>
</tr>
<tr>
<td>Hauhner et al., 2002</td>
<td>Sprague-Dawley</td>
<td>10% w/w soybean/olive oil blend diets with various DHA content</td>
<td>Day 2 gestation – end of lactation; diet maintained at weaning</td>
<td>Pup cerebrums at post-natal day 3, brainstem at post-natal day 31</td>
<td>Cerebrum post-natal day 3 (g/100 g): 1% DHA vs. 0% DHA 20:3n-6, DHA 22:5n-6</td>
<td>Significantly higher auditory brainstem conduction time in rat pups in 3% DHA group vs. 0% DHA at post-natal day 24 and 31. Auditory startle reflex appeared significantly later in the 3% DHA group vs. 0% DHA group</td>
</tr>
<tr>
<td>Schiefermeier and Yavin, 2002</td>
<td>Wistar</td>
<td>All 5% w/w content: 5% sunflower oil, 5% w/w soybean oil, 2.5% soybean oil + 2.5% DHA-TG</td>
<td>Day 15—20 gestation</td>
<td>Offspring at day 20, brain phospholipids (PE, PE, PS)</td>
<td>DHA supplemented vs. control (% fatty acids): 18:1n-9 (PC), DHA (PC, PE, PS) AA (PC, PE), 22:4n-6 (PC, PE, PS), 22:5n-6 (PC, PE, PS)</td>
<td>N/A</td>
</tr>
<tr>
<td>Innis and de la Presa Owens, 2001</td>
<td>Wistar</td>
<td>All 2% w/w content: Safflower oil, Soybean oil, High fish (tuna) oil</td>
<td>One week before mating and throughout gestation</td>
<td>Growth cone membranes from brains collected within 12 hours after birth</td>
<td>High fish (tuna oil) vs. soybean oil (g/100 g): DHA in growth cone PE, PS, PI ↓ LA in growth cone PC AA in growth cone PC, PE, and PS 22:4n-6, 22:5n-6 in growth cone PC, PE, PS, PI High fish (tuna oil) vs. safflower oil (g/100 g): DHA in growth cone PC, PE, PS, PI ↓ LA in growth cone PC, PE AA in growth cone PC, PE, and PS 22:4n-6, 22:5n-6 in growth cone PC, PE, PS, PI</td>
<td>High fish (tuna oil) vs. soybean oil: No significant differences in brain monoamine concentrations. High fish (tuna oil) vs. safflower oil ↓ dopamine and 3,4-dihydroxyphenylacetic acid</td>
</tr>
</tbody>
</table>
Amusquivar et al., 2000
Sprague-Dawley 10% w/w content: Fish oil Olive oil Throughout pregnancy and/or lactation Brain phospholipid, day 21 post-partum Fish oil vs. olive oil throughout pregnancy and lactation (g/100 g): DPA, DHA 14:0, AA Fish oil during pregnancy only vs. olive oil: No significant differences in brain phospholipids. Fish oil during lactation only vs. olive oil: 16:0, EPA 18:1n-9, LA, AA Fish oil vs. olive oil: Significant effects upon post-natal development indices. Significantly later eyelid and ear opening. Cross fostering indicates that diet during lactation period is most influential. Significantly later development of air righting and surface righting reflexes

Stockard et al., 2000
Sprague-Dawley DHA 2% total fatty acids DHA 4% total fatty acids DHA 6% total fatty acids Day 2 gestation – end of lactation, pups weaned onto corresponding maternal diet Total lipid extract of cerebrum at post-natal day 3 and 29 6% DHA vs. 2% DHA (g/100 g): Day 3: 18:1n-9, 20:3n-6, EPA, DPA, DHA AA, 22:4n-6, 22:5n-6 Day 29: 18:1n-9, LA, 20:3n-6, EPA, DPA, DHA 16:0, 18:0, AA, 22:4n-6, 22:5n-6 Dose-dependent increase in auditory brainstem conduction time at day 31

Glozman et al., 1999
Wistar Intra-amniotic administration of ethyl-DHA Administered at day 17 – 20 gestation Total lipid of fetal rat brain, brain PE at day 20 Et-DHA vs. control (% total fatty acids) ↑DHA in total lipid and PE Et-DHA reduced TBARS production (indicator of oxidative stress) in fetal brain after stimulation with Fe2+ up to 72 hr post injection. Et-DHA treatment reduced TBARS production following 20 minute ischemia episode (placental blood flow restriction)

Saste et al., 1998
Sprague-Dawley All 10% w/w content: Reference diet Synthetic diet – corn oil Synthetic diet – menhaden fish oil Day 2 gestation – end of lactation, pups weaned onto corresponding maternal diet Cerebrum collected post-natal day 3 Fish oil vs. corn oil (g/100 g): 18:1n-9, EPA, DPA, DHA LA, 20:3n-6, AA, 22:4n-6, 22:5n-6 Fish oil vs. corn oil: Significant effects upon brainstem auditory pathway. Auditory startle reflex appeared significantly later (mean day 12.5 vs. day 11.8). Significantly higher auditory brainstem conduction time at post-natal day 23 and 29

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<tr>
<td>Yeh et al., 1993</td>
<td>Sprague-Dawley</td>
<td>20% w/w fat content: Corn oil (20% w/w) Menhaden oil (20% w/w) + corn oil (1% w/w)</td>
<td>Day 2–12 post-partum</td>
<td>Brain phospholipids (PC, PI, PS and PE) in mitochondria, microsomes and synaptosomes</td>
<td>Menhaden oil vs. corn oil (weight %): Synaptosomes 16:0 (PS), EPA (PC, PS, PI, PE), DHA (PC, PS, PE) 18:0 (PS), LA (PI, PE), AA (PE) Microsomes 18:1n-9 (PC), EPA (PC), EPA (PS, PI, PE), DHA (PS, PE) 18:0 (PC, PI), 18:1n-9 (PS, PI), LA (PC, PE), AA (PE) Myelin 18:1n-9 (PC), LA (PS), EPA (PC, PS, PI, PE), DHA (PC, PI, PE) 16:0 (PI, PE), LA (PI), AA (PC, PS, PE) Mitochondria 18:1n-9 (PC), AA (PI), EPA (PC, PI, PE), DPA (PS, PE), DHA (PC, PS, PE) ↓ 16:0 (PI), 18:0 (PI), LA (PC, PS, PE), AA in (PS, PE)</td>
<td>N/A</td>
</tr>
<tr>
<td>Yonekubo et al., 1993</td>
<td>Wistar</td>
<td>10% fat w/w: Palm oil, lard oil, soybean oil &amp; coconut oil blend (control) 7% control oil + 3% w/w sardine oil</td>
<td>Throughout pregnancy and lactation/weaning</td>
<td>Brain phospholipids (PE, PC, PS, PI) at day 17, 19, 21 gestation, 2- and 7-week-old pups</td>
<td>Fish oil vs. control (mol % fatty acids): DHA in PE at day 19, 21 gestation and 2 weeks post-delivery. DHA in PS at day 19 and 21 gestation. AA in PE at 2 weeks post-delivery. AA in PE at day 19, 21 gestation and 7 weeks post-delivery. ↓ AA at day 19 and 21 gestation and 2 weeks post-delivery</td>
<td>N/A</td>
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</table>
also assessed a wide range of other outcome measures. The study by Trevizol et al. (2013) used a rat model of amphetamine-induced mania, and identified that fish-oil supplemented offspring had improved recognition memory, reduced brain reactive species production, and protein carbonyl levels, with an attenuated amphetamine-induced mania response, as measured by changes to exploratory activity. Four studies generated from one research group investigated the effect of long-chain omega-3 PUFA upon auditory development (Haubner et al., 2002; 2007; Saste et al., 1998; Stockard et al., 2000). It was identified that dietary long-chain omega-3 PUFA resulted in significantly longer auditory startle latency (Haubner et al., 2007), higher auditory conduction time (Haubner et al., 2002; Saste et al., 1998; Stockard et al., 2000) and later appearance of the auditory startle reflex (Haubner et al., 2002; Saste et al., 1998). These papers hypothesized that these impairments in auditory function were due to adverse effects of DHA upon myelination. Myelin is rich in cholesterol, saturated fatty acids, and sphingomyelin. Work by Yeh et al. (1993) specifically examined the fatty acid composition of brain myelin and, contrary to their expectations, found that dietary long-chain omega-3 PUFA could lead to a higher DHA content of myelin, with this change occurring during a critical phase of active myelination.

Only two studies investigated the effect of dietary long-chain omega-3 PUFA during lactation alone (Amusquivar et al., 2000; Yeh et al., 1993). While the work of Yeh et al. (1993) identified significant changes to the long-chain omega-3 PUFA and long-chain omega-6 PUFA content of brain synaptosomes, microsomes, myelin, and mitochondria, including higher DHA and lower AA, Amusquivar et al. (2000) found that while supplementation during lactation could induce higher EPA and lower AA brain content, it did not significantly alter brain DHA content (Table 24.2).

Several differences in brain EPA, DPA, and long-chain omega-6 PUFA content between the two diets were observed, but again with varied responses. A minority of studies reported that an ALA-rich diet resulted in significantly higher brain EPA (Fernandes et al., 2012) and DPA content (Sommer Hartvigen et al., 2004) compared to a long-chain omega-3 PUFA rich diet. The studies included here also describe mixed effects upon brain long-chain omega-6 PUFA status, with both lower (Fernandes et al., 2012; Sommer Hartvigen et al., 2004), and higher status (Alsted and Høy, 1992; Childs et al., 2010) reported with an ALA diet compared to a long-chain omega-3 PUFA diet. These differences between studies are likely to reflect differences in the mode of supplement delivery (i.e. direct gastric instillation was used in Valenzuela et al., 2004), the dietary dose of ALA (2–5% w/w) and long-chain omega-3 PUFA (0.2–1.84% w/w EPA + DHA) provided, and the relative dose of ALA:long-chain omega-3 PUFA selected for use in these studies for comparison (1.5–10:1).

Only one paper assessed other brain outcome measures, with performance in the Morris water maze not found to significantly differ between ALA and long-chain omega-3 PUFA supplemented rats (Sommer Hartvigen et al., 2004). Taken together the results indicate that direct provision of long-chain omega-3 PUFA has little advantage over dietary ALA in promoting brain DHA status in rats, but that differing effects upon other long-chain omega-3 PUFA and long-chain omega-6 PUFA are apparent. There is insufficient evidence to establish whether there are differences in functional outcomes between the two dietary interventions.

CONCLUSIONS

There is clear and convincing evidence that providing additional ALA or long-chain omega-3 PUFA to rats during pregnancy and/or lactation and early life can significantly influence brain fatty acid composition, and that this has the potential to induce measurable changes in cognitive performance (Fernandes et al., 2011; Joshi et al., 2004; Trevizol et al., 2013) and brain chemistry (Innis et al., 2001; Glozman et al., 1999; Roy et al., 2012; Trevizol et al., 2013) in rats. Data suggest that there are both potential risks and benefits of omega-3 PUFA supplementation during pregnancy upon offspring outcome measures. Altered patterns of fetal brain growth (Lenzi Almeida et al., 2011; Rao et al., 2007) and rates of neonatal maturation (Amusquivar et al., 2000) were observed, and evidence suggesting an adverse effect upon auditory development (Haubner et al., 2002; 2007; Saste et al., 1998;
### TABLE 24.3  Studies which Directly Compare Long-chain PUFA and ALA Supplementation.

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<tr>
<td>Fernandes et al., 2012</td>
<td>Sprague-Dawley</td>
<td>9% w/w fat content: Soybean oil (0.4% w/w ALA) Olive oil (0.04% w/w ALA) Fish oil (1.2% w/w EPA + DHA) Linseed oil (3.1% w/w ALA)</td>
<td>Day 1–12 gestation</td>
<td>Day 12 gestation, day 20 gestation, day 7 post-partum brain phospholipids</td>
<td>Linseed vs. fish oil (% fatty acids): ↓16:0, EPA ↓18:1n-9, AA, DHA Linseed oil vs. soybean oil (% fatty acids): ↓16:0, EPA, DPA ↓18:1n-9, AA, 22:5n-6 Fish oil vs. soybean oil (% fatty acids): DPA, DHA ↓22:5n-6</td>
<td>N/A</td>
</tr>
<tr>
<td>Childs et al., 2010</td>
<td>Wistar</td>
<td>Low fat (LF, 3% w/w): Soybean oil (0.1% w/w ALA) High fat (HF, 13% w/w): Soybean oil (0.9% w/w ALA) Linseed oil (5% w/w ALA) Salmon oil (1.3% w/w EPA + DHA) Sunflower oil (0.02% w/w ALA)</td>
<td>Day 1–20 gestation</td>
<td>Day 20 gestation, brain phospholipids (PC, PE)</td>
<td>HF linseed oil vs. HF fish oil (g/100 g): ↓AA, 22:5n-6 in PE HF linseed oil vs. HF soybean oil (g/100 g): ↑DPA in PE 22:5n-6 in PE HF fish oil vs. HF soybean oil (g/100 g): EPA, DPA in PE 22:5n-6 in PE</td>
<td>N/A</td>
</tr>
<tr>
<td>Sommer Hartvigsen et al., 2004</td>
<td>Wistar</td>
<td>20% fat w/w diets: High structured oil (2% w/w ALA) High linseed oil (2% w/w ALA) Low structured oil (0.4% w/w ALA) Low linseed oil (0.4% w/w ALA) Low salmon oil (0.2% w/w EPA + DHA) Control (1.26% w/w ALA, 0.5% w/w EPA + DHA)</td>
<td>Day 8 gestation – pup aged 13 weeks</td>
<td>Brain phospholipids (PE, PS) of pups at 1, 3, and 13 weeks of age</td>
<td>High linseed vs. low salmon oil (weight %): DPA in PS (1) 22:5n-6 in PE (1) and PS (1,3); 22:4n-6 in PE (0) and PS (13) High linseed vs. control (weight %): 22:4n-6 in PS (1); 22:5n-6 in PE (1) and PS (1,13) DPA in PS (1) Low salmon oils vs. control (weight %): No significant differences</td>
<td>Morris water maze: No significant differences in time taken to complete</td>
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<tr>
<td>Valenzuela et al., 2004</td>
<td>Habitual 8% w/w fat diet (0.26% ALA) Supplemented by gastric instillation with: 6 mg/kg DHA per day 60 mg/kg ALA</td>
<td>Wistar</td>
<td>40 days prior to mating and throughout gestation</td>
<td>Day 16 and 19 of gestation, 2 and 21 days post-delivery. Total lipids from frontal cortex, hippocampus, and cerebellum</td>
<td>No significant differences reported between ALA and DHA groups. DHA in frontal cortex at day 19 gestation and 2 and 21 days post-delivery. DHA in cerebellum at day 16 and 19 gestation and day 2 and 21 days post-delivery. DHA in hippocampus at 2 and 21 days post-delivery.</td>
</tr>
<tr>
<td>Alsted and Hay (1992)</td>
<td>20% w/w fat content: Fish oil diet (1.8% w/w EPA + DHA) Linseed oil diet (2.76% w/w ALA) Control diet (0.14% w/w ALA)</td>
<td>Female rats fed diet from weaning and throughout pregnancy and lactation until offspring aged 18 weeks</td>
<td></td>
<td>Brain PE, PS, PE, PIP, and PIP&lt;sub&gt;2&lt;/sub&gt; of offspring aged 18 weeks</td>
<td>Linseed oil vs. fish oil (weight %) of offspring: 16:0 (PIP&lt;sub&gt;2&lt;/sub&gt;, 16:1n-7 (PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), 18:1n-9 (PIP), LA (PS, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), 20:1n-9 (PI), 20:3n-6 (PE, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), EPA (PI, PE, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), 22:2n-6 (PIP), DPA (PI, PS, PE), 18:0 (PIP), AA (PE, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), 22:4n-6 (PS, PE), 22:5n-6 (PS)</td>
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<td>Fish oil vs. control (weight %) of offspring: 18:0 (PS, PIP), 20:1n-9 (PI), AA (PI, PS, PE, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), 22:4n-6 (PS, PE), 22:5n-6 (PS, PE) 16:0 (PIP&lt;sub&gt;2&lt;/sub&gt;), 16:1n-7 (PE, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), 18:1n-9 (PI, PIP), LA (PI, PS, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), 22:1n-9 (PS), 20:3n-6 (PE, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), EPA (PI, PE, PIP, PIP&lt;sub&gt;2&lt;/sub&gt;), 22:2n-6 (PIP), DPA (PI, PS, PE), DHA (PS)</td>
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Stockard et al., 2000). However, supplementary omega-3 PUFA was protective in models of toxic brain stimulation (Hogyes et al., 2003), hypoxia (Suganuma et al., 2010), ischemia (Glozman et al., 1999), and amphetamine-induced mania (Trevizol et al., 2013) and improved performance in measures of spatial awareness (Fernandes et al., 2011; Joshi et al., 2004), spatial memory (Fernandes et al., 2011), and recognition memory (Trevizol et al., 2013).

There are plausible mechanisms to support the pluripotent effects of omega-3 supplementation during pregnancy. AA status among premature infants has been demonstrated to be positively correlated to body weight, indicating that AA may have a growth-promoting effect (Koletzko and Braun, 1991). Provision of ALA or long-chain omega-3 PUFA has been demonstrated to significantly reduce circulating and tissue AA content (Childs et al., 2010) and may therefore be associated with a risk of impaired infant growth. The adverse effects observed upon neonatal auditory development in rats may be attributable to changes in the fatty acid composition of myelin at a critical phase of auditory development (Yeh et al., 1993). However, identifying whether these effects persist into an impaired auditory function in adulthood, or whether they reflect a transient delay in auditory development will require further research. The protective effect of omega-3 PUFA against acute or drug-induced brain injury is consistent with the role of DHA as a substrate for neuroprotectins. Neuroprotectins are potent anti-inflammatory and anti-apoptotic molecules (Serhan et al., 2004). Research into brain injury and aging has identified potential benefits of neuroprotectins in Alzheimer’s disease by blocking neurotoxicity in in vitro models (Lukiw et al., 2005), inhibiting white blood cell infiltration and inflammatory gene expression in a mouse model of stroke (Marcheselli et al., 2003), and improving neurological outcomes and reducing lesion size in a rat model of stroke (Bazan et al., 2012). Further research is required to confirm whether similar mechanisms underpin the protective effects observed in studies of acute brain injury during fetal development.

Data consistently indicate an increase in brain DHA content and a reduction in AA content with both ALA and long-chain omega-3 PUFA supplementation. In many studies, only the brain content of these two fatty acids is reported, but data suggest that the brain content of other long-chain omega-3 PUFAs and long-chain omega-6 PUFAs is also strongly influenced by diet, with significantly higher brain EPA and DPA and lower 22:4n-6 and 22:5n-6 reported in a large number of studies (Table 24.4).

Further studies will be required before firm conclusions are drawn regarding the relative efficacy of ALA versus long-chain omega-3 PUFA supplementation upon functional brain outcomes, but available data indicate that ALA is as effective at inducing higher brain DHA content in rats as a diet rich in long-chain omega-3 PUFA (Alsted and Høy, 1992; Childs et al., 2010; Sommer Hartvigsen et al., 2004; Valenzuela et al., 2004), but is also suggestive of a more modest effect in lowering brain long-chain omega-6 PUFA status. Further studies of whether omega-3 fatty acids may confer protection against hypoxia, ischemia, toxic brain injury, drug-induced mania or alter auditory function in humans, and whether ALA and long-chain omega-3 PUFA are equipotent in this regard, is worthy of further investigation.

**TABLE 24.4** Summary of Studies which Identify Changes to Brain Long-chain omega-3 and omega-6 PUFA after omega-3 Supplementation During Pregnancy and/or the Post-Natal Period in Addition to the Effects Observed upon AA and DHA.

<table>
<thead>
<tr>
<th>EPA</th>
<th>DPA mass</th>
<th>22:4n-6</th>
<th>22:5n-6</th>
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<tr>
<td>Alsted and Høy, 1992</td>
<td>Alsted and Høy, 1992</td>
<td>Alsted and Høy, 1992</td>
<td>Alsted and Høy, 1992</td>
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<td>Cheon et al., 2000</td>
<td>Childs et al., 2010</td>
<td>Childs et al., 2010</td>
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<td>Childs et al., 2010</td>
<td>Fernandes et al., 2011</td>
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<td>Fernandes et al., 2012</td>
<td>Guesnet et al., 1997</td>
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<td>Ikleno et al., 2009</td>
<td>Haubner et al., 2002</td>
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<td>Haubner et al., 2002</td>
<td>Lenz Almeida et al., 2011</td>
<td>Lenz Almeida et al., 2011</td>
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<td>Yeh et al., 1993</td>
<td>Sommer Hartvigsen et al., 2004</td>
<td>Sommer Hartvigsen et al., 2004</td>
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<td></td>
<td>Trevizol et al., 2013</td>
<td>Trevizol et al., 2013</td>
<td>Trevizol et al., 2013</td>
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<td>Yeh et al., 1993</td>
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OMEGA 3 FATTY ACIDS IN BRAIN AND NEUROLOGIC HEALTH
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References


Abstract

Dietary omega-3 fatty acids include α-linolenic acid (ALA) and the long-chain omega-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). Here we review available data from rat studies on the effect of omega-3 PUFA supplementation during pregnancy and/or early life upon brain composition and functional outcomes. Brain DHA content is significantly higher, and arachidonic acid (AA) content lower, after both ALA and long-chain omega-3 PUFA supplementation in the rat. Data suggest both potential risks and benefits of omega-3 PUFA upon offspring outcome measures, with adverse effects upon fetal brain growth, rates of neonatal maturation, and auditory development, but protection against toxic brain stimulation, hypoxia, ischemia, and drug-induced mania, and improved spatial awareness, spatial memory, and recognition memory. Further studies are required to directly compare the efficacy of ALA and long-chain omega-3 PUFA during pregnancy and/or lactation in influencing brain fatty acid composition and changes to functional outcome measures.

Keywords: Omega-3; brain; pregnancy; fetal