

Dietary (n-3) Fatty Acids and Brain Development^{1,2}

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Abstract

The (n-3) fatty acids are essential dietary nutrients, and one of their important roles is providing docosahexaenoic acid [22:6(n-3)] (DHA) for growth and function of nervous tissue. Reduced DHA is associated with impairments in cognitive and behavioral performance, effects which are particularly important during brain development. Recent studies suggest that DHA functions in neurogenesis, neurotransmission, and protection against oxidative stress. These functions relate to the roles of DHA within the hydrophobic core of neural membranes and effects of unesterified DHA. Reviewed here are some of the recent studies that have begun to elucidate the role of DHA in brain development and function. A better understanding of development and age-specific changes in DHA transfer and function in the developing brain may provide important insight into the role of DHA in developmental disorders in infants and children, as well as at other stages of the lifespan.

Introduction

Dietary (n-3) fatty acids and their metabolism. Docosahexaenoic acid [22:6(n-3)] (DHA)³ is the most abundant (n-3) fatty acid in the mammalian brain, and its levels in brain membrane lipids are altered by the type and amount of fatty acids in the diet, and with life stage, increasing with development and decreasing with aging (1–3). Mammals obtain DHA either as DHA itself or the precursor α -linolenic acid [ALA, 18:3(n-3)], and intermediates between ALA and DHA, including eicosapentaenoic acid [EPA, 20:5(n-3)]. Synthesis of DHA and EPA occurs in phytoplankton and animals, but not plants. DHA and EPA are absent from all vegetable fats and oils, including nuts, grains, and seeds and are also very low in ruminant fats, including milk and dairy products. The richest dietary sources are fish and sea foods, but poultry and eggs provide lower, but important, sources of EPA and DHA (4). The major dietary sources of ALA are soybean and canola oils; flax seed oils and some nuts are also high in ALA, but these latter sources are not usually consumed consistently or in large quantities.

Once obtained from the diet, ALA can be further metabolized by Δ -6 desaturation, elongation, and Δ -5 desaturation to EPA on the endoplasmic reticulum (4). The pathway generally accepted for further metabolism of EPA to DHA is that proposed by Sprecher and colleagues, which involves 2 sequential elongations of EPA to 24:6(n-3), followed by transport to the peroxisomes, and then a single cycle of β -oxidation to yield DHA, which is then transported back to the microsomes for incorporation into glycerolipids (5). Intermediate steps of translocation among cell compartments and their regulation, however, are unclear. Dietary deficiency of (n-3) fatty acids results in increased desaturation of (n-6) fatty acids to their 22 carbon chain metabolites, particularly (n-6) docosapentaenoic acid [DPA, 22:5(n-6)] (6). Infante and Huzagh have suggested that synthesis of DHA and (n-6) DPA occurs in the mitochondria via specific carnitine-dependent pathways, and with separate enzymes for the (n-6) and (n-3) fatty acids (7). However, even though the pathways involved in DHA synthesis are incompletely resolved, stable isotope tracer studies and interventions to increase dietary ALA intake concur that, when based on the appearance of ALA metabolites in blood lipids, ALA conversion to DHA is low in humans, with <1% dietary ALA converted to DHA (8). Although the conversion of ALA to DHA appears to be higher in women than in men, and increased in pregnancy (8), increased dietary intakes of ALA do not increase DHA in blood lipids of either pregnant women or their newborn infants (9). Early studies addressing the low blood (plasma and red blood cell membrane) lipid DHA in infants fed formula suggested low (immature) activity of fatty acid desaturase enzyme activity in newborns. More recent stable isotope tracer studies have now shown that ALA conversion to DHA is as high in preterm as in term gestation infants and is similar to that in adult men (8,10). Thus, low rates of DHA synthesis from ALA appear to be a general characteristic of human metabolism, with the slowest step in (n-3) fatty acid desaturation being at the conversion of EPA to DHA (8). Dietary DHA, however, is well absorbed and readily incorporated into plasma and blood cell lipids in humans (as shown in many studies relating DHA intake from fish and fish oils to cardiovascular disease risk endpoints and inflammatory mediators). Several animal studies have also shown that dietary DHA is readily incorporated into lipids of the developing brain, both before and after birth (1).

Before birth, DHA is transported across the placenta via pathways involving fatty acid binding, and transport proteins are then released to the fetal circulation (11,12). α -Fetoprotein is the major plasma transport protein before birth and has higher DHA than albumin (6). Observational and intervention studies concur that higher dietary intakes of DHA during pregnancy result in higher maternal-to-fetal transfer of DHA (11); knowledge that presents a paradox for placental transport mechanisms suggested to facilitate preferential DHA transfer to the fetus. After birth, the infant is provided with DHA in mother's milk (13). However, human milk levels of DHA vary from <0.1 to >1.0 g/100 g milk

¹ Research in the author's laboratory is supported by grants from the Canadian Institutes of Health Research (CIHR).

² Manuscript received 24 November 2006.

³ Abbreviations used: ALA, α -linolenic acid [18:3(n-3)]; ARA, arachidonic acid [20:4(n-6)]; DHA, docosahexaenoic acid [22:6(n-3)]; DPA, docosapentaenoic acid [22:5(n-3)]; EPA, eicosapentaenoic acid [20:5(n-3)]; EPG, ethanolamine phosphoglycerides; FABP, fatty acid binding protein; PC12, rat clonal pheochromocytoma cells; PS, phosphatidylserine; RXR, retinoid X receptor (RXR); RAR, retinoic acid receptor, SNARE, soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor.

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fatty acids, a result of differences in the amount of DHA in the mother's diet (13). As in adults, increasing the intakes of DHA, but not ALA, from human milk or milk substitutes results in higher blood lipid levels of DHA in the recipient infant (13). For example, infants fed formula with 0.4 or 2.4% energy from ALA had 2.3 ± 0.2 and 2.2 ± 0.3 g/100 g fatty acids as DHA in plasma phospholipids, respectively, despite the large difference in ALA intake, whereas infants fed formula with only 0.12% energy from DHA had plasma phospholipid DHA levels of 5.2 ± 0.2 g/100 g (14,15). Analyses of human infant autopsy tissue have shown lower brain cortex DHA, ~15% lower, in infants fed formula with no DHA than in breast-fed infants (16,17). Chronic dietary restriction of (n-3) fatty acid in developing animals results in reduced brain DHA, increased brain levels of (n-6) fatty acids, including DPA, and deficits in behavioral tasks of learning (1,4,6). Animal models addressing the role of DHA in the developing brain use dietary restriction of all (n-3) fatty acids, including ALA, EPA, and DHA, to overcome efficient conversion of ALA to DHA, particularly in rodents (1). Typically, brain DHA levels are 50–80% lower in (n-3) fatty acid-deficient animals than control animals, and this, together with usual issues relating to species differences, needs to be considered in extrapolating from studies with animals to humans.

Dietary (n-3) fatty acids: their origin and metabolism in brain development. High proportions of DHA in brain ethanolamine phosphoglycerides [(EPG), ethanolamine plasmalogen, and phosphatidylethanolamine] and phosphatidylserine (PS), reaching as high as 35% of fatty acids in synaptic plasma membranes, is a characteristic feature of the mammalian brain, even among herbivores, and regardless of low concentrations of DHA in plasma and hepatic lipids (1). Plasma lipid levels of DHA, on the other hand, are low in most terrestrial animals, including humans, suggesting the brain has particular mechanisms to concentrate DHA. Fatty acids at the glycerophospholipid C-1 and C-2 positions are continuously remodeled because of degradation and remodeling involving phospholipases and acyl CoA synthetases, and these remodeling steps seem likely to be a major route for fatty acid incorporation into membrane lipids (18).

In vitro, glia and cerebral endothelial cells, but not neurons, can form DHA from ALA and other precursor n-3 fatty acids (19), but whether this contributes meaningfully to brain DHA is uncertain. More likely, DHA is taken up from plasma, possibly involving concentration at the capillary endothelium, which also contains high amounts of DHA (6). There is no evidence that the developing brain is able to take up DHA over (n-6) DPA, yet recent studies point to important structural specificity for DHA in brain functions (20). Pathways for transfer of DHA to the brain involving unesterified fatty acids, lysophospholipids, and HDL have all been described (6). Further understanding of brain DHA uptake will provide much needed insight for interpretation of those plasma (n-3) fatty acid levels that pose risk of inadequate DHA transfer to the developing brain.

Dietary (n-3) fatty acids in brain development and function. Several hypotheses have been proposed to explain the role of DHA in the brain, which in general can be divided into properties conferred by lipid-bound DHA in the membrane bilayer and those related to unesterified DHA. Functions related to the membrane include those properties of the hydrophobic membrane core, such as conferring a high degree of flexibility and direct interaction with membrane proteins, thus impacting speed of signal transduction, neurotransmission, and formation of lipid rafts (21–23). Unesterified DHA, on the other hand, appears to

have roles in regulating gene expression, ion channel activities, and can be further metabolized to neuroprotective metabolites (24–26) in the brain. More recent studies also suggest that DHA is important in neurogenesis and also influences phospholipid synthesis and turnover (27–29).

Fatty acid-binding proteins (FABP) are a multi-gene family of small cytosolic proteins that function as cytoplasmic fatty acid transporters, playing key roles in fatty acid transfer to membranes, and mediating the effects of fatty acids on gene expression, and as precursors for synthesis of other metabolites. Among the FABP in brain, B-FABP is localized in the ventricular germinal cells and glial cells in the embryonic brain and in the astrocytes of developing and adult brains, whereas heart (H)-FABP is present in adult brains (30,31). B-FABP expression during development parallels that of early neuronal differentiation and is thought to be important early neurogenesis or neuronal migration. Mice with a null mutation in the B-FABP gene show decreased brain DHA in the neonatal period, and later enhanced anxiety and increased fear memory, which suggests an important role for DHA in early development for these behaviors (31). Spatial learning and memory are impaired in rodents fed an (n-3) fatty acid-deficient diet during development (1,6). However, B-FABP^{-/-} mice demonstrated no deficits in spatial learning or memory (31), suggesting later maturation, or a role of other DHA binding proteins in cellular delivery of DHA relating to these behaviors.

The (n-3) and (n-6) fatty acids are ligands for PPAR, a group of nuclear transcription factors that heterodimerize with the retinoid X receptor (RXR) and bind to specific regions of DNA to regulate transcription of target genes. PPAR γ is highly expressed in embryonic mouse brain and neural stem cells, in contrast to extremely low levels in adult brains, and appears to be important in regulating the early brain development, through effects that include regulation of stem cell proliferation (32). Recent studies have confirmed that DHA, as well as arachidonic acid [ARA, 20:4(n-6)], are ligands for brain RXR (33). Together with its retinoic acid receptor (RAR), RXR play key roles in many aspects of development, including neurogenesis during embryogenesis, morphological differentiation of catecholaminergic neurons, and activity-dependent plasticity. RAR and RXR are also highly expressed in the hippocampus, which may be relevant to understanding the role DHA adult brain function (33,34). Further, several studies have provided evidence that (n-3) fatty acid deficiency alters the expression of genes involved in the control of synaptic plasticity, cytoskeleton and membrane assembly, as well as signal transduction and ion channel formation (35), many of which are downstream targets of RAR-RXR signaling. Early changes in gene expression leading to altered molecular and morphological development could have long-term implications to brain function. However, resolving critical windows for DHA with respect to behavioral and cognitive development in humans will be challenging.

Recent studies also point to important roles for DHA in inhibition of oxidative stress-induced induction of proinflammatory genes and apoptosis in the brain and retina. Phospholipase A2 releases unesterified DHA, which is further metabolized to docosanoids, of which neuroprotectin D1 is a potent inhibitor of oxidative stress induced apoptosis and cyclo-oxygenase 2 (24). Consistent with this role, recent studies have shown that DHA has important free radical scavenging properties and protects against peroxidative damage of lipids and proteins in developing and adult brains, with attenuation of neuron loss and cognitive and locomotor deficits in animal models of ischemia-reperfusion brain injury (36–38).

The high proportion of DHA in neural membranes also raises the possibility that (n-3) deficiency may impair membrane

biogenesis, influencing such events as neurogenesis, neuronal migration, and outgrowth. DHA appears to be important for PS synthesis both in vivo and in vitro, which has broad implications, because of both the need for PS for membrane biosynthesis and also through the role of PS in apoptosis (29). Recent studies have suggested that (n-3) fatty acid deficiency decreases the mean cell body size of neurons in the hippocampus, hypothalamus, and parietal cortex, and decreases the complexity of dendritic arborizations on cortical neurons (39,40). Consistent with this line of investigation, DHA enhanced neurite outgrowth of hippocampal and cortical neurons and rat clonal pheochromocytoma (PC12) cells in culture (28,41,42). However, the usual caution is needed in extrapolating from studies with isolated cell types and transformed cells to the in vivo situation. Recently, we showed that maternal dietary (n-3) fatty acid restriction in gestation alters neurogenesis in the fetal rat cerebral cortex (27). The dentate gyrus and associated hilus of the hippocampal formation showed a consistent increased thickness in proliferative zones, with a decreased size of target regions, which could reflect an inhibition or delay of neurogenesis, or disruption in postmitotic migration (27). Other recent studies have shown that DHA promotes cell-cycle exit in retinal neuroprogenitor cells in culture (43) and promotes differentiation of neural stem cells into neurons by promoting cell-cycle exit and suppressing cell death (28). Restriction of (n-3) fatty acids leads to reduced DHA, increased (n-6) fatty acids, and increased dopamine in fetal brain cortex (44) and over-expression of dopamine receptor genes (45). Because dopamine D1 receptor activation reduces the entry of progenitor cells from the G1- to S-phase of the cell cycle, while D2 receptor activation promotes G1- to S-phase entry in embryonic lateral ganglionic eminence (27), altered monoaminergic neurotransmission could contribute to, as well as result from, disruption of neurogenesis associated with (n-3) fatty acid deficiency during development.

In addition to evidence that DHA may influence brain development through effects on gene expression, monoaminergic neurotransmission, or protection against apoptotic cell death, growth of neurite processes from the cell body is a critical step in neuronal development and involves a large increase in cell membrane surface area. This necessitates lipid biogenesis and membrane expansion through the fusion of transport organelles with the plasma membrane. Plasmalemmal precursor vesicle fusion into the cell membrane involves soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptor (SNARE) proteins, similar to that involved in interneuronal communication and information processing through release of neurotransmitter vesicles. Neurotransmitter secretion involves fusion of intracellular neurotransmitter storage vesicles with the plasma membrane and exocytosis, triggered by the influx of calcium into the nerve terminal, and occurring on a sub-millisecond timescale. This process requires formation of a tight ternary complex between the vesicular (v) SNARE, termed synaptobrevin (or VAMP, vesicle-associated membrane protein), and 2 membrane target (t)-SNARE, known as syntaxin and SNAP-25 on the cytosolic surface of the plasma membrane (46). Interestingly, recent studies have shown that reactive oxygen species impair acetylcholine release, independent of G-protein cascades, through presynaptic functioning of SNAP-25 as a reactive oxygen species sensor (47). Possibly, this may provide a link between the role of DHA in protection against oxidative stress (24) and in altering neurotransmission (23), including acetylcholine (48). Other recent studies have shown that the ability of syntaxin 3, which is essential for neurite growth in PC12 cells, to partner with other SNAREs strictly requires the binding of unesterified (n-3) or (n-6)

fatty acids, a role fulfilled by DHA (49), which thus provides a functional link between DHA and neurite outgrowth. Whether similar dependency occurs for syntaxin 1 in brain is not yet known.

Several studies have shown that monoaminergic and cholinergic systems are affected in rodents chronically deprived of (n-3) fatty acids during development (1,6,23,48). The changes described are complex, with effects at multiple levels, including synthesis, storage, release, and receptor-mediated uptake, with effects that also differ among different regions of the cortex. Chalon recently summarized extensive research on the effects of chronic (n-3) fatty acid deficiency on dopaminergic and serotonergic neurotransmission in rodents (23). Restitution of an adequate diet prior to weaning effectively restored several parameters of dopaminergic and serotonergic neurotransmission; however, the stimulated release of serotonin and dopamine, and the vesicular monoamine transporter 2 (VMAT₂), showed lasting deficits when an adequate diet was not fed until weaning. Similarly, recent studies with rhesus monkeys found that (n-3) fatty acid deficiency during only prenatal development resulted in lower amplitudes of cone and rod ERG a-waves when measured some 3 y after deficiency was corrected (50). These latter studies emphasize the potential long-term effects of inadequate supplies of DHA to meet the needs of the developing brain.

DHA in human brain development. Although there is no doubt that DHA is critical in the developing brain, the question of whether dietary DHA is important during human brain development remains unresolved. From the foregoing, it is clear that (n-3) fatty acid deprivation may affect brain development at multiple levels, from membrane biogenesis, through gene expression, protection against oxidative stress, and altered neurotransmission, with the effects differing, and the potential for recovery dependent on when the deficiency is imposed. Because DHA accretion relative brain weight is greatest during fetal development and early infancy, it is generally considered that this reflects a critical time during which deficiency of DHA may have long-term consequences for later brain function. A large number of clinical trials have been conducted on the effects of DHA supplementation in infants fed formula, and most find no advantage of enhanced DHA nutrition on mental and motor skill development in term infants (51,52). Studies by Birch et al. in Texas, however, have shown a benefit of dietary DHA in infants, whether fed from birth or later, after initial breast-feeding (53). Recently, attention has turned to DHA supplementation of pregnant and lactating women, again with most studies reporting no advantages to infant development during the first year after birth (52,54–58). However, regardless of the absence of differences between placebo and DHA intervention groups, a positive association between the infants' DHA status and neurodevelopmental outcome has been shown in several studies (54,55,58), mirroring the results of observational studies (4). Some longer-term follow-up studies are also emerging to suggest positive effects of early enhanced DHA nutrition on mental and motor skill development when measured in early childhood (59,60). Several explanations, and the complexity of such studies in humans, need to be considered. First, it remains possible that DHA is not required in humans appropriately nourished with the correct LA and ALA balance; individuals who adhere to vegetarian and vegan diets do synthesize small amounts of DHA, and evidence to indicate deficits in brain development among vegetarians has not been published. Next, DHA may be critical, but for reasons other than those as yet addressed; for example in protection against early ischemic insults, or aspects of behavior

currently beyond the scope of infant testing. Study design errors may also be important; for example, low intakes of (n-3) fatty acids or other nutrient deficiencies at the time of testing may overshadow effects stemming from insults earlier in life.

In conclusion, the role of (n-3) fatty acids in brain development and healthy brain aging is emerging as a field of intense scientific inquiry and of considerable public health importance. The evidence to show low rates of conversion of ALA to DHA and the demonstrated importance of DHA for brain function provide strong evidence that DHA is important for human brain development. The events in which DHA fulfils its essential roles, including neurotransmission, neurogenesis, and protection from oxidative stress are relevant throughout the lifespan and to maximizing cognitive potential in development and minimizing its loss with aging. A concerted effort is needed to better understand (n-3) fatty acid requirements to support optimal brain development and function and to elucidate those dietary conditions and diet-gene, or diet-disease, interactions that pose risk of inadequate brain DHA.

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