Alzheimer’s disease (AD) is a progressive irreversible neurodegenerative disorder and the most common cause of dementia in the elderly, currently affecting approximately 35 million worldwide (1). Clinically AD is characterized by a loss of short-term memory, intellectual performance, and disorientation with a complete loss of memory and mental functions in advanced stages of the disease (2).

The first notion on a possible involvement of lipids in AD dates back to Alois Alzheimer. By examining the brain of Auguste Deter, Alzheimer noticed, besides the infamous fibrillary and plaque pathology, lipid granule accumulation in the glia (3, 4). Almost a century later, Roses reported that the AD risk increased dramatically with increasing number of apoE4 alleles in families with late-onset AD (5), a finding immediately confirmed by several other groups (5–8). Among others, Sparks et al. (9) showed that cholesterol might represent a molecular risk factor for AD, reporting that cholesterol fed to rabbits increased cerebral amyloid-β (Aβ) deposition, a finding that was later successfully reproduced in AD transgenic mouse models (10, 11). Beyreuther and colleagues (12) found that cholesterol increases neuronal Aβ generation, which could be reversed by cholesterol depletion (13) and treatment with the cholesterol-lowering drug, simvastatin, which successfully lowered cerebral Aβ levels, including the relevant Aβ42 species (14) in guinea pigs. At the same time, epidemiological

Abstract In the last decade, it has become obvious that Alzheimer’s disease (AD) is closely linked to changes in lipids or lipid metabolism. One of the main pathological hallmarks of AD is amyloid-β (Aβ) deposition. Aβ is derived from sequential proteolytic processing of the amyloid precursor protein (APP). Interestingly, both, the APP and all APP secretases are transmembrane proteins that cleave APP close to and in the lipid bilayer. Moreover, apoE4 has been identified as the most prevalent genetic risk factor for AD. ApoE is the main lipoprotein in the brain, which has an abundant role in the transport of lipids and brain lipid metabolism. Several epidemiological approaches revealed changes in the lipid levels of cerebrospinal fluid or in post mortem AD brains. Here, we review the impact of apoE and lipids in AD, focusing on the major brain lipid classes, sphingomyelin, plasmalogens, gangliosides, sulfatides, DHA, and EPA, as well as on lipid signaling molecules, like ceramide and sphingosine-1-phosphate. As nutritional approaches showed limited beneficial effects in clinical studies, the opportunities of combining different supplements in multi-nutritional approaches are discussed and summarized.—Grimm, M. O. W., D. M. Michaelson, and T. Hartmann. Omega-3 fatty acids, lipids, and apoE lipidation in Alzheimer’s disease: a rationale for multi-nutrient dementia prevention. J. Lipid Res. 2017. 58: 2083–2101.

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Thematic Review Series: ApoE and Lipid Homeostasis in Alzheimer’s Disease

Omega-3 fatty acids, lipids, and apoE lipidation in Alzheimer’s disease: a rationale for multi-nutrient dementia prevention

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Abbreviations: Aβ, amyloid-β; AD, Alzheimer’s disease; ADAM, a disintegrin and metalloprotease; AICD, amyloid precursor protein intracellular domain; APP, amyloid precursor protein; BACE, β-secretase amyloid precursor protein cleaving enzyme; CSF, cerebrospinal fluid; FAD, familial Alzheimer’s disease; FC, Fortasyn Connect; GCS, glucosylceramide-synthase; GD3S, GD3-synthase; IDE, insulin-degrading enzyme; MCI, mild cognitive impairment; n3-FA, omega-3 FA; PE, phosphatidylethanolamine; PS, presenilin; ROS, reactive oxidative species; RXR, retinoic X receptor; S1P, sphingosine-1-phosphate; TR, targeted replacement.

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apoE exists as three different isoforms, termed apoE2, apoE3, and apoE4, of which apoE4 is the most prevalent genetic risk factor of AD (5, 19, 20). apoE is the main lipid-protein in the brain and plays an important role in intracellular transport of lipids and in brain lipid metabolism. The pivotal role of lipid-related mechanisms in the normal physiological function of apoE led to the suggestion that the pathological effects of apoE4 are driven by a lipid-related mechanism. This hypothesis is further supported by recent genome-wide association studies that identified genes involved in cholesterol metabolism or transport as AD susceptibility genes (21, 22). Genome-wide association analysis identified two novel loci, clusterin, also known as apol, on chromosome 8 and phosphatidylinositol binding clathrin assembly protein (PICALM) on chromosome 11 (21). Clusterin is a heterodimeric molecular chaperone involved in protein folding of secreted proteins and has been found to interact with the soluble form of Aβ (23, 24), whereas PICALM is important for clathrin-mediated endocytosis, an essential step in the intracellular trafficking of proteins and lipids. The evidence that supports this hypothesis and the mechanisms underlying the role of lipids in mediating the pathological effects of apoE4 will be presently discussed.

apoE4 AND LIPIDS IN AD

apoE4 inhibits serum lipolysis leading to decreased delivery of free FAs into the brain and their incorporation into glia and neurons (25, 26). Accordingly, a diet enriched with the omega-3 FAs (n-3 FAs), which are beneficial to non-apoE4 AD subjects, was found to be ineffective in the corresponding apoE4 subjects (27). Serum cholesterol levels are elevated in apoE4 carriers (28). Because elevated serum cholesterol levels at mid-life are associated with increased AD risk (29, 30), it has been suggested that serum cholesterol may play a role in mediating the lipid-related pathological effects of apoE4. Corresponding measurements of the total cholesterol and phospholipid levels in the cerebrospinal fluid (CSF) revealed that they are not affected by apoE4 (31). However, further studies that focused on distinct glycerolipids revealed specific effects of apoE4. Accordingly, the levels of sphingosine-1-phosphate (S1P) in the hippocampus are specifically lower in apoE4-positive AD patients than in corresponding non-apoE4 carriers (32) and the levels of phosphoinositol bisphosphate are reduced in post mortem brain tissue of apoE4 carriers as well, as in the brains of corresponding apoE4-targeted replacement (TR) mice (33).

The lipid-driven effects of apoE4 could be due to effects of the apoE genotype on the size, chemical composition, and structure of the apoE particles and/or to a lipid-related target with which apoE interacts. In the following, we discuss findings that show that the apoE isoforms differentially affect the size and extent of lipidation of the apoE particles. The first indication that apoE4 and apoE3 differed in their lipoprotein-related properties was obtained over 20 years ago by Weisgraber (38), who showed that, following incubation with serum lipoproteins, apoE4, but not apoE3, localizes preferentially with VLDL. Separation of human CSF apoE by density gradient ultracentrifugation revealed that apoE4 carriers have the highest level of lipid-depleted apoE particles, compared with the apoE2 and apoE3 groups, and that apoE2 has the highest levels of highly lipidated particles compared with the other groups (39). The effect of the apoE genotype on the size of the CSF apoE particle was assessed by nondenaturing gel electrophoresis, which revealed that the apoE2/E3 subjects had significantly larger apoE particles than apoE3/E3 subjects, who had significantly larger apoE complexes than apoE3/E4 and apoE4/E4 individuals (40). Similar results were obtained utilizing apoE4 and apoE3 TR mice in which brain apoE4 is associated with smaller less lipidated particles than apoE3 (40). Consistent with these findings, it was shown that viral mediated expression of the different apoE isoforms in apoE TR mice leads, in the case of apoE4, to the expression of the smallest and least lipidated apoE particles (41). Taken together, these observations show that brain and CSF apoE4 particles are smaller and less lipidated than those of non-apoE4 carriers (41). The mechanisms underlying the isoform-specific effects of apoE4 on the size and extent of lipidation of apoE and the role of this effect in mediating the pathological effects of apoE4 are addressed in the following sections.

ABCA1 AND THE LIPIDATION OF apoE4

ABCA1 is a transmembrane protein that translocates cholesterol and phospholipids to lipid-free lipoproteins.
leading to the generation of HDL-like particles (42). In the periphery, apoA-1 and apoE are the main substrates of ABCA1; whereas in the brain, apoE is its main substrate. Binding of the lipid free lipoprotein to ABCA1 is critical for initiation of lipid efflux (43). This is followed by translocation of the lipids from the plasma membrane and intracellular pools to the lipoproteins via ABCA1 and to the subsequent release of the lipidated HDL-like particles into the interstitial fluid. The ABCA1 lipidated particles can be further lipidated by the ABCG1 transporter, which, unlike ABCA1, lipidates partially lipidated, but not lipid-free, lipoproteins (44). An important development in the study of ABCA1 was the discovery of mutations in this protein that cause Tangier disease and lead to impaired cellular cholesterol efflux and low levels of HDL particles (42). A recent large scale genetic study established that a well-established loss-of-function mutation of ABCA1 is strongly associated with a higher risk of AD (45). The importance of ABCA1 for the lipidation of apoE in the brain was also highlighted by experiments utilizing ABCA1 knockout mice. This revealed that ABCA1 deficiency in mice reduced the amount of cholesterol in the CSF and that the corresponding CSF apoE-containing particles were smaller and contained less cholesterol and less apoE than those of ABCA1-containing mice (46, 47). Whereas ABCA1 deficiency decreases the level of the apoE protein, the corresponding mRNA was not affected by this treatment, suggesting that apoE is stabilized by ABCA1 (46). Interestingly, whereas ABCA1 is needed for the production of normally lipidated brain apoE, apoJ does not depend on ABCA1, consistent with the idea that apoE and apoJ reside on distinct lipoprotein particles (48).

The central role of ABCA1 in the lipidation of apoE led to the examination of the possibility that the hypolipidation of apoE4 could be reversed by pharmacological activation of ABCA1; this was first examined by treating apoE3 and apoE4 mice with the retinoic X receptor (RXR) ligand, bexarotene. This treatment increased the level of ABCA1 in the brains of the apoE4 and apoE3 mice and was associated with reversal of the hypolipidation of apoE4, but with no change in the lipidation of apoE3 (49). Similar results were obtained utilizing the peptide, CS-6253 (50), which was previously shown to directly activate ABCA1 in vitro (51). Bexarotene activates ABCA1 by increasing the expression of the ABCA1 and subsequently increasing the levels of the ABCA1 protein, whereas the effects of CS-6253 are mainly due to activation of ABCA1-driven lipid efflux. Although the molecular mechanisms underlying these processes are not fully worked out yet, the joint take home message is that the hypolipidation of brain apoE4 can be counteracted by activation of brain ABCA1 activity.

Measurement of the effects of the apoE genotype on apoE serum levels of apoE4 and apoE3 mice revealed that, like in the brain, the levels of apoE4 in the serum are lower than those of apoE3 and that they elute faster following gel permeation chromatography (49). Activation of ABCA1 reversed the elution profile of apoE4 and rendered it more similar to that of serum apoE3, which was not affected by this treatment (49).

**THE ROLE OF HYPOLIPIDATION IN MEDIATING THE PATHOLOGICAL EFFECTS OF apoE4**

This has been investigated by two complimentary approaches. The first focuses on the general in vivo neuronal and behavioral phenotype of apoE4 and on determination of the extent to which it can be counteracted by reversal of the hypolipidation of apoE4. The second approach focuses on specific a priori apoE4 driven mechanisms, such as cross-talk with Aβ and synaptic impairments.

**Counteracting the brain and cognitive effects of apoE4 by reversal of its hypolipidation**

Treatment of apoE4 and apoE3 TR mice with either bexarotene or the ABCA1 agonist, CS-6253, both of which reverse the hypolipidation of apoE4 (49), reversed the apoE4 driven accumulation of Aβ42 and hyperphosphorylated Tau in hippocampal neurons, as well as the associated synaptic impairment and cognitive deficits of these mice (49). These findings suggest that hypolipidization plays a pivotal role in mediating the pathological effects of apoE4 and point at ABCA1 as a promising therapeutic target. Beneficial effects of ABCA1 activation were also observed in the PDAPP mouse model where amyloid deposition was reduced by overexpression of ABCA1 (52). Cell culture studies revealed that neurite outgrowth is stimulated by PUFA-containing phospholipids, suggesting that PUFAs play an important role in mediating the lipidation-dependent effects of apoE (53). As the phenotype of apoE4 mice is complex and involves the AD molecules, Tau and Aβ, as well as synaptic molecules and apoE receptors, additional models that are more targeted are needed for assessing the cellular mechanisms and processes that drive the apoE4 hypolipidation-related effects of apoE4.

**apoE4 lipidation and Aβ**

In vitro binding studies revealed that human apoE binds to the Aβ isoform specifically in a lipid-dependent fashion. Accordingly, lipidated apoE3 binds to Aβ more avidly than the corresponding apoE4. The affinity of both apoE isoforms to Aβ and its apoE isoform specificity are both reduced following delipidation of apoE (54, 55). The finding that lipidated apoE3 binds more avidly to Aβ than apoE4 led to the suggestion that apoE3 is involved in the clearance of Aβ from the CNS and that this physiological mechanism is impaired in apoE4. This was confirmed by in vivo studies with mice that express the different human apoE isoforms together with the Aβ precursor, amyloid precursor protein (APP), which revealed that the t1/2 of Aβ in the brain was longer in apoE4 mice than in apoE3 mice and that, like in AD, this was associated with increased deposition of Aβ in the apoE4 mice (56–58). In view of these findings, it is possible that the accumulation of Aβ in hippocampal neurons of apoE4 mice (49, 59) is due to the hypolipidation of apoE4 and the resulting impaired clearance of Aβ. This suggests that delipidation plays an important role in mediating the Aβ-dependent apoE4-driven pathological effects. Although the in vitro data suggest that these effects are driven by direct interactions between apoE4 and apoE.
and Aβ, recent in vitro findings suggest that apoE4 and Aβ interact minimally under physiological conditions and that, consequently, indirect mechanisms may play a role in mediating the cross-talk between apoE and Aβ (57).

**apoE4 lipidation and synaptic pathology**

Neurite outgrowth requires a supply of lipids for membrane expansion. Indeed, neurite outgrowth by numerous cell types, including astrocytes and neurons, was activated following incubation with lipidated apoE3, whereas incubation with apoE4 either inhibited or had no effect on this process (60–63). However, as recombinant non-lipidated apoE4 has the same effect as lipidated apoE4 (61), it is possible that these neurite-related effects of apoE4 are also driven by a lipid-independent mechanism. Several other mechanisms are possible; apoE4 has been implicated, in addition to Aβ and the synaptic mechanism, in numerous other processes. These include apoE receptors, inflammation, apoE4 degradation, and the vascular system (64). The roles of apoE4 hypolipidation in mediating these apoE4 phenotypes remain to be determined.

**APP PROCESSING**

The main pathological hallmarks of AD are extracellular amyloid plaques composed of aggregated Aβ peptides (65, 66) and intracellular neurofibrillary tangles due to hyperphosphorylation of the microtubule-associated protein, Tau (67, 68). Aβ peptides are generated by sequential proteolytic processing of the APP, a large type I transmembrane protein (69). The amyloidogenic processing of APP is initiated by β-secretase APP cleaving enzyme (BACE)1 (70, 71), which cleaves APP within its extracellular/intraluminal N-terminal domain, shedding off β-secreted APP. The remaining membrane-tethered C-terminal fragment (βCTF/C83) is further cleaved by γ-secretase. This cleavage releases Aβ peptides in the extracellular space (72, 73) and the APP intracellular domain (AICD) in the cytosol. AICD is considered to translocate to the nucleus and to regulate gene transcription of different target genes (74–83). Aβ peptides vary in length from 37 to 43 amino acids, with the main products being Aβ38, Aβ40, and Aβ42 (73, 84–87). Although Aβ42 only accounts for approximately 10% of total secreted Aβ peptides, the Aβ42 species represents the major component of amyloid plaques. Due to the two additional amino acids, isoleucine and alanine, Aβ42 is more hydrophobic and aggregates faster than Aβ40 peptides (88, 89). Before their manifestation as amyloid plaques in vulnerable brain regions like hippocampus and cortex, these peptides form oligomers, protofibrils, and fibrils. Small oligomers of Aβ, up to 50 Aβ subunits, are proposed to be the most toxic variant of Aβ (90–93).

**APP SECRETASES**

The γ-secretase that generates the C terminus of Aβ peptides is a heterotetrameric protein complex consisting of presenilin (PS)1 or PS2, nicastrin, anterior-pharynx defective 1 a or b, and PS enhancer 2 (94). PS1 or PS2 represents the catalytically active components of the γ-secretase complex (95, 96). Mutations in the PSs cause early-onset familial AD (FAD) that is caused by an increase in Aβ42 peptides and a decrease in Aβ40 peptides (97–100). The γ-secretase complex belongs to the intramembrane cleaving proteases with the peculiar property to cleave transmembrane proteins within their hydrophobic transmembrane domains. Intramembrane cleaving proteases, including γ-secretase, are, themselves, membrane-embedded proteases with multiple transmembrane domains, emphasizing the importance of the lipid microenvironment of the membrane for proper function. Indeed, amyloidogenic processing of APP by β- and γ-secretase is proposed to occur within lipid rafts (101–104), membrane microdomains enriched in cholesterol and sphingolipids (13, 105, 106). The importance of lipids for AD is further strengthened by observations that several lipid classes can influence proteolytic processing of APP, including cholesterol, sphingolipids, PUFAs, plasmalogens, trans-FAs, and phytosterols (13, 107–117), and that several lipid classes have been found to be altered in AD post mortem brain (118–121). Alternatively to the initial cleavage by β-secretase, APP can be cleaved within its N-terminal domain by α-secretases. The α-secretases have been identified as members of the a disintegrin and metalloprotease (ADAM) family; they cleave APP within the Aβ domain, thus preventing the generation of Aβ peptides (122–126). Cleavage of the remaining C-terminal fragment, α-CTF/C83, by the γ-secretase complex liberates nontoxic p3 peptides (127). Interestingly the α-secretases, as well as BACE1, are also membrane-embedded proteases. In contrast to β-secretase processing of APP, which is proposed to be lipid raft associated, α-secretase cleavage of APP seems to take place outside of lipid rafts (128, 129). APP processing into Aβ, AICD, and other APP fragments is a key part of the physiological function of APP in lipid sensing and lipid regulation (76, 77, 130, 131), which provides a rationale why APP cleavage is very sensitive to alterations in the membrane lipid composition (78, 111, 132). Besides a possible direct effect of lipids on APP processing, lipids might be important regulators for lateral movement of proteins within the phospholipid bilayer and might, therefore, be critical for substrate/enzyme interaction and, thus, a correct balance between amyloidogenic and non-amyloidogenic APP processing (133). Moreover, disturbing or changing the lipid composition of the membrane might, therefore, play a crucial role in the pathogenesis and treatment of AD. The following section provides a brief overview of the role of the major lipids in AD.

**SM**

SM is a major constituent of cellular membranes and is one of the major lipids in the human brain. The structures of the discussed sphingolipids, which are changed in AD, are shown in Fig. 1. SM occurs in high concentrations in neuronal membranes and in the myelin sheets. Besides its role as a signaling molecule, it has a crucial function in the
structure of membranes. Due to its highly intermolecular interactions, which are mediated by the 2-amide group, the 3-hydroxy group, and partially by the 4,5-trans double bond of the sphingoid-base, SM has a crucial function in forming membrane microdomains. These microdomains, also called lipid rafts, are signaling platforms and are thought to include the secretases involved in the amyloidogenic pathway (13, 101–104). In the post mortem brains of patients suffering from AD, a reduction in SM and an elevation of ceramide have been reported, compared with age-matched control brains (134). Interestingly, SM is a precursor for ceramide, a reaction that is catalyzed by the action of the SMases. Acid SMase and acid ceramidase expression was accompanied by the observed change in the lipid pattern (134). Mechanistically, it has been shown that Aβ, especially Aβ42, can directly activate SMases in the picomolar range. In return, inhibition of SMases leading to an increased SM level resulted in a decrease of Aβ production (131). Accordingly, mutations in PS1 causing FAD showed increased SMase activity (131). Under pathological conditions, high Aβ42 concentrations resulting in oligomers, protofibrils, and fibrils and leading to oxidative stress and inflammatory mechanisms have been demonstrated to additionally activate nSMase (135, 136). Similar results have been found by Jana and Pahan (137, 138), showing that fibrillary Aβ peptides activate nSMases via NADPH oxidase-mediated mechanisms finally leading to the loss of neurons. A more recent report showed significantly increased nSMase activity not only in human post mortem brains but also in plasma and fibroblasts derived from AD patients. Notably, no elevation was found in samples from Parkinson’s disease patients, indicating that increased aSMase activity is a specific signature in AD (139). Elevated aSMase activity was also found in a transgenic AD mouse model consisting of mutated APP and PS1; these transgenic mice showed increased aSMase activity not only in neurons and brain but also in plasma and fibroblasts (139). Combining this transgenic mouse model with aSMase−/− mice revealed significantly reduced Aβ deposition and memory dysfunction (139). Recently, a convergent study was published; nSMase2-deficient mice combined with 5xFAD mice revealed improved cognition and showed an ameliorated AD pathology. Total Aβ42 and plaque burden and, additionally, Tau phosphorylation were decreased and the transgenic mice showed improved cognition in fear-conditioned learning tasks (140). Another connection between AD, SM, and SMases can be indirectly drawn from the fact that SM is decreased in depression, accompanied by an increase in aSMase activity (141). A quantitative meta-analysis revealed that depression is a major risk factor for the incidence of dementia, including AD and mild cognitive impairment (MCI) (142).

Analyzing different metabolites in biofluids of AD patients revealed that, in plasma, two ceramide species were increased and eight SM species were decreased in AD, accompanied by a total increase in ceramides, both in serum and CSF (143). Besides these unambiguous results, it should be mentioned that, in the case of biomarkers, the role of SM is not completely understood and controversial results are reported in the literature. For example, Koal et al. (144) report an increased SM species [SM (d18:1/18:0)] to be significantly enhanced in CSF samples of AD patients and suggest it as a biomarker for AD with a specificity of 76% and a sensitivity of 66% with a cut-off of 546 nM. Interestingly, this study also found an increased acyl-carnitine (C3-DC-M/C5-OH). Acyl-carnitines occur under physiological conditions in the cytosol and are used to transport FAs in the mitochondria by the carnitine-carrier system.

Fig. 1. Chemical structure of the sphingolipids that are altered in AD.
enhanced level of acyl-carnitines in CSF or in the extracellular space might indicate neuronal loss or cell death and might help to explain this finding (134). However, elevated SM levels have also been found in prodromal, mild, or moderate AD, compared with normal cognitive controls, suggesting that alterations in SM homeostasis may contribute to early AD pathological processes and are not only due to neuronal loss (145). Another study showed that, in contrast, many other sphingolipids, including SM39:1, SM41:1, and SM42:1, are decreased. However, this study clearly points out that SM alone is not sufficient as a biomarker and has to be accompanied by other lipids to increase specificity and sensitivity (146). Utilizing APP/PS1 transgenic mice, another study underlines the importance of SM in AD pathology, but also points out that alterations might be region specific, which might further help to explain different results in the literature (147). Summing it up, under physiological conditions, Aβ increases SMase activity. This increase is further enhanced under pathological conditions involving reactive oxidative species (ROS) and inflammatory processes. Consequently SM is found to be reduced at least in some specific regions of AD brain. In return, SM decreases Aβ production, suggesting that a decreased SM level is not only a consequence but also a cause in AD. With respect to biomarkers, the role of SM is not completely understood. Divergent results occur in the literature depending on the stage of disease or the biofluid that is analyzed.

CERAMIDE

The role of ceramide in cellular signaling processes, including cell differentiation, proliferation, and programmed cell death, is well known. Besides the above mentioned SMases, ceramide is also produced by de novo synthesis of serine-palmitoyltransferase and some additional enzymes, leading to dehydro-ceramide and ceramide in the endoplasmic reticulum. The concentration of ceramide is tightly linked to the action of the SMases, which were reviewed above. Therefore, it is not surprising that most studies found an increased ceramide level associated with AD (134, 148–150). Shotgun lipidomics approaches of early AD stages identified two ceramide species to be significantly lower and five other species that trended toward being decreased in plasma samples (151). Similar results were found by Mielke et al. (152), who reported that high baseline ceramides were associated with an increased risk of AD. Interestingly, a reduction in ceramide synthase 2 activity in Braak stages 1 and 2 in the temporal cortex and Braak stages 3 and 4 in the hippocampus and frontal cortex was found (153). Combining the facts that ceramide synthase 2 activity is proposed to generate very long chain ceramides and that very long chain ceramides are precursors for sulfatides and galactosylceramides typically found in myelin, these findings suggest a defect in myelin biosynthesis in the preclinical or early to moderate stages of AD (154). Moreover, pathway and network analysis revealed a role of ceramide in both inflammatory and anti-inflammatory pathways of AD, further illustrating the complex action and mechanisms mediated by different ceramides (155). Enhanced levels of ceramides have been shown to stabilize β-secretase and, therefore, increase the Aβ level (156). Ceramides have been shown to cause mitochondrial depolarization and permeabilization, cytochrome-C release, Bcl2-depletion, and caspase-3 activation, further leading to neuronal death (134). Besides the impact of ceramide in AD and APP processing, APP processing in return regulates ceramide level. It has been shown that the AICD is a regulator of the expression of serine-palmitoyl transferase, the committed step reaction in ceramide de novo synthesis spanning a complex regulatory cycle (76). All things considered, the link between ceramides and AD is widespread. It has to be kept in mind that ceramide function is highly dependent on the cellular localization of the ceramides and the ceramide species, especially on the FAs bound at the sn-2 position. Whereas ceramides with very long chain FAs are abundant in myelin and reported to be “good players” in respect to AD, other ceramide species trigger inflammatory and apoptotic processes. Therefore especially for ceramides, biomarkers have to be carefully chosen focusing not in general on the lipid class, the ceramides in total, but more precisely on the different ceramide species. Further studies are needed to fully understand the role of ceramides in the context of AD.

SPHINGOSINE AND S1P

As mentioned above, ceramidase has been reported to be altered in post mortem AD brains (134). Ceramidase catalyzes the degradation of ceramide to sphingosine by cleaving of one FA. By the action of sphingosine-kinase, sphingosine can be phosphorylated to S1P. In return, sphingosine-phosphatases can convert S1P to sphingosine. Additionally, S1P can be cleaved by S1P-lyase resulting in phosphoethanolamine and hexadecenal, which is an irreversible reaction. The ratio of S1P to ceramide is linked to several cellular processes, e.g., apoptosis, Ca homeostasis, or inflammation (157). As these biochemical processes are involved in neurodegeneration, a crucial role of sphingosine and sphingosine derivatives can be expected in the pathological events of AD. Indeed, it has been shown that S1P is able to bind to BACE1 and modulates its activity (158). Treatment of mouse neurons with sphingosine-kinase inhibitors, RNA interference leading to knockdown of sphingosine-kinase, or overexpression of S1P-degrading enzymes resulted in decreased BACE activity and reduced Aβ production. Another study demonstrates that increased S1P level caused by S1P-lyase deficiency results in the accumulation of APP and APP C-terminal fragments in lysosomal compartments and a reduction of γ-secretase activity (116). Besides its effect on APP processing, deficiency of S1P-lyase has been reported to increase hyperphosphorylation of Tau (159). However, the impact of S1P in AD is not completely understood. On the other hand, it has been shown that the S1P/sphingosine ratio declines with increased Braak stage. Additionally, the decrease of S1P was found in the brain regions that are most affected by AD pathology. Interestingly, the authors point out an association
of these results with the apoE status. The S1P/sphingosine ratio was 2.5-fold higher in the hippocampus of apoE2 carriers compared with apoE4 carriers (32). In line with these results, S1P enhances neuronal cell survival stressed by glucose deprivation and glucose reload stress (160) mediated by the activation of the S1P1- and S1P3-receptor signaling pathways. Recently, fingolimod, a drug already used in multiple sclerosis that is a functional S1P1 receptor antagonist, was used to treat 5xFAD transgenic mice (161). Decreased plaque density, accompanied by a decrease in soluble and insoluble Aβ, was found in fingolimod-treated mice. Additionally, fingolimod attenuated GFAP staining and microglial activation (161). In conclusion, sphingosine or sphingosine-related pathways seem to be a promising and interesting target in AD. Nevertheless, it has to be taken into consideration that S1P is tightly regulated in brain homeostasis in a low picomolar range and affects a broad network of cellular processes. Further studies are needed to elucidate the cross-talk between sphingosine or sphingosine derivatives and AD and to clarify the potential benefits in respect to the expected side effects. Furthermore, the above discussed relationship between apoE and sphingosine homeostasis suggests that not all patients might equally profit from a sphingosine-based therapy.

**GLYCOSYLATED SPHINGOLIPIDS**

By the addition of galactose or glucose to ceramide sulfatides and gangliosides, two other important lipid classes in brain are generated. In the case of sulfatides, cerебroside-sulfotransferase adds a sulfate to the galactosyl-ceramide, a reaction that can be reverted by arylsulfatase A. Sulfatides are not only present in the white matter and myelin, but also in the membranes of neurons, especially in the axon structure (162). A decrease in sulfatides has been reported in both gray matter and white matter of post mortem brains from AD subjects with mild dementia (163). Another study confirmed this finding and revealed that brain samples from subjects with Parkinson’s disease and dementia with Lewy bodies did not have changes or even higher sulfatide levels compared with control samples, suggesting that sulfatide reduction is a specific event in AD. Additionally, the authors suggest that Aβ accumulation is not a factor directly contributing to a decreased sulfatide level in AD (164). A decreased sulfatide level was also confirmed in transgenic AD mouse models. Interestingly, apoE was found to mediate sulfatide reduction in transgenic mouse models of AD (165) in an apoE isof orm-dependent manner. Therefore, besides S1P, sulfatides were also identified to cross-talk with apoE-mediated biochemical processes. A more recent study confirmed the reduction of sulfatide levels in preclinical stages of the disease (166).

Glucosylceramide-synthase adds glucose to ceramide generating the precursor for the gangliosides. Interestingly, it has been reported that glucosylceramide-synthase is regulated by APP processing (82). Similar results have been found for GD3-synthase (GD3S), another enzyme in the ganglioside pathway, converting α-series to β-series gangliosides (78). Importantly, GM3, the substrate for GD3S, decreases Aβ generation, whereas the product, GD3, enhances Aβ production (78). Accordingly, GD3S-deficient mice combined with a transgenic AD mouse model showed a reduced plaque burden and an improvement in cognitive abilities (129). A more recent study confirming the effect of GD3S-deficient mice also suggests an impact of the cholinergic-specific ganglioside, GT1ac, in memory retention (167). An increase of β-hexosaminidase, which is a catalytic enzyme in ganglioside homeostasis, revealed beneficial effects in cognitive tests with transgenic AD mouse models (168). The importance of this catalytic enzyme is also underlined by a study showing changed hexosaminidase-β-galactosidase in AD patients (169), probably linked with a dysfunction of lysosomal compartments, a known characteristic in AD (170, 171).

Another ganglioside, GM1, has been shown to enhance amyloidogenic pathways (172). Additionally, gangliosides have been shown to bind Aβ and, therefore, influence the aggregation of Aβ, leading to oligomers, protofibrils, and fibrils (173–178). Accordingly, ganglioside-Aβ complexes have been discussed to build aggregation seeds for oligomers and plaques (108). Aging itself changes the ganglioside homeostasis in the brain. Therefore, age-matched control brains compared to brains with AD pathology were used to elucidate pathological changes in ganglioside homeostasis caused by AD. These experiments revealed that the ganglioside homeostasis is affected by AD. However, the results seem to be brain region-specific and differ in the pathological stages of AD (96, 179–182), which explain the heterogeneous results found in the literature. For ceramides, it has already been discussed that the FA also determines the impact of the individual lipids in AD-relevant processes (153). Similar results have been found in gangliosides, further explaining the different results in the literature and emphasizing the need for exact and detailed lipidomic analysis, including not only the headgroup but also the FAs bound at the sn-1 and sn-2 positions.

**PLASMALOGENS**

Plasmalogens are ether-phospholipids characterized by a vinyl-ether bond at the sn-1 position and an ester linkage at the sn-2 position. The chemical structures of the plasmalogens are presented in Fig. 2. Several studies showed a decrease in phosphatidylethanolamine (PE)- and phosphatidylcholine-plasmalogens in AD post mortem brains (119, 121, 183). Analyzing PS mutation carriers, a study

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**Fig. 2.** Chemical structure of plasmalogens.
revealed a decrease in two plasmalogen species (PE34:2 and PE35:4) in the CSF, which correlated with an increased Tau level in CSF and an increased amyloid burden in the brain (184). Plasmalogens are also found to be decreased in erythrocytes of children with Down syndrome. As Down syndrome is a chromosomal aberration on chromosome 21 (trisomy 21) and APP is located on chromosome 21 (185), these results further strengthen the linkage between APP processing and plasmalogen homeostasis (186). As the vinyl-ether is highly susceptible for ROS and oxidation, the reduced plasmalogen levels could be caused by the increased oxidative stress occurring during AD pathogenesis. However, we could demonstrate that a reduced plasmalogen level is not just the consequence of pathological changes in the brain; plasmalogen, itself, is able to reduce γ-secretase activity in neuroblastoma cells and membranes derived from mouse brains (113). These results have recently been confirmed by another group, demonstrating that an increased PE-plasmalogen to PE ratio results in an inhibition of γ-secretase activity (187). Plasmalogens not only affect APP processing but also in return, APP processing tightly regulates plasmalogen homeostasis (77). AICD decreases the expression of the enzyme catalyzing the committed step reaction in plasmalogen synthesis, the alkyl-dihydroxyacetone-phosphate-synthase, leading to decreased plasmalogen synthesis. Results were confirmed in several transgenic mouse models lacking APP or only expressing a truncated APP construct devoid of a functional AICD domain (77, 188). Under physiological conditions, these results suggest a regulatory feed-back cycle in which plasmalogens inhibit APP processing and APP processing, in return, downregulates plasmalogen de novo synthesis. Under pathological conditions, this regulatory cycle is affected. Besides the reduction of plasmalogens by ROS, reduced alkyl-dihydroxyacetone-phosphate-synthase protein stability due to peroxisomal dysfunction was reported leading to a reduced plasmalogen level. The reduced plasmalogen level results in elevated Aβ production, further enhancing oxidative stress and peroxisomal dysfunction. Summing it up, the regulatory feed-back cycle, being necessary to adjust homeostasis, is converted to a feed-forward or futile cycle under pathological conditions enhancing Aβ production. Because of their potential benefits as a nutritional supplement in AD, efforts have been made to identify sources of plasmalogens in food, like marine invertebrates, blue mussels, or ascidians, and to extract them from these sources (189). Plasmalogens derived from these natural sources have already been shown to suppress activation of caspase-3 in SH-SY5Y cells, further implicating a beneficial effect in AD (189). Recently, at the First International Plasmalogen Symposium in Japan, results were presented suggesting beneficial effects of plasmalogens in clinical studies of patients with MCI.

EPA AND OTHER PUFAs

The n3-PUFA, EPA, is also present in the brain to a lower extent than DHA (190). Figure 3 illustrates the structures of DHA and EPA. Mechanistically, it has been shown that EPA is able to enhance Aβ degradation mediated by insulin-degrading enzyme (IDE). Like DHA, EPA is able to bind IDE and directly enhances IDE activity (136). Moreover, EPA increases IDE gene expression; in contrast to DHA, no alterations between the extracellular and intracellular IDE ratio compared with its corresponding saturated FA (20:0) was observed (136). It is discussed that EPA affects the gene expression of many inflammation-related genes in immune cells. A recent study revealed that the anti-inflammatory potency of EPA is comparable to DHA (191). Similarly, Hopperton et al. (192) reported that increasing brain n3-PUFAs decreases some aspects of the inflammatory response to Aβ. In this recent study, brain n3-PUFAs were increased by a transgenic approach using fat-1 transgenic mice carrying a transgene converting n6- to n3-PUFAs, or dietary means analyzing WT littermates that were orally administered a fish oil or safflower oil diet containing very low levels of n3-PUFAs. At 12 weeks of age, intracerebroventricular infusion of Aβ1-40 was performed. Compared with WT mice fed the safflower oil diet, fat-1 transgenic mice and WT mice fed the fish oil diet showed higher levels of brain DHA, whereas n6-PUFAs were decreased. Microglial activation was reduced in fat-1 transgenic mice at 10 days postsurgery. The WT mice fed the fish oil diet showed no effect on microglial activation, but revealed fewer degenerating neurons.

Additionally, n3-FA supplementation was reported to increase Aβ phagocytosis by macrophages and to influence brain amyloidosis. However, it has to be mentioned that, in this study, n3-PUFAs have been combined with antioxidants and resveratrol, so that no clear mechanistic conclusions to single compounds can be drawn and further experiments are needed, preferably with a higher n-number (193). In the context of inflammation and PUFAs, it should be noted that effects might also be mediated by dihydroxy- or trihydroxy-metabolites of n3-FA, which are called resolvins. Resolvin D1 was shown to be associated with increased Aβ phagocytosis (194) and might also inhibit fibrillar Aβ-induced apoptosis (195). Another enzymatic derivative of n3-FA, neuroprotectin D1, was suggested to downregulate Aβ production and pro-inflammatory gene expression and promote cell survival. In the anti-apoptotic bioactivity induced by neuroprotectin D1, PPARγ signaling may be involved and a shift from the amyloidogenic to the
non-amyloidogenic pathway is proposed to be responsible for the decreased Aβ production (196). Because of the complex effects of n3 derivatives like neuroprotectin in AD, we would like to refer to a previous review in the Journal of Lipid Research (197).

Although EPA is often combined with DHA in nutritional approaches (198, 199), mechanistic studies about EPA alone and AD-related processes are mainly not provided. Besides EPA, PUFAs have been shown to increase non-amyloidogenic processing of APP leading to increased α-secretase APP secretion (114, 200, 201). PUFAs have been shown to increase α-secretase activity in neuroblastoma cells and isolated membranes of both neuronal cell lines and human postmortem brains. Interestingly, for linolenic acid (18:3) and DHA, it was shown that these lipids can increase α-secretase activity utilizing purified ADAM10 enzyme (114). Given the findings that PUFAs are decreased in human postmortem AD brains (121, 202), the anti-inflammatory properties, and the effect on the nonamyloidogenic pathway and on Aβ clearance, other unsaturated FAs besides DHA might be interesting targets in AD, especially combined with other nutritional approaches. Remarkably, not only the desaturation but also the number and position of double-bonds of FAs seem to influence AD-relevant mechanisms. The conformation is also known to have an important impact. Under physiological conditions, double-bonds have a cis-conformation resulting in an angled nonlinear orientation of the FA in the biomembrane. The resulting angle especially determines physical properties like membrane fluidity. In contrast, trans-FAs have a linear orientation and the membrane is more condensed and tightly packed. The trans-FAs, which, for example, occur naturally in products of ruminants, enhance amyloidogenic and decrease non-amyloidogenic APP processing by influencing all three secretases (117). Moreover, trans-FAs seem to alter intracellular APP processing and enhance Aβ aggregation compared with the corresponding cis-conformation (117). Accordingly, epidemiological studies revealed that individuals who had an intake of trans-FAs of 4.8 g/day showed a 5-fold higher relative risk of developing AD than subjects consuming 1.8 g/day (203) and dietary intake of trans-FAs is associated with cognitive decline in the elderly population (204, 205).

DHA

As mentioned above, not only the headgroup of lipids but also the FAs are crucially linked with AD. DHA (DHA22:6), a polyunsaturated n3-FA, is the most prominent n3-FA in the CNS (206). Thirty to forty percent of the FAs in the phospholipids of neuronal plasma membranes and 8% of the human brain dry-weight are lipids containing DHA (206, 207). DHA synthesis is highly limited in humans; therefore, the major amount of DHA has to be taken up by diet (208). DHA and other n3-FAs are efficiently transported across the blood-brain barrier (209, 210). However, a recent study suggests that the apoE4 allele is associated with a decreased transport of DHA to CSF (211). Consequently apoE4, a known risk factor for AD (5, 212–214), is not only related to cholesterol homeostasis and transport but also influences the homeostasis of lipids tightly coupled to AD. As an example, we would like to underline the importance of apoE in sulfatide homeostasis, as reviewed above, and also in the transport of PUFAs across the blood-brain barrier, as indicated by the recent literature (211, 215).

After being incorporated into phospholipids, DHA determines the physical state of membranes, such as membrane fluidity (200, 216). Mechanistically, it has been shown that DHA increases the non-amyloidogenic processing resulting in an elevated α-secreted APP level by an increase in ADAM17 protein levels caused by upregulated gene expression and decreased protein degradation (201). Additionally, DHA attenuates amyloidogenic processing by both affecting β- and γ-secretase activity by independent mechanisms. Whereas BACE1 total protein levels were unchanged, a changed cellular distribution was observed leading to an accumulation of BACE1 at the cell-surface and a decreased intracellular BACE1 level in the endosomes (201). As BACE1 has its optimal activity at an acidic pH value, which is found in endosomes (217–220), the altered localization of BACE1 might act as an explanation for decreased BACE1 activity in the presence of DHA. For γ-secretase, a direct effect of DHA on the enzyme activity was reported, accompanied by a shift of PS1 from the raft microdomains to the non-raft microdomains of the membrane (201). As PS1 contains the active center of the γ-secretase complex (95), the change in PS1 distribution also leads to a shift in γ-secretase activity. Besides the direct effect of DHA on amyloidogenic and non-amyloidogenic pathways, we and others could demonstrate that cholesterol de novo synthesis, especially the activity of the enzyme catalyzing the committed step reaction, the HMG-CoA reductase, was decreased in the presence of DHA (201, 221). Cholesterol is known to increase amyloidogenic APP processing and decrease non-amyloidogenic APP processing (13, 107, 112). Therefore, a reduction in cellular cholesterol levels could also contribute to the pleiotropic DHA-mediated effects that all synergistically result in a decreased Aβ level. Aβ levels are not only dependent on Aβ production but also on Aβ catabolism. One of the major enzymes that is involved in Aβ degradation is the IDE (222). Phospholipids containing DHA (22:6) compared with phospholipids with the corresponding saturated FA (22:0) have been shown to directly bind to IDE (136). In addition, purified recombinant IDE enzyme showed elevated activity in the presence of DHA. Besides its direct effect, DHA enhances exosomal IDE secretion in the extracellular space and altered intracellular/extracellular protein level ratio (136). The effect of DHA on IDE results in increased Aβ degradation, further enhancing the effect of a decreased Aβ level in the presence of DHA (136). Notably, DHA increases the binding of Aβ1-42 to lipid rafts and it has been suggested that DHA might, therefore, contribute to the clearance of circulating Aβ by lipid raft-dependent degradation pathways (223).

Apart from its impact on Aβ homeostasis, another study revealed a critical role of DHA in the Act-mTOR-S6K pathway...
pathway in neuronal development and axon outgrowth (224). Moreover, DHA-containing phosphatidylcholine improved the cognitive deficits in an AD rat model. The authors suggest decreased phosphorylation of Tau in the cortex and hippocampal CA1 area as a possible mechanism (225). In vitro studies also indicate that DHA can inhibit and even reverse the formation of Aβ oligomers and, therefore, decrease Aβ-associated neurotoxicity (226–228), which was recently confirmed in an APP/PS1 rat model of AD, emphasizing that dietary DHA modulates Aβ oligomerization (229). Moreover, a recent study showed that DHA levels were associated with cerebral amyloidosis and preservation of entorhinal hippocampal volumes (215). Notably, the PUFA, DHA, has also been found to be an agonist of several nuclear receptors, e.g., the RXR (230, 231) and PPARα and -γ (232–234), a mechanism that might contribute to some of the beneficial effects of DHA in AD.

In mouse brain, DHA has been identified as an endogenous ligand for RXR (230, 231), a ligand-activated nuclear transcription factor important for reproduction, cellular differentiation, bone development, hematopoiesis, and pattern formation during embryogenesis. DHA was found to be specific for RXR, whereas DHA failed to activate the retinoic acid receptor, the thyroid receptor, or the vitamin D receptor (230). These data suggest that DHA influences neuronal function through the activation of a RXR signaling pathway. The ability to activate RXR does not seem to be exclusive for DHA, as additional unsaturated FAs, like docosatetraenoic acid, docosapentaenoic acid, arachidonic acid, and oleic acid have been found to bind and to activate RXR (230, 231). Recently, it has been shown that DHA in combination with bexarotene, a RXR agonist, strongly induces expression of the liver X receptor:RXR target genes, ABCA1 and apoE, involved in reverse cholesterol transport, in a 5xFAD mouse model (235). Furthermore, the dual therapy of bexarotene and DHA reduced amyloid pathology and inflammatory processes and restored the impaired working memory of these transgenic mice.

In the section dealing with SM, we have already pointed out that a connection between depression and AD, mediated by changes in aSMase or the SM/Cer ratio, exists. Notably n3-FAs, especially EPA and DHA, are also associated with major depressive disorder, independently suggesting a common lipid-based linkage between depression and AD from another related perspective (236).

It has to be pointed out that dietary DHA supplementation in the transgenic AD mouse model did not only increase the DHA level; other changes in FAs were found, for example, a reciprocal alteration of DHA and arachidonate was reported (237). Therefore, the effect of DHA should not be seen as an isolated event, but has to be interpreted in a complex context with other lipid changes (237) that include lipid-based hormonal factors, like estrogen, also being involved in AD (238). In this context, a recent publication showed that oxidized DHA does not only attenuate the beneficial effect of DHA but also reverses its action leading to increased amyloidogenic pathways (239). One percent oxidized DHA in the presence of 99% unoxidized DHA was sufficient to increase Aβ production in neuroblastoma cells. Interestingly, not just one species of oxidized DHA revealed a high amyloidogenic potency, all seven oxidized DHAs or lipid peroxidation products elevated the production of Aβ (239). These results strongly emphasize the need to prevent oxidative reactions of DHA, for example, by adding additional antioxidants. In this context, it has to be mentioned that the hydroxylated form of DHA (DHA-H) has recently been reported to improve behavioral motor function and survival of transgenic flies expressing human Aβ42 and Tau (240), whereas the ethyl ester form of DHA only showed moderate effects. Oral administration of DHA-H to 5xFAD mice for 4 months also improved the cognitive scores of these mice, as evaluated by the radial arm maze test. In human post mortem brain, an increased level of ROS is a well-known pathological characteristic of AD (241–244). Because of its structure, DHA is highly susceptible to oxidative stress. Further studies are needed, especially in advanced stages of AD, to evaluate whether dietary DHA is mainly oxidized and whether the beneficial effect of DHA or the controversial effect of oxidized DHA predominates. This also accentuates the question of at which stage of the disease DHA supplementation might be beneficial.

The important role of DHA during fetal neuronal development is well-documented, validating an actual need for DHA early in life (245). Later in life, an impact of DHA on cognition has been reported (246), but remains controversial, especially in respect to AD (247). DHA is required for neuronal function, signaling, and neuroprotection in general (248–250). In AD, this situation is aggravated because DHA is reduced (251, 252), at least in part, due to a reduced supply in liver and diet and a higher need and turnover due to the ongoing neurodegenerative process (253–255). Epidemiological studies highlighted that seafood consumption is associated with slower cognitive decline in apoE4 carriers (256), which is in agreement with the conclusion of a recent meta-analysis that reported that marine-derived DHA is associated with a lower risk for AD (257). AD clinical trials turned out negative; subgroup analysis, however, revealed interesting details. Mild to moderate AD patients who were apoE4 negative, but not apoE4 carriers, as well as patients with very mild AD, showed reduced cognitive decline (258, 259). Nevertheless, the benefit observed was very small, suggesting that the overall effectiveness of DHA is quite modest, or even absent, in the presence of additional factors that augment the disease process, like apoE4 or an advanced disease phase. This basically leaves two options, to initiate treatment earlier (260) and to enhance the effectiveness of DHA by other means.

MULTI-NUTRIENTS

Multiple dietary molecules have been suggested to target AD relevant pathologies: B-vitamins to reduce brain atrophy (261–264); EPA to boost or exceed the anti-depressive, anti-inflammatory, and vascular benefits of DHA (265); phytosterols to counteract the cholesterol effect (112, 115); choline and uridine to improve cognition (266); and vitamins E and C and selenium as candidates for AD intervention due to
their involvement in oxidative processes (267). Diet per se is a multi-nutrient. It is therefore plausible to combine several of these molecules mentioned above, like B vitamins or uridine with, for example, DHA and other nutrients, with the aim to achieve a neuroprotective effect built on the properties of each individual nutrient. That, in this context, increased effectiveness can indeed be achieved is evidenced by the combined treatment with choline and uridine, which, applied together, increase synaptic protein levels more than the individual molecules alone (268). A suitable starting point for an AD-targeting multi-nutrient diet could be DHA, as the candidate nutrients mentioned above may synergistically work together with DHA. DHA increases spine density, but this can be enhanced by addition of uridine monophosphate (250, 269). Similarly, enhancement of the cognitive and neuroprotective effects of choline and uridine in combination with DHA (266, 270) or of combined B/E/C-vitamins, selenium, choline, EPA, and DHA were noticed (271, 272). In Fortasyn Connect (FC), a multi-nutrient containing all of the nutrients listed above, a complex dietary formulation has been used, which often shows enhanced effects over single nutrients or less complex formulations in vivo. In an elegant in vitro assay on carbachol-induced membrane potential, Savelkoul et al. (273) showed that there was no response from treatment with selenium, choline, uridine, and phospholipids, only DHA and EPA induced a response. Nonetheless, when selenium, choline, or uridine, for example, were successfully added to DHA, the membrane potential change increased (273), providing an example of the important role of DHA to activate the synergistic potential of the above-mentioned molecules or nutrients and showing that the effectiveness of specific nutrients may depend on the dietary context in which they are provided (274).

A linear model has recently been proposed to explain this observation (275, 276). In this model, uridine and other nutrients serve as precursors and cofactors for DHA-enriched membrane synthesis. This then results in synapse fortification and, eventually, memory improvement, combining several biochemical and in vivo findings into a more complex model (275). In addition to possibly working through a single cascading biochemical pathway, multiple nutrients target a number of different cellular processes, some of which may be disease relevant. Koivisto et al. (115) recently compared fish oil (predominantly DHA and EPA), fish oil supplemented with the plant sterol, stigmasterol, or FC in a head-to-head study in 14-month-old APP/PS transgenic mice. Neither diet affected the APP/PS-induced hyperactivity, but all diets improved odor recognition. Only FC had an effect on spatial learning, while only stigmasterol improved memory performance (285, 286), increased neurophysiological measures of synaptic activity, and enhanced functional connectivity in the brain (286, 287), but did not slow cognitive decline in mild to moderate AD (288, 289). Results from the fourth clinical trial, which studies this intervention in prodromal (pre dementia) AD, have not yet been published.

It is now evident that lipids play an important role in AD. In multi-nutrient approaches, the presence of the apoE4 allele may provide serious challenges. Moreover, it must pointed out that the way toward successful implementation of these interventions in therapeutic approaches will require improving effectiveness over what has been achieved currently. Multi-nutrients, earlier intervention within the AD continuum, and combination with other pharmaceutical and nonpharmacological interventions may provide future steps in this direction.

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