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REVIEWS: CURRENT TOPICS

Experimental models and mechanisms underlying the protective effects of n-3 polyunsaturated fatty acids in Alzheimer's disease

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Abstract

Dementia such as Alzheimer's disease (AD) is a growing health problem in aging populations in many countries around the world. Currently, there is no cure for AD; consequently, alternative therapies are urgently needed. Recent studies suggest that nutritional intervention may have therapeutic benefits for AD. Specifically, an increased intake of n-3 polyunsaturated fatty acids (PUFA) from fish and marine oils may lower AD risk. This review will summarize the current body of knowledge regarding the association between n-3 PUFA and AD, including human studies and experimental models, and potential mechanisms of action.

Keywords: n-3 PUFA; Alzheimer's Disease; DHA; Fish oil; Mouse models

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease that commonly affects the elderly. The incidence rate in Canada is approximately 20 per 1000 person-years [1]. In the United States, 5.1 million people were estimated to be living with AD in 2007 and $148 billion is spent each year to provide health care for these individuals [2]. In addition, the prevalence of AD in the American population is expected to increase as the population of individuals over 65 years of age grows, and by 2050, it is estimated that there will be approximately 13.2 million cases [3].

There are two general types of AD. Familial AD is an autosomal dominant disease, usually early-onset, associated with mutations in amyloid precursor protein (APP), or presenilin-1 and 2, genes [4]. The sporadic form of AD occurs in the majority of cases, mostly after the age of 65 years, and is associated with the apolipoprotein E4 isoform (apoE4); however, it is unclear how apoE4 modulates AD pathology [4].

Both familial and sporadic forms of AD are characterized by loss of short-term memory and cognitive impairments. The cause of AD is unknown; however, the disease is associated with three major pathological traits. First, there is an overproduction and accumulation of β-amyloid (Aβ) peptide produced by the proteolytic processing of APP [5]. Aβ then folds incorrectly and aggregates to form senile plaques. Secondly, there is formation of neurofibrillary tangles caused by the aggregation of Tau protein within neurons [4]. Finally, there is neuronal cell loss. In addition to these three traits, there is growing evidence that inflammatory processes are important in AD progression [4].

Currently, there is no known cure for AD. However, there is growing interest in the role of diet in the prevention and treatment of AD. High antioxidant consumption of vitamin E and C and selected types of fats may be beneficial for AD prevention [6]. In particular, research has shown that n-3 polyunsaturated fatty acids (PUFA) from marine oils, containing eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) may have therapeutic utility for the prevention and treatment of AD. After a brief introduction to n-3 PUFA, this review will summarize recent findings investigating the association between n-3 PUFA and AD through an exploration of human and experimental studies, highlighting advances in experimental

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animal model systems, and potential mechanisms of action for future research.

2. N-3 PUFA

2.1. Structure of n-3 PUFA and dietary sources

n-3 PUFA are fatty acids with three or more double bonds. These fatty acids are characterized by the position of the first double bond which is between the third and fourth carbons from the terminal methyl end. This double bond cannot be formed de novo by mammals because, contrary to plants, they lack the necessary desaturase enzyme [7]. It is also impossible for them to convert n-6 PUFA to n-3 PUFA and vice versa [8]. Nutritionally, α-linolenic acid (ALA, 18:3n-3) is considered an essential fatty acid which must be obtained from the diet. In contrast, EPA and DHA can be consumed preformed in the diet or synthesized through the elongation and desaturation of ALA. However, <0.2% of ALA is converted to DHA [9].

ALA, EPA and DHA are the three major n-3 PUFA found in the human diet. DHA and EPA are predominantly found in fish oil and fatty fish like salmon, tuna and trout [10]. In contrast, ALA is mostly found in vegetable oils such as soybean, canola and flaxseed oils [10]. Moreover, walnuts are also good source of ALA [10]. ALA can also be found in other nuts, seeds, vegetables, legumes, grains and fruits in lower levels [10].

2.2. Relevance of n-3 PUFA in the brain

The brain is enriched in DHA, which accounts for about 8% of the dry weight of the brain [11]. DHA is incorporated into membrane phospholipids where it increases membrane fluidity [12]. DHA is also enriched in the retina and evidence suggests that n-3 PUFA play an important role in visual function as well as during fetal and infant neural development [13], leading to lower visual acuity or mental developmental scores in instances of deficiency or low n-3 PUFA intake [12]. Moreover, there is growing evidence demonstrating that n-3 PUFA remain important throughout our lifespan. A study done in adult mice showed that mice fed an n-3 PUFA-rich sardine oil diet for a year had better learning ability than mice fed a palm oil diet [14]. Furthermore, DHA was shown to be reduced in frontal cortex and hippocampus of 18-month-old rats fed 1200 mg of linoleic acid (LA, 18:2n-6) and 200 mg of ALA, compared to young 2-month-olds fed the same diet [15]. However, there is a crucial lack of human studies investigating changes in brain DHA levels during the normal aging process as well as the effect of DHA deficiency on the aging brain.

Even though ALA and EPA are not often detected in the brain [16], it has been hypothesized that they might have an indirect role on brain health through their conversion to DHA. Hepatic conversion of ALA and EPA to DHA may provide a source of brain DHA. Rats fed low-DHA diets are able to up-regulate de novo DHA synthesis from hepatic metabolism from circulating ALA [17]. However, this conversion has been shown to be inefficient in humans [9]; thus, the impact of the limited conversion on DHA status in the brain is unclear. The brain may also be able to synthesize limited amounts of DHA as brain astrocytes have been shown to synthesize DHA from ALA and EPA, therefore explaining the trace levels of ALA and EPA that may be detected [18].

ALA and EPA may also exert other indirect effects on brain health. Ketones are used as an energy source by the brain in replacement of glucose, especially in AD brains where glucose uptake is impaired. It has recently been shown that medium chain triglyceride consumption, which stimulates ketone body production, is associated with improvements in cognitive function in patients diagnosed with dementia [19]. Given that ALA is a good substrate for ketogenesis as well as β-oxidation, it has been hypothesized that ALA acts like medium chain triglycerides and improves cognitive function by providing the brain with a readily available source of energy [20]. ALA may also have effects on astrocyte maturation, as mustard and linseed oils containing ALA have been found to facilitate astrocyte maturation [21]. It is not clear however, whether the effects were due to conversion of ALA to DHA within astrocytes because DHA alone has also been shown to enhance astrocyte maturation [22].

Like ALA, EPA is believed to influence brain energy supply, but as a stimulator of fatty acid β-oxidation and ketogenesis [20]. In addition, EPA-fed rats have been shown to have lower levels of inflammatory cytokines in the brain as well as lower Aβ sensitivity [23], suggesting that EPA might act through inflammation-related pathways. Moreover, rats injected intracerebroventricularly with EPA or DHA have been shown to express higher levels of certain myelin proteins [24]. Because this effect was greater in EPA-treated rats than in DHA-treated rats, it could be argued that EPA has its own role in the brain. However, it is also not possible to rule out that some of the effects were due in part to brain synthesis of DHA [18].

It is evident that very little is known about the individual effects of n-3 PUFA, and therefore, more research is warranted to determine the mechanisms by which different n-3 PUFA (DHA, ALA and EPA) affect brain health. Nevertheless, there is growing interest in the role of n-3 PUFA in AD. The current state of knowledge of the biological effects and mechanisms by which n-3 PUFA modulate AD are reviewed in the following sections.

3. AD and n-3 PUFA studies

3.1. Human studies

As summarized in Table 1, 10 out of the 13 epidemiological studies to date report an inverse association between AD risk and n-3 PUFA status or intake. These studies were

Table 1
Epidemiological studies and n-3 PUFA

Type of study	Age/length of follow-up	Outcome	Summary	Ref.
Prospective cohort (Rotterdam Study)	55+/2.1 years	↑ Fish consumption is associated with ↓ AD risk	Inverse relationship	[29]
Prospective cohort (Rotterdam Study)	55+/6 years	No association between n-3 PUFA and AD	No relationship	[27]
Prospective cohort (PAQUID Study)	68+/2–7 years	Fish or seafood once a week ↓ risk of AD	Inverse relationship	[25]
Prospective cohort (Chicago Health and Aging Project)	65+/3.9 years	Fish once a week or more, ↓ risk of AD by 60% DHA and n-3 PUFA are associated with ↓ AD risk EPA is not associated with AD	Inverse relationship	[31]
Prospective cohort (Canadian Study of Health and Aging)	65+/5 years	↑ EPA in plasma PL of cognitively impaired cases ↑ n-3 PUFA and ↑ PUFA in dementia cases	Direct relationship	[30]
Cross-sectional (Canadian Study of Health and Aging)	65+/NA years	No difference in EPA, DHA, PUFA, n-3 and n-6 PUFA concentrations in plasma PL between controls and dementia or cognitively impaired cases	No relationship	[30]
Prospective (Cardiovascular Health Cognition Study)	65+/5.4 years	Fatty fish at least twice a week is associated with ↓ risk of AD by 41%	Inverse relationship	[28]
Prospective cohort (The Framingham Heart Study)	76/9.1 years	No effect for people with the APOE ε4 allele ↑ PC DHA levels in plasma (18 g/day of DHA or 3 servings/week of fish) is associated with ↓ dementia risk	Inverse relationship	[32]
Cross-sectional	NA	↓ Fish and n-3 PUFA intake for AD female patients ↑ n-6/n-3 ratio for male and female AD patients	Inverse relationship	[35]
Case-control study	76.5 years/NA	↓ Serum cholesteryl ester EPA and DHA in AD patients	Inverse relationship	[36]
Cross-sectional	82.7 years/NA	↓ 20:5n-3, DHA and total n-3 PUFA in plasma PL, PC and PE for AD patients ↑ total n-6 PUFA in plasma total PL for AD patients No difference in fatty acid levels of lysoPC	Inverse relationship	[34]
Prospective cohort (Zutphen Elderly Study)	70+/5 years	↓ 5-year Cognitive decline among fish consumers ↑ EPA+DHA intake is associated with ↓ 5 years cognitive decline	Inverse relationship	[33]
Prospective cohort (Atherosclerosis Risk in Communities)	50+/10 years	↑ AA and ↓ 18:2n-6 in plasma cholesteryl esters are associated with ↑ risk of cognitive decline ↑ Plasma EPA+DHA is associated with a lower risk of word fluency decline	Inverse relationship	[26]

PL, total phospholipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

carried out in elderly men and women. Nine were prospective [25–33], three were cross-sectional [30,34,35] and one was a case-control study [36]. The Mini-Mental State Examination was commonly used to assess cognitive function and AD status. Patients were seen by neurologists and neuropsychologists, and in some studies, patients underwent magnetic resonance imaging of the brain. Statistical analysis of risk was assessed using mainly Cox proportional hazards, logistic regression and linear regression. Six studies directly measured n-3 PUFA status in plasma or serum [26,30,32,34,36] and the remaining seven measured n-3 PUFA status with a food-frequency questionnaire [25,27–29,31,33,35]. Both methods have different strengths and limitations. Direct measurement in plasma or serum is not subject to under reporting issues like food frequency questionnaires, but may not represent the proportion of n-3 PUFA incorporated in the brain. As mentioned by the authors of the Canadian Study of Health and Aging, to explain the direct relationship observed between n-3 PUFA and dementia, higher n-3 PUFA in the plasma of dementia subjects might represent a low incorporation in the brain rather than a low consumption [30]. The authors also

mentioned that the actual difference in n-3 PUFA status was so small, it might not be clinically relevant even though statistically significant [30]. One limitation of the studies which used food-frequency questionnaires is the lack of discrimination between fish and plant derived n-3 PUFA sources. Almost all the studies measured fish or DHA and EPA separately, but very few reported ALA. The focus on many studies has been to assess EPA and DHA intake from fish. However, future studies are needed to evaluate the potential differential effect of ALA from plant sources (possibly through ketogenesis and fatty acid oxidation [20]) versus EPA and DHA from fish.

In addition to the Canadian Study of Health and Aging, the only other study to date that has reported no effect of n-3 PUFA is the 6-year follow-up to the Rotterdam Study [27,30]. However, this contradicts the earlier findings of the study, which showed a positive benefit of n-3 PUFA at the 2-year follow-up time point. The study authors suggest that the results of the 2-year study may have been too preliminary, and the incidence of dementia is too small to make definitive conclusions [27]. Alternatively, the results of the 2- and 6-year follow-up may reflect a progression in AD to a stage which is no longer responsive to n-3 PUFA in the diet. It is also important to note that neither of these studies reported negative effects of n-3 PUFA on AD outcome measures.

Despite promising findings from epidemiological studies, no effect of EPA or DHA on AD was observed in the three clinical studies conducted to date, which are summarized in Table 2. Overall, in epidemiological studies, the consumption of n-3 PUFA from all sources including ALA, EPA and DHA was similar to levels of total n-3 PUFA used in clinical trials. However, only EPA and DHA were used exclusively in the clinical trials; therefore, the type of n-3 PUFA may be a potential confounder. Consequently, the dose of EPA and DHA used in the clinical trials were higher than what is typically observed in epidemiological studies. Although there was a lack of effect of n-3 PUFA in the total population of subjects in the clinical trials, analysis of subgroups suffering from mild AD or mild cognitive dysfunction (MCI) showed benefit from DHA treatment [37,39]. The contrasting findings between epidemiological and clinical studies suggest that n-3 PUFA may only be effective when consumed prior to disease onset and possibly during the early stages of the disease when symptoms are mild.

Epidemiological and clinical studies have used blood levels of n-3 PUFA as a surrogate marker linking n-3 PUFA status in the brain to AD risk. However, direct analysis of brain fatty acid composition in postmortem studies of AD and healthy patients have not shown a consistent correlation between n-3 PUFA levels of DHA and AD pathology measured (Table 3). The only consensus among these studies is a decrease in hippocampal DHA. This is of interest because this is the first site affected by AD.

3.2. Cell culture studies

Cell culture studies shown in Table 4 have consistently shown lower Aβ, reduced apoptosis and markers of apoptosis, as well as higher cell viability in cells treated with DHA. It is also important to note that these studies were performed on a range of different neuronal cells originating from varied species including humans, mice and rats. Out of the five studies presented, three looked at Aβ levels and consistently found the level of Aβ peptides to be 20% lower in cells treated with DHA [40,44]. The other two studies focused on apoptosis, cell viability and their markers. Lower caspase-3 activation and cytoskeletal perturbations, markers of cell death, was reported in DHA treated cells [45,46]. In addition, lower apoptosis and higher cell viability was reported [45,46].

3.3. Animal studies

Table 5 summarizes rodent n-3 PUFA dietary studies. Four different Alzheimer's disease rodent models have consistently shown that DHA can protect against AD.

Table 2
Clinical trials and n-3 PUFA

Type of Trial	Population	Treatment	Outcome	Ref.
Randomized, double-blind and placebo controlled (OmegAD)	*n*=204 74 years	1.7 g DHA+0.6 g EPA per day for 6 months	No effect on cognitive decline ↓ Cognitive decline only for subgroup of patients with mild AD	[37]
Pilot study	AD patients *n*=20 65+ years AD patients	500 mg ethyl-EPA twice a day for 12 weeks	No effect on cognitive decline ↑ EPA, DPAn-3 and total n-3 in erythrocyte membrane	[38]
Randomized, double-blind and placebo controlled	*n*=8 67 years AD and MCI patients	240 mg AA and 240 mg DHA per day for 90 days	No effect on memory and attention for AD patients ↑ Immediate memory and attention for MCI patients	[39]

Table 3
Postmortem findings and n-3 PUFA

AD brain regions	Outcome in AD brains vs. controls	Ref.
Hip, Tem, Tha and Occ	↓ DHA and NPD1 (metabolite of DHA) in Hip and Tem No difference for DHA and NPD1 in Tha and Occ ↑ $cPLA_2$ and ↓ 15-LOX in Hip	[40]
PHC	↓ AA in PE No difference for AA in PC, PS, PI, CE No difference for DHA in PC, PE, PS, PI, CE	[41]
HPG, IPL, SMTG and Cer	↓ AA, DHA in PE of HPG; AA in PI and FFA of HPG ↓ DHA in PC of Cer, AA in PE of IPL No difference in SMTG for AA or DHA No difference for DHA in PC, PI and FFA of HPG, in IPL, and in PE, PI and FFA of Cer No difference for AA in PC of HPG, in PC, PI and FFA of IPL, and in Cer	[42]
FGM, WM, Hip and pons	↓ AA and DHA in PE of FGM, WM, Hip and pons ↓ AA in PC of FGM, WM and Hip; DHA in PC of FGM No difference for AA in PC of pons, and for DHA in PC of WM, Hip and pons	[43]

Hip, hippocampus; Tem, temporal lobe; Tha, thalamus; Occ, occipital lobe; PHC, parahippocampal cortex; HPG, hippocampus and parahippocampal gyrus; IPL, inferior parietal lobule; SMTG, superior and middle temporal gyri; Cer, cerebellum; FGM, frontal grey matter; WM, white matter; PI, phosphatidylinositol; PS, phosphatidylserine; CE, cholesteryl esters; FFA, free fatty acids.

Wistar rats infused with $A\beta_{40}$ mimic Aβ peptide over-production and aggregation seen in AD [49]. There are also several transgenic mouse models of AD. The Tg2576 mouse experiences cognitive impairments and Aβ plaques, usually around 9–12 months of age [54]; the APPswe/PS1dE9 transgenic mouse, with Aβ plaques just before 6 months of age [44] and the 3xTg-AD that develops both senile plaques and neurofibrillary tangles [55]. All these models experience Aβ deposition, which is a primary feature of AD, but some models like 3xTg-AD have also neurofibrillary tangles which make them more complete models of AD. It is also worthy to note the age at which the plaques appear. The APPswe/PS1dE9 mouse is a good model because plaques develop as soon as 6 months of age, in comparison to 9–12 months in the Tg2576 mouse.

DHA has been shown to decrease proapoptotic protein BAD (Bcl-2 antagonist of cell death) by 47% [48] and the secretion of Aβ by 30–70% [44,49,47,53], increase PI3-kinase neuroprotective pathway [48] and improve cognitive function as measured using the Morris water maze or an eight-arm radial maze [44,48–50]. DHA was incorporated in the diet of all the studies, except for one where EPA and arachidonic acid (AA, 20:4n-6) were also added [44]. The dose of EPA and DHA used in these animal studies range between 0.5% and 0.8% of lipid [44,47,48], which is approximately 2–4 times greater than typical human intake [56], estimated to be 0.2%. This 0.2% estimate was based on the assumption that humans typically consume 40% of energy from fat.

4. N-3 PUFA mechanism of action

4.1. Deficiency vs. adequacy

There is no clear evidence to demonstrate that low n-3 PUFA contributes to the onset or progression of AD. However, 10 out of 13 studies presented in Table 1 have shown an inverse association between n-3 PUFA status or intake and AD [25,26,28,29,31–36]. Nevertheless, as demonstrated in Table 2, n-3 PUFA supplementation interventions do not seem to improve cognitive function among patients with advanced AD, although it may be beneficial to those with mild AD [37], and as shown in Table 3, no consistent association between n-3 PUFA and AD has been observed in postmortem studies. DHA was lower in AD brains, but only in few lipid fractions and brain regions; no difference was observed in the other fractions

Table 4
Cell culture studies and n-3 PUFA

Type of cells	Treatment and length	Outcome	Ref.
Human neural cells	50 nM DHA for 4–8 weeks	↓ $A\beta_{40}$ and $A\beta_{42}$, ↑ NPD1	[40]
COS-7/pCEP-SP-C99 (high Aβ levels)	0.5% DHA for 12 h[a]	↓ Aβ[b]	[44]
IMR-32 neuroblastoma	20–60 μM of DHA for 48 h	Dose-dependant ↓ $A\beta_{40}$	[44]
Neuro 2A (mouse neuroblastoma)	25 μM DHA for 48 h	↑ Phosphatidylserine, and translocation and phosphorylation of Akt ↓ Caspase-3 activation	[45]
Cortical neurons (from fetuses of rats) treated with Aβ	0.5 μM DHA for 48 h	↑ Cell viability ↓ Apoptosis, perturbation of cytoskeleton and caspase-3 and caspase-9 activation	[46]

[a] % DHA is expressed in % of lipid.
[b] Compared to a high saturated fat (40%) treatment and a high saturated fat (40%)+1% cholesterol treatment, but not to a low saturated fat (15%) treatment.

Table 5
Animal studies and n-3 PUFA

Animals	Age and treatment	Outcome	Ref.
Tg2576 mice	17–19 months 0.6% DHA for 3–5 months[a]	↓ Total Aβ ↓ $A\beta_{42}$ in cortex ↓ Plaque burden ↓ α-CTF, β-CTF and APP	[47]
Tg2576 mice	17 months 0.6% DHA for 3–5 months[a]	↑ PI3-kinase pathway, pAkt, pBAD and Morris water maze performances ↓ BAD (proapoptotic)	[48]
Wistar rats infused with $A\beta_{40}$	20 weeks 300 mg DHA/kg of body weight by day for 12 weeks	↓ Aβ and cholesterol in lipid rafts fraction of the brain cortex ↓ Reference and working memory errors ↓ Learning impairment ↓ Lipid peroxidation ↑ Synaptosomal membrane fluidity	[49–52]
APPswe/ PS1dE9 mice	6 months 0.4% DHA, 0.4% EPA and 0.2 % AA for 3 months[a]	In hippocampus: ↓ $A\beta_{42}$ No effect on plaque number	[44]
APPswe/ PS1dE9 mice	6 months 0.5% DHA for 4 months[a]	No effect on spatial learning in the water maze ↓ $A\beta_{40}$ and ↓ $A\beta_{42}$[c] No effect on average plaque load	[44]
3xTg-AD	3 months 1.3% DHA for 3–9 months[b]	↓ $A\beta_{40}$ and Tau at 3, 6 and 9 months; and ↓ $A\beta_{42}$ at 3 and 6 months ↓ PS1 levels (unit of γ-secretase complex) at 3 and 9 months	[53]

PI3, phosphatidylinositol-3; PS1, presenilin 1.
[a] % DHA is expressed in % of lipid.
[b] % DHA is expressed in % wt of diet.
[c] Compared to a diet with no DHA and 1% cholesterol.

and regions. Because each study analysed different lipid fractions and brain regions, it is very difficult to find confirmation of these observations. Overall, these data suggests that n-3 deprivation may play a role in the early stages of AD.

There is no consensus on the level of n-3 PUFA that humans should consume in their diet. Recommendations may be very general, such as eating fish two times per week [10,57], or much more precise, such as 0.7% of energy as ALA and at least 500 mg per day of DHA and EPA combined [10]. Moreover, the World Health Organization recommendation is to consume 1–2% of energy from n-3 PUFA [10]. The general population does not appear to meet these recommended intakes. In fact, a recent study of 641 Belgian women showed that less than 0.7% of their energy was coming from n-3 PUFA [58]. In the United States, 0.7% of energy is estimated to come from n-3 PUFA intake (1.6 g per day), while EPA and DHA intake is only about 0.1–0.2 g per day [56]. Consequences of low n-3 PUFA intake can be very serious, including learning and vision impairments in the young [7].

4.2. Cholesterol-lowering effects and apolipoprotein E isoforms

High plasma cholesterol levels have been linked to AD pathology in epidemiological studies while higher Aβ production and deposition into amyloid plaques has been observed in animals fed a high cholesterol diet [59]. Moreover, the ε4 form of apolipoprotein E gene (APOE ε4), which is a potential cholesterol transporter within the brain, has been strongly associated with increased risk of AD [59]. As shown in Table 5, cholesterol is reduced in lipid rafts of Aβ-infused rats pretreated with dietary DHA [49], suggesting that DHA-lowering effects on brain cholesterol is a potential mechanism explaining the therapeutic effect of n-3 PUFA in AD. However, this cholesterol-lowering effect of n-3 PUFA might not take place in individuals with the APOE ε4 allele. In fact, in Table 1, it is noted that the Cardiovascular Health Cognition Study found no effect of fish consumption on AD risk among people with the APOE ε4 allele [28].

4.3. Anti-inflammatory effects in brain

AA, an n-6 PUFA, and DHA, an n-3 PUFA, are both present in cell membrane phospholipids. AA is implicated in inflammatory events. It is cleaved and released from the plasma membrane by phospholipase A_2 (PLA_2). Then, it is transformed into proinflammatory lipid mediators such as prostaglandins by COX-2 [60]. Furthermore, calcium-dependant cytosolic PLA_2 ($cPLA_2$) and COX-2 enzymes have been shown to be elevated in AD brains [61,62], and DHA as well as neuroprotectin D1 (NPD1, derived from DHA) have been shown to lower mRNA levels of COX-2 and nuclear factor κB [63]. Recently, it has been shown that dietary n-3 PUFA deprivation in rats for 15 weeks increases the expression of enzymes associated with the AA inflammatory pathway such as $cPLA_2$ and COX-2 [64]. Therefore, the effect of n-3 PUFA observed on AD pathology is potentially related in part to the anti-inflammatory effect of n-3 PUFA through its effect on $cPLA_2$ and COX-2.

4.4. Neuroprotection

n-3 PUFA are also believed to act as neuroprotective molecules that can increase cell survival [63]. Tables 4 and 5 summarize cell culture and animal studies investigating three

different pathways of neuroprotection. First, Lukiw et al. [40] showed that NPD1 derived from DHA via a 15-lipoxygenase (15-LOX)-mediated pathway reduces cell apoptosis in the presence of $A\beta_{42}$. They also showed that NPD1- and DHA-treated cells have higher gene expression levels of antiapoptotic proteins of the Bcl-2 family [40]. Furthermore, Akbar et al. [45] explored the effect of DHA on the PI3-kinase pathway. They have shown that DHA increases the concentration of phosphatidylserine, which helps to increase the translocation and phosphorylation of Akt. Then, Akt inhibits caspase-3 activity leading to an increased cell survival [45]. Moreover, inhibition of caspase-3 activity through DHA pretreatment was confirmed in another cell culture study by Florent et al. [46], and the higher activity of the PI3-kinase pathway leading to a higher phosphorylation of Akt was also observed in a mouse model of AD consuming DHA [48]. Finally, the brain derived neurotrophic factor (BDNF) has been associated with increased cell survival [65]. Rao et al. [65] examined the effect of n-3 PUFA deprivation in rats on BDNF levels. They found that p38 MAPK (mitogen-activated protein kinase) activity was reduced, leading to lower phosphorylation levels of the transcription factor CREB (cyclic AMP response element binding), resulting in lower levels of BDNF in the brains of n-3 PUFA-deprived rats [65].

4.5. Caveolae and lipid rafts in cellular signalling and APP processing

It is hypothesized that processing of APP is a major determinant of AD pathology [4]. As a result, most of the cell culture and animal studies presented in Tables 4 and 5 have examined the effect of n-3 PUFA supplementation on Aβ levels. These studies have consistently found lower levels of Aβ in animal's brains and cells treated with DHA (see Tables 4 and 5).

Proteolytic cleavage of APP competitively follows an amyloidogenic or a non-amyloidogenic pathway (Fig. 1). Cleavage by β-secretase leads to a βAPP soluble (βAPPs) fragment and the β-carboxy terminal fragment (β-CTF).

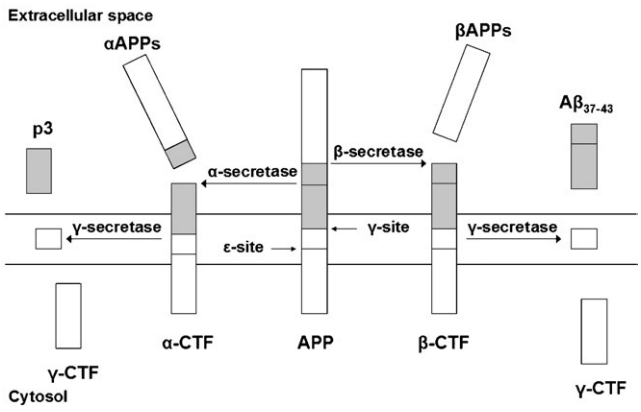

Fig. 1. Proposed mechanism for APP processing. Adapted from *J Neurochem*. 93 (2005) 769–792 with permission.

Then, β-CTF is cleaved at two sites (γ and ε) by a γ-secretase to form the γ-CTF and the Aβ peptide. γ-CTF localizes to the nucleus to interact with transcription factors while Aβ peptides are secreted into the extracellular space, accumulate and aggregate. Aβ is a small peptide about 37–43 amino acids long, but the $A\beta_{40}$ and $A\beta_{42}$ forms are the most common. The amyloidogenic pathway refers to the more neurotoxic $A\beta_{42}$, which aggregates more easily than $A\beta_{40}$. The non-amyloidogenic pathway occurs when APP is cleaved by α-secretase within the Aβ sequence to form α-CTF- and αAPP-soluble fragment. α-CTF is then cleaved by the γ-secretase at γ and ε sites to produce p3 and γ-CTF [5].

APP processing is believed to occur in membrane microdomains known as lipid rafts and caveolae. Highly controversial lipid rafts and caveolae are likely two distinct types of membrane microdomains. Lipids rafts are specialized compartments of the plasma membrane, approximately 10–200 nm in size and highly enriched in cholesterol, sphingolipids and saturated fatty acids. They compartmentalize proteins and cellular processes such as signal transduction, protein trafficking and proteolytic processing [66]. Caveolae are similar to lipid rafts, but contain caveolin-1, a scaffolding protein required for caveolae formation that produces cave-like invaginations at the plasma membrane [67]. The α-secretase processing of APP (non-amyloidogenic pathway) is believed to occur in caveolae because APP, α-secretase and caveolin have been found in these microdomains [68]. Whereas, the amyloidogenic pathway is localized in lipid rafts which contain APP, β-secretase, γ-secretase and the β-amyloid peptide [69–71]. These findings suggest that a treatment affecting the relative proportion of caveolae or lipid rafts could also affect the processing of APP in a beneficial or harmful way. Recent evidence has shown that n-3 PUFA can incorporate and modify the lipid composition, cholesterol content and structure of lipid microdomains in mouse colon, breast cancer cells, rat brain and model membranes [49,72–74]. n-3 PUFA influence membrane fluidity within these microdomains, which can affect signalling protein localization and function. This effect in brain would likely be attributable to DHA, given that it is the major n-3 PUFA present in brain, EPA and ALA being found only in trace amounts. Although this hypothesis has yet to be tested, effects on brain lipid microdomains may be another potential mechanism. Additional research is needed to further explore this potential relationship.

5. General discussion and conclusion

The relation between n-3 PUFA and AD is not yet understood. Ten out of 13 epidemiological studies presented in this review have shown an inverse relationship between n-3 PUFA intake or status and AD pathology. However, postmortem findings have been inconsistent [75] and clinical studies have shown no

beneficial effect of n-3 PUFA in patients with AD, except in subgroups of patients with mild AD or MCI. Because epidemiological studies have observed an association between n-3 PUFA and AD, whereas clinical studies have failed to demonstrate a benefit, this suggests that the effect of n-3 PUFA on AD might only be seen after long-term treatment, i.e., lifetime consumption. The only beneficial effects seen in clinical studies are in groups of patients with mild AD or MCI, which also points toward a preventive effect of n-3 PUFA rather than a potential treatment modality. In addition, the type of n-3 PUFA used in epidemiological (fish) versus clinical studies (fish oil/EPA/DHA) might also explain the discrepancies. For example, the Chicago Health and Aging Project, as many others, reported that DHA consumption is inversely associated with AD risk, but not EPA [31]. Furthermore, animal and cell culture studies have more consistently shown a positive effect of DHA on AD pathology markers like the Aβ peptide. As mentioned in this review, several mechanisms are currently being investigated, including the neuroprotective, the anti-inflammatory and the cholesterol-lowering effects of n-3 PUFA, as well as their effect on APP processing. It is highly probable that not one but several of these mechanisms contribute to the effects of n-3 PUFA on AD pathology observed in animal and cell culture studies. However, these effects remain to be confirmed in clinical trials. Preclinical studies leading to a better understanding of the mechanism of action by which DHA and other n-3 PUFA affect the progression of AD are warranted.

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