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Omega-3 polyunsaturated fatty acids in the brain: metabolism and neuroprotection

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1. ABSTRACT

Omega-3 polyunsaturated fatty acids (n-3 PUFAs) are a group of essential fatty acids that serve as energy substrates and integral membrane components, and therefore play crucial roles in the maintenance of normal neurological function. Recent studies show that n-3 PUFAs display neuroprotective properties and exert beneficial effects on the cognitive function with aging. The brain's need of n-3 PUFAs is predominantly met by the blood delivery due to their limited synthesis in the brain. The present review focuses on the metabolism of n-3 PUFAs in the brain, including their accumulation and turnover. We also highlight the current understanding of the neuroprotective effects of n-3 PUFAs against cerebral ischemia and neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease.

2. INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are series of fatty acids with more than one carbon-carbon double bonds. Based on the localization of the first double-carbon bond from the methyl end of the chains, polyunsaturated fatty acids are mainly divided into two classes, omega-3 (n-3) and omega-6 (n-6). The n-3 fatty acid family is comprised of alpha-linolenic acid (ALA, C18:3 n-3), eicosapentaenoic acid (EPA, C20:5 n-3), docosapentaenoic acid (DPA, C22:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3); while the n-6 PUFA includes linoleic acid (LA, C18:2 n-6), arachidonic acid (AA, C20:4 n-6) and docosapentaenoic acid (DPA, C22:5 n-6). ALA and LA are the so-called "parent" fatty acids for the PUFAs because most tissues can produce other n-3 and n-6 PUFA from them. For example, 20-carbon PUFAs (EPA and AA) and 22-carbon PUFAs (DHA and

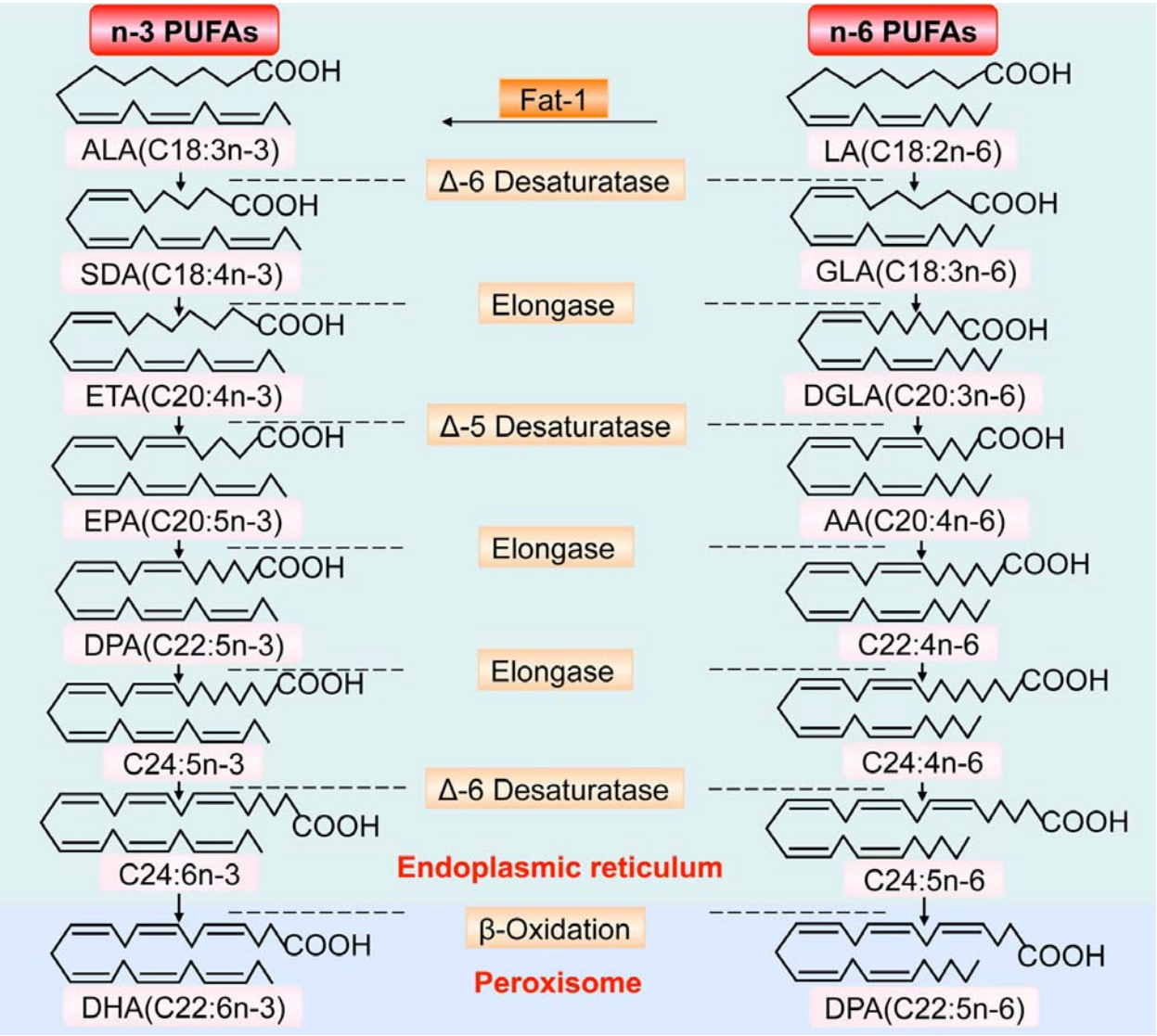

Figure **1.** Biosynthesis of long chain unsaturated fatty acids from 18-carbon precursors in mammals. Most of tissue could construct 20-carbon unsaturated fatty acids (like EPA and AA) and 22-carbon unsaturated fatty acids (like DHA and DPA) from the corresponding 18-carbon fatty acids precursors by elongation and desaturation. This process mainly occurs in the endoplasmic reticulum, followed by the final oxidation in the peroxisome. Fat-1 transgenic mice carry an n-3 fatty acid desaturase gene from *Caenorhabditis elegans*, which can synthesis n-3 PUFAs from n-6 PUFAs to increase the concentration of n-3 PUFAs in tissues, including brain.

DPA) can be generated from the ALA or LA by elongation and desaturation in the endoplasmic reticulum, followed by final beta-oxidation in the peroxisome (Figure 1).

Mammals do not have necessary desaturases to construct ALA and LA; therefore, PUFAs cannot be synthesized *de novo* in vertebrate tissue and have to be obtained from dietary sources. ALA and LA are therefore called essential fatty acids. Recently, a transgenic mouse expressing the *Caenorhabditis elegant*-derived *fat-1* gene was developed to evaluate the health effect of n-3 PUFAs (1, 2). The *fat-1* gene, which is absent in mammals, encodes an n-3 fatty acid desaturase that is able to add a double bond into n-6 fatty acids at the n-3 position to subsequently produce n-3 fatty acids. *Fat-1* transgenic mice exhibits elevated amount of n-3 fatty acids and higher n-3/n-6 ratio compared with the non-transgenic counterparts (2, 3). Among all the tissues, liver is the primary site for lipid metabolism and PUFA production from their dietary precursors. Adipose tissue is the major site for PUFA storage, which can be released to plasma in case of intake deficiency.

3. METABOLISM OF N-3 PUFA IN THE BRAIN

3.1. Accumulation of n-3 PUFAs in the brain

The brain is one of the organs mostly enriched with long-chain PUFAs, especially DHA (4-6). Despite of

their PUFAs abundance, neurons and glias could not perform the desaturation of fatty acids, which are necessary for the synthesis of DHA and other PUFAs from their precursors (7). Instead, microvascular endothelial cells in the brain provide a substantial amount of elongation/desaturation products of 18-carbon precursors to neurons. They predominantly produce and supply 20-carbon AA (n-6) and EPA (n-3) from their corresponding precursors; and astrocytes complete their subsequent conversion into 22-carbon DPA (n-6) and DHA (n-3), respectively. A recent publication demonstrated that hippocampal neurons may possess the capability to convert low amount of precursor PUFAs into DHA and AA; however the neuron-generated DHA or AA may not be representative of neuronal function in the brain (8). Therefore, astrocytes are the major provider of DHA in the brain although endothelium and neurons are involved in converting parental PUFAs into DHA. The released DHA and AA from astrocytes are rapidly taken up by neurons and incorporated into phospholipids on plasma membranes (7, 9, 10). However, the rate of *in situ* DHA and AA conversion is very low in the brain due to the prompt beta-oxidation of ALA and LA upon their entry into brain, and they are not the major source of PUFAs (11-13).

Ultimately, cerebral concentration of long-chain PUFAs depends on their dietary supply and peripheral synthesis in the liver from precursor essential fatty acids. Dietary restriction of n-3 PUFAs significantly increases the transcriptional activity of enzymes related to the elongation of ALA to DHA in the liver (4, 14, 15). Despite of the elevated synthesis in the liver, the DHA level in the liver is still apparently lower than that in the brain tissue, suggesting that the DHA synthesized in the liver is transported to the brain to maintain the cerebral DHA levels (15-17). Long-term deprivation of n-3 PUFAs decreases DHA concentration in the brain, particularly in oligodendrocytes, myelin, synapsomes and astrocytes; however, the DHA level in neurons is only slightly affected, indicating the preferential neuronal supply of PUFAs. Chronic dietary DHA deficiency also decreases n-3 PUFAs level in the liver and plasma; these decreases in the liver and plasma can be corrected by DHA supplements in 2 weeks, while cerebral DHA doesn't return to normal until 8 weeks later, suggesting a slower accumulation and recovery of DHA in the brain (18). Thus, although the cerebral DHA contents tend to be preferentially preserved during short-term deprivation of n-3 PUFAs, long-term dietary deprivation will result in ultimate loss of DHA in the brain, which takes relatively longer time to recover after n-3 PUFA replenishment.

As the n-3 and n-6 families share the same elongation/desaturation enzymes for their synthesis, the deprivation of long-chain n-3 PUFAs will promote the production of n-6 PUFAs. Their metabolic cascades are altered reciprocally by the change of dietary long-chain PUFAs (19). Hence, the decrease of brain DHA (C22:6 n-3) could be compensated by the increase of DPA (C22:5 n-6), with the loss of a double bond at n-3 carbon (20, 21). Compared with n-6 DPA, the n-3 DHA is more flexible because of the additional double-carbon bond, and isomerizes with shorter fatty acids. The incorporation of DHA into neuronal membrane decreases the total cholesterol fraction, leading to the elevated membrane fluidity and the affinity of receptors in the synapse. Whereas the loss of n-3 double in DPA can lead to a more even distribution of chain densities along the normal bilayers, which could influence the activity and/or the distribution of integral membrane proteins (22).

Considering the dietary dependence of the brain on long-chain PUFAs, efficient brain uptake of plasma-derived PUFAs plays an essential role in the accumulation and maintenance of PUFAs level in the brain (23). Astrocytes and endothelial cells, two major components of the blood-brain barrier, only play a minor role in the production of DHA or AA, however, they may be important in the transport of PUFAs into the brain tissue. Based on the extensive studies on the uptake of PUFAs, two possible mechanisms have been proposed: 1) passive diffusion and 2) saturable transport processes. The transportation of PUFAs is mediated by lipid transportation proteins, such as FAT/CD36, caveolin-1, fatty acid binding proteins (FABPs) and fatty acid transportation proteins (FATPs). Once liberated from the albumin and circulating lipoproteins, the PUFAs accumulate on the luminal surface of the endothelial membrains, with the help of membrane bounding protein FAT/CD36 and FABPpm. Following protonization, PUFAs integrate into the external phospholipid bilayers as uncharged molecules, and subsequently translocate to the inner leaflet of the phospholipid bilayers by flip-flop. At the inner surface of endothelial membranes, a small portion of these fatty acids is delivered into the subcellular compartments for further metabolism, while most of the fatty acids may diffuse into the cytosol with or without the aid of FABPs or caveolin-1. Subsequently, these fatty acids repeat the flip-flop process and go through the abluminal membrane of endothelia with the aid of transportation proteins (Figure 2) (24, 25). Among the molecules involved in the PUFAs transportation, fatty acid transport-related proteins play an important role, although the mechanism remains unclear and deserves further studies.

3.2. Turnover of n-3 PUFAs in the brain

As a component of glycophospholipids, DHA mainly takes the sn-2 position, especially in phosphotidylserine (PS), phosphotidylethanolamine (PE) and phosphatidylcholine (PC); while AA is also incorporated into the sn-2 position, majorly in phosphatidylinositol (PI) and phosphoatidylcholine (PC). Different from DHA and AA, ALA, LA and EPA have lower incorporation rate into the phopholipids, and are prone to be oxidized in the brain (11, 12, 26). Upon hydrolysis by selective phospholipidase A_2 (PLA_2), DHA and AA are rapidly released from the glycophopholipids and subsequently take part in the downstream signaling transduction or lipid recycle through Land's pathway. Most of the released fatty acids (about 97% of AA and 90% of DHA) will be reesterfied into phospholipids to maintain the stability of the membrane. The remainder (5%) will be beta-oxidized or catalyzed by cytosolic enzymes, including lipoxygenase, cyclooxygenase and cytochrome P450. These enzymatic reactions can produce eicosanoids, such as prostaglandins, leukotrienes, thromboxanes, resolvins, docosatrienes, lipoxin from AA

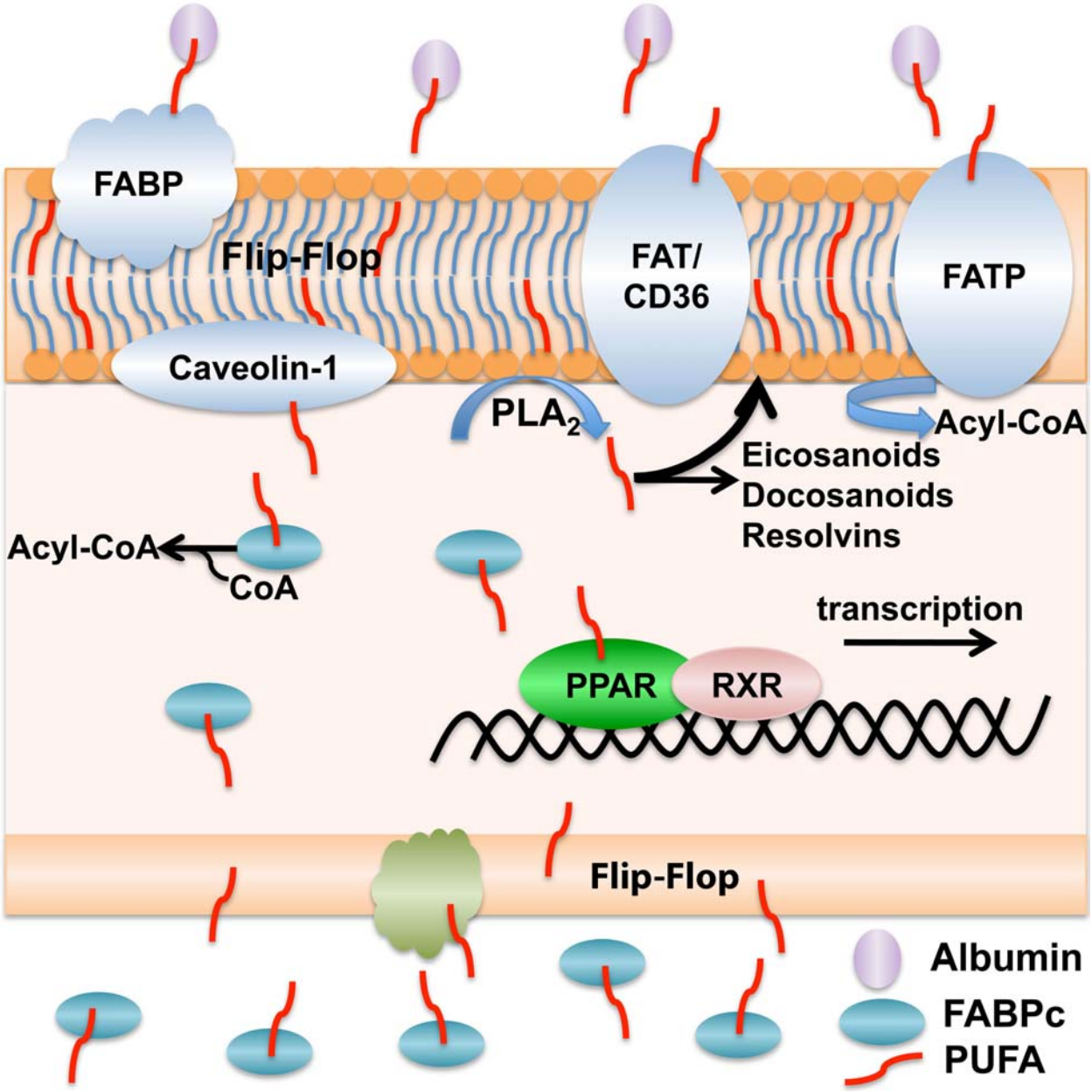

Figure **2.** Uptake and turnover of PUFAs in brain. FABPpm, FAT/CD36 and FATP facilitate the liberation of free fatty acids from albumin, and the subsequent integration of these fatty acids into external phospholipids bilayers. Fatty acids then translocate from outer leaflet into inner leaflet of phospholipids bilayers by flip-flop. Most of fatty acids repeat the flip-flop process or take advantage of the transportation related proteins to go across the endothelial transluminal membranes. Some of them is combined with caveolin-1 or FABPc and delivered to endoplasmic reticulum for acylation, or to nucleus to regulate the cell signaling transduction. Membrane incorporated PUFAs is rapidly catalyzed by phospholipase A_2 (PLA_2) and released into cytosol on stimulations. Most of free long-chain fatty acids are recycled through Land's pathway, whereas less than 5% of them undergo downstream metabolism to produce eicosanoids and/ or docosanoids.

and protectins (neuroprotectins) from DHA (Figure 2). In response to inflammation, oxidative stress or ischemia, over-activated PLA_2 can release more DHA and AA, which subsequently disturb the stability of the membrane and enhance the production of downstream metabolites, including pro-inflammatory and anti-inflammtory factors. These factors can influence the fate of the cells.

PUFAs are essential in the central nervous system, particularly in neuronal cells, either as precursors for the synthesis of membrane lipids or as anti-oxidation mediators maintaining cellular homeostasis (27). In addition, PUFAs can influence the functions of membranes, enzymes,

receptors, ion channels and synapses (28-30). Also, it can regulate gene expressions via nuclear transcription factors, such as peroxisome proliferator-activated receptor (PPAR), retinoid X receptor (RXR) and nuclear factor kappa B (NF-kappaB) (31-33). Considering the significance of PUFAs in central nervous system, we will mainly focus on their roles in several neurological diseases in the following part.

4. N-3 PUFA IN CEREBRAL ISCHEMIA

Previous MRI study showed that modest consumption of fish with higher EPA and DHA content was associated with lower prevalence of subclinical infarcts and white matter abnormities (34). Recent studies confirmed the beneficial effect of n-3 PUFAs in the focal cerebral ischemia model with improved neurological and histological outcomes (35-37). As a major n-3 PUFAs in the body, DHA is predominantly esterified at the sn-2 position of membrane phospholipids. Various stimulations can trigger the rapid release of DHA from the plasma membrane as discussed above. In the following session, we will specify the biological function of DHA and its metabolites in stroke.

Cerebral ischemia-reperfusion triggers the activation of PLA_2, leading to the disruption of cellular membrane stability and enhanced production of free long-chain PUFAs. The polyunsaturated diacyl molecular species, which can be degraded into PC and PE, are more rapidly degraded than other saturated and monounsaturated molecular species in the early phase of ischemia. In addition, PE, which is rich in AA, is degraded more rapidly than PC (38). Consequently, catabolism of phospholipids induced by transient cerebral ischemia results in marked biphasic accumulation of free fatty acids (FFA) in the brain. As in the permanent cerebral ischemia, AA is the main free fatty acid in the first phase accumulation, which began at 30 minutes and reached a peak at 1 hour; while DHA is the major free fatty acid in he second phase, which occurred at 24 hours following injury (39, 40). The acute breakdown of phospholipids results in the instability of plasma membrane.

In addition to the activation of PLA_2, cerebral ischemia also induces the massive calcium influx and degradation of lysosomes, which can lead to the profound perturbation of biomembranes. The elevated intracellular calcium further activates the Ca^{2+}-dependent enzymes, including $cPLA_2$. This enzyme subsequently translocates from the cytosol into the nuclear, endoplasmic reticulum, and plasma membranes. Once being phosphorylated, $cPLA_2$ calcium-dependently moves to its membrane binding sites. Subsequently, PLA_2 releases corresponding PUFAs from membranes, resulting in membrane degradation and accumulation of unesterified FFA and lysophospholipids in cytosol. It has been shown that Na(+)/Ca(2+) exchange inhibitors block the activation of PLA_2 and sustain the stability of cell membranes, resulting in significant reduction of DHA and AA in the brain after ischemia-reperfusion injury (41).

Due to its rapid breakdown, FFA is greatly needed to preserve the bioactivity and stability of plasma membrane. Albumin administration leads to an increased level of systemic circulation FFA, which can leave the plasma as the blood crosses the brain and incorporates into the membrane phospholipids (42). This finding suggests that albumin treatment may contribute to the functional recovery by the mechanism of the replenishment of PUFA loss after cerebral ischemic insult (43). Sufficient membrane DHA exerts neuroprotective effect, at least in part, by preserving the membrane stability after ischemia, which is supported by the findings that inhibition of Ca^{2+}-independent PLA_2 protects against neurodegeneration, whereas inhibition of prostaglandin production is ineffective (44).

The addition of DHA increases the concentration of phosphatidylserine (PS) on the membrane, which facilitates the activation of PI3-K/Akt pathway to protect neurons against staurosporine-induced apoptosis (45, 46). Similarly, EPA, precursor of DHA, was also demonstrated to confer neuroprotective effect via increasing the phosphorylation of Akt and suppressing the activity of caspase-3 (47). This result is confirmed by our research on neuroprotection of PUFA in neonatal hypoxic-ischemic brain damage. Sufficient supply of n-3 PUFAs is critical to maintain the fluidity of biomembranes to protect neurons against ischemic-like injury (fig3). Physically, FFA is uptake from the cerebral blood flow, and subsequently esterified and incorporated into the cell membrane. This process, which is probably more active in the collaterally perfused cortical region than in the core of the ischemic insult, contributes to the restoration of the membrane phospholipids loss after ischemic brain damage (39).

Inflammatory response after ischemic injury contributes to neuronal damage. PUFAs are able to influence immune system and modulate inflammatory responses. Interestingly, opposite actions of n-3 PUFAs and n-6 PUFAs on inflammation have been reported, with n-3 PUFAs being anti-inflammatory (48) and n-6 PUFAs being pro-inflammatory (49). Due to the activation of PLA_2, the excessive production of AA results in the accumulation of n-6 PUFAs-derived prostaglandins, leukotrienes, and thromboxanes, which contribute to the inflammatory response after cerebral ischemia. In contrast, n-3 PUFAs exert fundamental role on inhibition or modulation of eicosanoid pathways, which lead to alteration of inflammatory responses. The beneficial impact of n-3 PUFAs has been shown in many human inflammatory related diseases, such as inflammatory bowel disease (50), rheumatoid arthritis (51), cardiovascular disease (52, 53) and stroke (54). DHA inhibits the activation of LPS-induced nuclear factor kappa B (NF-kappaB) and attenuates the synthesis of proinflammatory cytokines, such as IL-1beta and TNF-alpha in microglia (55). Our research further confirmed the anti-inflammatory functions of PUFAs in neonatal hypoxic-ischemic brain injury (31). This anti-inflammatory effect of DHA is, at least partially, related to the DHA integration into the injured membrane of microglia to affect the presentation of CD14 and toll-like receptor-4, and effectively inhibit the production of cytokines (56).

As one of the target of oxidative injury, DHA is prone to be oxidized under oxidative stress conditions in the cell. However, pre-administration of DHA promotes its anti-oxidative effects, demonstrated by decreased malondialdehyde

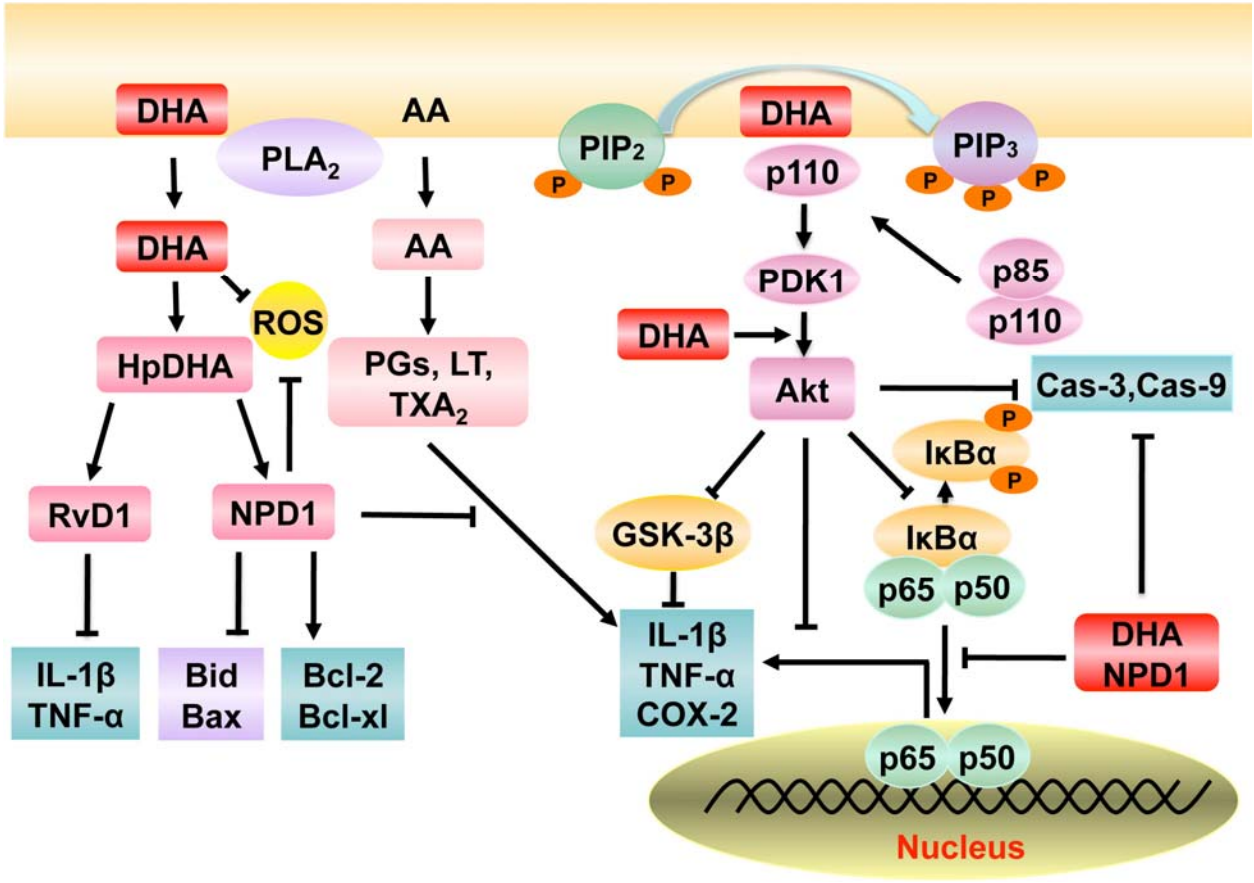

Figure **3.** The role of n-3 PUFAs in cerebral ischemia. Membrane enriched n-3 PUFAs preserves the activation of PI3-K to promote the phosphporylation of PIP2 and the production of PIP3 on the inner membrane. PIP3 facilitates the phosphorylation of PDK1 and its downstream target protein Akt. Activated Akt prohibits the activation of apoptosis related proteins, such as caspase-3 and caspase-9 as well as the nuclear translocation of transcription factor NF-kappaB. It also enhances the phosphorylation of GSK-3β (inactivation of GSK-3beta), which eventually inhibits the expression of pro-inflammatory mediators. Meanwhile, cerebral ischemia-induced overload of calcium stimulates the activation of PLA_2, which catalyze PUFAs from sn-2 position of phospholipids. Free docosahexnoic acid (DHA) and arachidonic acid (AA) produce resolvin D1, neuroprotectin D1 (NPD1) and prostaglandins (PGs), leukotrienes (LTs), thromboxane A_2, respectively. AA-derived metabolites lead to the inflammatory reaction through upregulating the production of inflammatory factors. However, metabolites from DHA suppress the inflammation and oxidative stress induced by cerebral ischemia. DHA could also promote the expression of anti-apoptotic Bcl-2 family proteins Bcl-2 and Bcl-xl, and suppress the expression of pro-apoptotic Bax and Bad to inhibit neuronal apoptosis.

(MDA) production, increased superoxide dismutase activity and glutathione level compared with DHA post-treatment (55). DHA is initially converted to 17S-hydroperoxy-DHA (HpDHA), then further enzymatically converted to resolvin D1 (RvD1) and protectin D1 (10,17S-docosatriene, NPD1/PD1) (57-59). Reslovin (resolution-phase interaction product) is first introduced to signify the new endogenous mediators that have potent anti-inflammatory and immunomodulatory activities (60). RvD1 is able to block TNF-alpha-induced IL-1beta transcription in microglia, and to limit PMN infiltration in animal models. Another resolvin, RvE1, derived from EPA, displays potent counter-regulatory actions that protect against leukocyte-mediated tissue injury and excessive pro-inflammatory gene expression in several animal models of inflammatory diseases (61, 62). Resolvins are generated in the ischemic cerebral tissue (63), but the studies on its role in cerebral ischemia are still at the early stage of research.

NPD1, another DHA-derived peroxidation product, is extensively studied in ischemic brain damage in the past decades. NPD1 attenuates oxidative-stress-induced apoptosis and DNA fragmentation *in vitro* by stimulating anti-apoptotic Bcl-2 protein expression (58). In experimental stroke, endogenous NPD1 synthesis is found to be upregulated, which coincides with the release of DHA from the membrane. Extra supply of DHA enhances the accumulation of NPD1 in the ipsilateral hemisphere (42, 64). DHA perfusion promotes neuroprotection through inhibiting leukocyte infiltration, NF-kappaB activation, and cyclooxygenase-2 induction in experimental stroke, which may be related to the elevated production of NPD1 (64). NPD1 can directly suppress the IL-1beta-stimulated expression of COX-2, and upregulate the anti-apoptotic Bcl-2 family proteins, such as Bcl-2 and Bcl-xl. It also suppresses the expression of pro-apoptotic Bax and Bad (Figure 3) (58). Overall, the production of NPD1 attributes to the DHA-mediated neuroprotection in cerebral ischemia.

Released free DHA can bind fatty acid binding proteins (FABP) in the cytosol and avoid oxidation. This binding also facilitates the translocation of DHA from the cytosol into the nucleus and regulates signal transduction and protein expression of the cell (65). PUFAs and their oxidation derivatives have already been recognized as ligands of several nuclear transcription factors, such as PPAR-gamma and RXR-alpha. There are plenty of evidences suggest that n-3 PUFAs regulate the expression of proteins, including inflammatory factors in a PPAR-dependent way (32, 33). Until now, four PPAR isoforms have been reported: alpha, beta, delta and gamma, each have different functions and complicated interrelations. In cardiomyocytes, dietary supplementation of n-3 PUFAs leads to its accumulation in the nucleus, which further promotes the expression of PPAR and enhances the binding of PPAR-beta/delta to DNA (66, 67). Similarly, n-3 PUFAs stimulate the transcriptional activity of PPAR-alpha, PPAR-gamma and RXR-alpha, as well as suppress the expression of inflammatory genes of diverse functions (68). Nevertheless, the activation of PPAR exerts different physiological functions depending on cell types. For example, n-3 PUFAs activates PPAR-gamma to abrogate the neovescularization and retinal angiogenic activation in proliferative retinopathy (69), whereas the selective stimulation of PPAR-alpha and PPAR-gamma promotes the angiogenesis in a VEGF-dependent mechanism in type 2 diabetes (70). In central nervous system, previous researches have demonstrated that the activation of PPAR-gamma confers functional neuroprotection against ischemia-induced brain injury via inhibiting excessive production of inflammatory mediators and suppressing over-activation of oxidative stress pathways (71, 72). Specific ablation of PPAR-gamma in neurons increases their susceptibility to ischemic injuries (73). Whether the neuroprotective effect of PUFAs against cerebral ischemia could be ascribed to its activation of nuclear receptor PPARs is under further investigation.

5. N-3 PUFA IN NEURODEGENERATIVE DISEASES

5.1. n-3 PUFAs in Alzheimer's disease

Alzheimer's disease (AD) is characterized with the formation of senile plaques (SPs). SPs in AD brains are predominantly composed of the beta-amyloid protein (Abeta) and neurofibrillary tangles (NFT) (74). The level of SPs correlates with the degree of neuronal damage, cognitive impairment and memory loss in AD patients. In western countries, around 10% of people older than 65 years old suffer from AD, and this number is still increasing with general aging of the population. Although the cause and progression of AD are not well understood so far, epidemiological studies have shown that low serum levels of DHA and dietary intake of n-6 PUFAs-rich foods are associated with increased risk of dementia and AD (75-78), while routine consumption of fish may reduce the risk of AD (79-81). The critical role of n-3 PUFAs was further supported by the decreased DHA concentration in AD patients compared with age-matched healthy control (82), and the increased formation of oxidative product F4-isoprostanes, which is caused by the deficiency of DHA in brain (83). Lipid analysis revealed that aging has no effect on the fatty acid compositions, while the pronounced decrease of phosphatidylethanolamine (PE)-derived and phosphatidylinositol (PI)-derived PUFAs is detected in the hippocampus of AD subjects, suggesting that the relative abundance of certain fatty acids may involve in AD (84, 85).

Abeta is derived from proteolysis of the beta-amyloid precursor proteins (APP) by the beta- and gamma-secretases. The excessive production of hydrophobic Abeta1-40 and Abeta1-42 enhances the formation of amyloid plaques, leading to the progression of AD (86). DHA suppresses the amyloidgenic pathway, leading to reduced extracellular and intracellular Abeta levels and concomitant increase of membrane full-length APP to counteract the amyloid burden (87, 88). However, another research on 3xTg-AD animals indicates that DHA had no effect on alpha- or beta-amyloid precursor protein processing. The reduced production of soluble Abeta is more correlated with decreased stability of presenilin1 (PS1) in DHA treated transgenic mice (89).

Amyloidogenic APP processing primarily takes place in the lipid rafts of the synaptic membrane where the key proteins in Abeta formation are also localized (90). Cholesterol, one of major component of lipid raft, may contribute to the pathogenesis of AD by suppressing the production of Abeta and regulating the intracellular signal transduction (91-94). Previous researches show that reduced cholesterol promotes the nonamyloidogenic alpha-secretase pathway to produce neuroprotective soluble APP (95). Cholesterol depletion disrupted APP, beta-secretase and PS1 compartmentalization within lipid rafts, resulting in the decrease of Abeta (92, 93, 96). Aberrant low level of n-3 PUFAs in lipid rafts of AD brain suggests the importance of n-3 PUFAs in modulating lipid rafts (97, 98). DHA results in the decreased affinity of cholesterol for phospholipid and in turn facilitates its transfer from cholesterol-rich regions (such as the plasma membrane) to cholesterol-poor regions (such as the endoplasmic reticulum) (99). Finally, DHA reduces cholesterol concentration in the detergent-insoluble membrane fractions and downregulates the proteolytic processing of APP (100), indicating a potential role of DHA in the decreased production of Abeta (88, 101). Lipid rafts also facilitate the deposition of neurotoxic Abeta (102), which could also be attenuated by DHA through the downregulation of cholesterol on plasma membrane (103). In the case of n-6 PUFAs, AA is adopted to produce DPA and compensate the deficiency of DHA (104). The loss of a single double bond in DPA results in decreased flexibility of membrane and more ordered packing of hydrocarbon chains. This change of bilayer properties may alter the lateral movement of detergent-insoluble lipid rafts. It also affects activities of proteins as well as ion channels (22) (Figure 4).

Preformed Abeta monomer rapidly aggregated to form multimeric complex from low molecular weight dimmers, trimers to protofibrils and fibrils (105, 106). Abeta1-40 is the major Abeta found in the cerebrospinal fluid of AD patients, while Abeta1-42 is the minor component (107, 108). However, Abeta1-42 are the major components of senile plaques, more hydrophobic and more toxic than Abeta1-40. Abeta1-42 is more copious than Abeta1-40 in AD brains (109). The fibrillation kinetics of

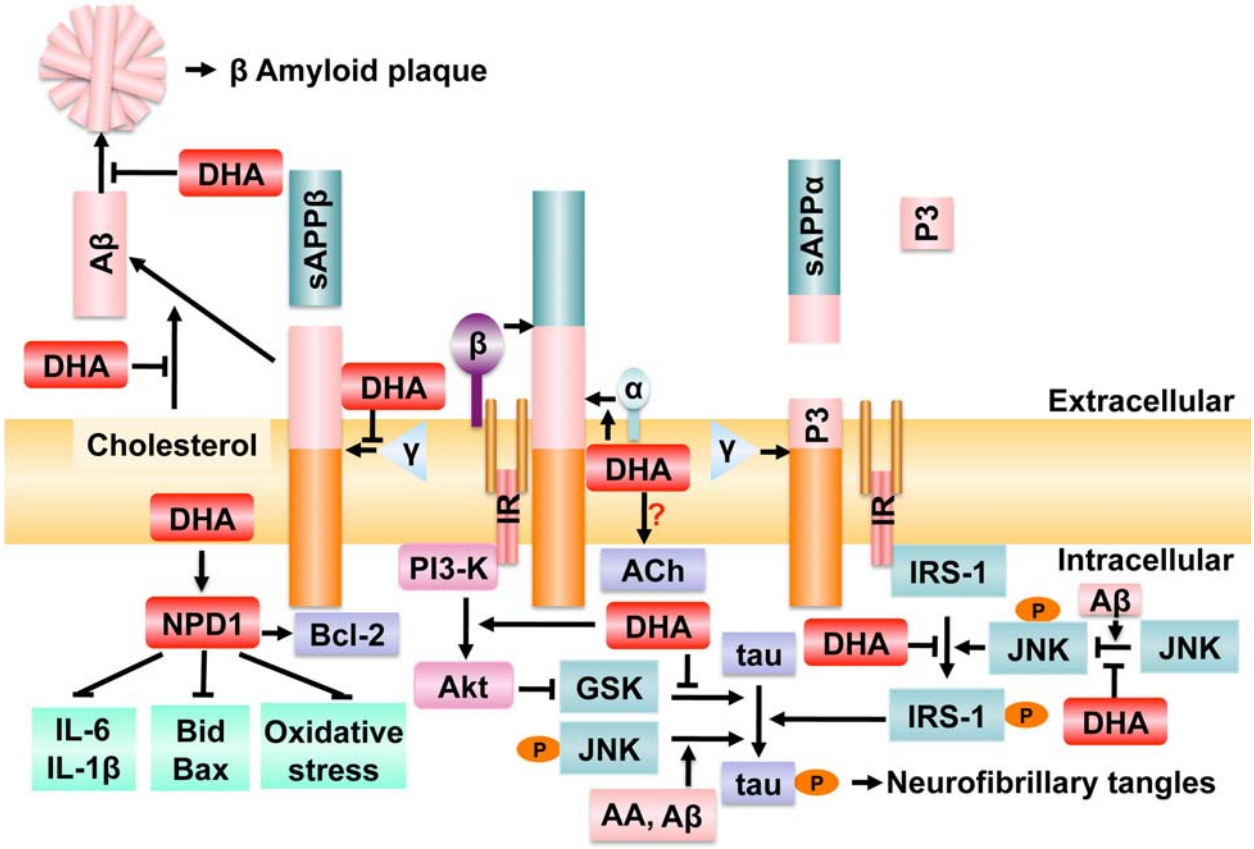

Figure **4.** The role of n-3 PUFAs in Alzheimer's disease. DHA modulates the metabolism of APP by suppressing the amyloidgenic pathway and promoting non-amyloidgenic pathway, which is represented by the reduced extracellular and intracellular Abeta levels. DHA counteracts the high cholesterol-induced accumulation of Abeta by promoting the nonamyloidogenic α-secretase pathway and interrupting the deposition of formed Abeta. Two major tau kinases, GSK-3beta and JNK can be induced by Abeta and facilitate the phosphorylation of tau. JNK also leads to the phosphorylation of insulin receptor substrate-1 (IRS-1), which transmits insulin/insulin receptor-mediated intracellular signals by interacting with downstream SH2 domain-containing molecules, triggering the phosphorylation of tau and promoting the formation of neurofibrillary tangels. DHA suppresses Abeta and AA-induced phosphorylation of tau via enhancing the activation of PI3-K/Akt pathway. DHA, together with one of its metabolite NPD1, also represents anti-inflammatory, anti-oxidation and anti-apoptosis effects in Alzheimer's disease. Furthermore, DHA preserves the deficit of ACh synthesis and the loss of cholinergic neurons in AD brain with unclear mechanism.

Abeta1-42 is also higher than Abeta1-40, indicating the critical role of Abeta1-42 in the progression of AD (110). DHA administration inhibited fibrillation of both Abeta1-40 and Abeta1-42 by suppressing oligomerization and subsequent elongation into mature fibrils, and counteracted the neuronal toxicity of Abeta (103, 111).

Another AD pathological feature is the intracellular neurofibrillary tangle, resulted from the aggregation of hyperphosphorylated tau. Although the underlying mechanism of tau phosphorylation is not fully understood, evidence shows that Abeta may play a role in the enhanced phosphorylation of tau (112). Two major tau kinases, GSK-3beta and JNK, could be activated by Abeta (113, 114). JNK activation results in the phosphorylation of insulin receptor substrate-1 (IRS-1), which relays insulin/insulin receptor-mediated intracelluar signals by interacting with downstream SH2 domain-containing molecules (115-117). Elevated phospho-IRS-1 was accompanied by the rapid degradation of IRS-1 and IRS-2, downregulation of insulin signaling, and the formation of neurofibrillary tangles in AD brains (118-121). On the other hand, the activation of JNK induces age-dependent amyloid deposition and loss of synaptophysin following tau phosphorylation in transgenic AD mouse models (122). Saturated fatty acids and n-6 PUFAs enhance the activation of JNK and the phosphorylation of IRS-1 and tau in 3xTg-AD transgenic mice. In contrast, n-3 PUFAs counteracts the activation of JNK and the related phosphorylation, and preserves the expression of IRS-1, which may contribute to the reduced phosphorylation and fragmentation of tau (89, 113, 114).

IRS-1 can phosphorylate phosphatidylinositol-3 kinase (PI3-K) on its p85 regulatory subunit, which subsequently activates the glucose transport and Akt, help to preserve the membrane integrity and cell viability (123-125). Glycogen synthase kinase-3beta (GSK-3beta) is a substrate of PI3-K/Akt. It is also identified as a brain microtubule-

associated tau kinase, whose activation leads to the phosphorylation of tau and the disruption of microtubule (126-129). GSK-3beta is implicated in APP processing and Abeta production; it is also associated with learning and memory functions (130, 131). Abeta-mediated neurotoxicity can be alleviated through the inhibition of GSK-3beta (132). Additionally, exposure of neurons to Abeta results in the inactivation of PI3-K and subsequent enhancement of GSK-3beta activity, supporting a potential role of GSK-3beta in Abeta-induced phosphorylation of tau (133). DHA depletion causes AD-like increase of caspase activity and downregulation of PI3-K-mediated insulin signaling (134, 135). DHA and EPA suppress the neurotrophic factor withdrawal-induced cell death and reversed the synaptic dysfunctions by enhancing the activation of PI3-K/Akt pathway (46, 47). Thus, it is not surprising that DHA administration inhibits the activation of GSK-3beta. Taken together, n-3 PUFAs not only suppresses the activation of JNK, but also facilitates the activation of PI3-K/Akt and subsequently inhibits the GSK-3beta activity, which ultimately limits the phosphorylation of tau in AD (fig4).

Following the deposition of senile plaques and neurofibrillary tangles, additional structural change and functional alterations ensue, such as synaptic dysfunction, inflammatory responses and oxidative stress (106). Synaptic function, particularly of the cholinergic system, is severely affected in the brains of AD patients (136, 137). Cholinergic neurons provide the major source of cholinergic innervations to the cerebral cortex, hippocampus, and amygdala, all of which are closely related to the memory. Dramatic loss of acetylcholine (ACh) due to the reduced chonline acetyltransferase (ChAT) was found in the cortex of AD brains (138). Abeta peptide (1-40) and tau-containing neurofibrillary tangles are both involved in the cholinergic neocortical pathway, which may enhance significant degeneration of cholinergic system. Among subcellular fractions of the brain, synaptosomal membranes, synaptic vesicles and growth cones contain the highest levels of DHA, suggesting its essential role in synaptic functions (17). Deficiency of n-3 PUFAs leads to impaired cholinergic neurotransmission in the brain, particularly in the hippocampus (139). DHA pre-administration preserves the activity of ChAT and prevents degeneration of cholinergic system challenged by beta-amyloid protein infusion, which eventually protects the brain against the loss of synapses (140, 141). ChAT is expressed in cortical neurons that are insulin and IGF-1 receptor-positive. However, their colocalization is reduced in the AD model, suggesting the possible relationship between insulin signaling pathway and the activity of acetyltransferase in the pathological progress of AD. As previously illustrated, DHA had regulatory effect on the activation of IGF-1 receptor; and ChAT expression was increased with the stimulation of insulin or IGF-1. However, whether DHA contributes to the preservation of ChAT activity through the insulin pathway is still unknown.

Increased production of reactive oxygen species, together with their following attack to DHA and other PUFAs, contributes to the pathophysiology of neurodegenerative diseases. Brain damage induced by oxidative stress is exacerbated by the decrease of DHA, one of the prime lipid peroxidation targets. The oxidation-induced loss of DHA is further confirmed by the concomitantly accumulated peroxidation products in AD patients (83, 142, 143). The antioxidative effect of DHA has been demonstrated in Abeta-infused rats. The production of lipid peroxide and reactive oxygen species was suppressed in the cerebral cortex and hippocampus, which may contribute to improve spatial cognition learning ability of the Abeta-infused rats (144).

Membrane DHA is liberated by a highly regulated PLA_2 and is subsequently converted into 10, 17S-docosatriene (Neuroprotectin D1) via a 15-lipoxygenase-like (15-LOX-like) enzyme. The sAPP (soluble APP), one of a neurotrophic peptide, strongly promotes the biosynthesis of NPD1, which further upregulates the expression of neuroprotective members of the Bcl-2 gene family, including Bcl-2, Bcl-xl and Bfl-1. NPD1 downregulates the expression of proapoptotic proteins like Bax and Bid (145, 146). In addition to its anti-apoptosis effect, nanomolar quantities of NPD1 have been shown as a potent inhibitor of pro-inflammatory gene expression and as a repressor of COX-2, IL-1beta (147, 148). In AD brains, unesterified DHA, as well as its downstream product NPD1, is significantly decreased, partially resulting from the abnormal expression of PLA_2 and/or 15-LOX enzymes (145). AD patients treated with DHA enriched n-3 PUFAs supplementation were reported to have increased plasma concentrations of DHA (and EPA), which were associated with reduced release of IL-1beta, IL-6 and granulocyte colony-stimulating factor from PBMCs (147).

EPA, another important n-3 PUFAs, tend to be oxidized once absorbed (26). Despite of its trace amount in the brain, EPA is able to abolish the IL-1beta-stimulated production of IL-6, partially via interacting with PPAR-gamma, showing its anti-inflammatory effect (149). EPA helps to preserve the acetylcholine (Ach) release and the expression of NGF, which can alleviate the memory deficits induced by IL-1beta (150). Dietary pre-administration of EPA could ameliorate the impairment of spatial cognitive learning ability induced by intracerebroventricular injection of Abeta, possibly by modulating the synaptic plasticity and facilitating the activation of PI3-K/Akt pathway (Figure 4). Taken together, n-3 PUFAs preserve the synaptic and neuronal functions and slow down the progress of neurodegeneration in AD via multiple mechanisms, including reducing the production and extracellular aggregation of amyloid peptide plaques, inhibiting hyperphosphorylation of tau protein, normalizing the activity of choline acetyltransferase, as well as inhibiting cell death process induced by oxidative stress and inflammation (151).

5.2. n-3 PUFAs in Parkinson's disease

Parkinson's disease (PD) is a common neurodegenerative disease characterized by bradykinesia, rigidity, resting tremor and postural instability. It is pathologically featured by cell loss or dysfunction of dopaminergic neurons in the substantia nigra pars compacta (152). The neuropathologic hallmark of PD is the neuronal aggregation of Lewy bodies composed mostly of alpha-synuclein and ubiquitin. Several recent observations reported

an association of high dietary consumption of saturated fatty acids, cholesterol and low intake of unsaturated fatty acids with high risk of PD (153-155). However, the literature regarding both n-3 PUFAs intake and PD risk is very limited. Some preclinical research provided evidence that DHA administration showed neuroprotective effects in animal models of PD (156, 157). However, other research emphasized the detrimental effect that DHA enhances 6-OHDA-induced dopamine reduction in the mouse striatum, causing increased susceptibility to peroxidation (158). Furthermore, elevated DHA was observed in brain areas containing α-synuclein inclusions in PD and DLB (dementia with Lewy bodies) patients (159). It was also detected in the cerebral cortex prior to alpha-synuclein deposition in incidental Lewy body disease (160). The controversial reports imply the complicated roles of n-3 PUFAs in the pathogenisis of PD. For example, oxidative stress, evidenced by increased lipid hydroperoxides, is a major contributory factor in the pathogenesis of PD (161-163). Enrichment of PUFAs in the brain may contribute to the formation of the neurotoxic peroxidation products and thus be detrimental to the PD brain. On the other hand, the deficiency of DHA results in the instability of membrane, causing impaired related biological functions of the membrane, which, apparently, would also be harmful to the PD brain.

As a major component of Lewy bodies, alpha-synuclein is predominantly expressed at presynaptic nerve terminals (164). It was suggested to be involved in synaptic plasticity and regulation of dopamine neurotransmission and act as a chaperone (165-167). Recently, PUFAs were found to interact with alpha-synuclein, promoting the formation of highly soluble oligomers. This precedes the formation of insoluble aggregates that are associated with neurodegeneration (159, 168). Researches on DHA further indicated that it rapidly triggers the α-helical conformation in both recombinant and native alpha-synuclein. Also, prolonged DHA exposure resulted in the assembly of alpha-synuclein into amyloid-like fibrils (169). However, with the current knowledge on the interaction of PUFAs with alpha-synuclein, it is hard to come to a conclusion how PUFAs regulate the pathogensis of PD.

Latest researches reported that lipid hypoperoxides, the primary peroxidative products, could react with dopamine and subsequently synthesize dopamine adducts, including succinyl dopamine (SUD), propanoyl dopamine (PRD), hexanoyl dopamine (HED) and glutaroyl dopamine (GLD). These dopamine adducts are derived from DHA and AA, respectively. Among them, HED, one of the AA-derived dopamine adduct, significantly induces a monoamine transporter-mediated ROS generation and apoptosis in the SH-SY5Y cells (170). The formation of dopamine adducts could not only contribute to the dopamine deficiency, but also exacerbate the oxidative stress in PD. Until now, little is known about the role of less toxic n-3 PUFAs-derived dopamine adducts in the brain. However, n-3 PUFAs supplementation is likely beneficial in PD by potentially counteracting the activity of AA and suppressing the production of AA-derived dopamine adduct (171).

In summary, research spanning decades supports the argument that n-3 PUFAs display multiple benefits in the prevention and treatment of cerebral ischemia and neurodegenerative diseases. The intriguing results should serve to spur much needed research on its neuroprotective mechanisms, which may, in turn, open new avenues for the therapeutic application of n-3 PUFAs.

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Abbreviations: PUFA and PUFAs: polyunsaturated fatty acids; DHA: docosahexaenoic acid; AA: arachidonic acid; EPA: eicosapentaenoic acid; ALA: alpha-linolenic acid; DPA: docosapentaenoic acid; NPD1: neuroprotectin D1; PPAR: peroxisome proliferator-activated receptor; FABP: fatty acid binding proteins; FATP: fatty acid transportation proteins; SPs: senile plaques; NF-kappaB: nuclear factor kappa B; APP: beta-amyloid precursor proteins; ChAT: chonline acetyltransferase; IRS: insulin receptor substrate; PI3-K: phosphatidylinositol-3 kinase; GSK-3beta: glycogen synthase kinase-3beta.

Key Words: DHA, ischemia, Alzheimer's disease, Parkinson's disease, Review

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