REVIEW

Dietary lipids and their oxidized products in Alzheimer's disease

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Alzheimer's disease (AD) is the commonest form of dementia in the elderly, characterized by memory dysfunction, loss of lexical access, spatial and temporal disorientation, and impaired judgment. A growing body of scientific literature addresses the implication of dietary habits in the pathogenesis of AD. This review reports recent findings concerning the modulation of AD development by dietary lipids, in animals and humans, focusing on the pathogenetic role of lipid oxidation products. Oxidative breakdown products of ω-6 polyunsaturated fatty acids (ω-6 PUFAs), and cholesterol oxidation products (oxysterols), might play a role in favoring β-amyloid deposition, a hallmark of AD's onset and progression. Conversely, ω-3 PUFAs appear to contribute to preventing and treating AD. However, high concentrations of ω-3 PUFAs can also produce oxidized derivatives reacting with important functions of nervous cells. Thus, altered balances between cholesterol and oxysterols, and between ω-3 and ω-6 PUFAs must be considered in AD's pathophysiology. The use of a diet with an appropriate ω-3/ω-6 PUFA ratio, rich in healthy oils, fish and antioxidants, such as flavonoids, but low in cholesterol-containing foods, can be a beneficial component in the clinical strategies of prevention of AD.

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1 Introduction

Various neurodegenerative disorders have been described, which induce a progressive and irreversible cognitive decline (dementia); Alzheimer's disease (AD) accounts for 45–60% of cases of dementia [1]. AD affects about 20–30 million people worldwide [2] and nearly half of those above 85 years [3]. Histopathological features of AD in the brain comprise neuronal and synaptic loss, extracellular deposition of amyloid β (Aβ) peptide in senile plaques and the formation of neurofibrillary tangles due to intraneuronal precipitation of hyperphosphorilated tau proteins [4]. Clinically, AD is characterized by memory dysfunction, loss of lexical access, spatial and temporal disorientation, and impaired judgment.

The pathogenesis of AD is still not fully understood, and no curative treatments are yet available [1]. However, a growing body of literature points to an important role of nutrition in the development of AD: changes in the metabolism of lipids, such as cholesterol and ω-6 polyunsaturated fatty acids (PUFAs), with the accumulation of their oxidized derivatives, might contribute to the onset and progression of this disease [5, 6]. This would provide mechanistic support for the widely held opinion that appropriate dietary supplementation with ω-3 PUFAs [7–10] and antioxidants

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Abbreviations: AA, arachidonic acid; **Aβ,** amyloid β; **AD,** Alzheimer's disease; **ALA,** α-linolenic acid; **Apo E,** apolipoprotein E; **ApoE4,** ε4 allele Apo E gene; **APP,** amyloid precursor protein; **BACE,** β-site APP-cleaving enzyme; **BBB,** blood–brain barrier; **COXs,** cyclooxygenases; **CTF,** C-terminal fragment of APP; **DHA,** docosahexaenoic acid; **EOAD,** early onset AD; **EPA,** eicosapentaenoic acid; **F2-IsoP,** F2-isoprostane; **HHE,** 4-hydroxyhexenal; **HNE,** 4-hydroxynonenal; **LA,** linoleic acid; **LOAD,** late onset AD; **MDA,** malonildialdehyde; **NPD1,** neuroprotectin D1; **24-OH,** 24-hydroxycholesterol; **27-OH,** 27-hydroxycholesterol; **PG,** prostaglandin; **PS,** presenilin; **ROS,** reactive oxygen species

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[11–13], which counteract lipid oxidation products, is helpful in preventing or delaying the development of AD. Figures 1 and 2 show the ω-3 and ω-6 PUFAs of interest in human nutrition, as well as cholesterol, together with the lipid oxidation products possibly implicated in AD.

The aim of this report is to comprehensively review recent animal and human studies focused on the potential role of PUFAs and cholesterol-oxidized products in the molecular pathogenesis of AD.

2 Molecular mechanisms involved in the pathogenesis of AD

2.1 The amyloid hypothesis and oxidative damage

Amlyoid β (Aβ) is generated through sequential cleavage of the transmembrane amyloid precursor protein (APP) by a group of proteases, namely, α-, β- and γ-secretase. APP is a 695–770 amino acid transmembrane protein, with a large hydrophilic *N*-terminal extracellular domain, a single hydrophobic transmembrane domain of 23 amino acids and a small C-terminal cytoplasmic domain [14, 15]. α-Secretase comprises three multidomain proteins located in the cell membrane, named ADAMs (a-disintegrin-and-metalloprotease) and is considered to down-regulate Aβ generation [16]. On the contrary, the synthesis of Aβ appears dependent upon the activity of β-secretase (also called BACE, β-site APP-cleaving enzyme), which is a particular protease belonging to the pepsin family of aspartyl proteases [17]. Finally, γ-secretase is a complex of enzymes, namely, presenilin (PS)1 and PS2, nicastrin, anterior pharynx-defective phenotype and PS enhancer [18, 19].

The cleavage and processing of APP can be subdivided into non-amyloidogenic and amyloidogenic pathways. The non-amyloidogenic pathway is initiated by α-secretase, which cleaves APP within the amyloid peptide domain, hampering Aβ generation. Alternatively, APP can be cleaved, in an amyloidogenic manner, by β-secretase and the *N*-terminus of the Aβ peptide is generated [17]. The α-secretase cleavage produces a soluble extracellular segment, sAPPα, and an intracellular fragment bound to the cell membrane, i.e. the α-C-terminal fragment (CTF) of APP. Conversely, APP cleavage by β-secretase leads to production of the β-soluble sAPPβ and the membrane-bound β-CTF. α-CTF and β-CTF are substrates for

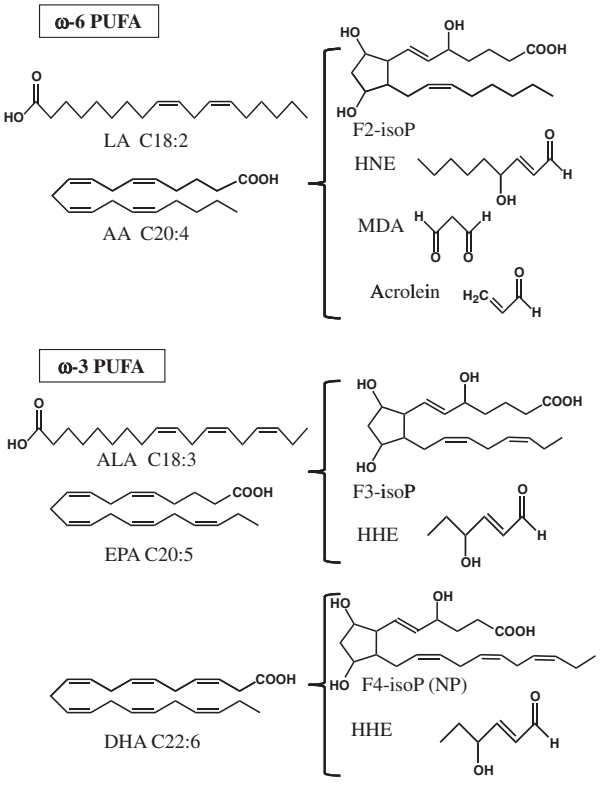

Figure 1. Main PUFAs and their oxidized derivatives in AD. PUFAs can be attacked by radicals and can undergo cleavage to yield peroxyradicals, which can be further cyclizated to form endoperoxides and degraded to different isoprostanes and aldehydes. The most implicated ω-6 PUFA in the progression of AD are LA and AA, whose decay leads to the formation of F2-IsoP and the shortest aldehydic derivatives such as HNE, MDA and acrolein. On the other hand, the most interesting dietary ω-3 PUFAs involved in AD are ALA, EPA and DHA acids. EPA degradation can generate F3-IsoP, whereas DHA produces F4-IsoP, also called neuroprostane (NP) because of its high concentration in the brain. Both EPA and DHA are cleaved to form HHE.

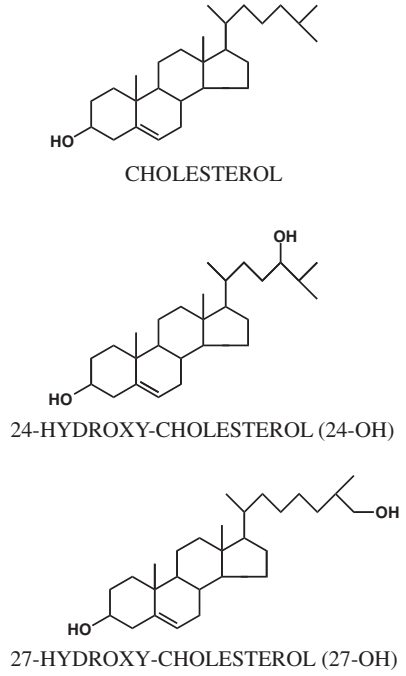

Figure 2. Structures of cholesterol and of the more common oxysterols involved in brain cholesterol metabolism. Cholesterol, 24-OH and 27-OH are shown. The two brain-derived oxysterols are more polar than cholesterol, cross the BBB readily and facilitate the elimination of cholesterol from the brain.

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γ-secretase. α-CTF cleavage due to γ-secretase results in a truncated non-amyloidogenic peptide (p3), whereas β-CTF cleavage leads to the amyloidogenic Aβ. Further, β-CTF can be cleaved at different sites by γ-secretase, respectively, forming the main variants 40 or 42 amino acid peptides, Aβ40 or Aβ42. Because of its increased hydrophobicity, Aβ42 forms aggregates and represents the predominant type of Aβ in senile plaques [20]. Figure 3 shows the two APP cleavage pathways and the different sites potentially targeted by dietary and genetic factors.

The non-amyloidogenic pathway is the commonest APP cleavage route, and elicits both neurotrophic and synaptotrophic effects [21]. Conversely, Aβ peptides activate neurotoxic pathways, leading to neuronal cell dysfunction. These events include enhanced cytotoxicity, increasing calcium neuronal influx and activation of microglia, which trigger the overproduction of reactive oxygen species (ROS) and pro-inflammatory cytokines [22–25].

Of the large volume of in vitro and in vivo studies investigating the potential modulation of APP processing and trafficking by nutrients [26, 27], only a small number has examined dietary lipids, mainly PUFA and cholesterol; these have been considered as potential sources of oxidants and inflammatory cytokines [28, 29].

Oxidative stress, i.e. the biochemical consequence of a net imbalance between production of ROS and their removal by cellular antioxidants, has consistently been suggested to play a key role in the onset of AD [30, 31]. Cell lipids are particularly susceptible to oxidative reactions, especially PUFA and cholesterol, leading to an increased production of oxidized derivatives; these are often much more reactive than the parent non-oxidized compounds. Interestingly, the ω-6 PUFA arachidonic acid (AA) is the main substrate for both membrane lipid peroxidation and the enzymatic production of inflammatory mediators through cyclooxygenases (COXs), which lead to brain damage [32, 33].

Cholesterol oxidation products, i.e. oxysterols, have recently been reconsidered for their pro-inflammatory, pro-apoptotic and pro-fibrogenic effects [34, 35]. In SH-SY5Y human neuroblastoma cells, 27-hydroxycholesterol (27-OH) appeared to enhance Aβ 1–42 production, up-regulating APP and BACE1 protein levels [36]. Conversely, in the same cell line, 24-hydroxycholesterol (24-OH) stimulated α-secretase activity [36, 37].

Taken together, these observations suggest that a variety of lipid oxidation products might be involved in the pathogenesis of cognitive decline and the neuronal loss that characterizes neurodegenerative diseases, including AD.

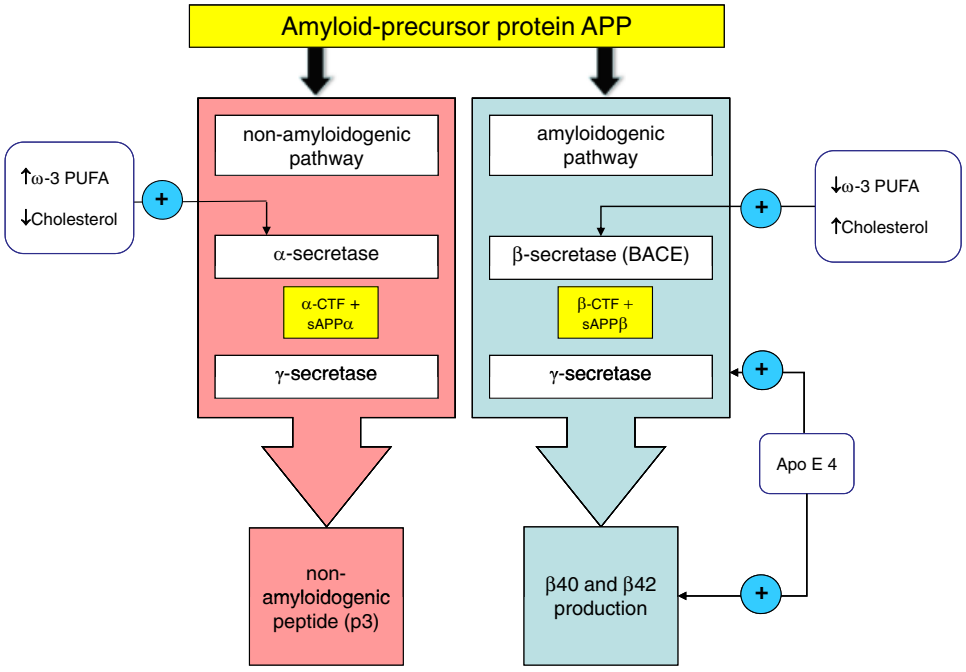

Figure 3. The non-amyloidogenic and amyloidogenic pathways of APP processing. The influence of dietary and genetic factors. The non-amyloidogenic pathway of APP is initiated by α-secretase, while in the amyloidogenic pathway APP is cleaved by β-secretase. α-Secretase cleavage produces a soluble extracellular segment (sAPPα) and an intracellular fragment bound to the cell membrane, α-CTF. β-Secretase cleaves APP into an sAPPβ and β-CTF. Subsequently, α-CTF and β-CTF are cleaved by another secretase, namely, γ-secretase: the product of α-CTF degradation is a non-amyloidogenic peptide (p3), while Aβ40 or Aβ42 are produced by β-CTF cleavage. Consequently, Aβ42 is capable of forming aggregates that might generate senile plaques. α-Secretase is the more active of the two enzymes in the low-cholesterol, high-PUFA content cell membranes (left panel). Conversely, β-secretase activity is more pronounced in cells having a high content of cholesterol and a low PUFA content (in particular ω-3) in their membranes. An adequate cholesterol/PUFA ratio might be a possible therapeutic approach (right panel). ApoE4 promotes the formation of amyloid fibers, both activating γ-secretase and increasing β40 and β42 production.

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2.2 Genetics of AD

An important feature in AD pathogenesis is the implication of a number of gene mutations: gene-environmental interactions may thus significantly influence the course of the disease.

There are two main forms of AD: ''early onset'' (EOAD) and ''late onset'' (LOAD) AD, the two forms having different, although not yet fully defined, etiology. EOAD (before 65 years) often has a positive family history [38]; this form has been linked to mutations of the genes for APP on chromosome 21, PS1 on chromosome 14 and PS2 on chromosome 1. These mutations affect the metabolism or stability of Aβ, and are responsible for 40% of cases of EOAD [39]. LOAD accounts for 95% of all AD cases. A number of possible susceptibility-enhancing genes have been implicated as risk factors for LOAD [40, 41]; the most widely studied is apolipoprotein E (Apo E) gene, on chromosome 19, encoding for the cholesterol transport protein. The ε4 allele Apo E gene (ApoE4) has been identified as a risk factor in both familial and sporadic AD [39]; subjects who carry one or two ApoE4 genes have a considerably higher risk of developing AD [42], whereas carriers of the ε2 allele appear to be relatively more protected against its development.

Apo E is known to play a key role in cholesterol and phospholipid metabolism and transport in many cells, but it also appears to be implicated in brain development, brain regeneration after injury and the synaptic plasticity [43, 44]; an increasing number of functions are now ascribed to Apo E as regards neuronal cells and astrocytes, which provide this lipoprotein to the brain [45]. As regards AD, the ApoE4 isoform has been shown to stimulate Aβ deposition, enhancing the amyloidogenic processing of APP and reducing clearance of the Aβ peptide [46]. ApoE4 is present in Aβ plaques, and probably acts as a pathological chaperone, promoting β sheet conformation of soluble Aβ. There is also some in vitro evidence that Apo E directly promotes amyloid fibril formation, increasing both the rate of fibrillogenesis and the total amount of amyloid produced [47].

3 The role of diet in AD

Diet may influence both the development and the prevention of AD [48, 49]. Here, the attention will be concentrated on the role played by PUFAs, cholesterol and relevant oxidative metabolites.

3.1 PUFAs

A suitably balanced intake of ω-3 and ω-6 PUFA is essential for brain homeostasis [50], while a significantly reduced ω-3/ω-6 PUFA ratio appears to contribute to the onset of AD [51, 52]. The ω-3 PUFAs mainly involved are α-linolenic acid (ALA, 18:3), eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6), while the ω-6 PUFAs chiefly comprise linoleic acid (LA, 18:2) and AA (20:4). PUFAs are the constituents of phospholipid membranes, and play significant roles in cellular membrane fluidity and enzyme activities. They are also precursors of active pro-inflammatory metabolites (prostaglandins (PGs), prostacyclins, thromboxanes and leukotrienes), produced enzymatically from the 20-carbon or 22-carbon long-chain PUFAs, known as eicosanoids or docosanoids, respectively.

3.2 ω-6 PUFAs

These are most likely involved in the pathogenesis of many diseases, including neurodegenerative diseases; this in particular applies to AA, from which are derived inflammatory molecules that can cross the blood–brain barrier (BBB), stimulate specific cell surface receptors, and that exert potent autocrine and paracrine activities. The two main groups of enzymes involved in the formation of these inflammatory mediators are the COXs, which catalyze the formation of PGs from free AA, and the lipoxygenases, which catalyze the oxygenation of PUFAs to form lipid hydroperoxides, i.e. hydroperoxyeicosatetraenoic acids, and finally leukotrienes [53].

AA is distributed in several different cell types in the brain, both in the gray and white matter, and appears to be implicated in a number of neuronal functions, for example, synaptic signaling, memory and learning; AA may for instance contribute to important functions in neurons, including the propagation of cell signals related to synaptogenesis [54]. However, in humans, a decreased AA concentration in the brain is frequently associated with the aging process and AD onset [55]. This enhanced consumption of ω-6 PUFAs would indirectly suggest an excessive production of pro-inflammatory eicosanoids stemming from enzymatic oxidation of AA [56]. The pro-inflammatory effects resulting from an increased utilization of membrane AA in the brain of AD patients could be competitively counterbalanced by the presence of the anti-inflammatory EPA [57].

In transgenic mice with memory impairment and Aβ deposition, a dietary regimen poor in ω-3 PUFA and high in ω-6 PUFA leads to a significant decrease of *N*-methyl-D-aspartate (the postsynaptic receptor complex in the brain, thought to regulate learning and memory) and to a net potentiation of programmed cell death through caspase-3 activation. These changes, which may contribute to the cognitive damage afflicting AD patients, was found to be amplified in transgenic mice expressing a mutated form of the APP human gene, known as the Swedish mutation (APPswe) [58].

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3.3 ω-3 PUFAs

ALA, DHA and EPA also contribute to cell structure and function in the nervous system. While ALA can only be obtained from the diet, DHA and EPA can also be synthesized in the body, by desaturation of ALA [59]. Hepatic conversion of ALA and EPA to DHA is probably an additional source of brain DHA: this is suggested by experimental studies on rats fed an ω-3-deficient diet, in which increased synthesis of DHA in the liver occurred, and it reached the brain. However, this particular biochemical reaction is related to normal metabolism in healthy humans, and there is no substantial proof of its involvement in AD patients [60, 61].

A deficit of dietary ω-3 PUFAs appears to contribute to inflammatory signaling, apoptosis, and neuronal dysfunction, and is associated with age-related cognitive decline and neurological disorders [62]. EPA is the substrate for the production of the so-called anti-inflammatory PGE_3. Although not yet fully understood, the anti-inflammatory properties of EPA are thought to play a neuroprotective role during aging, since EPA competes with AA for incorporation into cell membrane phospholipids, and for the active site on the COX enzymes. This competition, and the resulting production of PGE_3, might result in decreased levels of pro-inflammatory PGE_2 [63, 64].

Studies on the onset and progression of AD have demonstrated that dietary consumption of EPA is associated with a lower risk of AD and cognitive decline [44, 65]. A longitudinal study found higher plasma EPA concentration and lower ω-6/ω-3 ratio to be associated with a lower incidence of dementia, especially in depressed patients [9].

Another ω-3 PUFA is DHA, which is abundant in the brain, where it is mainly localized in the neuronal membranes [53]. It represents more than 17% by weight of total fatty acids in the brain of adult rats [66]. DHA is a key player in conferring fluidity to human axons, and to neuronal membranes; the latter effect would favor the processing of APP via the non-amyloidogenic pathway.

Several experimental studies support a positive effect of DHA supplementation in quenching the development of AD. For instance, dietary pre-administration of DHA showed beneficial effects on learning ability in an experimental Aβ peptide-produced AD model in Wistar rats [66]. Apparently, this nutritional supplement increases cortico-hippocampal glutathione (GSH) levels and glutathione reductase activity, with a net reduction of lipid peroxides and ROS in the cerebral cortex and hippocampus of treated animals. Consistent with the likely implication of oxidative stress in the onset of AD, dietary DHA supplementation has been found to lower Aβ deposition levels in APPswe (Tg2576) transgenic mice [67].

DHA might influence the APP processing pathways: it has been hypothesized that DHA reduces the risk of AD onset by inducing the neuronal sorting protein LR11 [68], which is reduced in LOAD. LR11 is a member of the ApoE-low-density lipoprotein receptor family, which reduces APP, trafficking to secretases that generate Aβ [69]. This ω-3 PUFA might influence membrane bilayer properties by changing biophysical properties of lipid rafts. Amyloidogenic APP processing is believed to occur at the level of the lipid rafts in the synaptic membrane, where β- and γ-secretases are located [70]. DHA may reduce Aβ generation, both by displacing cholesterol from the lipid raft and by diminishing the PS1 protein concentration present in raft microdomains, thus reducing γ-secretase processing of APP [71].

Measurement of the impact of DHA on pre- and post-synaptic marker vulnerability showed that it has important effects on dendritic structural elements and signaling pathways; all these crucial activities could explain why low DHA availability in the brain is accompanied by behavioral deficits. APP transgenic mice fed on high doses of DHA showed improved spatial memory [72, 73]. Similar results were obtained in heterozygous APPswe/PS1ΔE9 transgenic mice that co-express human PS1 bearing the ΔE9 deletion mutation and a chimeric mouse–human APP. DHA supplementation of these animals markedly decreased cortical and striatal plaque deposition compared to transgenic mice fed a normal diet [8].

DHA, besides having a generic anti-inflammatory action, is the source of derivatives, namely, docosanoids, which include the neuroprotectin D1 (NPD1). This bioactive lipid mediator is, for example, synthesized by retinal pigment epithelial cells in response to oxidative stress, and it protects brain and retina against cellular oxidative damage [74]. Interestingly, NPD1 content was shown to decrease in the hippocampus of Alzheimer's patients. Overall, NPD1 is a modulator of signaling pathways that promote brain cell survival through the induction of antiapoptotic and neuroprotective gene-expression programs, and the suppressing of Aβ42 production and neurotoxicity. Thus, NPD1 elicits potent cell-protective, anti-inflammatory, pro-survival, repair signaling [74, 75]. Alongside the increasing body of experimental reports supporting a protective role of ω-3 PUFAs in AD prevention, a recent study by Arendash et al. is worth mentioning, which found no protective role played by ω-3 PUFA or fish oil supplementation, in the AD transgenic mouse model APPsw+PS1 [76].

As far as humans are concerned, many epidemiological analyses have addressed the relationship between ω-3 PUFA intake and development of dementia, with controversial results. On one hand, the longitudinal Canadian Study of Health and Aging, with multiple follow-ups, did not find any relationship between blood concentration of the ω-3 PUFAs EPA and DHA and the incidence of dementia or AD [77]. Similar results emerge from the Rotterdam Study, after a 6-year follow-up [78]. Conversely, various other studies report an inverse correlation between continual ω-3 PUFA consumption with the diet and incidence of dementia and AD [65, 79, 80]. Multiple factors may influence the outcome of these epidemiological investigations, such as the

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non-uniform etiopathogenesis of AD, different study settings and different PUFA intake with the food. In addition, EPA and DHA can be cleaved non-enzymatically, producing the aldehyde 4-hydroxyhexenal (HHE), whose high concentration could be deleterious for normal function of nervous cells (see below).

The few clinical studies available thus far about ω-3 PUFA supplementation and possible amelioration of cognitive decline appear to indicate a positive effect of DHA supplementation, but only in patients with organic brain lesions or mild cognitive impairment, and not in AD patients [81]. A similar conclusion was reached by a randomized, double-blind, placebo-controlled clinical trial, which ranked very mild, mild and moderate AD patients by the Mini Mental State Evaluation. Administration of DHA and EPA was shown to delay the rate of cognitive decline only in the group of patients with a very mild form of AD [82].

4 PUFA-derived oxidation products

It is widely accepted that non-enzymatic peroxidation of AA yields peroxyl radical intermediates, which are unstable and can undergo cyclization to form endoperoxides. The insertion of a second oxygen and further reduction give rise to different F2-isoprostanes (F2-IsoPs), while further chain cleavage yields several aldehydes, such as malonildialdehyde (MDA) and acrolein, or hydroxyalkenals [83–86].

Increased PUFA peroxidation in the brain suggests an association between AD development and the production of F2-IsoPs and aldehydes [87–89].

The non-enzymatic oxidation of EPA and DHA leads to the formation of analogs of F2-IsoPs, i.e. F3-IsoPs and F4-IsoPs, respectively. F4-IsoPs are also called neuroprostanes (F4-NPs), DHA being the most abundant PUFA in the brain. [90]. The chemical structures of the main PUFAs and of their non-enzymatic oxidized derivatives are reported in Fig. 1.

F2-isoPs and F4-NPs have been shown to be elevated in the frontal, temporal, parietal and occipital lobes, and in the hippocampus of AD patients, compared with controls. However, it has been found that isoprostanes are increased to the same extent in patients with mild cognitive impairment, and in late-stage AD patients, without significant differences between these two stages of dementia [91]. Thus, isoprostanes are enhanced in AD and might be active players in its pathogenesis, but there is not enough proof to consider them indices of disease progression [92].

Although MDA is one of the most widely used markers of oxidative stress, this aldehyde cannot be considered a specific marker of AD, as it is also enhanced in mild cognitive impairment and in the healthy elderly [93, 94].

Acrolein has been hypothesized as molecular mediator in AD pathogenesis [95], since increased levels of this aldehyde, as well as of 4-hydroxynonenal (HNE), have been detected in post-mortem brain samples from AD patients [96]. Increasingly, reports are indicating a potential role of HNE, a major hydroxyalkenal end-product of ω-6 PUFAs (LA and AA) in the pathogenesis of neurodegenerative diseases, including AD [97]. Increased levels of HNE have been detected in the ventricular fluid and brain of AD patients [98]. HNE is highly reactive with amino and thiol groups of proteins, and the AD brain shows increased levels of protein-bound HNE; these HNE–protein adducts might contribute to the formation of neurofibrillary tangles and to the alteration of different enzymes in the brain [99, 100]. HNE has been shown to deactivate neprilysin, a protease implicated in the removal of Aβ [101, 102]. Moreover, HNE also induces the production of short Aβ oligomers, at the same time inhibiting the conversion of these protofibrils into long and straight amyloid fibrils, probably less involved than protofibrils in the pathogenesis of AD. Most likely, a vicious circle occurs, in which oxidative stress leads to HNE-induced oligomers of Aβ that, in turn, amplify the oxidative stress condition and HNE production [103]. Of note, the up-regulation of Aβ production by neuronal cells challenged with HNE was very recently shown to depend on the overexpression of β-secretase, induced by this aldehyde [104, 105].

The biological relevance of ω-3 PUFA-derived hydroxyalkenals is much less widely documented than is HNE's activity; the compound of this class that has been most studied to date is HHE. Despite the fact that ω-6 PUFAs are considered the most abundant in biomembranes, larger amounts of HHE, derived from ω-3 PUFAs, than of HNE have been detected in various tissues, included the brain, where a high concentration of ω-3 DHA is present [97, 106]. An uncontrolled dietary supplementation of ω-3 PUFAs may anyway be deleterious, yielding high doses of HHE. Recently, elevated amounts of free and protein-bound HHE have been found in the hippocampus/parahippocampal girus of brain specimens from human autopsies of subjects in preclinical or late-stage AD [107].

Although conclusive proof of the involvement of HNE, and possibly also of other hydroxyalkenals, including HHE, in the pathogenesis of AD has yet to be obtained, there is no doubt that HNE is a very likely candidate molecule for the role of triggering and sustaining a large variety of biochemical events that underlie the development of this neurodegenerative process.

5 Cholesterol and its oxidation products

The brain is the organ with the highest concentration of cholesterol, mainly localized in neuronal and glial membranes, where it is essential to maintain normal fluidity, permeability and function [108]; brain cholesterol derives from de novo synthesis by astrocytes, since plasma lipoproteins cannot cross the BBB [3]. Conversely, the BBB is permeable to oxysterols, i.e. cholesterol oxidation derivatives; thus excess cholesterol in the brain may, under normal

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physiological conditions, be excreted in the blood [109]. The major oxysterol involved in this excretion mechanism appears to be 24-OH, almost exclusively synthesized in the brain by the enzyme cholesterol 24-hydroxylase (cytochrome P450-46A1) [110, 111]. Another oxysterol of major interest in brain pathophysiology is 27-OH; this compound is produced in the brain by the cytochrome P450-27A1, but can also flow into the brain from the blood circulation [112]. Chemical structures of cholesterol and of the two oxysterols 24-OH and 27-OH are shown in Fig. 2.

Hypercholesterolemia is unanimously recognized to be a risk factor for sporadic AD, a form that accounts for the great majority of cases [3, 113]; of note, in hypercholesterolemic individuals, the percentage of oxysterols, in particular that of 27-OH, is significantly elevated [114]. The latter oxysterol can then easily cross the BBB, where it may alter the balance between oxysterols and cholesterol in the brain. In addition, the hypercholesterolemic condition is strongly suspected to derange the integrity of the BBB in some way, also allowing unoxidized blood cholesterol to flow into the brain. The pathological accumulation of cholesterol and oxysterols in the central nervous system, with the consequent change in the brain cholesterol/oxysterol balance, may thus be the missing link between hypercholesterolemia and AD.

In this connection, an abnormal pattern of cholesterol hydroxylases has been observed in the AD brain, with a prominent expression of 24-hydroxylase in the astrocytes and around the amyloid plaques [115]. Increased availability of cholesterol in the brain, for instance provoked by hypercholesterolemia, together with increased conversion of the sterol to 24-OH, could favor the neurotoxicity of Aβ peptide accumulating in the vicinity. Indeed, 24-OH has recently been shown to markedly enhance Aβ-induced necrotic death of cultured human neuronal cells [116].

Cholesterol and certain oxysterols appear to influence not only the toxic effect of Aβ, but also its synthesis. An excessive amount of cholesterol within the membrane bilayer of neurons appears to enhance the affinity of β- and γ-secretases for APP, thus greatly enhancing Aβ production [117]. This hypothesis is supported by evidence that Aβ-positive nerve terminals of AD patients contain higher concentrations of cholesterol than do Aβ-negative nerve terminals [118]. Similarly, ''in vitro'' studies have demonstrated that a reduction of the cholesterol content of neurons lowers the amount of Aβ synthesized by these cells [119]. Again in support of the possibility that oxysterols impact on Aβ synthesis, preliminary results obtained in our laboratory suggest that 24-OH and 27-OH may up-regulate expression and synthesis of BACE1 (unpublished data).

In conclusion, excess dietary cholesterol could in principle contribute to the multifactorial pathogenesis of AD, and reports linking hypercholesterolemia to AD pathogenesis are steadily mounting. Oxysterols appear to interfere with cholesterol metabolism in the central nervous system, but only those deriving from enzyme-dependent oxidation of the lateral chain of cholesterol are involved. Conversely, the oxysterols introduced into the body with the diet are of non-enzymatic origin, and show hydroxylic or epoxidic groups in the sterol ring. At least to present knowledge, they do not appear to be involved in the impaired cholesterol/oxysterol equilibrium observed in the AD brain.

6 Nutritional considerations

It is reported that the daily intake of ω-3 PUFAs among the world population is significantly lower than that recommended by the World Health Organization (1–2% of energy from ω-3 PUFAs) [120, 121]. It cannot be excluded that the incidence and prevalence of AD are increased in populations with a dietary intake of EPA and DHA below the recommended doses. Because of the likely beneficial effects that a suitable dietary content of ω-3 PUFAs would confer against the incidence and development of cardiovascular and neurodegenerative diseases in humans, it would be of great importance that, in each country, the National Academy establish reference dietary intakes, at least for EPA and DHA.

In Western countries, consumers' demand for foods or food supplements that help to prevent or manage disease processes, including AD, is continuously increasing [122]. A dietary regimen including the intake of a recommended amount of ω-3 PUFA or, better, an appropriate ω-6/ω-3 PUFA ratio, could certainly help to prevent cognitive impairment and, in patients with early AD, would help to delay progression of the disease. The ''Mediterranean diet'', with an appropriate ω-3/ω-6 PUFA ratio, rich in healthy oils, fish and antioxidants but low in cholesterol-containing foods, has been shown to be a beneficial component of the clinical treatment of AD [101, 123]. Further, a recent epidemiological study shows a relation between higher fish consumption and improved cognitive function in later life [124].

As regards cholesterol oxidation products, they might, at least in part, explain how hypercholesterolemia may markedly promote the development of atherosclerosis [125]. Moreover, an altered cholesterol metabolism is most likely implicated not only in the pathogenesis of vascular dementia but also in the onset of AD and of the mixed degenerative and vascular types of dementia [3, 113]. Over the last few years, the molecular signaling triggered by oxysterols of interest in human pathophysiology has been investigated in depth, and the involvement of certain phosphorylation pathways clearly elucidated. In particular, oxysterols appear to modulate levels and activities of various kinases of the protein kinase C (PKC) and the mitogen-activated protein kinase (MAPK) families [125] and also those of the transcription factor NF-κB [126]; further, 24-OH has been shown to strongly enhance generation of NADPH oxidase-dependent ROS in human neuronal cell cultures [116]. In relation to this latter process, the demonstrated inhibition of oxysterol-dependent ROS production when

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neuronal cells were pre-treated with either genistein or quercetin, two strongly-antioxidant flavonoids, is very important. These two flavonoids also prevent the neurotoxicity exerted by 24-OH [116]. This very recent report provides further support for the emerging opinion that certain flavonoids should be included in nutritional strategies of prevention and early treatment of AD and other neurodegenerative diseases. Several epidemiological studies indicate that the consumption of flavonoids, such as quercetin and the catechins, is associated with a lower incidence of Parkinson's disease [127] and of AD [128, 129]. These and other polyphenols can apparently cross the BBB, due to their relatively high lipophilicity [130] and their interaction with specific efflux transporters present in the BBB [131]. It thus appears likely that the quenching of NADPH- and mitochondria-dependent oxidative stress, as well as of the inflammatory and neurotoxic reactions induced by these cholesterol oxidation compounds, are the protection mechanisms afforded by flavonoids against AD-related oxysterols. The neuroprotective effects of flavonoids probably do not only depend on their antioxidant properties but also on non-antioxidant mechanisms, such as modulation of intracellular signaling cascades and gene expression that regulate neuronal survival, death and differentiation [132].

In conclusion, considerable experimental data suggest the importance, in the pathogenesis of AD, of persistent oxidative stress associated with the presence of oxidation end-products. The balance between ω-3 and ω-6 PUFAs and cholesterol surely plays a crucial role in AD's onset, but it must be weighed with many other factors, including genetic factors, education and lifestyle considerations. In addition, clinical and epidemiological studies have yet to provide conclusive results, probably because the discrepancies in dietary supplementation, and the differences in the lipids investigated, are too wide. However, it may be said that a dietary regimen low in ω-6 PUFA and cholesterol, together with the use of antioxidants, including polyphenols, is valuable within a general strategy of prevention of neurodegenerative diseases.

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