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A Diet Enriched with the Omega-3 Fatty Acid Docosahexaenoic Acid Reduces Amyloid Burden in an Aged Alzheimer Mouse Model

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Epidemiological studies suggest that increased intake of the omega-3 (n-3) polyunsaturated fatty acid (PUFA) docosahexaenoic acid (DHA) is associated with reduced risk of Alzheimer’s disease (AD). DHA levels are lower in serum and brains of AD patients, which could result from low dietary intake and/or PUFA oxidation. Because effects of DHA on Alzheimer pathogenesis, particularly on amyloidosis, are unknown, we used the APPsw (Tg2576) transgenic mouse model to evaluate the impact of dietary DHA on amyloid precursor protein (APP) processing and amyloid burden. Aged animals (17–19 months old) were placed in one of three groups until 22.5 months of age: control (0.09% DHA), low-DHA (0%), or high-DHA (0.6%) chow. Dietary DHA also decreased Aβ42 levels below those seen with control chow. Image analysis of brain sections with an antibody against Aβ revealed that overall plaque burden was significantly reduced by 40.3%, with the largest reductions (40–50%) in the hippocampus and parietal cortex. DHA modulated APP processing by decreasing both α- and β-APP C-terminal fragment products and full-length APP. BACE1 (β-secretase activity of the β-site APP-cleaving enzyme), ApoE (apolipoprotein E), and transthyretin gene expression were unchanged with the high-DHA diet. Together, these results suggest that dietary DHA could be protective against β-amyloid production, accumulation, and potential downstream toxicity.

Key words: DHA; polyunsaturated fatty acid; Aβ; APP; secretase; Alzheimer

Introduction

The essential fatty acids, which include the omega-6 (n-6) and omega-3 (n-3) fatty acids, are crucial components of the diet. Docosahexaenoic acid (DHA) is an n-3 polyunsaturated fatty acid (PUFA) found predominantly in marine fish and algae. Although it has a low conversion rate, it can also be biosynthesized in vivo in mammals from dietary n-3 sources, notably α-linolenic acid (Salem et al., 1996b). It is essential for prenatal brain development and normal maintenance of brain function and vision in adults (Mitchell et al., 1998). Deficiencies in the level of DHA, such as low serum levels as well as high dietary intake ratios of n-6/n-3 fatty acids, have been linked to cognitive impairment (Connor et al., 1990; Suzuki et al., 1998; Kyle et al., 1999; Gamoh et al., 2001; Ikemoto et al., 2001; Catalan et al., 2002) and Alzheimer’s disease (AD). The brain membranes of AD patients have been found to be deficient in DHA (Soderberg et al., 1991; Prasad et al., 1998). The loss of DHA in AD may reflect its propensity for free radical-mediated lipid peroxidation (because of its six double bonds), resulting in its conversion to neuroprostanes (F-4 isoprostanes), which are elevated in AD (Nourooz-Zadeh et al., 1999; Montine et al., 2002). Decreased dietary intake and increased oxidative stress could contribute to brain DHA depletion and low blood levels in AD patients. Several epidemiological studies show a protective effect associated with increased fish consumption and intake of unsaturated fats leading to low n-6/n-3 fatty acid ratios (Kalmijn et al., 1997; Barberger-Gateau et al., 2002; Grant et al., 2002; Yamada et al., 2002; Morris et al., 2003; Kalmijn et al., 2004). Thus, it seems that n-3-rich diets may be beneficial in reducing risk for AD, but it is unclear how n-3 impacts AD pathogenesis and whether DHA is the preventive dietary factor in fish oil.

Feeding n-3-depleted diets to multiple generations of animals

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limits their learning ability, but learning is restored when they are switched to diets supplemented with DHA (Connor et al., 1990; Suzuki et al., 1998; Gamoh et al., 2001; Ikemoto et al., 2001; Moriguchi and Salem, 2003). This adverse effect on CNS function in the absence of neurodegenerative pathology may contribute to increased AD risk. DHA or its enzymatically generated metabolites may be neuroprotective by reducing \( \beta \)-amyloid (\( \beta \)42) toxicity (Mukherjee et al., 2004). Consistent with this view, preadministration of DHA in \( \beta \)-amyloid-infused rats protected against neuronal apoptosis and was beneficial in reducing \( n \)-6/\( n \)-3 ratios and the decline of learning ability (Hashimoto et al., 2002). Furthermore, DHA depletion caused AD-like phosphatidylinositol 3 kinase (PI3K) deficits and enhanced behavioral deficits in a transgenic (Tg) model (Calon et al., 2004). It is unknown whether some of these beneficial effects of DHA occur because of direct effects on \( \beta \)42, the causal factor in AD, such as limiting \( \beta \)42 production, aggregation, and accumulation. Therefore, the purpose of this study was to evaluate the impact of DHA on \( \beta \)42 production and amyloid precursor protein (APP) processing in the Tg2576 mouse.

### Materials and Methods

**Animal treatment groups.** Seventeen- to 19-month-old male and female Tg2576 mice were placed on one of three diets: (1) control chow (Purina 5015 breeder chow; Purina Mills, St. Louis, MO) was the standard chow on which all animals were raised (\( n \)-6/\( n \)-3 ratio of 7:1); (2) a \( \beta \)-amyloid-depleting low-\( n \)-3 PUFA diet adequate in all other nutrients that we called "low-DHA" diet (\( n \)-6/\( n \)-3 ratio of 85:1; TD05225; Harlan Teklad, Madison, WI) with 6% fat as safflower oil; or (3) low-DHA diet supplemented with 0.6% DHA (Marteck Bioscience, Columbia, MD), which we referred to as "high-DHA" diet (TD01200) (Calon et al., 2004). Mice were fed control chow (0.09% DHA; \( n \)= 8), low-DHA chow (0% DHA; \( n \)= 6), or high-DHA chow (0.6% DHA; \( n \)= 6) for an average of 103 ± 5 d before being killed at 22.5 months of age. After animals were perfused with HEPES buffer, brain regions were dissected from one hemisphere as described previously (Lim et al., 2000). Biochemical measurements were performed in the residual cortex (cortex region without frontal, entorhinal, or piriform areas, which were used for confirmation of DHA depletion and the absence of presynaptic marker loss). The other brain hemisphere was fixed in 4% paraformaldehyde and processed for immunohistochemistry.

**Tissue preparation.** Tissue samples were processed in TBS (solute fraction) and lysis buffer (membrane fraction) containing protease inhibitor mixture as described previously (Lim et al., 2001; Calon et al., 2004). Briefly, brain tissue was homogenized and sonicated in TBS. The insoluble pellet was then sonicated in lysis buffer (150 mM NaCl, 10 mM NaH2PO4, 1 mM EDTA, 1% Triton X-100, 0.5% SDS, and 0.5% deoxycholate) containing the same protease inhibitor mixture. The resulting homogenate was subjected to ultracentrifugation, and the lysis-soluble supernatant was collected and frozen. To analyze the detergent-insoluble \( \beta \)42, the lysis-insoluble pellet was sonicated in 8 vol of 5 M guanidine and 50 mM Tris-HCl and solubilized by agitation at room temperature for 3–4 h.

**Fatty acid analysis.** Fatty acid analysis in the frontal cortex was performed using the Folch method (Moriguchi et al., 2000) and gas chromatography with flame ionization detection, as reported previously (Salem et al., 1996a; Calon et al., 2004).

**\( \beta \)42 levels.** The sandwich ELISA for total \( \beta \)42 has been described previously (Lim et al., 2000) and \( \beta \)42-depleting low-\( n \)-3 PUFA test diet adequate in all other nutrients that we called "low-DHA" diet (\( n \)-6/\( n \)-3 ratio of 85:1; TD05225; Harlan Teklad, Madison, WI) with 6% fat as safflower oil; or (3) low-DHA diet supplemented with 0.6% DHA (Marteck Bioscience, Columbia, MD), which we referred to as "high-DHA" diet (TD01200) (Calon et al., 2004). Mice were fed control chow (0.09% DHA; \( n \)= 8), low-DHA chow (0% DHA; \( n \)= 6), or high-DHA chow (0.6% DHA; \( n \)= 6) for an average of 103 ± 5 d before being killed at 22.5 months of age. After animals were perfused with HEPES buffer, brain regions were dissected from one hemisphere as described previously (Lim et al., 2000). Biochemical measurements were performed in the residual cortex (cortex region without frontal, entorhinal, or piriform areas, which were used for confirmation of DHA depletion and the absence of presynaptic marker loss). The other brain hemisphere was fixed in 4% paraformaldehyde and processed for immunohistochemistry.

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The absence of contamination was checked using reverse transcription (RT) products made without Moloney murine leukemia virus reverse transcriptase. The R² of relative standard curves in TaqMan PCR was above 0.99. For optimum reliable comparisons, only sample values from the same PCR plate were statistically analyzed and are shown here.

## Results

### CNS fatty acid profile reflects expected changes with DHA depletion and repletion

DHA deficiency can be confirmed by assessing the levels of other C22 fatty acids that coordinately change in rodent brain in response to DHA depletion and repletion. For example, docosapentaenoic acid (DPA) (22:5 n-6) shows a compensatory increase in rodent brain with DHA-deficient diets (Youyou et al., 1986; Salem et al., 2001). We reported previously that the DHA content in the frontal cortex of Tg2576 mice was depleted when animals were placed on this low-DHA diet paradigm, which resulted in DPA increasing 3.1-fold \( (p < 0.001) \), but with DHA supplementation resulted in an 8.4-fold decrease \( (p < 0.0001) \) (Calon et al., 2004). Another 22 carbon fatty acid, adrenic acid, C22:4 (n-6), is also increased by DHA depletion and restored to normal levels by DHA supplementation (Ikemoto et al., 2001; Greiner et al., 2003). Hence, we measured the levels of adrenic acid in frontal cortex of all animals (Table 1). Adrenic acid was significantly increased by 15% in transgenic mice on low-DHA diets \( (p < 0.01) \) compared with control diet; there was no significant increase in nontransgenic animals. When placed on high-DHA diets, adrenic acid levels decreased by 29–37% \( (p < 0.0001) \). Therefore, adrenic acid changes were consistent with transgene- and diet-dependent DHA depletion that was restored with DHA supplementation.

### DHA lowers insoluble Aβ, but not soluble Aβ, in cortex

To evaluate whether DHA can change amyloid levels, insoluble Aβ was measured in the guanidine-soluble extract. One-way ANOVA demonstrated a significant treatment effect between animals on low-DHA diet and high-DHA diet, in which DHA lowered the level of amyloid by 77% \( (p < 0.05) \) (Fig. 1A). Despite high DHA decreasing insoluble amyloid levels compared with low DHA, levels in the low-DHA group were not significantly different from levels seen in the control group \( (p = 0.06) \). Soluble Aβ was also measured from the TBS-soluble fraction of the same three groups. There was a 38% reduction in soluble Aβ in the high-DHA group when compared with low-DHA mice, but statistical analysis showed that this difference was not significant \( (p = 0.50) \) (Fig. 1B).

### Aβ42 and Aβ40 levels are also lowered by DHA treatment

C-terminus antibodies specific for Aβ42 and Aβ40 were used to determine how DHA was affecting Aβ40 and Aβ42 in the cortex. One-way ANOVA analyses of both Aβ40 and Aβ42 in the guanidine-soluble fraction showed treatment effects between low-DHA and high-DHA animals. Aβ42 levels were significantly reduced by 49.1% in the high-DHA group compared with the low-DHA group \( (p < 0.01) \) (Fig. 1C) and reduced by 53.6% comparing high DHA with control group \( (p < 0.001) \). However, low-DHA and control group Aβ42 levels were not significantly different, as was observed with total insoluble Aβ results. Interestingly, Aβ40 levels were significantly increased by 65% in the low-DHA group when compared with the control group \( (p < 0.01) \) (Fig. 1D). High-DHA diet decreased Aβ40 by 47.5% \( (p < 0.01) \), comparable with levels seen in the control group.

### DHA lowers plaque burden

To determine whether DHA was reducing plaque burden, tissue sections from low-DHA and high-DHA mice were stained with an antibody against Aβ1−13. Image analysis was performed on hippocampus, frontal cortex, parietal cortex, entorhinal cortex, and perirhinal cortex regions. ANOVA showed a significant overall treatment effect, in which plaque burden was lowered by

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**Table 1. Effect of dietary treatment on docosatetraenoic (22:4n-6) acid levels in Tg2576 mice**

<table>
<thead>
<tr>
<th>Diet group</th>
<th>Dietary PUFAs</th>
<th>Brain DTA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHA % w/w</td>
<td>n-6/n-3 ratio</td>
</tr>
<tr>
<td>Control ( (n = 8) )</td>
<td>0.09</td>
<td>7:1</td>
</tr>
<tr>
<td>Low DHA ( (n = 6) )</td>
<td>&lt;0.01</td>
<td>85:1</td>
</tr>
<tr>
<td>High DHA ( (n = 6) )</td>
<td>0.6</td>
<td>5:1</td>
</tr>
</tbody>
</table>

Units are mean percentage of total fatty acids. * \( p < 0.05 \) compared with control chow group; ** \( p < 0.001 \) compared with low-DHA group. DTA, Docosatetraenoic acid.

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**Figure 1.** Detergent-insoluble amyloid (total Aβ, Aβ42, and Aβ40) is reduced in cortex of animals fed DHA-enriched diets. **A.** Detergent-insoluble amyloid measurements in cortex. Total Aβ ELISA was performed on guanidine-soluble amyloid from cortices of low-DHA \( (n = 6) \) and high-DHA \( (n = 6) \) groups. Error bars represent SE. * \( p < 0.05 \) compared with low-DHA group. **B.** Soluble amyloid measurements in cortex. Total Aβ ELISA was performed on TBS-soluble fractions \( (24 \mu g) \) of the same two groups of animals. Error bars represent SE. **C.** Aβ42 measurements in cortex. Aβ42 ELISA \( (Biosearch) \) was performed on guanidine-soluble fractions in cortices of animals fed control \( (n = 8) \), low-DHA \( (n = 6) \), and high-DHA \( (n = 6) \) diets. ** ** \( p < 0.01 \) compared with low-DHA group; *** \( p < 0.001 \) compared with control diet group. Error bars represent SE. **D.** Aβ40 ELISA \( (Biosearch) \) was used to measure levels of Aβ40 in cortices of the same three groups of animals. *** \( p < 0.01 \) compared with control group; ** ** \( p < 0.01 \) compared with low-DHA group. Error bars represent SE.
DHA may regulate Aβ clearance by modulating expression of amyloid binding proteins TTR and ApoE. Therefore, the cortical TTR and ApoE mRNA levels were measured as a function of diet, but, surprisingly, no changes were found (data not shown).

DHA can alter levels of full-length APP and β- and α-secretase products

Because DHA reduced the detergent-insoluble amyloid and plaque burden, we evaluated whether DHA altered APP processing because secretase pathways are influenced by their lipid environments. To determine whether DHA changed the amount of secreted full-length APP, immunoblots from the TBS-soluble and lysis fractions of mice on control, low-DHA, and high-DHA diets were probed with 22C11 APP antibody that recognizes an extracellular N-terminal domain. Bands identified as secreted full-length APP were increased in low-DHA mice compared with control mice, consistent with increased proteolytic activity (Fig. 3A). Supplementing DHA in the diet restored levels comparable with those seen with control diet. Conversely, less APP was present in the membrane fraction of low-DHA mice, which was consistent with APP being secreted into the cytosolic fraction (Fig. 3B). Together, these data indicated that there was increased APP secretase processing in animals on low-DHA diets, which was reduced in animals supplemented with DHA.

We then sought to determine whether amyloidogenic (β-secretase, BACE1) or nonamyloidogenic (α-secretase) pathways were selectively affected by DHA. β- and α-secretase activities were indirectly assessed by examining the levels of APP C-terminal fragment products (C99 and C83, respectively) in the membrane fraction in all three groups of mice. Two bands at 11 and 9 kDa were revealed on blots probed with a polyclonal antibody targeting the C-terminal 20 residues (751–770) (Fig. 3C). Cell lysate from B5 cells transfected with the β-secretase generated C-terminal fragment of APP (C99) was used to identify the 11 kDa band as the product of β-secretase (Fig. 3C, lane 4). This rate-limiting β-secretase product was significantly increased by 71% in low-DHA-treated animals compared with those on control chow (p < 0.01) (Fig. 3D) and may contribute to increased levels of AB40 in these same mice. Supplementing DHA reversed this effect and significantly decreased the protein by 38.3% when compared with low-DHA diet (p < 0.01). The effect of DHA on the α-secretase 9 kDa CTF was even greater (Fig. 3E). Depleting DHA significantly increased the 9 kDa fragment by 96% compared with animals on control chow (p < 0.001). Adding DHA to the diet significantly reduced the level of the 9 kDa band by 54.9% when compared with those on low-DHA diets (p < 0.001).

Additional evidence of an association between DHA levels and secretase activity was found by performing a simple regression analysis between DHA levels (mean percentage of fatty acids) and βAPP-CTF (11 kDa) and αAPP-CTF (9 kDa) ratio levels. Significant negative correlations were found between increasing levels of DHA and reduced amounts of both the βAPP-CTF (R² = 0.37; p < 0.01) and αAPP-CTF (R² = 0.47; p = 0.002) (Fig. 4). Together, these results suggest that dietary DHA limits amyloid production but reduces both amyloidogenic and nonamyloidogenic pathways in these transgenic mice.

BACE expression was unchanged by dietary PUFA

Because secretase products were elevated with DHA depletion and reduced by dietary DHA, we then investigated effects on secretase expression. In particular, whereas α-secretase expression appears more or less constitutive (Sisodia, 1992; Lammi-Webb et al., 1999; Lopez-Perez et al., 2001), BACE1 expression in neuronal cells has been reported to be increased by both proinflammatory cytokines (Sastre et al., 2003) and oxidative damage (Tamasgno et al., 2002). n-3 PUFAs have been reported to be anti-inflammatory (De Caterina et al., 1994; Raederstorff et al., 1996; Simopoulos, 2002) and to reduce oxidative damage (Komatsu et al., 2003), consistent with protein carbonyl reduction with high-DHA diets (Calon et al., 2004). Therefore, BACE1 mRNA levels in animals on low- and high-DHA diets were analyzed by real-time PCR.
time quantitative PCR. ANOVA analysis revealed no treatment differences in BACE mRNA expression between the two diet groups (data not shown). Together, these data and our results showing a DHA impact on total APP and βCTFs argue for a DHA effect on APP trafficking or secretase activity rather than BACE expression.

**Discussion**

In this study, we report that an adequate DHA intake can significantly reduce detergent-insoluble amyloid, plaque burden, and APP processing pathways in aged transgenic animals. Because genetic and environmental factors can impact AD risk, our data point to a causal role for DHA in epidemiological studies, in which sufficient DHA intake is associated with reduced AD risk (Kalmijn et al., 1997; Barberger-Gateau et al., 2002; Morris et al., 2003). Many Aβ-lowering treatments, such as Aβ vaccinations, nonsteroidal anti-inflammatory drugs, vitamin E, and statins, were highly efficacious when administered in young APP animals (Lim et al., 2000; Das et al., 2001; Refolo et al., 2001; Jantzen et al., 2002; Sung et al., 2004), but Aβ vaccine and vitamin E were shown not to work well in older animals. Our data demonstrate that interventions introduced as late as 17 months of age are effective at reducing amyloid burden. Previously, we showed that DHA also has profound effects on synaptotoxicity (Calon et al., 2004). Postsynaptic marker loss and recovery in the DHA-deficient and DHA-treated animals, respectively, could be a result of Aβ accumulation because increased amyloid, particularly Aβ42, is linked to synaptic loss (Mucke et al., 2000; Chin et al., 2004). However, in our studies, synaptic loss occurred without subsequent increases in either total amyloid or Aβ42 (Fig. 1) in low-DHA mice and were not likely a direct result of total Aβ accumulation.

We determined previously that brain DHA levels in the Tg2576 mice are reduced 16% with DHA-depleted diets (Calon et al., 2004). A 16% DHA loss is reasonable for a 3–5 month treatment, because other studies report that large (50–80%) (Weisinger et al., 2002) decreases in brain DHA require multiple generations of animals on DHA-depleted diets (Salem et al., 2001). Fatty acid analysis also revealed compensatory increases in another C22 fatty acid, docosapentanoic acid (DPAn-6), a phenomenon typically seen when brain DHA is depleted (Salem et al., 2001). In this current study, we show that adrenic acid levels (docosatetraenoic; 22:4n6) were increased by 15% with DHA depletion, consistent with previous rat studies (Bourre et al., 1989; Ikemoto et al., 2001; Moriguchi et al., 2001). Whereas DPAn-6 levels have not been evaluated in AD brains, adrenic acid levels are increased threefold to fourfold in gray matter from AD patients (Skinner et al., 1993), consistent with DHA depletion.

DHA depletion would result in specialized biophysical effects on neuronal membrane structure (Salem and Niebylski, 1995). Recent nuclear magnetic resonance studies directly confirmed that DHA-phospholipids have greater flexibility and less ordered packing of hydrocarbon chains than those containing DPAn-6 (Eldho et al., 2003). One of the best established DHA effects is the
enhancement of retinal G-protein coupling by increasing lateral mobility (Niu et al., 2004). These bulk bilayer properties may alter the lateral movement of proteins, ion channels, and detergent-insoluble lipid rafts. Amyloidogenic APP processing is believed to occur in lipid rafts in the synaptic membrane in which β- and γ-secretases are located (Kawai et al., 2004). Hence, increasing brain DHA could modify processing pathways by affecting lateral mobility collision rates between APP and secretases. Our data imply that brain DHA content influences APP processing and are summarized in Figure 5. Immunoblot results indicate that high DHA decreased soluble APP (APPs) in the cytosol but increased membrane full-length APP compared with the low-DHA group (Fig. 3). These data are complimentary, suggesting that proteolytic processing of the membrane-bound APP is reduced with DHA. Furthermore, high DHA decreased β- and α-CTFs (Fig. 3), implying that DHA may downregulate Aβ generation by altering APP trafficking to secretase-containing compartments of the membrane or secretase enzymatic activity itself.

Our findings demonstrate that DHA supplementation decreased total insoluble Aβ, including both Aβ40 and Aβ42. Although older animals were used in this study, the Aβ-lowering effect was much larger compared with what we reported with chronic ibuprofen or curcumin treatment beginning at 16 months of age (Lim et al., 2000, 2001). Although the difference was not significant, DHA showed a trend to reduce soluble Aβ (Fig. 1) and may have an effect on this potentially toxic form of amyloid. Interestingly, insoluble and soluble Aβ are reduced in low-DHA mice, but Aβ40 levels were increased by 65% compared with control mice. These and other inconsistencies between control and low-DHA chow may be attributable to other differences in the chow other than DHA, such as total fat or cholesterol. Clearly, however, chows that were most closely matched (low and high DHA) showed very consistent results. DHA is highly enriched in the neuronal or synaptic membrane (Salem, 1989), and its depletion can lead to increased n-6/n-3 ratios, resulting in inflammation (De Caterina et al., 1994; Raederstorff et al., 1996; Simopoulos, 2002) or excessive oxidative stress (Komatsu et al., 2003), two conditions that enhance Aβ production (Sastre et al., 2003) and are present in AD and transgenic mice (Akaike et al., 2000; Lim et al., 2000; Pratico et al., 2001; Grundman et al., 2002). Inflammatory cytokines and oxidative stress induce BACE expression and activity (Tamagno et al., 2002; Sastre et al., 2003). DHA supplementation reduces oxidative stress because it decreased oxidized protein levels by 57% in the Tg2576 mouse (Calon et al., 2004). Although our data rule out an effect on global BACE expression, they do not exclude the possibility that oxidative damage influences local BACE expression or activity around plaques because BACE activity is upregulated in aging Tg2576 and AD (Fukumoto et al., 2004). Therefore, increased brain DHA content could alter Aβ production by lowering plaque-associated oxidative stress and inflammation.

ELISA revealed that Aβ42 levels in high-DHA mice were lower compared with control and low-DHA chow groups, whereas Aβ40 levels were comparable (Fig. 1). DHA could modulate γ-secretase activity by suppressing GSK3α (glycogen synthase kinase) (Phil et al., 2003). Both GSK3β and GSK3α are negatively regulated through inhibitory phosphorylation of the PI3K pathway, which is suppressed with DHA depletion (Calon et al., 2004). In this study, we found reduced inhibitory GSK3β (ser9) phosphorylation in low-DHA mice that was restored with high DHA (data not shown) but could not get reliable data on GSK3α and related γ-secretase CTF products. Extensive testing of GSK3α and γCTFs will require new groups of aged mice.

Aβ reduction could occur via increased Aβ clearance mechanisms, such as upregulated expression of Aβ-cleaving enzymes such as insulin-degrading enzyme (IDE) or upregulation of Aβ chaperones such as transthyretin (discussed below). IDE can degrade amyloid and its production, and expression is regulated by the insulin signaling pathway via pAkt and PI3K. DHA-depleted diets and in vitro conditions can downregulate pAkt and PI3K (Akbar and Kim, 2002; Taouis et al., 2002; Calon et al., 2004). Furthermore, we reported recently that decreased PI3K correlates with reduced IDE in Alzheimer brain and in Tg2576 mice on low-DHA diet, and decreased IDE was associated with increased Aβ monomer levels in low-DHA mice (Zha et al., 2004). Thus, upregulation of IDE by DHA could reduce Aβ accumulation in our mouse model.

Interestingly, RT-PCR assays revealed no significant changes in TTR and ApoE expression as a function of diet. ApoE modulates Aβ metabolism and deposition in transgenic mouse models (Fagan et al., 2002; Holtzman, 2004). DHA could regulate ApoE expression by inducing the transcriptional activity of LXR/RXR (liver X receptor/retinoid X receptor) (de Urquiza et al., 2000), which modulates ApoE expression (Liang et al., 2004; Rebeck, 2004). However, we did not find significant changes in ApoE mRNA levels in high-DHA mice. The absence of TTR induction was unexpected because TTR is markedly elevated in Tg2576 mice (Stein and Johnson, 2002), and its mRNA is induced by dietary fish oil in aged rats (Puskas et al., 2003). These TTR inductions were present in the hippocampus, in which TTR protein is prevalent. Unfortunately, in our study, RNA material was only available from cortex, so it is quite possible that upregulation of TTR by DHA may have occurred in the hippocampus of our mice. It is also possible that eicosapentaenoic acid, the other prominent omega-3 fatty acid in fish oil, induced TTR mRNA levels in aged rats and may partially explain the absence of TTR induction in cortex in our high-DHA mice.

In summary, our data show that altering dietary DHA intake can have profound effects on total insoluble Aβ and Aβ42 levels. These Aβ changes were associated with decreased plaque burden and coordinate changes in full-length membrane APP, APPα, and APP-CTF levels. Together, it is plausible that brain DHA can limit APP secretase processing, which may occur at multiple levels, including control of membrane order and lateral mobility, oxidative stress, and through inhibition of PI3K and GSK3α. Our data correlate well with epidemiological studies that point to reduced risk of AD with proper n-3 PUFA intake. Currently, the safety and tolerability of n-3 PUFA (4 g/d) in mild to moderate AD patients is being tested in Sweden (Y. Freund-Levi, personal communication).
communication), and two double-blind, placebo-controlled clinical studies are underway to evaluate whether n-3 PUFAs are effective in treating mild AD patients (J. F. Quinn, personal communication and http://www.alzheimer.ca/english/treatment/trials-listing.html).

References


