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; ADAM, a disintegrin and metalloprotease; AICD, APP intracellular domain; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE, β-secretase APP cleaving enzyme; CSF, cerebrospinal fluid; EPA, eicosapentaenoic acid; FAD, familial Alzheimer's disease; FC, fortasyn; GCS, glucosylceramide-synthase; GWAS, genome wide association study; HMGCR, 3-

hydroxy-3-methyl-glutaryl-CoA-reductase; I-CLIPs, intramembrane cleaving proteases; IDE, insulin-degrading enzyme; LDL, low densitiy lipoprotein; MCI, mild cognitive impairment; NPD1, neuroprotection D1; PE, phosphatidyl-ethanolamine; PS, presenilin; ROS, reactive oxidative species; sAPPα, α-secreted APP; sAPPβ, β-secreted APP; SPT, serin-palmitoyl transferase; S1P, sphingosin-1-phosphat

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 which cleave APP close to and in the lipid bilayer. Moreover, apolipoprotein E4 (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 lipidomic approaches revealed changes in the lipid levels of CSF 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, docosahexaenoic acid and eicosapentaenoic acid as well as on lipid signaling molecules like ceramide and sphingosine-1-phosphat. As nutritional approaches showed limited beneficial effects in clinical studies, the opportunities of combining different supplements in multinutritional approaches are discussed and summarized.

Key words:

Alzheimer's disease, apolipoproteins, lipids, nutrition, sphingomyelin, ceramide, DHA, amyloid-β; multi-nutrients, n3 fatty acids

Introduction

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 apolipoprotein E4 (apoE4) alleles in families with late onset AD (5), a finding immediately confirmed by several other groups (5-8). Among others, Sparks *et al.* showed that cholesterol might represent a molecular risk factor for AD reporting that cholesterol fed to rabbits increased cerebral Aβ deposition (9), a finding that was later successfully reproduced in AD transgenic mouse models (10, 11). Beyreuther and colleagues found that cholesterol increases neuronal Aβ generation (12), which could be reversed by cholesterol depletion (13) and treatment with the cholesterol lowering drug simvastatin successfully lowered cerebral Aβ levels, including the relevant Aβ42 species (14) in guinea pigs. At the same time epidemiological evidence supported the presence of an AD – cholesterol – statin link (15). Cholesterol was identified as an early risk factor for AD (16), indicating that cholesterol plays an important role approximately at the same time when amyloid deposition initiates. Clinical trials however found no benefit of atorvastatin or simvastatin treatment in mild to moderate AD (17, 18).

ApoE

Apolipoprotein E (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 lipoprotein in the

brain and plays an important role in intercellular 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 (GWA) studies that identified genes involved in cholesterol metabolism or transport as AD susceptibility genes (21, 22). GWA analysis identified two novel loci, clusterin (CLU), also known as ApoJ, on chromosome 8 and phosphatidylinositol binding clathrin assembly protein (PICALM) on chromosome 11 (21). CLU 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 support 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 fatty acids (FAs) into the brain and their incorporation into glia and neurons (25, 26). Accordingly, a diet enriched with the omega-3 fatty acid (n3-FA) which is 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). Since 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 sphingosin-1-phosphate 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 carrier as well as in brains of corresponding apoE4 targeted replacement (TR) mice (33).

TR mice in which the endogenous mouse apoE has been replaced be either human apoE4 or human apoE3 have been used extensively to study the mechanisms underlying the pathophysiological effects if apoE4 and the role of lipids in mediating them. Measurements of the effects of apoE2, apoE3 and apoE4 on the brain and plasma levels of glycerolipids and cholesterol did not however reveal a pronounced effect of the apoE genotype on these lipids (34, 35). Nonetheless there was a pronounced effect of diet on the magnitude of the apoE4 phenotype such that the brain pathological phenotype and cognitive effects of apoE4 were reduced by high DHA diet and accentuated by a high cholesterol (34, 36) and carbohydrate diets (37). These human and animal model studies support an involvement of lipids in the pathological effects of apoE4.

Lipidation of apoE4

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 affect differentially the size and extent of lipidation of the apoE particles. The first indication that apoE4 and apoE3 differ in their lipoprotein related properties was obtained over 20 years ago by Weisgraber and colleagues who showed that following incubation with serum lipoproteins, apoE4 but not apoE3 localizes preferentially with vLDL (38). Separation of human CSF apoE by density gradient ultra-centrifugation revealed that apoE4 carriers have the highest level of lipid depleted apoE particles compared with the apoE2 and apoE3 groups and that apoE2 had the highest levels of highly lipidated particles compared to the other groups (39). The effect of the apoE genotype on the size of the CSF apoE particle was assessed by non-denaturing 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). Consistently 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

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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 being addressed in the following sections.

ABCA1 and the lipidation of apoE4

The ATP binding cassette transporter A1 (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 were 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 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 aposE4 can be counteracted by activation of brain ABCA1 activity.

Measurement of the effects of the apoE genotype on apoE serum levels of the 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 apriory apoE4 driven mechanisms such as cross talk with Aβ and synaptic impairments.

Counter acting the brain and cognitive effects of apoE4 by reversal of its hypolipidation: nd Treatment of the 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 hypolipidation 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 were amyloid deposition was reduced by overexpression of ABCA1 (52). Cell culture studies revealed that neurite outgrowth is stimulated by PUFA containing phospholipids suggesting that PUFA play an important role in mediating the lipidation dependent effects of apoE (53). As the phenotype of the apoE4 mice is complex and involves the AD molecules Tau and Aβ as well as synaptic molecules and apoE receptors, additional more targeted models are needed for assessing the cellular mechanisms and processes which drive the apoE4 hypolipidation related effects of apoE4.

ApoE4 lipidation and Aβ: *In vitro* binding studies revealed that human apoE binds to 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 central nervous system and that this physiological mechanism is impaired in apoE4. This was confirmed by *in vivo* studies with mice which express the different human apoE isoforms together with the Aβ precursor APP, which revealed that the $t_{1/2}$ of Aβ in the brain is longer in the apoE4 than the apoE3 mice and that, like in AD, this was associated with increased deposition of Aβ in the apoE4 mice (56-58). nIn view of these findings it is possible that the accumulation of Aβ in hippocampal neurons of the apoE4 mice (49, 59) is due to the hypolipidation of apoE4 and the resulting impaired clearance of Aβ. This suggests that dislipidation 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 Aβ, recent *in vitro* findings suggest that apoE4 and Aβ interact minimally under physiological conditions and that consequently indirect mechansims 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 inhibits or has no effect on this process (60-63). However as recombinant non lipidated apoE4 has the same effect

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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 synaptic mechanism, with 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.

Amyloid precursor protein (APP) processing

Main pathological hallmarks of AD are extracellular amyloid plaques composed of aggregated amyloid-β (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 amyloid precursor protein (APP), a large type I transmembrane protein (69). The amyloidogenic processing of APP is initiated by β-secretase BACE 1 (70, 71) which cleaves APP within its extracellular/intraluminal N-terminal domain, shedding off β-secreted APP (sAPPβ). The remaining membrane-tethered C-terminal fragment (βCTF/C99) is further cleaved by γ-secretase, to release Aβ peptides in the extracellular space (72, 73) and the APP intracellular domain (AICD) in the cytosol which is discussed 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 manifested 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 discussed to be the most toxic variant of Aβ (90-93).

APP secretases

The γ-secretase which generates the C-terminus of Aβ peptides is a heterotetrameric protein complex consisting of Presenilin 1 or 2 (PS1 or PS2), nicastrin, anterior-pharynx defective 1 a or b and presenilin enhancer 2 (94). PS1 or PS2 represent the catalytically active components of the γ-secretase complex (95, 96). Mutations in the presenilins cause familial early onset Alzheimer's disease caused by an increase in Aβ42 peptides and a decrease in Aβ40 (97-100). The γ-secretase complex belongs to the intramembrane cleaving proteases (I-CLIPs) with the peculiar property to cleave transmembrane proteins within their hydrophobic transmembrane domains. I-CLIPs 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 discussed 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, polyunsaturated fatty acids (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 ADAM family (a disintegrin and metalloprotease), 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 non-toxic 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 discussed 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 und lipid regulation (76, 77, 130, 131), which provides a rational why APP cleavage is very sensitive to alterations in the membrane lipid composition (78, 111, 132). Beside a possible direct effect of lipids on APP processing, lipids might be important regulators for lateral movement of proteins within the phospholipid bilayer and are therefore 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 treatmentof AD. The following text provides a brief overview of the role of the major lipids in AD.

Sphingomyelin

Sphingomyelin (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 figure 1. It 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, 3-hydroxy group and partially by the 4,5-*trans* doublebond of the sphingoid-base, SM has a crucial function in forming membrane microdomains. These microdomains, also called lipid rafts, are signalling platforms and are discussed to include the secretases involved in the amyloidogenic pathway (13, 101-104). In *post mortem* brains of patients suffering from AD a reduction in SM and an elevation of ceramide has been reported compared to age-matched control brains (134). Interestingly SM is a precursor for ceramide, a reaction which is catalyzed by the action of the sphingomyelinases (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, inibition of SMases leading to an increased SM level resulted in a decrease of Aβ production (131). In line, mutations in PS1 causing familial Alzheimer's disease (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 *et al.* showing that fibrillary Aβ peptides activate nSMases via NADPH oxidase mediated mechanisms finally leading to the loss of neurons (137, 138). A more recent paper showed a significantly increased nSMase acitivity 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 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; especially in neurons and brain but also in plasma and fibroblasts these transgenic mice showed an increased aSMase activity (139). Combining this transgenic mouse model with an aSMase +/- mouse revealed a significantly reduced Aβ deposition and memory dysfunction (139). Recently a convergent study was published; nSMase 2 deficient mice combined with 5xFAD mice revealed improved cognition and showed an ameliorated AD pathology. Total Aβ42 and plaque burden but also additionally Tau phosphorylation was decreased and the transgenic mice showed an 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 incidence of dementia including AD and mild cognitive impairment (MCI) (142).

Analysing 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). Beside these unambiguous results it should be mentioned that in case of biomarkers the role of SM is not completely understood and controversial results are reported in literature. For example Koal *et al.* report an increased SM species (SM(d18:1/18:0)) to be significantly enhanced in CSF samples of AD patients and are suggested as a biomarker for AD with a specificity of 76% and a sensitivity of 66% with a cut-off of 546nM (144). 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. An 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 to 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 the sphingomyelins 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. In respect to biomarkers the role of SM is not completely understood. Divergent results occur in literature depending on the stage of disease or the biofluid which is analyzed.

Ceramide

The role of ceramide in cellular signaling processes including cell differentiation, proliferation and programmed cell death is well known. Beside the above mentioned SMases, ceramide is also produced by *de novo* synthesis of the serine-palmitoyl transferase (SPT) and some additional enzymes leading to dehydro-ceramide and ceramide in the endoplasmatic reticulum. The concentration of ceramide is tightly linked to the action of the SMases which are already reviewed above. Therefore it is not surprising that most studies found an increased ceramide level associated with AD (134, 148-150). Shot gun lipidomics approaches of early AD stages identified two ceramide species to be significantly lower, additionally five other species trend to be decreased in plasma samples (151). Similar results were found by Mielke *et al.* reporting that high baseline ceramides were associated with an increased risk of AD (152). Interestingly a reduction in Ceramide synthase 2 activity in Braak stage 1,2 in temporal cortex and Braak stage 3,4 in hippocampus and frontal cortex was found (153). Combining the facts that ceramide synthase 2 activity is discussed 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 increasing Aβ level (156). Ceramides have been shown to cause mitochondrial depolarisation and permeabilisation, cytochrom-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 APP intracellular domain (AICD) is a regulator of the expression of the SPT, the committed step reaction in ceramide *de novo* synthesis spanning a complex regulatory cycle (76). Giving a resumee the link between ceramides and AD is widespread. It has to be keepen in mind that ceramide function is both highly dependent of the cellular localisation of the ceramides and the ceramide species especially on the FA bound at the sn-2 position. Whereas ceramides with very long chain FAs are abundant in myelin and discussed 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 Sphingosine-1-phosphate

As mentioned above Ceramidase has been reported to be altered in *post mortem* AD brains (134). Ceramidase catalyses the degradation of ceramide to sphingosine by cleaving of one FA. By the action of sphingosine-kinase sphingosine can be phosphorylated to sphingosine-1-phoshat (S1P). In return, sphingosine-phosphatases can convert S1P to sphingosine. Additionally S1P can be cleaved by S1P-lyase resulting in phosphoethanolamin and hexadecenal which is an irreversible reaction. Especially 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 knock-down 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). Beside 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 that the S1P/sphingosine ratio is declined with increased Braak stage. Additionally the decrease of S1P was found in brain regions which are mostly affected by AD pathology. Interestingly the authors point out an association of these results with the apoE status. The S1P/sphingosine ratio was 2.5fold higher in hippocampus of apoE2 carriers compared to apoE4 carriers (32). In line with these results S1P enhances neuronal cell survival destressed by glucose deprivation and glucose reload stress (160) mediated by the activation of the S1P1- and S1P3-receptor signalling pathways. Recently Fingolimod, a drug already used in multiple sclerosis which is a functional S1P1 receptor antagonist, was used to treat 5xFAD transgenic mice (161). A decreased plaque density accompanied by a decrease in soluble plus insoluble Aβ was found in Fingolimod-treated mice. Additionally Fingolimod attenuated GFAP staining and microglia 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 crosstalk between sphingosine or sphingosine derivatives and AD and to clarify the potential benefits in respect to expected side effects. Furthermore, the above discussed relation between apoE and sphingosine homeostasis suggests that not all patients might equally profit by 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 case of sulfatides the cerebrosid-sulfo-transferase adds a sulfate to the galactosyl-ceramide, a reaction which can be reverted by the arylsulfatase A. Sulfatides

are both present in the white matter, in the myelin but also in membranes of neurons, especially in the axon structure (162). For both, in grey matter and white matter of *post mortem* brains from AD subjects with mild dementia, a decrease of sulfatides has been reported (163). Another study confirmed this finding and revealed that brain samples from subjects with Parkinson disease and in dementia with Lewy Bodies has no changes or even higher sulfatide levels compared to 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 could also be confirmed in transgenic AD mouse models. Interestingly apoE was identified to mediate sulfatide reduction in transgenic mouse models of AD (165) in an apoE isoform dependent manner. Therefore beside S1P also sulfatides as a second lipid class were identified to crosstalk with apoE-mediated biochemical processes. A more recent study confirmed the reduction of reduced sulfatide levels in preclinical stages of the disease (166).

The glucosylceramide-synthase (GCS) adds glucose to ceramide generating the precursor for the gangliosides. Interestingly it has been reported that GCS is regulated by APP processing (82). Similar results have been found for the GD3-synthase (GD3S), another enzyme in the ganglioside pathway, converting *a*- series to *b*-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 the cognitive abilities (129). A more recent study confirming the effect of GD3S deficient mice suggests also an impact of the cholinergic specific ganglioside GT1Aalpha (167) in memory retention. An increase of the β-hexosaminidase which is a catabolic enzyme in ganglioside homeostasis revealed beneficial effects in cognitive tests with transgenic AD mouse models (168). The importance of this catabolic enzyme is also underlined by a study showing a changed hexosaminidase and 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) and additionally gangliosides have been shown to bind Aβ and therefore influencing the aggregation of Aβ leading to

oligomers, protofibrils and fibrils (173-178). In line ganglioside-Aβ complexes have been discussed to build aggregation seeds for oligomers and plaques (108). Not only during aging but also by comparing brain samples of AD patients with age-matched controls revealed a complex change in ganglioside homeostasis. However it has to be pointed out that the heteregenous results in literature might be explained that changes seem to be brain region specific and differ in the pathological stages of AD (96, 179-182). For ceramides it has already been discussed that the FA also determines the impact of the individual lipid in AD relevant processes (153). Similar results have been found in respect to gangliosides further explaining different results in literature and emphasizing the need of exact and detailed lipidomic analysis including not only the headgroup but also the FA bound at the sn-1 and sn-2 position.

Plasmalogens

Plasmalogens are ether-phospholipids characterized by a vinylether bond at the sn-1 position and an ester linkage at the sn-2 position. The chemical structure of the plasmalogens are presented in figure 2. Several studies showed a decrease in phosphatidylethanolamin- and phosphatidylcholin-plasmalogens in AD *post mortem* brains (119, 121, 183). Analysing PS mutation carriers, a study revealed a decrease in two plasmalogen species (PE34:2, PE35:4) in the CSF which correlated with an increased Tau level in CSF and an increased amyloid burden in the brain (184). Pasmalogens are also found to be decreased in erythrocytes of children with Down syndrom. As Down syndrom is a chromosaml aberation on chromosome 21 (Trisomie 21) and APP is located on chromosome 21 (185), these results further strenghten the linkage between APP processing and plasmalogen homeostasis (186). As the vinylether is highly susceptible for reactive oxidative species and oxidation, the reduced plasmalogen levels could be caused by the increased oxidative stress occuring during AD pathogenesis. However, we could demonstrate that a reduced plasmalogen level is not only 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 been recently confirmed by another group demonstrating that an increased PE-plasmalogen to PE ratio results in an inhibition of γ-

secretase activity (187). Plasmalogens do not only affect APP processing but also in return APP procesing tightly regulates plasmalogen homeostasis (77). AICD decreases the expression of the enzyme catalyzing the comitted step reaction in plasmalogen synthesis, the alkyl-dihydroxyacetonephosphate-synthase (AGPS), leading to a 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). These results suggest under physiologogical conditions 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. Beside the reduction of plasmalogens by ROS, a reduced AGPS protein stability due to peroxisomal dysfunction was reported leading to a reduced plasmalogen level. The reduced plasmalogen level results in an elevated Aβ production further enhancing oxidative stress and peroxisomal dysfunction. Summing it up the regulatory feed-back cycle, beeing necessary to adjust a homeostasis, is converted to a feed-forward or futile cycle under pathological conditions enhancing Ab production. Because of its potential benefits in AD as a nutritional supplement, efforts have been made to identify sources of plasmalogens in food like marine invertebrates or 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 on the 1st International Plasmalogen Symposium in Japan results were presented suggesting beneficial effects of plasmalogens in cinical studies of patients with MCI.

Eicosapentaenoic acid and other polyunsaturated fatty acids

Also in a lower extent than DHA, the n3 PUFA eicosapentaenoic acid (EPA) is present in brain (190). Figure 3 illustrates the structure of DHA and EPA. Mechanistically it has been shown that EPA is able to enhance Aβ degradation mediated by 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 to 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.* reported that increasing brain n3-PUFAs decreases some aspects of the inflammatory response to Aβ (192). 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 analysing wt littermates which were orally administered to 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 to wt mice fed with the safflower oil diet, fat1 transgenic mice and wt mice fed with the fish oil diet showed higher levels of brain DHA whereas n6-PUFAs were decreased. Microglia activation was reduced in fat1 transgenic mice at 10 days post-surgery. The wt mice fed with the fish oil diet showed no effect on microglia activation but revealed fewer degenerating neurons.

Additionally n3-FA supplementation was reported to increase Aβ phagocytosis by makrophages and influences brain amyloidosis. However it has to be mentioned that in this study n3-FAs have been combined with antioxidants and reservatol, so that no clear mechanistical conclusions to single compounds can be drawn and further experiments are needed preferable with a higher n-number (193). In context of inflammation and PUFAs it shoud be discussed that effects might also be mediated by dihydroxy- or trihydroxy-metabolites of n3-FAs which are called resolvins. Resolvin D1 was shown to be associated with an increased Aβ phagocytosis (194) and might also inhibit fibrillar Aβ induced apoptosis (195). Another enzymatic derivative of n3-FA, neuroprotectin D1 (NPD1), was suggested to downregulate Aβ production, pro-inflammatory gene expression and promotes cell survival. In the anti-apoptotic bioactivity induced by NPD1, PPAgamma signalling may be involved and a shift from the amyloidogenic to the non-amyloidogenic pathway is discussed to be resposible 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 this journal (197).

Although EPA is often combined with DHA in nutritional approaches (198, 199) mechanistical 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 an increased sAPPα 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 *post mortem* brains. Interestingly for linolenic acid (18:3) and DHA it was shwon that these lipids can increase α-secretase activity utilizing purified ADAM10 enzyme (114). Together with the result that PUFAs are decreased in human *post mortem* AD brains (121, 202), the anti-inflammatory properties, the effect on the non-amyloidogenic pathway and on Aβ clearance, also other unsaturated FAs than DHA might be interesting targets in AD especially combined with other nutritional approaches. Remarkably, not only the desaturation, the number and position of double-bonds of FAs seem to influence AD relevant mechanisms. Also the conformation is known to have an important impact. Under physiological conditions double-bonds have a *cis*-conformation resulting in an angled, non-linear orientation of the FA in the biomembrane. Especially the resulting angle determines physical properties like membrane fluidity. In contrast *trans*-FAs have a linear orientation and the membrane is more condensed and tightly packed. *Trans*-FAs, which e.g. naturally occur in products of ruminents, 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 to the corresponding *cis*-conformation (117). In line epidemiological studies revealed that individuals who had an intake of *trans*-FAs of 4.8g/day showed a 5fold higher relative risk to develop AD than subjects consuming 1.8g/day (203) and dietary intake of *trans*-FAs is asscoiated with cognitive decline in the elderly population (204, 205).

Docosahexaenoic acid

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). 30 – 40 % 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 again to underline the importance of apoE in sulfatide homeostasis as reviewed above, but also in the transport of PUFAs across the blood-brain-barrier indicated by the recent literature (211, 215).

After being incorporated in 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 sAPPα (α-secreted APP) level by an increase in ADAM17 protein levels caused by an upregulated gene expression and a 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 e.g. in the endosomes (201). As BACE1 activity has its optimal activity at an acidic pH-value which is found in endosomes (217-220), the altered localisation of BACE1 might act as an explanation for a decreased BACE1 activity in presence of DHA. For γ-secretase, both, 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 comitted step reaction, the 3-hydroxy-3-methyl-glutaryl-CoA-reductase (HMGCR), was decreased in presence of DHA (201, 221). Cholesterol is known to increase amyloidogenic and to decrease non-amyloidogoenic APP processing (13, 107, 112). Therefore a reduction in cellular cholesterol levels also contribute to the pleiotropic DHA-mediated effects which all synergistically result in a decreased Aβ level. Aβ level are not only dependent on Aβ production but also on Aβ catabolism. One of the major enzymes being involved in Aβ degradation is

the insulin-degrading enzyme (IDE) (222). Phospholipids containing DHA (22:6) compared to phospholipids with the corresponding saturated FA (22:0) have been shown to directly bind to IDE (136). In addition purified recombinant IDE enzyme showed an 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 an increased Aβ degradation further enhancing the effect of a decreased Aβ level in 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 in neuronal development and axon outgrowth (224). Moreover, DHA-containing phosphatidyl-choline improved the cognitive deficits in an AD rat model. The authors suggest as a possible mechanism a decreased phosphorylation of Tau in cortex and hippocampal CA1 area (225). *In vitro* studies also indicate that DHA could inhibit and even reverse the formation of Aβ oligomers and therefore decreases Aβ-associated neurotoxicity (226-228), which could be recently confirmend in an APP/PS1 rat model of AD emphasizing that dietary DHA modulates Aβ oligomerisation (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 been also found to be an agonist of several nuclear receptors, e.g. the retinoic X receptor (RXR) (230, 231) and peroxisome-proliferator receptor (PPAR) α 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 endogenous ligand for RXR (230, 231), a ligand-activated nuclear transcription factor, important for reproduction, cellular differentiation, bone development, hematopoesis and pattern formation during embryogenesis. DHA was found to be specific for RXR, whereas DHA failed to activate the retinoic acid receptor (RAR), 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 seems to be not exclusive for DHA, as additional unsaturated fatty acids like docosatetraenoic acid,

docosapentaenoic acid, arachidonic acid and oleic acids 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 (LXR):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, inflammatory processes and restored the impaired working memory of these transgenic mice.

In the paragraph 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 and especially EPA and DHA are also associated with major depressive disorder suggesting independently 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 transgenic AD mouse model did not only increase the DHA level; further other changes in FAs were found, e.g. 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), also including 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 even reverses its action leading to increased amyloidogenic pathways (239). 1% oxidized DHA in presence of 99% unoxidized DHA was sufficient to increase Aβ production in neuroblastoma cells. Interestingly not only one species of oxidized DHA revealed a high amyloidogenic potency, all seven oxidized DHA or lipid peroxiadtion products elevated the production of Aβ (239). These results strongly emphasize the need to prevent oxidative reactions of DHA e.g. by adding additional antioxidants. In this context it has to be mentioned that the hydroxylated form of DHA (DHA-H) has been recently 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. Orally administration of DHA-H to 5XFAD mice for 4 months also improved cognitive scores of these mice evaluated by the radial arm maze (RAM) 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 to evaluate if dietary DHA given especially in advanced stages of AD are mainly oxidized and if the beneficial effect of DHA or the controversial effect of oxidized DHA predominates. This also accentuates the question at which stage of disease DHA supplemenation 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). An impact of DHA later in life 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 reduced supply trough 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 overall effectiveness of the 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, e.g. B-vitamins to reduce brain atrophy (261-264), eicosapentaenoic acid (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), vitamins E/C and selenium as candidates for AD intervention due to their involvement in oxidative processes (267). Diet *per se* is a multi-nutrient. It therefore is plausible to combine several of those molecules with the aim to achieve a

neuroprotective effect build on the properties of each individual nutrient. That in this context increased effectiveness can indeed be achieved is evidenced e.g. 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 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 (FC), a multi-nutrient containing all of those nutrients listed above, a complex dietary formulation has been used, which often showed *in vivo* enhanced effects over single nutrients or less complex formulations. In an elegant *in vitro* assay on carbachol-induced membrane potential Savelkoul and colleagues showed that for treatment with either of the above-mentioned vitamins, selenium, choline, uridine and phospholipids none showed any response. Only DHA and EPA did. Nonetheless, when these molecules were successively added to DHA the membrane potential change increased (273), providing an example for the important role of DHA to activate the synergistic potential of those dietary molecules and 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 these molecules serve as precursors and co-factors for DHA-enriched membrane synthesis. This would then result 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 trough a single cascading biochemical pathway, multiple nutrients will target a number of different cellular processes, some of which may be disease relevant. Koivisto 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 (115). 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 habituation and suppressed microglial activation. In another approach treatment with DHA+EPA+uridine largely, and with FC almost entirely, ameliorated white and gray matter structural

integrity. FC was more effective on aspects of neuronal repair and maintenance, neurogenesis and anxiety-related behavior. Other AD related pathologies targeted by this multi-nutrient approach include cerebral blood flow and perfusion, secretase activity, amyloid pathology, hippocampal neurotransmission and apoE4 related pathologies (36, 115, 277-281). Taken together these results indicate that lipid based multi-target based approaches have the potential to be more effective than treatment with single lipids. While dietary habits have been extensively studied in AD and concluded that healthy dietary patterns, e.g. adherence to a Mediterranean diet or regular fish consumption may reduce the dementia risk (257, 282), few clinical trials studied specific multi-nutrient intervention. B-vitamin supplementation in patients with mild cognitive impairment in a post-hoc analysis resulted in reduced brain atrophy and increased cognitive improvement, which was limited to that subgroup of patients which had the highest blood-level of n3-FAs, especially DHA (264, 283, 284). A set of four clinical trials based on the FC multi-nutrient formulation, showed 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 been published thus far.

It is now evident that lipids play an important role in AD. Presence of the apoE4 allele may provide serious challenges as well as on the way towards Successful implementation 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 non-pharmaceutica interventions may provide future steps in this direction.

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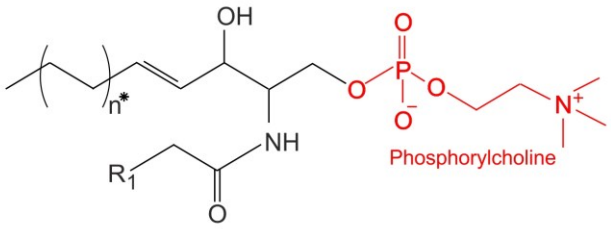
Figure 1:

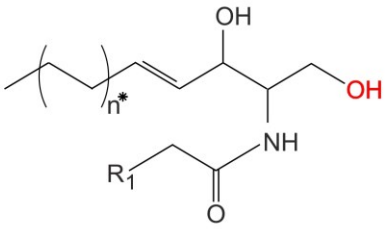
Sphingomyelin (SM):



R_1 = different acyl chains

n*= in many cases 6

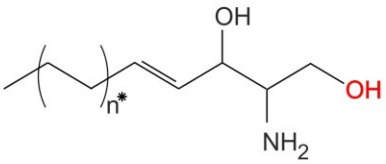
Ceramide (Cer):



R_1 = different acyl chains

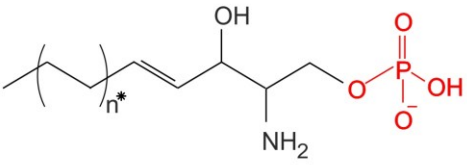
n*= in many cases 6

Sphingosine (Sph):



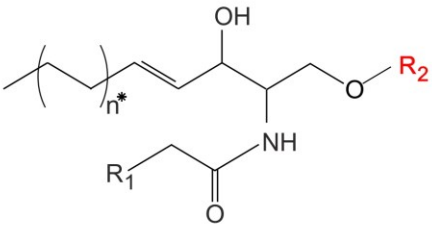
n*= in many cases 6

Sphingosine-1-Phosphate (S1P):



n*= in many cases 6

Gangliosides



R_1 = different acyl chains

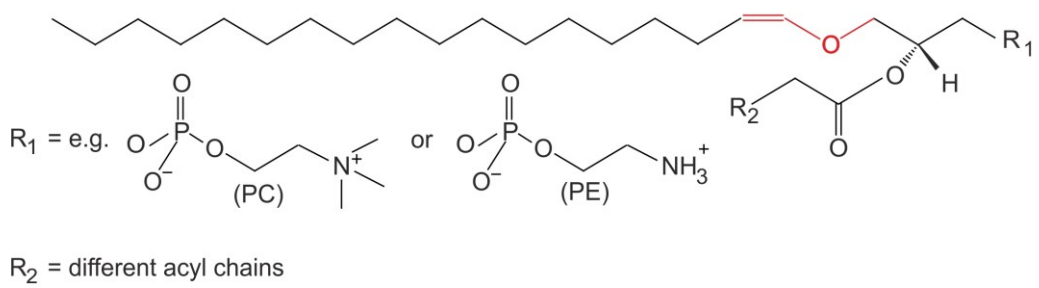
n*= in many cases 6

R_2 = different carbohydrates containing one or more sialic acids and derivates and different sugars

Figure 1: Chemical structure of the sphingolipids which are altered in AD.

Figure 2:

Plasmalogen (PL):

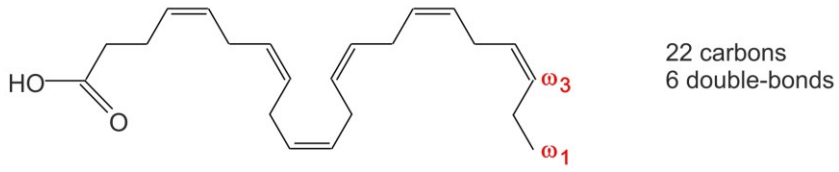


R2 = different acyl chains

Figure 2: Chemical structure of plasmalogens

Figure 3:

DHA:



EPA:

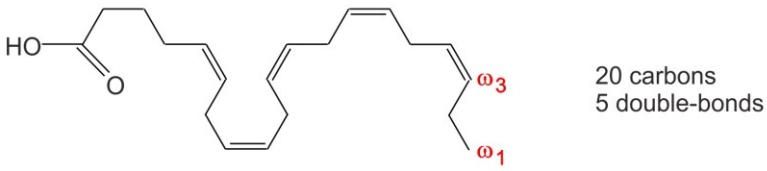


Figure 3: Chemical structure of the fatty acids DHA and EPA